AHP-0001

Allied Health Professionals (Including Nurses & Dieticians)

PREVENTION OF PAEDIATRIC REFEEDING SYNDROME: A NATIONWIDE SURVEY OF CURRENT PRACTICE AMONGST PAEDIATRIC DIETITIANS

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Objectives and Study: To date no randomised controlled trials for the prevention of paediatric refeeding syndrome have been performed. There is no consensus agreement for the preventative management of patients at risk of paediatric refeeding syndrome amongst medical professionals in the UK. Refeeding syndrome has been described as severe electrolyte and fluid shifts associated with metabolic abnormalities in malnourished patients undergoing refeeding, whether orally, enteraly or parenterally. (Crook et al 2001) The clinical sequelae of refeeding syndrome may adversely affect nearly every organ system and include cardiac dysrhythmias, heart failure, acute respiratory failure, nephropathy, liver dysfunction, coma and paralysis. The biochemical and clinical sequel of the refeeding syndrome are reported in the literature in a number of clinical specialities including in Anorexia Nervosa, critical illness and gastrointestinal disease.

Methods: A nationwide survey of dietetic practice on the management of paediatric patients thought to be at risk of developing refeeding syndrome. State registered Paediatric Dietitians self completed a questionnaire. Responses were obtained from 138 paediatric dietitians working in the acute centre 76% (n = 105) and community setting 19% (n = 26).

Results: Of Paediatric Dietitians surveyed 87% (n = 120) reported to have no protocol for clinical prevention of refeeding syndrome in the workplace and 66% rely on varied clinical judgement rather than using published guidance. 97% (n = 134) of those surveyed felt it would be useful to have national guidance in place for the management of refeeding syndrome. Practice varied with gradual feed introduction (full feeds being reached after a median of 5 days (range 1 day to 10 days). Of those surveyed 25% (n = 34) reported to have seen clinical, and 63% (n = 87) biochemical consequences of refeeding syndrome in their patients. This exceeds levels of biochemical imbalances reported in the literature. (Ashworth A et al, 2004).

Conclusion: The survey data suggest inconsistencies in dietetic practice and approaches to managing patients at risk of the refeeding syndrome. The development of a guidance document for the clinical management of these patients would help standardise care, prevent unnecessary underfeeding in malnourished patients and reduce the risk of serious complications of refeeding syndrome occurring in paediatric care.


Disclosure of Interest: None declared.

AHP-0002

Allied Health Professionals (Including Nurses & Dieticians)

PATIENT-DRIVEN LEARNING AND SYMPTOM MONITORING USING HANDHELD TECHNOLOGY: A NEW PERSPECTIVE ON EDUCATION AND COUNSELING IN THE MULTIDISCIPLINARY PEDIATRIC INFLAMMATORY BOWEL DISEASE TEAM

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Objectives and Study: When faced with a life-changing event such as a diagnosis of inflammatory bowel disease (IBD), young patients and their families are frequently overwhelmed by the volume and complexity of the information given to them. A recent audit conducted within our Division has identified important deficits in the knowledge of patients and parents with regards to disease location and previous investigation results (Benchimol et al. IBD 2010). Reporting of IBD-related symptoms and compliance with medication are particularly troublesome in the teenage years (Hommel et al. IBD 2009), thus impeding the delivery of adequate IBD care. Our aims were to design a novel way of empowering young patients and their families to come to terms with the diagnosis of IBD, to enable patient-driven
1. Benchimol et al. References activity and compliance. patient-driven learning and real time recording of disease use on handheld devices. This app has enabled ongoing has resulted in the development of a dedicated application for Conclusion: for a transition to adult care.

2. Hommel et al. Results: Until now, IBD-related information was most often delivered to young patients and their families at the time of diagnosis or during disease flares using printed material. Consequently, IBD-education was mostly directed at parents/guardians with children/teenagers often too unwell to make full use of the provided counseling, in spite of the increased time commitment by particularly IBD Nurse Specialists worldwide. This application has given our young patients and their families the opportunity to preview/review the information given during the face-to-face meeting with a member of our IBD-team. Thus, the time spent with the health professional can be more focused on answering questions. Within the same app, we have included a feature to monitor disease activity and treatment compliance in real time. This has allowed our young patients to take control of their symptom reporting, to generate a clinical summary-pdf containing an IBD-video-academy, a dedicated IBD-educational game and a real time recording feature of disease activity and compliance with medication, which will be beta-tested during the Spring of 2011 and presented at the meeting. Conclusion: Our innovative approach to pediatric IBD-care has resulted in the development of a dedicated application for use on handheld devices. This app has enabled ongoing patient-driven learning and real time recording of disease activity and compliance.

References

Disclosure of Interest: None declared.

AHP-0003

Allied Health Professionals (Including Nurses & Dieticians)

SPECIALIST NURSE AND DIETITIAN CARE PATHWAY FOR EXCLUSIVE ENTERAL NUTRITION IN PAEDIATRIC CROHN’S DISEASE— A TERTIARY CENTRE EXPERIENCE

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Objectives and Study: Exclusive enteral nutrition (EEN) is effective in inducing remission in paediatric patients with Crohn’s disease. Our centre uses an 8 week course of EEN in the treatment of Crohn’s disease in children. This can be a challenging therapy for the patient and their family. Previously clinical review and decision for steroid therapy was medical. It was also our perception that patients and families received inadequate support. We developed a pathway to co-ordinate the patient journey when EEN was commenced. The specialist nurse (SN) and specialist dietitian (SD) provide support and clinical review during the 8 week course. We describe the first 12 months of this service.

Methods: Following initiation of EEN, the patient and family were telephoned twice a week for 2 weeks, once by the SN and once by the SD. Calls assessed feed tolerance, compliance and symptom control. If full volumes were not achieved orally by day 3 of therapy, the patient was admitted and a nasogastric tube (NGT) passed. The SN and SD also aimed to address non-medical and social issues. A 4 week review comprised history and examination by the SN. The SD assessed weight change and adequacy of nutritional intake. Feed volume and concentration were altered accordingly. Bloods were taken if there was clinical suspicion of treatment failure, therefore PCDAI was only recorded in this instance. The decision for inpatient admission, NGT or treatment with oral steroids was made independently by the SN, a non-medical prescriber, and the SD. Calls assessed feed tolerance, treatment with oral steroids was made independently by the SN, a non-medical prescriber, and the SD. 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Objectives and Study: Hospital, London, United Kingdom. most presenting with vomiting or abdominal pain and feed-

DIFFICULTIES GORD is a common problem in infants and children, with GORD and MDT management by a feeding team consisting of gastroenterologist, dietician, Speech-Language Therapist and Nutrition Nurse Specialist. Our aim is to assess the role of MDT in the management of infants and children with GORD.

Methods: An invitation letter to fill in a questionnaire was sent to all caregivers whose children had been examined by endoscopy during Jan 1- Oct 30, 2010. 78 children underwent gastroscopy and 51 children underwent colonoscopy. The questionnaire was sent by letter and included a secured link on the Internet leading to the questionnaire. We asked if the caregivers would have wanted to stay instead of leaving their sleeping child in the examination room, and what they would expect as benefits and drawbacks from staying.

Results: The responses for gastroscopy were 19 for gastroscopy (G) and 14 for colonoscopies (C). Distribution in age groups were 1–3 years 21% (G) 7% (C), 4–6 years 32% (G) 7% (C), 7–10 years 16% (G), 28% (C), 11–13 years 21% (G), 14–18 10% (G) 57% (C). 31% of G and 46% of C stated that they would have liked to be present during the endoscopic procedure. The motives to stay were “to watch how the procedure is performed” 58% (G), 28% (C) “get instant information of results” 25% (G) 71% (C) “less worried about what happens to my child” 41% (G) 28% (C) and “maintain control of what goes on” 50% (G), 43% (C). The motives for not wanting to stay were “unpleasant to watch my child under GA” 62% (G) 67% (C); “unpleasant to watch the procedure” 69% (G) 44% (C); “worried to immediately get a distressing result” 55% (C).

Conclusion: We conclude that caregivers may be invited to stay during endoscopy procedures on their anesthesised child, however there may be reluctance among caregivers of young children. A planned prospective study aiming to recruit a higher response rate may add further information.

Disclosure of Interest: None declared.

AHP-0005

Allied Health Professionals (Including Nurses & Dieticians)
MULTIDISCIPLINARY MANAGEMENT OF CHILDREN WITH GASTRO OESOPHAGEAL REFLUX DISORDER AND BEHAVIOURAL FEEDING DIFFICULTIES—A SINGLE-CENTRE EXPERIENCE
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Objectives and Study: Gastro oesophageal reflux disorder (GORD) is a common problem in infants and children, with acid related symptoms but food refusal can be difficult to manage, often causing the families significant levels of anxiety. We have a multi disciplinary team (MDT) approach to manage children with FD and our team consists of a Consultant Paediatric Gastroenterologist, Dietician, Speech-Language Therapist and Nutrition Nurse Specialist. Our aim is to assess the role of MDT in the management of infants and children with GORD.

Results: All patients were referred by a paediatrician or neonatologist, with FD and a diagnosis of GORD. Total of 21 children was seen during this period and 66% of children children were seen more than once, with maximum of 6 appointments (median 2) per patient. The median age at presentation was 17months (range 6–36 months) and median duration of GORD symptoms prior to the first appointment was 13 months with a range from 6 – 35 months. 33% of children seen in clinic were discharged after the initial assessment following advice for behavioral FD alone and others needed changes in the management of GORD in addition to advice. Anti reflux medications were started approximately 2 months (median 2) after the onset of GORD symptoms and 80% of children were treated with more than one medication for GORD. Domperidone (80%) with a combination of omeprazole (71%) or ILansoprazole (14%) was the commonest choice. Overall improvement in severity of FD over the duration of clinic care was 97%. There was an agreement in objective scale rating (median 2, range 1–6) for severity of FD by professionals and parents in 66% of children. Parental anxiety was a major problem and 23% of children from our clinic were referred to the psychology services for further help.

Conclusion: Behavioural FD is common in children with GORD and MDT management by a feeding team consisting of gastroenterologist, dietician, Speech-language pathologist, and nutrition nurse specialist will improve the outcome of children with FD. Involving psychologist will help to alleviate parental anxiety and behavioural difficulties.

Disclosure of Interest: None declared.

AHP-0006

Allied Health Professionals (Including Nurses & Dieticians)
BEHAVIOURAL FEEDING DIFFICULTIES IN CHILDREN: A SURVEY INTO CURRENT PRACTICE AND PROVISION PROVIDED BY SPEECH-LANGUAGE THERAPISTS IN THE SOUTHEAST OF ENGLAND

www.jpgn.org
hoe the majority of SLTs working in the field of SLTs in the field of paediatric behavioural feeding difficulties was circulated among 175 SLTs attending the London Paediatric Dysphagia Special Interest Group. The aim was to survey how many SLTs working within Paediatric Dysphagia see children with BFDs, what the service provision for these children is and what access they have to the relevant MDT members. The completed questionnaires were collated with the quantitative data statistically analysed to quantify current practice and identify significant correlations between variables. Qualitative data was compiled to provide insight into the views of SLTs on working with this client group.

Results: Completed surveys were collected from 144 SLTs (82%), of which 123 (85%) indicated that the SLT works with children with BFDs. The primary reason for not working with these clients is that this area is not within the remit of the SLT. 41 (33%) SLTs working with children with BFDs take the sole professional lead in coordinating care for these children and an additional 25 (20%) manage these cases as part of an MDT. 42 (34%) SLTs indicate that coordination of care for children with BFDs is uncertain or follows not set pathway. Significant positive correlations were found between the inclusion of a psychologist on the MDT and provision of a psychology service to children with BFDs separate to that provided by the SLT (r=0.46***), although 23 (19%) SLTs working with children with BFDs have no access to psychology. 41 (35%) SLTs see children with BFDs in an MDT clinic, with a significant positive correlation of the number of SLTs working in a team and those working in the acute setting (r=0.235*).

Conclusion: The majority of SLTs working in the field of paediatric dysphagia provide a service for children with BFDs, whilst many services lack a clear care pathway for managing this client group. SLTs are most likely to see children with BFDs as part of an MDT when working in the acute setting, and SLTs have greater access to support from a psychologist when a separate service is provided by psychology. The literature suggests that an MDT approach is necessary to manage the often complex underlying causes of behavioural feeding difficulties, however results from this study suggest that only a small minority of cases are currently dealt with in this way.

Disclosure of Interest: None declared.

Objectives and Study: To quantify aspects of current practice among Speech and Language Therapists (SLTs) in the management of children with Behavioural Feeding Difficulties (BFDs) via a questionnaire based survey to highlight the role of the SLT within the field.

Methods: A questionnaire devised by a working group of SLTs in the field of paediatric behavioural feeding difficulties was circulated among 175 SLTs attending the London Paediatric Dysphagia Special Interest Group. The aim was to survey how many SLTs working within Paediatric Dysphagia see children with BFDs, what the service provision for these children is and what access they have to the relevant MDT members. The completed questionnaires were collated and the quantitative data statistically analysed to quantify current practice and identify significant correlations between variables. Qualitative data was compiled to provide insight into the views of SLTs on working within this client group.

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Disclosure of Interest: None declared.

PL-G-0007

Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori

4-AMINOBUTYRATE AMINOTRANSFERASE (ABAT): GENETIC AND PHARMACOLOGICAL EVIDENCE FOR AN INVOLVEMENT IN GASTROESOPHAGEAL REFUX DISEASE

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Methods: Thirty-six families demonstrating dominant transmission of GERD were subjected to whole genome microsatellite genotyping and subsequent linkage analysis. We collected two additional independent GERD patient cohorts, one consisting of 219 trios (affected child with parents) and the other an adult GERD case control cohort consisting of 256 cases and 485 controls. These additional patient cohorts were collected in order to identify and validate genes through SNP genotyping and association analysis. Functional validation was performed in dogs using pharmacological intervention.

Results: Five linked regions were identified in the family collection. Two families shared a linkage region on chromosome 16 with a combined logarithm of the odds (LOD) score of 5.5. To investigate if this region harbors a GERD associated gene, 66 single nucleotide polymorphism (SNP) markers distributed over the nine genes present in the linked region were genotyped in the independent GERD trio cohort. Transmission disequilibrium test analysis followed by multiple testing adjustments for all 66 SNPs genotyped revealed significant genetic association for one SNP located in an intron of the gene 4-aminobutyrate aminotransferase (ABAT) (Pcorr=0.027). The selective ABAT inhibitor Vigabatrin (g-vinyl GABA) reduced transient lower esophageal sphincter relaxations (TLESRs) in dogs by 57.3 ± 11.4% with a p-value of 0.007 and the reflux events from 3.1 ± 0.4 to 0.8 ± 0.4 with a p-value of 0.007.

Conclusion: The overlapping linkage peak identified in two families led us to investigate the nine genes in this linked region. Association analysis in the trio cohort identified ABAT as a GERD associated gene. Pharmacological inhibition of ABAT enzyme function significantly reduced TLESRs and acid reflux in dogs. Our results demonstrate the direct involvement of ABAT in pathways affecting lower...
epithelial sphincter control and identify ABAT as a genetic risk factor for GERD.

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**Disclosure of Interest:** None declared.

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**PL-G-0008**

**Food Allergy**

**CD25+CD4+ TREGS MEDIATE THE PROTECTION FROM ORAL PEANUT-INDUCED ESOPHAGEAL LESIONS OF SENSITIZED MICE TREATED BY EPICUTANEOUS IMMUNOTHERAPY**

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**Objectives and Study:** In mice sensitized to peanut, sustained oral ingestion of peanut protein extracts (oral PPE) induces eosinophilic infiltration of the esophageal mucosa comparable to eosinophilic esophagitis (EoE) and villous atrophy in jejunum. When sensitized mice are previously treated by epicutaneous immunotherapy (EPIT), lesions decrease dramatically, together with a decrease of IL5, IL13 and eotaxin expression and an increase of FoxP3 expression in the esophageal mucosa (Mondoulet, ESPGHAN 2010). These findings suggest a role for T regulatory cells (Tregs) in EPIT. The aim of this study was to confirm the EPIT-induced Treg mechanism in the prevention of EoE occurring in sensitized mice following oral PPE.

**Methods:** First, 2 groups of 8 Balb/c mice were orally sensitized by PPE with cholera toxin during 6 weeks, then treated by EPIT or sham using a patch (Viaskin) with 100 μg of PPE 48h once a week for 8 weeks. In one group, Tregs were blocked by injection of anti-mouse CD25 antibody (αCD25) once a week for 8 weeks. The 2 groups (EPIT and EPIT+αCD25) were compared to sensitized untreated (Sham) and naïve groups after oral PPE. Second, CD25+CD4+ Tregs were sorted from spleen cells of EPIT and Sham treated mice and transferred to PPE-sensitized mice which then received oral PPE. At the end of experiments, esophageal samples were taken for histology and mRNA analysis.

**Results:** As previously described, oral PPE induced EoE in Sham treated mice and increased eotaxin expression, which were prevented in EPIT treated mice (respectively \(36.39 \pm 5.61 \) vs \(13.30 \pm 4.07 \) eosinophils per mm\(^2\)(E/mm\(^2\)), and \(2.11 \pm 0.29 \) vs \(1.17 \pm 0.15 \) eotaxin relative mRNA (rmRNA), \(P < 0.05\)). Also, the esophageal expression of FoxP3 increased in EPIT compared to Sham (respectively \(2.04 \pm 0.49 \) vs \(0.85 \pm 0.15 \) rmRNA, \(P < 0.05\)). αCD25 reversed the effects of EPIT in the esophagus (\(38.93 \pm 7.13 \) E/mm\(^2\), \(2.35 \pm 0.62 \) eotaxin rmRNA and \(0.71 \pm 0.18 \) FoxP3 rmRNA; \(P < 0.05\) vs EPIT). Transferring Tregs from EPIT treated mice to PPE sensitized mice submitted to oral PPE prevented eosinophil infiltration and eotaxin expression, and induced FoxP3 in esophagus whereas cell transfer from Sham treated mice demonstrated no effect.

**Conclusion:** Induction of Tregs by EPIT is able to prevent EoE in mice sensitized to peanut confirming the Treg mediated mechanism of EPIT.


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**PL-G-0009**

**Inflammatory Bowel Disease**

**INDUCIBLE T-CELL CO-STIMULATOR LIGAND (ICOSLG) INFLUENCES CROHN’S DISEASE SUSCEPTIBILITY IN THE SCOTTISH PAEDIATRIC INFLAMMATORY BOWEL DISEASE (IBD) POPULATION**

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**Objectives and Study:** Inducible T-cell costimulator (ICOS) and its ligand ICOslg are involved in the proliferation and differentiation of T-lymphocytes. A locus on Chr. 21q22 harbouring the ICOSLG gene is associated with susceptibility to adult and paediatric Crohn’s disease (CD) and ulcerative colitis (UC). Our aim was to perform a gene-wide haplotype-tagging association study of the ICOSLG gene to delineate the region of strongest association to guide future deep sequencing studies.

**Methods:** 416 IBD patients <17yrs at diagnosis (278 CD, 101 UC, 37 IBD-unspecified) and 735 parents (276 complete trios) were genotyped for 4 Single Nucleotide Polymorphisms (SNPs) tagging the two haplotype blocks encoding ICOSLG as well as the region extending to rs762421 which achieved genome-wide significance in the paediatric IBD Genome-wide association study.(1) Detailed phenotypic characteristics of this cohort were previously described.(2) SNPs were selected using HapMap data. Detailed single marker and haplotype analysis by transmission disequilibrium testing (ParentTDT) was carried out using Haploviz (permutation analysis, n = 100,000).

**Results:** The two-marker haplotypes (rs762421A/G - rs8126734A/G and rs283529G/C - rs4818890C/A (both located within the 3’UTR of ICOSLG) showed weak
associations with overall IBD susceptibility ($P < 0.05$) which did not retain significance after permutation analysis. However, the strength of this association increased substantially when we focused our analysis on childhood-onset CD. After stringent permutation analysis, the rs8126734A allele showed significant overtransmission to CD patients ($P = 0.02$, $D'$ and $r^2$ with rs762421 was 0.78 and 0.21, respectively). The two-marker haplotype rs762421A-rs8126734G also showed significant distortion of transmission ($P = 0.03$). Using a sliding-2 marker haplotype analysis, we found that association signals do not extend upstream from rs8126734, thus implicating the 3' interval between rs762421 and rs8126734 as a target region for deep sequencing.

**Conclusion:** We have applied the first family-based association analysis of ICOSLG in childhood onset CD, thus minimising the effect of population stratification. We demonstrated that the signal at the 21q22 locus is due to germline variation at the 3' end of ICOSLG. Deep sequencing of the 3' UTR of the ICOSLG gene is now warranted to identify causative variants, potentially affecting mRNA stability.

**References**

**Disclosure of Interest**
None declared.

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**PL-G-0010**

**Coeliac Disease and Enteropathies**

**SPECIFICITY OF DOUBLE COLOUR IMMUNOFLUORESCENCE STAINING FOR INTESTINAL IGA-TRANSGLUTAMINASE DEPOSITS: COMPARISON WITH PHAGE DISPLAY ANTIBODY LIBRARY**

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**Objectives and Study:** Coeliac disease (CD) is a multifactorial disease characterized by the synthesis of anti-transglutaminase 2 (anti-tTG) antibodies (Abs) VH5 gene family dependent in the small bowel mucosa. Moreover their presence seems predictive of future CD even in the absence of intestinal damage and serum endomysial (EMA) and anti-tTG Abs.

The aims of our study were to verify the specificity of the direct double colour immunofluorescence staining to detect intestinal IgA anti-tTG Abs by means of the phage display VH5 antibody libraries that is considered highly specific for CD dependent humoral response; to confirm the effectiveness of these techniques for the diagnosis of CD especially for that cases tested negative for the current CD diagnostic markers.

**Methods:** We studied 225 subjects who underwent to intestinal biopsy: 149 for clinical suspicion (sera anti-tTG Abs, mucosal histology, symptoms) of CD and 76 for other diseases (30 Crohn, 26 ulcerative colitis, 20 esophagitis) (control group). From each subject we obtained 2 frozen biopsies: one for phage library and one for double immunofluorescence staining. All the biopsies were investigated for tTG-targeted intestinal auto-Ab deposits while the phage display assay was used for those cases negative for CD related Abs with normal mucosal histology.

**Results:** 76 control samples were negative (100% specificity) and 149 positive for IgA-tTG deposits. Out of 149 positive subjects 144 were coeliac patients with villous atrophy and anti-tTG Abs positive and 5 with normal villous structure and anti-tTG Abs negative. From all these 5 patients we were able to isolate mucosal VH5 anti-tTG Abs by selection of the phage antibody libraries.

**Conclusion:** Both the used methods were completely in full agreement confirming the high specificity of the double immunofluorescence technique which is easier and faster than phage display assay.

The investigation of intestinal celiac IgA auto-antibodies against tTG by immunofluorescence staining or phage display VH5 restricted antibody libraries resulted very useful to identify all the cases in which the usual diagnostic markers are not clearly present.

These results support the hypothesis that small intestinal anti-tTG Abs occur in the early stages of disease prior to manifest mucosal lesion and before auto-Ab appear in the serum. Thus it should drive clinicians to take account of intestinal IgA-tTG deposits as a predictive marker of forthcoming CD.

**Disclosure of Interest**
None declared.

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**PL-G-0011**

**Coeliac Disease and Enteropathies**

**ARE TG2 INHIBITORS ABLE TO DECREASE GLIADIN-INDUCED TOXICITY RELATED TO CELIAC DISEASE—A PROOF-OF-CONCEPT STUDY**

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**Objectives and Study:** Celiac disease is an autoimmune-mediated enteropathy characterized by an immune response to dietary gluten in wheat, rye and barley in genetically susceptible individuals. Gluten-derived gliadin peptides are deamidated by transglutaminase 2 (TG2), which leads to immune response in the small-intestinal mucosa. Therefore, it has been suggested that TG2 inhibitors could substitute the gluten-free diet as a treatment for celiac disease in the future. The aim of this study is to investigate whether TG2 inhibitors can prevent the toxic effects of gliadin in vitro and ex vivo.
Methods: Caco-2 cells used in in vitro studies were pre-treated with two TG2 inhibitors, R281 (extracellular) and R283 (intracellular), and thereafter treated with peptic-tryptic digested gliadin (PT-gliadin). The change of permeability in response to different compounds was analyzed by measuring transepithelial resistance. Effects on cytoskeleton were defined with fluorescence staining. Small-intestinal biopsies used in ex vivo studies were derived from celiac disease patients who were either untreated or on gluten-free diet. Biopsies were pre-treated with R281 or R283 and cultured for 24 h or 48 h with or without PT-gliadin. Culture media were collected for measuring the secreted autoantibodies and snap-frozen biopsies were stained with CD25 antibody to quantitate activated lymphocytes and Ki-67 antibody to assess epithelial cell proliferation.

Results: Pretreatment with TG2 inhibitors abolished the PT-gliadin-induced decrease of Caco-2 cell transepithelial resistance and actin cytoskeletal rearrangement measured as membrane ruffling. Ex vivo celiac patient small bowel mucosal biopsy organ culture experiments showed that TG2 inhibitors inhibited the gluten-induced increase of CD25-positive lymphocytes and augmented epithelial cell proliferation. However, the TG2 inhibitors were not able to prevent PT-gliadin induced secretion of celiac-specific autoantibodies into the culture medium.

Conclusion: TG2 inhibitors alleviate the toxic innate effects of gliadin on intestinal Caco-2 epithelial cells. Moreover, the inhibitors are able to prevent the gliadin-induced increase of CD25-positive lymphocytes and Ki-67 positive proliferative epithelial cells in celiac patient mucosal biopsies but they do not affect the secretion of celiac disease-specific autoantibodies. Taken together, our results would suggest that TG2 inhibitors decrease some, but not all, of the toxic effects of gliadin. Therefore, further studies are needed to test the suitability of TG2 inhibitors as an alternative future treatment form for celiac disease.

Disclosure of Interest: None declared.

PL-G-0012

Coeliac Disease and Enteropathies

THE FREQUENCY OF COELIAC DISEASE (CD) IN HIGH-RISK YOUNG CHILDREN FROM FAMILIES WITH CD: THE PREVENTCD COHORT

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Objectives and Study: The frequency of CD in first degree relatives has been reported as 10–15%, but it has never been prospectively studied in young children. Aim of the study was to elucidate the frequency of CD in the first three years of life, in a prospective cohort of high risk young children from families with CD.

Methods: In the context of the EU-funded PreventCD project (www.preventcd.com) 1319 infants belonging to families with a first degree case of CD were recruited. 906 of them, HLA-DQ2 and/or DQ8 positive, were prospectively followed-up for the development of CD. Biopsies to confirm the diagnosis were performed if symptoms appeared and/or if two or three consecutive samples were positive for anti-tissue transglutaminase (a-tTG) or anti-gliadin (a-gli) antibodies, respectively.

Results: At the 21st of December 2010, 787, 450, and 207 infants were older than 12, 24, 36 months, respectively. 47 biopsies were performed in 46 children. In 33 cases symptoms were present; in 33 elevated a-tTG and in 4 elevated only a-gli antibodies were the reason for the biopsy. 31 CD diagnoses were made (CD group). Of the 207 children who reached the age of 36 mo, 14 were diagnosed between the 2nd and 3rd year (prevalence = 6.76%). Of the 243 children with age 12–24 m 15 new cases occurred (6.17%). 2 cases were diagnosed before the age of 12 m. In the CD group, symptoms were present in 20; only elevated a-tTG in 6 cases and elevated both antibodies (a-tTG and a-gli titres) in 25 cases. Median titres were >100 and 56.1, respectively. In the non-CD group, 14 in total, median age of biopsy was 22.5 months, similar to CD group. Symptoms were present in 13, elevated a-tTG and elevated only a-gli titres were found in 2 (potential CD) and 4 cases. Median titres were 0.1 and 1.9, respectively.

Conclusion: These preliminary observations suggest a high incidence of CD in at-risk infants, at a quite early age. 2/3 had symptoms. All had major damage at the intestinal mucosa (from Marsh3a to 3c). Anti-tTG antibodies showed a high predictive value.

Disclosure of Interest: None declared.

SP-G-0013

Inflammatory Bowel Disease

A RANDOMIZED, MULTICENTER, OPEN-LABEL PHASE 3 STUDY TO EVALUATE THE SAFETY AND EFFICACY OF INFlixIMAB IN PEDIATRIC PATIENTS WITH MODERATE TO SEVERE ULCERATIVE COLITIS

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Objectives and Study: To evaluate the efficacy of IFX in inducing clinical response in pediatric patients with moderately to severely active UC and the safety of IFX during induction and maintenance.

Methods: Patients aged 6–17yrs, with active UC (Mayo score 6–12, including endoscopic subscore ≥2) who failed to respond to/tolerate 6-MP, AZA, corticosteroids, and/or 5-ASA were enrolled. Patients received IFX 5mg/kg at wks 0, 2, and 6. Primary endpoint, clinical response at wk8, was defined as decrease from baseline in Mayo score ≥30% and ≥3 points, with decrease in rectal bleeding subscore of ≥1 or rectal bleeding subscore of 0/1. A positive study was prospectively defined as the lower limit of the 95% CI for the proportion of patients in clinical response >40% (upper limit of CI from pooled placebo group in ACT 1&2, which evaluated IFX in adult UC). Patients who achieved clinical response at wk8 were randomized to IFX 5mg/kg q8wks through wk46 or q12wks through wk42. Non-responders were discontinued from study agent.

Results: 60 patients were enrolled. Baseline demographics and disease characteristics were generally comparable across the groups: 53% female, median age 14.5 yrs, weight 50.8kg, disease duration 1.4 yrs, CRP 0.3 mg/dL, median Mayo score 8.0, median PUCAI score 55. IFX induced clinical response at wk8 in 73.3% (44/60) patients [95% CI 62.1%–84.5%] and met the criteria for a positive study. At wk8, 40.0% (24/60) were in clinical remission by Mayo score and 33.3% (17/51) were in remission by PUCAI. At wk8, 68.3% (41/60) patients achieved mucosal healing (endoscopic subscore of 0/1). At wk54, among patients who continued treatment, a numerically greater proportion of patients were in remission in the 5 mg/kg q8wk group (38.1% [8/21]) vs 5 mg/kg q12wk group (18.2% [4/22]) though this did not reach statistical significance (P = 0.146). More patients on corticosteroids at baseline were in remission and off corticosteroids at wk54 in the q8wk maintenance group (38.5% [5/13]) vs q12wk maintenance group (0.0% [0/13]). IFX was generally well tolerated. The proportions of patients experiencing serious AEs and infusion reactions were similar across maintenance groups. No deaths, malignancies, opportunistic infections, TB or delayed hypersensitivity reactions were reported.

Conclusion: IFX is effective and safe in the treatment of pediatric patients with moderately to severely active UC with results comparable to the ACT trials. At wk54, twice as many patients were in remission following q8wk vs q12wk therapy.

Disclosure of Interest: None declared.
Food Allergy
TECHNOLOGICALLY PROCESSED SOLUBLE FACTORS OF LACTOBACILLUS RHAMNOSUS ARE PROTECTIVE AGAINST ALLERGIC AIRWAY INFLAMMATION IN NEONATAL MICE

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Objectives and Study: Over the past decades, the incidence of allergic diseases such as asthma is on the rise in infants and children. In experimental models of allergic airway inflammation in rodents, a protective effect of probiotic supplementation on the development of allergic disease has been shown. We previously reported that soluble factors from Lactobacillus rhamnosus GG (LGG) may also exert this beneficial effect. In potential applications certain technological processing aspects like spray-drying, ultra-filtration and lyophilisation may affect bioactivity of soluble factors from LGG. Therefore, the aim of this study was to validate the effects of differentially processed LGG supernatant supplementation on allergic airway inflammation in a neonatal mouse model in vivo.

Methods: Newborn Balb/c mice were orally supplemented with LGG or LGG supernatants, from day two until six weeks of age. Subsequently, acute allergic airway inflammation was induced by sensitization and challenge with ovalbumin. On day 71, airway reactivity was measured, and animals were sacrificed on day 72. Bronchoalveolar lavage (BAL) cell counts, BAL cytokines, serum ovalbumin-specific immunoglobulin isotypes as well as lung histology were analyzed.

Results: Confirming earlier findings, LGG supplementation caused a significant decrease in BAL eosinophils, BAL IL-5 and both inflammation and goblet cell scores, but did not have an effect on airway hyperresponsiveness. Similar results were obtained after supplementation with ultrafiltered and lyophilized LGG supernatant, with an additional increase in BAL IL-10 and decrease in IL-9. In contrast, supplementation with the raw unprocessed LGG supernatant and LGG supernatant that was desalted using G25 column chromatography and subsequently filtered and lyophilized did not show similar effects.

Conclusion: Dietary supplementation of newborn mice with ultrafiltered, lyophilized LGG supernatant induces comparable protective effects on the development of allergic airway inflammation as viable LGG bacteria. Specific preparation of the soluble factors may retain bioactivity and thus may be suitable for application in nutritional formulations.


Food Allergy
DIETARY INTERVENTION WITH SYNBiotics PROTECTS AGAINST ALLERGIC DISEASE VIA INDUCTION OF GALECTIN-9 SECRETION BY INTESTINAL EPITHELIAL CELLS

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Objectives and Study: Intestinal epithelial cells (IEC) abundantly express galectins, which are known to modulate T cell responses. In this study, immune modulation and epithelial expression of galectin-9 (Gal9), induced by a galacto/fructooligosaccharide mixture (scGOS/lcFOS) and TLR9 ligand, and its relevance for the suppression of allergic disease were determined both in vitro and in vivo.

Methods: Human IEC were grown on transwell inserts and apically exposed to 0.5% scGOS/lcFOS together with TLR ligands and co-cultured with activated healthy donor. After 24 h, cytokines and immune cell phenotype were measured. In vivo, mice were sensitized orally to whey, while being fed a diet containing scGOS/lcFOS and Bifidobacterium breve M-16V (GF/Bb). Gal9 expression was determined by immunohistochemistry in the intestine and measured in serum by ELISA. In addition, in a double-blind, placebo-controlled multicentre trial, Gal9 levels were measured in sera of 90 infants with atopic dermatitis that received hydrolyzed formulae with or without GF/Bb for 12 weeks.

Results: Apical exposure of IEC to scGOS/lcFOS and a synthetic TLR9 ligand or genomic DNA from Bifidobacterium breve M-16V enhanced IFN-γ secretion by co-cultured PBMC and resulted in increased percentages of T_h1 and T_reg cells. IEC-derived Gal9 mRNA, protein expression, and basolateral secretion increased after combined addition of scGOS/lcFOS and TLR9 ligand in the co-culture model. Furthermore, development of T_h1 and T_reg cells was enhanced in Gal9 treated PBMC, resulting in increased IL-10 and IFN-γ, but suppressed IL-17 secretion. In vivo, the GF/Bb diet resulted in reduced acute ear swelling response upon dermal challenge with allergen and lower mouse mast cell protease-1 levels in serum. Furthermore, the GF/Bb diet enhanced serum Gal9 levels, which correlated with decreased allergic symptoms and immunohistochemistry revealed specific basolateral Gal9 expression on IEC. In addition, infants suffering from atopic dermatitis receiving the GF/Bb diet also showed enhanced Gal-9 levels in serum, which coincided with less severe allergic symptoms.

Conclusion: These data indicate that dietary supplementation with scGOS/lcFOS has significant implications for the prevention of allergy through TLR9-induced Gal9 secretion by IEC.

Disclosure of Interest: Employee of: Mead Johnson Nutrition, H. Garn: None declared.
Disclosure of Interest: This study/work was performed within Dutch Top Institute Pharma, project T1–214.S.de Kvit: None declared, E. Saeland: None declared, A. Kraneveld: None declared, H. van de Kant: None declared, B. Schouten Employee of: Scientist at Danone, B. van Esch Employee of: Scientist at Danone, J. Knol Employee of: Director Microbiology at Danone, A. Sprikkelman: None declared, L. Knippels Employee of: Group Leader Immunology and Allergy at Danone, J. van Kooij: None declared, L. Willemsen: None declared.

Objectives and Study: The role of vitamin D in the development of type 2 diabetes (T2DM) has been described in adults. Pancreatic fat fraction (PFF) appears to be related to the development of impaired insulin secretion in obese adolescents. Serum vitamin D levels are known to be decreased in obese subjects. The aim of our study was to explore the relationship between serum vitamin D levels, PFF, and metabolic parameters in obese and lean adolescents.

Methods: We recruited 25 lean and 24 obese adolescents (mean age 13.6±1.5 yrs). Pancreatic fat fraction (PFF) and visceral adipose tissue (VAT) were determined using MRI. We measured 25-OH vitamin D, fasting glucose, insulin, leptin and lipids levels. Obese subjects underwent an oral glucose tolerance test.

Results: Vitamin D was significantly different between lean and obese subjects (18.5±7.4 vs. 9.9±6.4, P < 0.001). As vitamin D was strongly related to VAT, we performed linear and multiple regressions adjusted for this variable. Results showed that vitamin D was only associated with PFF (R²-change: 0.061, P = 0.039). In obese subjects vitamin D was related to fasting insulin (R²-change: 0.261, P = 0.007), HOMA-IR (R²-change: 0.215, P = 0.0017) and inversely to PFF (R²-change: 0.358, P = 0.001). In lean controls vitamin D was only related to serum ALT (R²-change: 0.241, P = 0.011) but not to PFF or insulin level.

Conclusion: Vitamin D concentration is very low in obese adolescents probably due to its accumulation in visceral adipose tissue. Obese adolescents with the lowest vitamin D concentration show the highest pancreatic fat deposition and the lowest fasting insulin level. These findings may suggest a novel mechanism for the development of obesity-related glucose intolerance.

Disclosure of Interest: None declared.

Clinical Nutrition

VITAMIN D IS RELATED TO PANCREATIC FAT FRACTION AND FASTING SERUM INSULIN IN OBESE ADOLESCENTS

V. A. McLin, VITAMIN D IS RELATED TO PANCREATIC FAT FRACTION AND FASTING SERUM INSULIN IN OBESE ADOLESCENTS

Clinical Nutrition

SP-N-0018

Clinical Nutrition

VITAMIN D INSUFFICIENCY IN PRETERM VERY LOW BIRTH WEIGHT INFANTS

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Objectives and Study: To assess vitamin D status in preterm very low birth weight (VLBW) infants, determine predictors of vitamin D status, and evaluate response to prolonged vitamin D supplementation in those with low vitamin D status.

Methods: We prospectively assessed serum 25-hydroxyvitamin D (S-25-OHD) levels in preterm VLBW infants (born ≤32 weeks’ gestation or ≤1.5 kg) admitted to our tertiary referral neonatal unit (n = 274). We collected demographic data (including gestational age, birth weight, gender, ethnicity, season of birth, and day of life at time of S-25-OHD assessment) and recorded serum total alkaline phosphatase (ALP), corrected calcium and phosphorous levels. In those with S-25-OHD <50 nmol/L, we continued augmented vitamin D intake (≥400 IU/day) from fortified feeds and supplements long-term and evaluated the response (n = 148).

Results: At a median (interquartile range) of 18 (11–28) days of life, and according to Institute of Medicine (IOM) thresholds, 13.9% were at risk of deficiency (S-25-OHD <30.0 nmol/L), 64.6% were within the target range (S-25-OHD 30.0–49.9 nmol/L), and 21.5% were above the target range (S-25-OHD ≥50 nmol/L).

Multivariable analysis determined that the two predictors of S-25-OHD were duration of vitamin D supplementation and gestational age at birth (r²=0.215; P<0.001). S-25-OHD correlated with secondary indices of deficiency: serum total ALP (r=−0.123, P=0.47), calcium (r=0.226, P<0.001) and phosphorus (r=0.263, P<0.001). In the follow-up survey with prolonged supplementation, 86.5% achieved S-25-OHD ≥50 nmol/L, 10.1% met our criteria for poor response, and 26.4% had levels ≥100 nmol/L.

Conclusion: Vitamin D insufficiency is an issue for preterm VLBW infants warranting early intervention to prevent and correct. The only predictors of vitamin D status in these infants were duration of supplementation and gestational age at birth; birth weight, ethnicity, gender or season of birth was not. In those with low status, oral vitamin D intake ≥400 IU daily achieves target S-25-OHD levels in the majority; however some reached supra-optimal S-25-OHD levels suggesting a lower intake should suffice once the target level is achieved.

Disclosure of Interest: None declared.
SP-H-0019

**Hepatology**

RELATIONSHIP AMONG CELLULAR FIBROSIS MARKERS AND IRON CONCENTRATION AND STAGE OF FIBROSIS IN THE LIVER OF BETAL THALASSEMIAS PATIENTS WITH TRANSFUSIONAL IRON OVERLOAD

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Objectives and Study: Patients with thalassaemia major (TM) develop iron overload mainly caused by regular transfusions of packed red cells. Enhanced oxidative stress within the liver is associated with the development of fibrosis that may progress to cirrhosis. The interactions between various resident hepatic cell populations and immune cells that lead to the establishment of fibrosis are complex. Despite the major relevance of metalloproteinase to the pathophysiology of liver fibrosis, little information is available so far with respect to the possible alterations in serum levels and tissue expressions of fibrosis markers and contribution to the liver fibrosis in TM.

Methods: This study was run in Turkey following the completion of the ICL670A0107E extension. TM patients who completed core phase and continued with deferasirox or switched to deferasirox during 4 year extension were included after consenting for participation in this locally run study. Liver biopsy specimens and simultaneously collected frozen serum samples of those patients was used. Serum concentrations of tenascin, collagen IV, tissue inhibitors of metalloproteinase (TIMP-1), and matrix metalloproteinase (MMP-1) levels were measured with commercial enzyme-linked immunoassay kits. Liver iron concentrations (LIC) were measured by AAS. Fibrosis stage and inflammation grade were assessed in a blinded fashion by a single pathologist according to the Ishak (score 0–6, grade 0–18) and Sciotto (0–4×3). Paraffin sections from formalin fixed material were immunostained with antibodies against alfa-SMA, Collagen-4, TIMP-1 and MMP-1. The intensity of immunostaining in entire representative slides was semiquantitatively graded from 0 to 4.

Results: A total of 198 liver biopsy specimens and serum samples from 66 patients aged between 2.5 and 28 (mean 12.5 ± 6.2) years were included. LIC was significantly correlated with liver fibrosis and inflammation ($P = 0.000$). Although, there was a highly significant correlation between LIC and iron deposition in hepatocytes, Kupffer cells and portal field ($P = 0.000$), fibrosis was only correlated with hepatocytes and portal iron but not iron settled in Kupffer cells. Serum collagen4 and TIMP-1 were significantly correlated with LIC ($P = 0.0002$, $P = 0.004$) but not with the stage of fibrosis. However, portal expression of collagen4 and TIMP-1 were significantly correlated with fibrosis stage ($P = 0.009$, $P = 0.02$). Further, portal aSMA also showed a significant correlation with fibrosis ($P = 0.001$).

Conclusion: This study first revealed the role of hepatic stellate cell activation leading TIMP-1 upregulation.


SP-H-0020

**Hepatology**

HAEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS SHOULD BE CONSIDERED IN DIFFERENTIAL DIAGNOSTIS OF ACUTE LIVER FAILURE BEFORE MAKING THE DECISION ON LIVER TRANSPLANTATION

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Objectives and Study: Diagnostic and therapeutic management of children affected with acute liver failure due to haemophagocytic lymphohistiocytosis (HLH) remains a challenge. Acute liver failure (ALF) may be the first clinical presentation of HLH. There are case reports in literature on liver transplantation (LTx) in patients with familial lymphohistiocytosis (FHL) and other genetic causes of HLH which resulted in disease reactivation and patient’s death. The aim of our study was to describe difficulties in making diagnosis of HLH in children presenting with ALF.

Methods: From 2005 to 2010 we diagnosed and treated 8 children (5 girls + 3 boys), aged from 10 days to 4 years, with ALF complicating HLH. In a newborn, who died despite performed LTx, the diagnosis of HLH was made after death. Molecular diagnostics confirmed genetic HLH (due to MUNC13–4 gene mutation) only in one child out of 5 tested; genetic examination in the youngest child is ongoing. Finally 6 children were given the diagnosis of secondary HLH. In the same time period we treated 71 patients affected with ALF and performed LTx for ALF in 25 children.

Results: All children presented with fever, jaundice and hepatosplenomegaly. Neurological symptoms included irritability, seizures or coma. We used diagnostic protocol HLH-2004 to establish the final diagnosis. Laboratory examination revealed anaemia, thrombocytoaenia, coagulopathy (INR
1.8–4.0, low fibrinogen) and elevated serum ferritin in all patients. Hyperferritinaemia was difficult to interpret in 3 cases because of previous blood transfusion. Increased haemophagocytosis in bone marrow was finally found in all children but one. Cytotoxic activity of NK cells, evaluated in 6 children, was low in 4. Perforin expression in NK cells was normal in 6 tested patients. Treatment according to HLH-2004 protocol (dexamethasone, cyclosporin A, etoposide) was implemented in 6 children; a girl with multi-organ failure was given only DXM; a newborn suspected of neonatal hemochromatosis underwent LTx. The girl treated with DXM and the boy with EBV-HLH died from multi-organ failure. 2 girls died from HLH-reactivation; one boy died after MUD-HSCT. A newborn, who underwent LTx, died 3 days after surgery. Only 2 children are alive and free of symptoms.

Conclusion: HLH should be considered in differential diagnostics of acute liver failure, especially before making the decision on liver transplantation. HLH diagnostic and therapeutic protocols should be used in liver centers.

Disclosure of Interest: None declared.

PA-N-0021

Pediatric Nutrition

NO INFLUENCE OF BREAST-FEEDING ON ADIPOSY IN ADOLESCENTS: THE HELENA study


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Objectives and Study: The protective effect of breast-feeding on body composition and obesity remains controversial, especially during adolescence which is a turning point for nutrition. The main objective of this study was to assess the relationship between breast-feeding and adolescents’ body composition.

Methods: HELENA is a cross sectional study conducted in 3910 adolescents (aged 12.5–17.5 years) in 10 countries. We used questionnaires for breast-feeding, smoking status, parental socioeconomic status (SES) and parental adiposity. Adolescents were measured for weight and height, fat mass (skinfolds, circumferences) and fat free mass index (FFMI) (impedance). Breast-feeding (never vs ≥4 months) was studied by sex for each body composition parameter with adjustment (smoking status, age, fitness, sedentariness, parental body composition, city, sexual maturation, SES) 1/ by covariance analysis (propensity score adjustment) 2/ by multivariante quantile regression.

Results: With adjustment on propensity score, the analysis did not found any significant effect of breast-feeding on body composition parameters (BMI: \(P = 0.55\); FFMI: \(P = 0.73\); sum of skinfolds: \(P = 0.18\); waist/height ratio: \(P = 0.08\)). However, boys adjusted analysis revealed a trend for beneficial effect of breast-feeding on subcutaneous \(P = 0.065\) and visceral adiposity \(P = 0.057\). Quantile analysis raised crescent effect toward the highest percentiles of adiposity. This effect was not found in girls who in majority finished puberty.

Conclusion: This first European study including a large number of confounders thanks to the propensity score shows no effect of breast-feeding on adolescent’s body composition. For boys’ subcutaneous and visceral adiposity, a beneficial effect of breast-feeding is suggested; this effect seems more pronounced in the highest adiposity percentiles.


PA-N-0022

Clinical Nutrition

INCREASING EARLY PROTEIN INTAKE IS ASSOCIATED WITH A REDUCTION IN THE INCIDENCE OF INSULIN-TREATED HYPERGLYCAEMIA IN VERY PRETERM INFANTS

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Objectives and Study: We have previously shown a standardised, concentrated neonatal parenteral nutrition regimen (scPN1) can increase protein intake in infants <29 weeks’ gestation. Protein intake and nitrogen balance can be enhanced further by very early amino acid introduction. The potential effects of this strategy on glucose control and insulin use have not been reported in this population. Controlling neonatal hyperglycaemia with insulin infusions is now routine practice despite the limited evidence base and potential risks. Our aim was to measure carbohydrate, protein and fat (macronutrient) intake and metabolic...
tolerance using a modified regimen (scNPN2) and compare to the previous regimen.

Methods: Local audit committee approval was obtained. The local electronic data management system was used to collect complete 14-day biochemical and detailed fluid/drug infusion data for infants <29 weeks gestation receiving scNPN2 using methodology identical to that in the previous study (scNPN1). This allowed evaluation of metabolic stability and actual daily parenteral and enteral macronutrient intake to be calculated and compared. Both regimens had identical formulations, intravenous glucose regimens and protocols (using insulin) for managing hyperglycaemia but scNPN2 introduced protein more rapidly.

Results: Data from the first 38 consecutive infants: median (range) birthweight 935 g (440–1350 g), gestation 26 (23–28) days. No clinically important violations of hyperglycaemia were identified in either group. Similar in both groups with a peak in insulin use between 5–10 days. The pattern of glucose intolerance was associated with fewer infants treated with insulin (P<0.001) partly achieved by starting PN sooner after birth (P<0.001). This was associated with fewer infants treated with insulin (P<0.01) over the first 14 days. The pattern of glucose intolerance was similar in both groups with a peak in insulin use between 5–10 days. No clinically important violations of hyperglycaemia but scNPN2 increased stability and actual daily parenteral and enteral macronutrient intake to be calculated and compared. Both regimens had identical formulations, intravenous glucose regimens and protocols (using insulin) for managing hyperglycaemia but scNPN2 introduced protein more rapidly.

Table 1: Protein intake (median [IQR]) and insulin usage

<table>
<thead>
<tr>
<th>PN start (hours)</th>
<th>Protein intake (g/kg/7d)</th>
<th>Infants on insulin</th>
<th>Insulin days</th>
</tr>
</thead>
<tbody>
<tr>
<td>scNPN1 (2006)</td>
<td>23 (14–31)</td>
<td>11.9 (10.7–13.5)</td>
<td>20/38</td>
</tr>
<tr>
<td>scNPN2 (2009–10)</td>
<td>3.5 (2.5–6)</td>
<td>16.0 (15.2–17.4)</td>
<td>10/38</td>
</tr>
</tbody>
</table>

Conclusion: The scNPN2 regimen successfully improved actual early protein intake. This was associated with a clinically important fall in insulin treated hyperglycaemia that requires further study.

Disclosure of Interest: None declared.

PA-N-0023

Clinical Nutrition

DHA AND/OR 5-MTHF SUPPLEMENTATION DURING PREGNANCY AND LONG-TERM EFFECTS ON THE PATTERN REVERSAL VEPc IN OFFSPRING 5 YEARS OLD

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Objectives and Study: Cortical Visual Evoked Potentials (VEPc) correspond to the electrophysiologic registration of the cortical response to a luminic stimulus. We analyze the long-term effect of different nutritional supplements received by healthy pregnant women during pregnancy (NUHEAL Project: Placebo; DHA: 500 mg/day; 5-MTHF: 400 mg/day; or both) on the pattern-reversal VEPc performed in 91 healthy German and Spanish children at 5 years old. For infants not fully breastfed, formulas were available with/without supplementary DHA according to the nutritional code assigned.

Methods: Pattern with progressive diminishing visual angle (2°, 1°, 30′, 15′, and 7.5′) was presented in a screen disposed in front of the children. Latency (Lat) (ms) and Amplitude (Amp) (mV) of the P1 component were analyzed. DHA content in plasma phospholipids (PL), and in erythrocyte membrane (phosphatidylycholine (PC) and phosphatidylethanolamine (PE)) (% of weight of total fatty acids) were determined in the mother during pregnancy and delivery, and in their offspring at delivery. DHA (mg/L) content in human milk was also determined. General linear model for repeated measures and correlation study were done (SPSS Version 16.0).

Results: There were no differences between the 4 groups in Lat or Amp. DHA PL in umbilical cord was inversely correlated with latency in all explorations. DHA-PL in the mother at 20 weeks of pregnancy and at delivery were inversely correlated with latency 1° of arc (r=−0.226, P=0.043; r=−0.256, P=0.023, respectively) and at 30° of arc (r=−0.262, P=0.018; r=−0.271, P=0.015, respectively) in their children; DHA-PC in mothers at 30 wks of pregnancy were closely negatively correlated to latency in all examinations, and at delivery with latency at 30° of arc (r=−0.285, P=0.039) and 7.5° (r=−0.323, P=0.020). DHA-PE at delivery was correlated inversely with the latency at 30° of arc (R=−0.313, P=0.013). So, the latency at 30° of arc was closely and inversely correlated with the DHA content in plasma phospholipids, in PC and PE in the mother at delivery. There was also correlation between DHA content in human milk and latency at 2° of arc.

Conclusion: DHA content in plasma phospholipids, in PC and PE in the mother at delivery and the DHA-PL concentrations in umbilical cord will play an important role of long-term retinal (so brain) development at 5 years.

Funding: This work is part of the EARNEST 6th EU Framework Program, FOOD-CT-2005-007036.

Disclosure of Interest: None declared.

PA-N-0024

Pediatric Nutrition

A NEW LIQUID HUMAN MILK FORTIFIER IMPROVES LINEAR GROWTH IN PRETERM INFANTS

www.jpgn.org
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Objectives and Study: Few studies have evaluated current generations of human milk fortifiers (HMF) in very immature infants with birth weight (BW) ≤1250 g. This third party-blinded, controlled study was designed to evaluate the growth of premature infants fed a new ultraconcentrated liquid HMF that provides 1.8 g protein/4 vials added to 100 ml of milk. By design, to further characterize the risk to benefit ratio we present the data of those infants that followed the most stringent use of the HMF (i.e., received ≥80% of energy from breast milk + HMF).

Methods: Preterm infants fed their mother’s and/or donor breast milk were randomized to receive milk with added powder HMF (Control, 1.1 g protein/4 sachets, Mead Johnson Nutrition) or liquid HMF (LHMF, 1.8 g protein/4 vials) for 28 days. Serum prealbumin and BUN were measured and analyzed by Kruskal-Wallis. HCO3− (measured on days 6 and 14), pH (measured on days 6 and 14), and growth [weight (analysis of covariance), length, head circumference (HC)] were monitored and compared using ANOVA, unless otherwise stated.

Results: In this subset of infants (Control=36, LHMF=40), LHMF had significantly higher serum prealbumin (day 14, P = 0.048) and BUN (day 6, P = 0.017; day 14, P < 0.001, day 28, P = 0.006) than Control. Conversely, LHMF had significantly lower pH (day 6, P = 0.002; day 14, P = 0.001) and HCO3− (day 6, P = 0.001; day 14, P < 0.001) than Control. By day 28, LHMF had significantly greater achieved weight, length, and HC than Control (Table). There was no difference in weight or HC growth rate between groups (P = 0.232 and P = 0.432, respectively), but length growth rate was significantly higher in LHMF than Control (Table 1).

<table>
<thead>
<tr>
<th>TABLE 1. Achieved growth (weight, length, HC) at study days 14 and 28 and length growth rate (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
</tr>
<tr>
<td>Day 14</td>
</tr>
<tr>
<td>Day 28</td>
</tr>
<tr>
<td>Length (cm)</td>
</tr>
<tr>
<td>Day 28</td>
</tr>
<tr>
<td>HC (cm)</td>
</tr>
<tr>
<td>Day 28</td>
</tr>
<tr>
<td>Length growth rate (cm/day)*</td>
</tr>
</tbody>
</table>

*There was a significant gender by group interaction (male, Control = 0.151 ± 0.009, LHMF = 0.153 ± 0.009; female, Control = 0.138 ± 0.008, LHMF = 0.176 ± 0.008).

Conclusion: A new ultraconcentrated liquid HMF with higher protein provides better growth (as indicated by achieved growth, length growth rate, prealbumin, and BUN) with minimal impact on metabolic stress (as indicated by pH and HCO3−).


PA-G-0025

Immunology

EFFECTS OF EXTENSIVELY HYDROLYZED SOY PROTEIN FORMULA ON CYTOKINES’ PRODUCTION IN INFANTS WITH COW’S-MILK ALLERGY

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Objectives and Study: Cytokines are produced by immunologic cells which stimulate the proliferation of specific effectors cells and mediate the systemic inflammation. Considering the importance of cytokines on control of immunological response, as its relation to symptoms exacerbation and oral tolerance development, the aim of this study was to verify the effect of the hydrolyzed soy protein (HSF) formula on the pro- (IL-4 and IL-13) and anti-inflammatory cytokines (IL-10 and TGF-β), in infants with cow’s-milk allergy.

Methods: A Prospective controlled study with infants with cow’s-milk allergy (CMA) has been conducted at the Clinic of Allergy, Immunology and Gastroenterology of Pediatrics Department, University of Medicine of ABC. Fourteen infants fed hydrolyzed soy protein (HSF) formula (Alergomed, ComidaMed from Germany) during 120 days, with age between 8.41 ± 3.87 months were evaluated. The CMA diagnose was made with total and specific IgE levels quantification, clinical parameters, elimination diet, skin prick test and challenge test. After CMA diagnose, the infants were treated with cow’s milk and derivatives elimination diet and its replacement for HSF formula. Blood samples were collected before initiation of dietetic treatment, in the presence of symptoms (T0) and after 120 days without allergy symptoms (T1). The samples were immediately frozen at −80°C to cytokines determination from serum. The data were shown as mean and standard error. Statistics analysis was performed by paired t test with P < 0.05 adopted as significant levels.

Results: 54% of infants with CMA were IgE-mediated and 46% non IgE-mediated. The pro-inflammatory cytokine levels, IL-13, were significantly decreased by dietetic treatment (0.61 ± 0.18 to 0.29 ± 0.04) (P = 0.04). Although the IL-4 levels were detected only in 50% of the sample, a significant decrease was also observed from T0 to T1 (8.52 ± 3.92 to 0.72 ± 0.26) (P = 0.04). The anti-inflammatory cytokines levels, IL-10 and TGF-β, were significantly increased by dietetic treatment (IL-10: from 12.11 ± 2.70 to
Objectives and Study: The data show that the treatment with HSF formula increases the anti-inflammatory cytokines, IL-10 and TGF-β and decreases the pro-inflammatory cytokines, IL-13 and IL-10. As the imbalance between these cytokines has an important role in the symptoms exacerbation hindering the development of oral tolerance, this study shows that the appropriate dietetic treatment, as HSF, is able to alter inflammatory mediators leading to clinical symptoms reduction.


PA-G-0026

Food Allergy
A SPECIFIC MIXTURE OF NONDIGESTIBLE OLIGOSACCHARIDES ENHANCES THE TOLERIZING CAPACITY OF A PARTIAL WHEY HYDROLYSATE IN A MOUSE MODEL FOR COW’S-MILK ALLERGY

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Objectives and Study: Hypoallergenic infant formulas (HA) are considered a good alternative for infants at high risk for developing allergy if breast-feeding is not possible. Dietary intervention studies with HA combined with nondigestible oligosaccharides, mimicking oligosaccharides present in human milk, have been shown to reduce allergic symptoms in these children. However, the mechanisms by which these nondigestible oligosaccharides exert their effect are yet to be explored. In a mouse model of cow’s-milk allergy, the contribution of a specific oligosaccharides mixture on the tolerizing capacity of a partial whey hydrolysate (WH) in relation to effects on intestinal regulatory T cell and CD103+ DC was investigated.

Methods: Mice were sensitized orally once a week for five weeks with whey using cholera toxin as adjuvant. Prior to sensitization mice were pre-treated orally with 50 mg partial WH or PBS (as control), or a specific non-digestible oligosaccharide mixture containing sc-Galacto-, lc-Fructo- and Acidic-oligosaccharides (9:1:1) with or without the partial WH. After challenge, the acute allergic skin response, the mast cell mediator mMCP-1 and whey-specific antibodies were measured. The presence of Foxp3+ T-cells and CD103+ DC were determined in mesenteric lymph nodes.

Results: Oral pretreatment of mice fed the partial WH induced tolerance as reflected by a reduced acute allergic skin response and suppressed mMCP-1 release without affecting whey-specific IgE levels. This effect coincided with increased CD103+ DC and Foxp3+ regulatory T-cell numbers. Interestingly, a combination of both completely abolished the acute allergic skin response and mMCP-1 release. In addition, a tendency towards decreased IgE levels and a further increase in intestinal CD103+ DC numbers was observed.

Conclusion: A specific mixture of non-digestible oligosaccharide, mimicking oligosaccharides present in human milk, enhanced the capacity of a partial WH to induce oral tolerance. This effect was associated with increased numbers of CD103+ DC in the mesenteric lymph nodes, known to play a role in tolerance induction, suggesting an important mechanistic role of these cells in the observed tolerance inducing capacity of non-digestible oligosaccharides combined with partial WH.


PA-G-0027

Immunology
SINGLE-CENTRE EXPERIENCE OF HEMATOPOEITIC STEM CELL TRANSPLANTATION FOR IPEX SYNDROME


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Objectives and Study: Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome with mutations in FOXP3 presents with severe autoimmune enteropathy, endocrinopathy, haematological cytopenias and other autoimmune manifestations. Symptoms can be ameliorated with immunosuppression, but hematopoietic stem cell transplantation (HSCT) is curative.

Methods: A retrospective study of patients with IPEX syndrome with mutations in FOXP3 who underwent HSCT at Newcastle General Hospital, 1 of 2 nationally designated centres for such procedures in the UK, was performed.

Results: 5 patients were identified who fulfilled the inclusion criteria. All had severe enteropathy and were parenteral nutrition (PN) dependent. Regarding autoantibodies, 3 had anti-islet, 1 anti-enterocyte, 1 anti-smooth muscle, and 1 anti-adrenal. Four were Coombs positive, 3 had an eosinophilia >2x10^9/L, and 3 IgE >2000 kU/L. The FOXP3 mutations were c.1157G>A, c. AAUA/AAC> AAU-GAA, c.758T>C, c. IV57 + SG/A, c.1037T>C. Transplant
TABLE.

<table>
<thead>
<tr>
<th>Case</th>
<th>Donor-sibling</th>
<th>Conditioning C-1H</th>
<th>Complications</th>
<th>PN stopped day 27. Normal diet and development. Ht 0.4–2C, wt 2–9C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HLA match 10/10</td>
<td>1 mg/kg</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>FU 7 y</td>
<td>Source-marrow</td>
<td>Bu16/Cy200</td>
<td>Complications</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Unrelated donor</td>
<td>Conditioning C-1H</td>
<td>Lung disease</td>
<td>PEG feeds. Myopathic facies.</td>
</tr>
<tr>
<td>Case 3</td>
<td>HLA match 10/10</td>
<td>1 mg/kg</td>
<td>None</td>
<td>Motor delay, ADHD, Ht 9–25C, wt 25–50C.</td>
</tr>
<tr>
<td>FU 5.5y</td>
<td>Source-cord</td>
<td>Bu16/Cy200</td>
<td>Complications</td>
<td>Grade II GvHD, Hypotonia.</td>
</tr>
<tr>
<td>Case 5</td>
<td>HLA match 10/10</td>
<td>1.6 mg/kg</td>
<td>None</td>
<td>Motor delay, ADHD, Ht 9–25C, wt 25–50C.</td>
</tr>
<tr>
<td>FU 2</td>
<td>Source-cord</td>
<td>Treo2/Cy200</td>
<td>Complications</td>
<td>Grade II GvHD, nephrotic syndrome.</td>
</tr>
<tr>
<td>6</td>
<td>DQB1 mismatch</td>
<td>0.6 mg/kg</td>
<td>None</td>
<td>Motor delay, ADHD, Ht 9–25C, wt 25–50C.</td>
</tr>
<tr>
<td>Case 7</td>
<td>Unrelated donor</td>
<td>Conditioning C-1H</td>
<td>Complications</td>
<td>Grade II GvHD, nephrotic syndrome.</td>
</tr>
<tr>
<td>8</td>
<td>HLA match 10/10</td>
<td>0.3 mg/kg</td>
<td>None</td>
<td>Motor delay, ADHD, Ht 9–25C, wt 25–50C.</td>
</tr>
<tr>
<td>FU 1.4y</td>
<td>Source-cord</td>
<td>Treo36/Flu150</td>
<td>Complications</td>
<td>Grade II GvHD, nephrotic syndrome.</td>
</tr>
</tbody>
</table>

Characteristics as below. Only cases 2 and 3 have 100% chimerism. All are alive, have resolution of their enteropathy and have discontinued PN.

Conclusion: HSCT is curative in IPEX syndrome, and patients should be referred early for assessment.

Disclosure of Interest: None declared.

PA-G-0028

Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori

THE EVALUATION OF Lansoprazole AS a PROBE FOR THE ASSESSMENT OF CYTOCHROME P450 2C19 ACTIVITY AND GENOTYPE-PHENOTYPE CORRELATION IN CHILDHOOD

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Objectives and Study: Proton pump inhibitors (PPIs) have been widely used in children for the management of acid-related diseases. Interindividual differences in PPI metabolism have been shown to influence pharmacokinetics, pharmacodynamics and clinical outcome of PPI therapy. Cytochrome P450 2C19 (CYP2C19) is the major enzyme that is responsible for the metabolism of PPIs. CYP2C19 exhibits marked genetic polymorphisms and distribution of these polymorphisms varies among different ethnic groups. Drugs used for the determination of CYP2C19 activity are not ideal probes and there is limited data regarding their use in children. In this study, lansoprazole was evaluated as an in vivo phenotyping probe for the assessment of CYP2C19 activity in children.

Methods: The CYP2C19*2, *3 and *17 polymorphisms were determined by PCR-RFLP method in 244 patients with ages between 2 to 18 years. Plasma lansoprazole and 5-hydroxy lansoprazole concentrations were analyzed by an HPLC method.

Results: The CYP2C19*17 was the most frequent variant allele (24.4 %) among the analyzed polymorphisms. The frequency of CYP2C19*2 which is the main defective allele was 10.0 %. CYP2C19*3 was not detected in the study population. The group with CYP2C19*17+17 genotype had a 70 % lower (P<0.05) mean lansoprazole plasma concentration compared to the CYP2C19*17+1 genotype group (90.7±106.1 ng.mL⁻¹ vs. 299.9±233.6 ng.mL⁻¹ as mean±SD) while the CYP2C19*1+2 group had about 7 fold higher (P<0.01) mean lansoprazole plasma concentration compared to the same genotype group (2062.5±536.8 ng.mL⁻¹ vs. 299.9±233.6 ng.mL⁻¹). Lansoprazole metabolic ratios (lansoprazole/5-hydroxy lansoprazole) were found to be significantly lower in the *17+17 (2.8±2.1) group and higher in the *1+2 group (63.5±12.2) compared to that of the *1+1 genotype group (6.1±4.5). Frequency distribution histogram of lansoprazole metabolic ratios showed a bimodal distribution with a visually determined antimode of about 1.6. Lansoprazole metabolic ratios showed a statistically significant correlation with omeprazole metabolic ratios in 19 patients (r₂=0.74, P=0.0003).

Conclusion: According to our results from a Turkish pediatric population, lansoprazole is a suitable probe drug for the phenotyping of CYP2C19. The CYP2C19*17 is the most frequent variant allele demonstrating increased metabolism of lansoprazole in our population. The CYP2C19*2 and *17 variants should be taken into consideration in predicting the clinical outcome of therapies with proton pump inhibitors in pediatric population.

Disclosure of Interest: None declared.
PA-G-0029

Inflammatory Bowel Disease

MATHEMATICAL WEIGHTING OF THE PEDIATRIC CROHN’S DISEASE ACTIVITY INDEX AND COMPARISON WITH ITS OTHER SHORT VERSIONS


Objectives and Study: The PCDAI has become the standard outcome measure in pediatric Crohn’s disease (CD). However, weighting of the items has never been subjected to mathematical modeling. Shorter versions have been proposed but without systematic evaluation. We aimed to mathematically weight the PCDAI items on the largest pediatric cohort to date. We systematically compared this mathematically weighted PCDAI (wPCDAI) with the original PCDAI, abbreviated PCDAI (abbrPCDAI), short PCDAI (shPCDAI) and modified PCDAI (modPCDAI) with respect to feasibility, validity and responsiveness.

Methods: The raw data from 4 prospectively collected datasets were used, totaling 437 children with CD (including the REACH and budesonide trials, growth study and the North American Registry). The beta-score of each PCDAI item in the multivariate modeling on the derivation cohort, guided their weights. Discriminant validity utilized physician global assessment (PGA), and construct validity- the correlation with PGA and laboratory tests. Feasibility and face validity were ascertained by a Delphi survey of 33 worldwide experts in pediatric CD. Responsiveness was assessed on the first 2 visits in each cohort, utilizing diagnostic utility statistics.

Results: The mathematical modeling yielded a newly weighted wPCDAI (range 0–125 points) which excluded three redundant items: two (height velocity and abdominal examination) had low feasibility and two (abdominal examination and hematocrit) had low frequency of endorsement in the datasets studied. The wPCDAI had better performance than the PCDAI in construct validity and responsiveness and it discriminated better between the disease activity categories (AUC of ROC 0.97 (95% CI 0.95–0.99)). It was the only version that differentiated moderate from severe disease activity (0.87 (0.82–0.92)). In comparison with the original PCDAI, the non-invasive versions (abbrPCDAI and shPCDAI) had lower face, construct and discriminant validity but were significantly more feasible. The modPCDAI performed well in the construct validation but was consistently inferior in all other parameters. Cutoffs corresponding to remission, response and gradations of disease activity were determined for each version.

Conclusion: The new wPCDAI performed better than the original PCDAI and is more feasible. The non-invasive versions (shPCDAI and abbrPCDAI) are inferior to the wPCDAI and the full PCDAI, but when needed in retrospective studies, either may be equally used.

Disclosure of Interest: None declared.

PA-G-0030

Coeliac Disease and Enteropathies

APPLICATION OF THE NEW DIAGNOSTIC CRITERIA IN COELIAC DISEASE IN A COHORT FROM SOUTHERN ITALY

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Objectives and Study: ESPGHAN diagnostic criteria for Coeliac Disease (CD) are undergoing a profound revision. There is growing evidence that high serum levels of anti-transglutaminase (TG2) antibodies predict the presence of gluten-dependent villous atrophy. It has then been suggested that in the presence of suggestive symptoms, anti-TG2 antibodies ≥10 times the upper limit of normal (ULN) and a compatible HLA, CD can be diagnosed without intestinal biopsy.

Methods: We have assessed the efficacy of this algorithm, examining the records of 1155 patients that underwent an intestinal biopsy from January 2007 to May 2010. 128 were HLA typed.

Results: 406/1155 (35%) patients had anti-TG2 ≥ 10 ULN and gluten-dependent symptoms. 387 of the 406 (95%) showed different degrees of villous atrophy. All those HLA typed (40/387) resulted to be DQ2 and /or 8 positive. Everybody was successfully treated with a gluten-free diet.

Conclusion: The presence of anti-TG2 serum levels ≥ 10 times upper normal limit together with symptoms and compatible HLA would have avoided intestinal biopsy in approximately one third of patients presenting with the suspicion of CD. However, 5% of them turned out not to have villous atrophy. High serum levels of anti-TG2 antibodies do not always predict enteropathy. Enteropathy is probably not necessary to diagnose CD.

Disclosure of Interest: None declared.

PA-G-0031

Inflammatory Bowel Disease

INTESTINAL ALPHA-DEFENSIN EXPRESSION IN PAEDIATRIC INFLAMMATORY BOWEL DISEASE

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Objectives and Study: Reduced alpha-defensin expression has been reported in the terminal ileum (TI) of adult patients with ileal Crohn’s disease (CD). However, it remains unclear whether this is causative or due to epithelial cell loss in long standing disease. Moreover, little is known about alpha-defensin expression in children with chronic inflammatory bowel disease.

Methods: A total of 283 intestinal biopsies were obtained from children with CD, ulcerative colitis (UC) and healthy controls. Absolute mRNA copy numbers for HD5, HD6, IL-8, Villin 1 and Tcf-4 were analyzed by RT-PCR. HD5 immunostaining was performed on biopsy sections and patients genotyped for NOD2 mutations.

Results: Equal expression levels of HD5 and HD6 were found in TI biopsies of children with ileal CD (L1+L3) compared to patients with isolated colonic disease (L2) and healthy controls. In contrast, we found significantly higher levels of alpha-defensins in the TI of children with UC. Reduced expression of Tcf-4 was observed in the duodenum and TI of CD patients with L1+L3 phenotype. We demonstrate significant up-regulation of HD5 and HD6 by metaplastic Paneth cells in the inflamed colon of children with IBD.

Conclusion: In this study no difference in alpha-defensin expression was found in the TI of CD children and controls. However, significant reduction of Tcf-4 in L1+L3 phenotype suggests that impaired PC differentiation may lead to altered HD5 and HD6 expression at a later stage of disease. Additionally, substantial upregulation of alpha-defensins in the inflamed colonic mucosa raises the question for their potential involvement in modulating inflammation in pediatric colonic IBD.

Disclosure of Interest: None declared.

PA-G-0032

Immunology

MOLECULAR BASIS OF IMMUNOSUPPRESSIVE TREATMENT IN AUTOIMMUNE ENTEROPATHY

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Objectives and Study: IPEX syndrome is a very severe autoimmune disease caused by regulatory T cell dysfunction, resulting in severe enteropathy and variable other autoimmune manifestations. Treatment options are immunosuppressive therapy and for extremely severe disease presentations stem cell transplantation, if an appropriate HLA identical donor is available. We observed over the last years, that immunosuppressive therapy with rapamycin is as efficacious as tacrolimus in treating autoimmune enteropathy, albeit less toxic. To further improve the care of children with IPEX syndrome and autoimmune enteropathy, we analyzed the molecular mechanisms of immunosuppressive therapy in these patients.

Methods: Data of a single center series of eight children with IPEX syndrome were collected and analyzed. All patients had a complete gastroenterological and immunological work-up. PBMC and purified CD4+ T cell lines of IPEX patients and healthy controls were cultured in the presence of immunosuppressors tacrolimus or rapamycin (10–100 ng/ml). T cell survival and proliferation were analyzed using standard methods (Annexin V assay, 3H thymidine incorporation). Cytokine expression was quantified by ELISA.

Results: Upon CD3/CD28 stimulation, effector T cells were highly overreactive in IPEX patients compared to healthy controls (increased cytokine production). Whereas rapamycin had a strong anti-proliferative effect, tacrolimus did not interfere with T cell proliferation or survival. Both immunosuppressors, rapamycin and tacrolimus were potent suppressors of T cell function, analyzed by their potential to inhibit Th1, Th2, and Th17 cytokine production. The strongest effect of both tacrolimus and rapamycin was observed on IFN-gamma and IL17, with a comparable inhibitory potential of either immunosuppressor.

Conclusion: This study provides the first molecular arguments for the good clinical results obtained with rapamycin in treating children with IPEX syndrome. Comparable to the effect of tacrolimus, rapamycin potently blocks T effector overstimulation which is crucial in the control of the inflammatory reactions. There is convincing evidence to consider rapamycin as alternative immunosuppressor in the treatment of autoimmune enteropathy.

Disclosure of Interest: None declared.

PA-H-0033

Hepatology

OUTCOME OF LIVER INVOLVEMENT IN CONGENITAL DISORDER OF GLYCOSYLATION TYPE IB

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Objectives and Study: Congenital disorder of glycosylation type Ib (CDGib) is a rare inborn error of metabolism related to Phosphomannomerase isomerase deficiency and is the only treatable CDG. Patients display a relatively consistent clinical presentation, characterized by a protein-losing enteropathy (PLE), hyperinsulinemic hypoglycaemia (HH) and liver involvement. Mannose therapy has been proven to improve the general condition and the digestive symptoms in
all reported patients. The outcome of liver involvement remains uncertain. The aim of this retrospective study was to better define the outcome of liver involvement in children with CDG1b under mannose therapy.

Methods: Medical records of children diagnosed with CDG1b in France were reviewed. Of a total of 7 children, one died prematurely before mannose therapy was initiated. The remaining 6 children were included in the study. Treatment with mannose was initiated at diagnosis of CDG1b within 2 months and 4 years of age, with a follow-up (FU) of 3 to 16 years.

Results: All children presented with digestive symptoms, HH, and hepatomegaly (HM). Splenomegaly was noted in 2 and cytolysis in 4. Digestive symptoms (including PLE), HH, and cytolysis resolved under therapy within a few months in all. With respect to liver involvement, the outcome was rather good in 3 children after a FU of 3, 9 and 16 years: no signs of liver disease in one, and only a firm HM in the 2 others, 1 of whom underwent a liver biopsy showing proliferating dysplastic and dilated bile ducts, and prominent portal fibrosis, akin to CHF. In the remaining 3 children, the HM became nodular at imaging. Signs of portal hypertension (splenomegaly and signs of hypersplenism) developed at 5 months, 6 months and 5 years of age. Two children developed esophageal varices (EV) at 5 months and 3 years of age and bled at 18 months and 4 years of age respectively. The third child had no EV after 11 y FU. A liver biopsy performed in 2 children (age 2 and 3 years) showed characteristic features of CHF with nodular fibrosis. None presented with signs suggestive of bacterial cholangitis during FU.

Conclusion: Chronic liver disease appears a main feature in CDG1b, related to an absence of remodelling of the foetal ductal plate akin to CHF which to date has been reported in all patients in whom a liver biopsy was performed. Mannose therapy does not prevent or cure the liver disease that is most probably established at birth. Regular screening for signs of portal hypertension and avoidance of salicylic acid and non steroid anti-inflammatory drugs since diagnosis of CDG1b are recommended.

Disclosure of Interest: None declared.

PA-H-0034

Transplantation

INTESTINAL TRANSPLANTATION, 16 YEARS DOWN THE ROAD: LESSONS AND FUTURE


Objectives and Study: To describe the mid- and long-term results of intestinal transplantation (Tx), in order to discuss the indications and ways for improvement. From 1994 until 2010, 88 children received 94Tx: 53 isolated small bowel Tx (SBTxs), 38 liver-small bowel Tx (L-SBTx, 2 with pancreas), 2 multivisceral Tx (from stomach to colon, pancreas and liver) including 1 with kidneys, 1 modified multivisceral Tx (without liver). Indications were: 30 short bowel syndromes, 26 congenital enteropathies, 28 motility disorders, 7 retransplantations, and 2 other diagnosis.

Methods: Retrospective study of medical data. Follow-up is 4 months to 16 years (median 8 y).

Results: Of 62 children (67 Tx) transplanted more than 5 years ago, 21 (31%) have a functional graft, 2 after retransplantation: 11/19 and 4/11 L-SBTx for more than 10 y and 5–10 y respectively; 1/13 and 5/19 SBTx for more than 10 y and 5–10 y. Of 27 Tx since 2006, 14 are functional. After SBTx, 27/53 grafts (51%) were removed, mostly in the 1st year, but 7 (13%) 2 to 9 y post-Tx, for acute or chronic rejection. The mortality rate is 35% (31/88): 10 children died after SBTx, 21 after L-SBTx (2 after retransplantation, 3 after resection of the transplanted bowel), 25 in the year after Tx, five 2–10 y later of Tx-related complications, 1 in an accident.

Conclusion: These results are similar to the data of the International Registry for graft survival, better for patient survival. Early mortality is high for L-SBTx, but long-term graft survival is better than after SBTx. Intestinal transplantation is still a difficult procedure, and its indications are limited to the failures of long-term parenteral nutrition. Recent improvements have decreased the early mortality and detransplantations. Improvements have to be made on the understanding and control of late graft losses and complications.

Disclosure of Interest: None declared.

PA-H-0035

Transplantation

EARLY DETECTION OF LYMPHOPROLIFERATIVE DISORDERS (PTLD) IN PAUCISYMPTOMATIC PEDIATRIC LIVER TRANSPLANT RECIPIENTS BY ADENOTONSILLAR HISTOLOGY

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Objectives and Study: PTLD is a severe complication of transplantation linked in most cases to EBV infection. Prevalence in pediatric liver transplant recipients is 5–7% and mortality over 50%. In most cases PTLD is recognized at the stage of lymphoma because less aggressive variants are a/paucisymptomatic. Standardized, simple and non invasive tests to rule out these disorders are lacking.

Methods: We prospectively evaluated for PTLD all liver transplanted children with symptoms of nasal obstruction and/or intermittent diarrhea and/or unexplained failure to thrive. Adenotonsillar tissue was obtained by rhinofibroscopy and biopsy or by adenotonsillectomy.
Results: Among 120 liver transplant pediatric recipients 18 (9 males) presented rhinolalia and snoring or recurrent upper airway infection; 4 had recurrent diarrhoea, with weight loss in 3. Median age at evaluation was 4.6 years (2.5–15.5), median age at liver transplant (OLT) was 1.1 years (0.3–11.25), median time from OLT was 3.25 years (0.75–6.5). 5 children underwent adentonsillectomy and 13 adenontonsillary biopsy. PTLD was diagnosed in 16 (89%), in 8 identified as polymorphic PTLD, in 8 as “early lesion.” There were not significant differences between these two groups in terms of age at diagnosis or time from OLT. 6 patients underwent gastrointestinal endoscopy. In all, PTLD was found also to involve gastrointestinal tract with the same histological grading of adenotonsillar tissue in 5. 15 patients with PTLD were EBV naïve at the time of OLT and 13 showed signs of infection after OLT. At diagnosis EBV DNA on peripheral blood mononuclear cells (PBMC) was positive in 10 patients: median value 140/10⁵ PBMC (15–1950) but EBER RNA was detected in lymphoid tissue in 15. Therapeutic approach consisted in decreasing doses of tacrolimus in patients with “early lesions” PTLD and in shift from tacrolimus to rapamycin in the others. After a median follow-up of 6 months (1–52) all are alive without signs of progression to aggressive variants of PTLD.

Conclusion: Waldeyer ring lymphoid tissue hypertrophy is strictly associated to low grade variants of PTLD in liver transplanted children. EBV naïve status seems to be a common feature. At diagnosis EBV DNA in PBMC is highly variable and even absent. Adenotonsillar biopsy is an easy and cost effective procedure to achieve diagnosis. Histological picture is consistent with gastrointestinal lymphoid tissue. We believe that early diagnosis and better management of immunosuppression might reduce the rate of progression toward aggressive variants and allow a reduction of mortality.

Disclosure of Interest: None declared.

PA-H-0036

Hepatology

EFFICACY OF SBC-102, A RECOMBINANT ENZYME REPLACEMENT THERAPY, ACROSS A BROAD RANGE OF DOSES IN AN IN VIVO MODEL OF LYSOSOMAL ACID LIPASE DEFICIENCY

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Objectives and Study: Lysosomal acid lipase (LAL) deficiency is a rare recessive disorder that leads to the accumulation of lipids, predominately cholesterol esters (CE) and triglycerides (TG) in a number of tissues. Early onset disease (Wolman) is characterized by profound malabsorption and is usually fatal within the first year of life. In later onset disease (CESD), hepatomegaly and type II hyperlipidemia dominate the clinical picture. We have recently established that 4 weekly doses of 5 mg/kg of rhLAL (SBC-102) decreases lipid substrate accumulation in key tissues and corrects clinically relevant phenotypic abnormalities in a rat model of LAL deficiency. The aim of this study was to investigate the dose-response relationship of SBC-102.

Methods: SBC-102 at dosages of 0.2 mg/kg to 5 mg/kg or vehicle was administered by IV injection beginning at 4 weeks of age to LAL deficient rats through to 8 weeks of age. Efficacy parameters included body weights, organ weights, histopathology, and tissue cholesterol and triglyceride levels.

Results: Abnormalities in this model of LAL deficiency resembles the human disease with increased hepatocyte lipid, aggregates of foamy macrophages and disruption of liver architecture. In addition there is extensive lipid accumulation in the lamina propria of the small intestine, which is a feature of early-onset disease in humans. Dose-dependent improvements were observed in key efficacy endpoints at 8 weeks of age. SBC-102 treated LAL-deficient rats gained more weight than vehicle-treated animals at dosages of 0.35 mg/kg qw and above. The percent increase in body weight during treatment with higher levels of SBC-102 was similar to weight gain observed in the wild-type (LAL+/+) rats. Favorable responses were also observed with regard to organomegaly, with reduction in the size of all the affected organs examined. Consistent with the expected mechanism of action of SBC-102, tissue levels of LAL-substrates were reduced after 4 weeks of treatment. The decreases in substrate accumulation were associated with a marked reduction in Oil Red-O staining.

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Liver % BW</th>
<th>Spleen % BW</th>
<th>Jejunum % BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>100.9±17.6</td>
<td>11.50±1.62</td>
<td>0.86±0.16</td>
<td>3.67±0.35</td>
</tr>
<tr>
<td>0.35 mg/kg qw</td>
<td>113.6±12.6</td>
<td>6.95±0.65</td>
<td>0.58±0.06</td>
<td>3.60±0.19</td>
</tr>
<tr>
<td>1 mg/kg qw</td>
<td>149.9±22.4</td>
<td>6.24±0.49</td>
<td>0.47±0.05</td>
<td>2.59±0.22</td>
</tr>
<tr>
<td>5 mg/kg q2w</td>
<td>157.8±58.5</td>
<td>6.01±0.8</td>
<td>0.50±0.19</td>
<td>2.75±1.29</td>
</tr>
</tbody>
</table>

Conclusion: These studies demonstrate efficacy of SBC-102 across a broad range of doses in an animal model that mimics LAL Deficiency in humans. Given that for ERTs, animal models are highly predictive of clinical effectiveness, SBC-102 warrants further investigation as a new treatment for patients with CESD due to LAL deficiency.


PL-H-0037

Hepatology

ANTI-HUMAN ALPHA-ENOLASE ANTIBODIES ARE HIGHLY PREVALENT IN AND SPECIFIC FOR BILIARY ATRESIA

E20
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Objectives and Study: A recent study (Gastroenterology 2010), using rabbit α-enolase as target, shows anti-α-enolase reactivity in a murine model of biliary atresia (BA) and in children with BA. We have investigated prevalence and specificity of anti-human-α-enolase (anti-Hu-α-enolase) antibodies prospectively in a large cohort of patients with BA and pathological controls.

Methods: 587 serum samples were tested: 304 from 80 patients with BA, comprising 38 tested at diagnosis, before and after liver transplant (LT) (median follow-up 12 years, range 6–17; median 6 samples/patient, range 2–6) and 42 non transplanted tested at diagnosis and at last follow up (7 years, 2–15; 2 samples/patient); 99 samples from 29 age matched children with α-antitrypsin deficiency (α1-ATD, all PiZZ) tested at diagnosis, before and after LT (follow-up 9 years, 5–14; 3 samples/patient, 2–5); 165 from patients with other liver diseases at presentation, comprising 31 progressive familial intrahepatic cholestasis (PFIC), 29 Alagille syndrome (AGS), 16 idiopathic giant cell hepatitis (GCH), 41 autoimmune hepatitis type 1 (AHI-1), 33 autoimmune hepatitis type 2 (AHI-2), 15 autoimmune sclerosing cholangitis (ASC); and 19 healthy controls. Anti-Hu-α-enolase reactivity was investigated with an in house ELISA using full-length recombinant human α-enolase (Abcam) as target. A rabbit polyclonal anti-α-enolase antibody (Abcam) was used as positive control.

Results: Overall, anti-Hu-α-enolase reactivity on at least one occasion was observed in 51% (41/80) BA patients, but only in 6% pathological controls [13/194, (3 α1-ATD, 3 PFIC, 3 AGS, 2 GCH, 1 AHI-1 and 1 AHI-2] and in none of 19 healthy controls (P < 0.05 for both). Prevalence of anti-Hu-α-enolase was similar in transplanted (52%) and non transplanted BA patients (50%). Of the 38 BA patients who required LT, 12 (31%) had anti-Hu-α-enolase antibodies at diagnosis, of whom 5 remained persistently positive during follow up and 7 lost reactivity after LT. Of the 26 negative at diagnosis, 8 developed anti-Hu-α-enolase antibodies after LT, while 20 remained negative. Of the 42 nontransplanted patients, 18 (42%) had anti-Hu-α-enolase antibodies at diagnosis, of whom 4 remained persistently positive during follow up and 14 became negative. Of the 24 negative at presentation, 3 developed anti-Hu-α-enolase reactivity over time and 21 remained negative.

Conclusion: This study shows that autoantibodies against human α-enolase are highly prevalent in and specific for biliary atresia, supporting the notion of an autoimmune component in the pathogenesis of this condition. Positivity for anti-Hu-α-enolase does not predict early LT requirement.

Disclosure of Interest: None declared.

PL-H-0038

Hepatology

Hepatology

ATP7 B EXPRESSION MEASUREMENT IMPROVES PICK UP RATE OF NEWLY DIAGNOSED PATIENTS WITH WILSON DISEASE

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Objectives and Study: Standard investigations to diagnose Wilson’s disease (WD; ceruloplasmin, serum and urine copper, liver histology and –copper, Kayser-Fleischer ring and mutation analysis in ATP7B gene) fail in some cases. More than 400 mutations in the ATP7B gene have been currently known. Given the difficulties of searching for mutations in a gene spanning more than 80 kb of genomic DNA data suggested that at least 10–15% of mutations might still be unidentified. Mutations in the ATP7B gene lead to the production of a dysfunctional gene product, the so called Wilson protein. We aim to evaluate measurement of specific Wilson protein mRNA as a new diagnostic tool to improve time and pick up rate of patients with newly diagnosed Wilson’s disease. ATP7B expression in human liver tissue from WD patients was never analyzed before.

Methods: Total RNA was extracted from frozen liver tissue using standard procedures. Briefly the tissue was processed in liquid nitrogen in a mortar and subsequently crushed using a pestil. RNA was extracted from the tissue powder using Trizol (Invitrogen, Darmstadt, Germany). As extraction kit Quiaien RNeasy kit was used. After CDNA synthesis real time PCR was performed using a LightCycler 2.0 (Roche, Mannheim, Germany). TATA Box binding protein (TBP) gene was used as housekeeping gene. The calculation of expression of the ATP7B gene was performed after the Delta Delta CT Method. Total RNA from liver biopsy specimens was isolated from 15 patients with WD. Gene expression was compared with a number of controls: Control group 1 was RNA from 25 different hepatocellular cell lines. Control group 2 consisted liver biopsy specimens from 12 children with end stage, cholestatic biliary atresia (n = 12).

Results: ATP7B mRNA expression was significantly decreased in liver tissue from patients with WD (median 1.54; range 0.45–4.31) compared to control group 1 (median 6.14; range 3.06–12.0). Furthermore ATP7B expression was significantly lower in patients with WD than in age-matched children with biliary atresia (control group 2, median 4.39; range 3.31–18.53).

Conclusion: Our findings suggest that measurement of the ATP7B gene product (mRNA) may shorten the time to diagnosis in patients with suspected Wilson’s disease. Prospective studies in larger patient cohorts are necessary to validate our results for clinical practice. Additional the results of this study will give new insights into the regulation of the ATP7B gene in patients with WD.

Disclosure of Interest: None declared.
PL-H-0039

Hepatology
WHOLE-EXOME-SEQUENCING-BASED DISCOVERY OF NOVEL SYNDROMIC FORM OF NEONATAL CHOLESTASIS
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Objectives and Study: Two cousins from a consanguineous family presented with low gamma glutamyl transferase (GGT) cholestasis, trichorrhexis nodosa (TN) and severe hypoglycaemia which required diazoxide to stabilise. One child also had life threatening diarrhoea necessitating parenteral nutrition, which suggested the possible diagnosis of trichohepatoenteric syndrome (THES). However screening of the THES gene (TTC37) excluded mutations. The objective of this study was to identify the molecular genetic defect in this family and hence further understanding of unexplained cholestasis within a multisystem disorder.

Methods: We used a novel combination of autozygosity mapping combined with whole-exome-sequencing (WES). An Affymetrix 250K SNP chip genome-wide linkage scan was used to identify common regions of shared homozygosity. SureSelect human All Exon kit (Agilent Technologies) and Illumina GaIIx was used for WES of both individuals. Single nucleotide substitutions and small insertion deletions were identified. Filtering of variants for novelty was performed by comparison to dbSNP131 and 1000 Genomes pilot SNP calls (March 2010) and variants identified in 40 control exomes sequenced and analysed by the same method described above.

Results: The largest overlapping autozygous regions were at chromosome 7, 16, 20, 12 and 4. The whole exome sequencing identified 17,844 and 17,867 variations in patients 1 and 2 respectively. Of these only 3 homozygous nonsynonymous variants and 1 frameshift variant were found in both patients in the identified homozygous regions. The frameshift was a homozygous single base G deletion (c.587delG) in exon 6 of AKR1D1 which mapped within the candidate homozygous region in chromosome 7. The variant results in a frameshift at amino acid 196 leading to a premature stop codon 11 amino acids downstream (p.Cys196SerfsX11). AKR1D1 encodes the enzyme \( \Delta^2 \)-3-oxosteroid 5β-reductase that is required for the synthesis of chenodeoxycholic and cholic acids important for normal bile flow. Mutations in AKR1D1 have previously been described in patients with severe neonatal liver disease.

Conclusion: In conclusion we have extended the clinical features of bile salt synthesis disorders resulting from mutations in AKR1D1 to include a severe form of low GGT cholestasis, TN and severe hypoglycaemia which may be amenable to treatment with bile salt supplementation. Combining the technique of whole genome linkage mapping and WES creates a powerful tool to elucidate the molecular basis of uncharacterised genetic disorders.

Disclosure of Interest: None declared.

PL-H-0040

Hepatology
IL28B GENE POLYMORPHISMS IN CHILDREN WITH CHRONIC HEPATITIS C
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Objectives and Study: Polymorphisms upstream of the IL28B gene are predictors of outcome in adults with hepatitis C (HCV). We evaluated these polymorphisms in children.

Methods: Subjects were 92 cases of paediatric chronic HCV. Route of infection was mother-to-child in 71 (77%). HCV genotype (g) was 1 in 75 (81.5%), g2 in 1, g3 in 11, g4 in 5. Only 2 showed advanced disease.

Genotyping of the IL28B rs12979860 and rs8099917 polymorphisms was performed by sequencing. IL28B genotypes were studied in relation to route of infection, HCV genotypes and Knodell staging. Polymorphisms upstream of the IL28B gene are predictors of outcome in adults with hepatitis C (HCV). Study of these polymorphisms was performed in children.

Results: The distribution of IL28B rs12979860 genotype was: CC = 24 (26%), CT = 50 (54%), TT = 18 (19.5%). That of rs8099917 was: TT = 41 (44.5%), TG = 48 (52%), GG = 3 (3.2%). CC rs12979860 associated with TT rs8099917 (P < 0.0001), 23 patients had both. CC rs12979860 patients, compared to non-CC. They had similar gender distribution, route of infection, HCV genotype, ALT values and Knodell. Viral load was higher (above mean value: CC = 83%, non-CC = 35%, P = 0.0001). Response to any treatment was better (SVR: CC = 59% vs non-CC = 25%, P = 0.01) while none of clinical data influenced response. TT rs8099917 patients, compared to non-TT: TT was more frequent in subjects with parenteral HCV (12/17), higher than average viral load (P = 0.03) and responders (SVR TT = 52% non-TT = 21%, P = 0.009). d) LKM antibody (n = 7 g1) associated with CC rs12979860 (n = 5, P = 0.01) and TT rs8099917 (n = 6, P = 0.05). e) Thyroid dysfunction induced by treatment was not associated with IL28B polymorphisms.

Conclusion: Favourable IL28B polymorphisms were found in 26% (CC rs12979860) and 44% (TT rs8099917) in a population of children with chronic HCV. As described in adults, they associated with higher viral load and with increased response to treatment. Relationship to LKM+ is a new finding. IL28B genotyping keep unable to predict treatment-induced thyroid disturbances.

Disclosure of Interest: None declared.
Objectives and Study: Evaluate the evolution of the prognosis of biliary atresia (BA) since liver transplantation (LT) became widely available.

Methods: The charts of all patients diagnosed with BA, born between 1986 and 2009, and living in France, were reviewed in 45 centers. Survivals were calculated with the Kaplan-Meier method and were compared using the log rank test.

Results: 1107 BA children were identified: 990 born in metropolitan France (incidence 1/18400 live births), 88 overseas, and 29 abroad. 14 children (1.3%) without BA underwent a Kasai operation were not included. Kasai operation or its variants were performed in 1044 BA patients (94.3%). Survival with native liver after Kasai operation was 40%, 36% and 30% at 5, 10 and 20 years. These results did not progress over years. 587 children underwent LT; 1 to 4 times (692 transplants). Mortality without transplantation was 16%, 7% and 4% in the cohorts 1986–96, 1997–2002 and 2003–2009, respectively (P<0.001). Survival after transplantation was 83%, 82% and 77% at 5, 10 and 20 years in the whole series. Survival 5 years after transplantation progressed from 75% in the 1986–96 cohort to 90% in the 1997–2002 and 2003–2009 cohorts (P<0.001). In the whole series, overall BA patient survival was 81%, 80% and 77% at 5, 10 and 20 years. 5 year BA patient survival progressed from 72% in the 1986–96 cohort, to 88 and 89% in the 1997–2002 and 2003–2009 cohorts (P<0.001).

Conclusion: With the sequential treatment of Kasai operation and liver transplantation if needed, 9/10 BA patients can live, and 3/10 reach the age of 20 years without transplantation. The prognosis of BA has improved in recent years, mainly due to a better access to LT, and better results after LT.

Disclosure of Interest: None declared.

Objectives and Study: Stem cell transplantation is a promising treatment for human liver inborn errors of metabolism diseases. Organ shortage stimulated further research on alternative stem cell sources. Umbilical cord matrix stem cells demonstrated interesting properties and differentiation potential towards hepatic lineage. Therapeutic use of umbilical cord matrix stem cells (UCMSC) relies on the demonstration of their stabilized properties during long-term culture. During large scale ex vivo cell expansion, cell characteristics may be altered. We therefore investigated in vitro and in vivo genetic stability of these cells cultured up to senescence.

Methods: UCMSC were isolated from fourteen healthy term newborns. Cells were characterized by measuring cytoplasmic and cell surface markers expression by flow cytometry, immunofluorescence and qPCR. We followed the growth, cell morphology and anchorage dependence at each passage. Hepatic differentiation potential was assessed by the analysis of key hepatic metabolic functions. Long-term genotype stability was investigated by performing karyotype, telomere length, measure of telomere activity and gene expression related to tumorigenesis. Tumorigenic potential was investigated after injection of 1.10^7 cells in a xenograft model.

Results: Proliferative capacity was similar between cell cultures. Cells reached senescence after 27.6±1.6 corresponding to a culture period of 160.9±6.9 days. Cells expressed high levels (>95%) of CD73, CD90, CD105, CD44 and CD29, and maintained their original phenotype up to senescence. UCMSC could acquire mature hepatic metabolic functions after differentiation, nearly reaching the potential of human hepatocytes concerning urea production and Cyp3A4 activity. UCMSC remained cytogenetically stable during long-term culture. The cells did not express telomerase activity or alternative telomere lengthening mechanisms. Human telomerase reverse transcriptase expression was not detected. Levels of cell cycle related genes such as p53, p16, p21 and pRb were typically correlated with progressive cell senescence as shown by positive senescence associated beta-galactosidase staining. UCMSC did not display tumorigenic potential in vivo or in vitro. Anchorage dependence was conserved in vitro. Nude mice subcutaneously injected with UCMSC did not develop tumors.

Conclusion: UCMSC is an inexhaustive, uncontroversial and easily accessible cell source. They can be expanded in vitro while maintaining a stable phenotype, genotype and differentiation capacity. UCMSC therefore represent safe and effective candidates for liver regenerative medicine.

Disclosure of Interest: None declared.
Endoscopy: Diagnosis and Therapeutic Surgical Procedures

DEEP SEDATION WITH PROPOFOL FOR UPPER AND LOWER GASTROINTESTINAL ENDOSCOPY IN CHILDREN, ADMINISTERED BY SPECIALLY TRAINED PEDIATRICIANS

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Objectives and Study: Evidence is accumulating that non-anesthesiologists administered propofol sedation is safe and effective. However, limited data exists on this practice for gastrointestinal endoscopy in children. The aim of the present study was to assess the safety and efficacy of procedural sedation with propofol administered by specially trained pediatricians for upper and lower gastrointestinal endoscopy in children.

Methods: Data on procedural sedation with propofol administered by specially trained pediatricians for gastrointestinal endoscopy in children between January 1, 2000 and December 31, 2010 was prospectively recorded. Particular attention was given to the incidence and type of adverse events and the frequency between the former groups (0.96 and 0.91, respectively, P < 0.001), but there was no difference between the latter groups (0.96 and 0.91 µm vs 0.55, respectively, P < 0.001). AET did not differ between the two groups (ERD: 7.6 ± 3.9, NERD: 6.6 ± 4.5, NS) as well as TN (ERD: 105.5 ± 54.16, NERD: 89.08 ± 35.91, NS). The two groups did not differ neither for total numbers of AR, Wac and Walk refluxes (ERD: 55.8 ± 38.49, AR: 16.5 ± 11.91, Wac: 11.7 ± 10.41 Walk; NERD: 45.2 ± 26.22 AR, 12.76 ± 17.16 Wac, 8.64 ± 9.8 Walk, respectively; NS nor for the total number of reflux reaching the proximal, mid, and distal esophagus (ERD: 56.55 ± 39.72, 17.85 ± 7.9, 9.6 ± 9.7; NERD: 40.36 ± 23.17, 14.4 ± 8.8, 5.08 ± 5.06, respectively;

Conclusion: Administration of propofol by trained pediatricians for procedural sedation during gastrointestinal endoscopy was successful and relatively safe. Constant and prompt availability of anesthesiologists is however mandatory.

Disclosure of Interest: None declared.

OSOPHAGEAL MUCOSAL DILATED INTERCELLULAR SPACES (DIS): IS A REAL ULTRASTRUCTURAL MARKER OF GERD PHENOTYPE? A CHILDREN POPULATION STUDY

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Objectives and Study: Esophageal mucosal dilated intercellular spaces (DIS) have been reported to be an early sign of mucosal impairment in adult patients with both erosive (ERD) and nonerosive reflux disease (NERD). No data are available in children. In population of children with gastro-esophageal reflux disease (GERD) with different phenotypic expression, we assessed the relationship between esophageal mucosa ultrastructural changes and reflux pattern.

Methods: Twenty-four patients (median age 9 years) with NERD, 20 patients (median age 8.8 years) with ERD and 10 controls (median age 9.7 years) were prospectively enrolled. All patients and controls underwent upper endoscopy. Biopsies were taken at 3–5 cm above the esophagogastric junction and intercellular space diameters were measured on transmission electron microscopy photomicrographs. Both NERD and ERD patients underwent 24-h multichannel intraluminal impedance (pH)-pH monitoring; the following variables were analyzed: acid exposure time (AET), total number of reflux episodes (TN), number of acid (AR) (pH<4), weakly acidic (WAc) (pH>4<7), and weakly alkaline (Walk) (pH>7) reflux episodes, and height of reflux episodes.

Results: The median value of intercellular space diameter was significantly higher in both NERD and ERD groups as compared with controls (P < 0.001), but there was no difference between the former groups (0.96 and 0.91 µm vs 0.55, respectively, P < 0.001). AET did not differ between the two groups (ERD: 7.6 ± 3.9, NERD: 6.6 ± 4.5, NS) as well as TN (ERD: 105.5 ± 54.16, NERD: 89.08 ± 35.91, NS). The two groups did not differ neither in total numbers of AR, Wac and Walk refluxes (ERD: 55.8 ± 38.49 AR, 16.5 ± 11.91 Wac, 11.7 ± 10.41 Walk; NERD: 45.2 ± 26.22 AR, 12.76 ± 17.16 Wac, 8.64 ± 9.8 Walk, respectively; NS nor for the total number of reflux reaching the proximal, mid, and distal esophagus (ERD: 56.55 ± 39.72, 17.85 ± 7.9, 9.6 ± 9.7; NERD: 40.36 ± 23.17, 14.4 ± 8.8, 5.08 ± 5.06, respectively;
were less frequently implicated than expected. H. pylori infection (27%) and gastrotoxic medications (23%) referred for upper GI endoscopy. A pilot study suggested that H. pylori was involved in gastric and duodenal ulcers or erosions among children.

**Objectives and Study:**

**PROSPECTIVE EUROPEAN MULTICENTRE EPIDEMIOLOGIC CASE-CONTROL STUDY ON RISK FACTORS OF GASTRIC AND DUODENAL ULCERS OR EROSIONS IN CHILDREN**

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**Objectives and Study:**

To analyse risk factors associated with gastric and duodenal ulcers or erosions among children referred for upper GI endoscopy. A pilot study suggested that H. pylori infection (27%) and gastrotoxic medications (23%) were less frequently implicated than expected.

**Methods:**

Open, prospective, case-control study. Data anonymously reported for patients and 2 controls that immediately follow the index case, cross-matched for age groups. Study carried out between Jan 2008 and Dec 2009 in 11 European countries.

**Results:**

244 patients (153 with erosions alone and 91 with ulcers) and 488 controls were included. Median age comparable between patients and controls (11.2 y – 0.1–17.8 y vs 11.1 y – 0.2–17.9 y). Ulcer/erosions were more frequent in children >10 y (OR 2.5 – P < 0.0001). Peptic lesions were significantly related to male gender (OR 1.4, P = 0.04), non-steroidal anti-inflammatory drugs (NSAIDs, OR 1.6, P = 0.05), alcohol consumption (OR 1.9, P = 0.05), and tobacco use (OR 7.4, P < 0.0001). H. pylori infection was present in 63/244 patients and 81/488 controls (OR 1.9, P < 0.001). H. pyloristatus was considered as not valid in 26 patients and 34 controls (recent use of antibiotics). H. pylori infection was strongly related to duodenal ulcer (OR 5.0, P < 0.0001) and duodenal erosions (OR 2.3, P = 0.02), whereas no association was observed between H. pylori infection and gastric lesions. On the contrary heartburn, chronic cough, chronic lung disease, and coeliac disease were significantly more frequently reported in controls than in patients. The use of steroids, immune-suppressive drugs, antibiotics, antacids, H2-blockers and PPIs are equally distributed. No significant differences were reported for socioeconomic, lifestyle factors and other symptoms or chronic diseases. No known risk factors for PUD were observed in 141/244 (57.8%) cases.

**Conclusion:**

This study confirms that H. pylori infection is a risk factor for duodenal, but not for gastric lesions in children. Male gender, age (older than 10 y), NSAID, alcohol and tobacco use are independent risk factors for gastric and duodenal ulcer/erosions in children. A high proportion of children have primary ulcer/erosions with no identifiable risk factors.

**References:**


**Disclosure of Interest:** None declared.

**PA-G-0046**

**Coeliac Disease and Enteropathies**

**CELLIAC DISEASE RISK IN BIRTH COHORTS THAT DIFFER WITH RESPECT TO INFANT FEEDING**

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**Objectives and Study:**

An epidemic of celiac disease (CD) in children below 2 years of age was experienced in Sweden from 1984 to 1996, partly explained by changes in infant feeding. The ETICS study—Exploring the Iceberg of Celciacs in Sweden—is part of the EU-funded PrevenCD project. The overall aim is to resolve if primary prevention of CD is possible by favourable infant feeding. In this study we compared CD prevalence, including symptomatic and screening-detected cases, in 12-year-olds from two birth cohorts (1993 and 1997) exposed to different infant feeding.

**Methods:**

A two-phased cross-sectional CD screening in 2005–2006 (phase I) and 2009–2010 (phase II) involving all 6th graders in the same well delineated geographical areas...
across Sweden. During phase I 10041 children were invited with 7567 (75%) consenting, and blood samples from 7207 (72%). During phase II 8282 children were invited with 5711 (69%) consenting, and blood samples from 5456 (66%). All samples were analysed for anti-human tissue transglutaminase-IgA (tTG) with cut-off 4U/mL (Celikey, Phadia GmbH, Freiburg, Germany). When an intermediate tTG level (2–4U/mL), anti-endomysial-IgA (EMA) was also analysed with cut-off 1:5 dilution (The Binding Site, Birmingham, UK). All children with elevated markers were referred for small bowel biopsy. Previously diagnosed CD cases were identified through the consent form, and verified by the National CD Register and/or medical records.

**Results:** In the epidemic cohort of 1993 (phase I in 2005–2006) the prevalence of elevated CD markers was 27 per 1000 (95% CI 23–30), and the biopsy-verified prevalence was 21 per 1000 (95% CI 18–24). Out of 187 children with elevated markers 167 had elevated tTG, and 29 had intermediate tTG and positive EMA. The prevalence of previously diagnosed CD was 8.9 per 1000 (95% CI 6.7–11), based on 66 cases. In the post-epidemic cohort of 1997 (phase II in 2009–2010) the prevalence of elevated CD markers was 19 per 1000 (95% CI 16–23), and small bowel biopsies are underway. Out of 104 children with elevated markers 88 had elevated tTG, and 19 had intermediate tTG and positive EMA. The prevalence of previously diagnosed CD was 5.3 per 1000 (95% CI 3.8–7.7), based on 33 cases.

**Conclusion:** The CD prevalence in 12-year-olds from birth cohorts of both the epidemic (1993) and post-epidemic (1997) periods were unexpectedly high. A comparison of the prevalence of CD enteropathy between these 2 cohorts, which differ with respect to infant feeding, has to await completion of the small bowel biopsies.

**Disclosure of Interest:** None declared.

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**PA-G-0047**

**Endoscopy: Diagnosis and Therapeutic Surgical Procedures**

**DOUBLE-BALLOON ENTEROSCOPE—A TERTIARY CARE EXPERIENCE**

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**Objectives and Study:** Double-balloon enteroscopy (DBE) has become a preferred method for management of small bowel disorders in adult population. Experience in paediatric population remains limited with regards to utility, therapeutics and safety profile of DBE.

**Methods:** Thirty-seven procedures were performed on 35 patients (21 M; 14F) from January 2004 to December 2010 at Sheffield Children’s Hospital, with median age of 12.7 years (range 1–18) and median weight of 39.8 kg (range 8–95).

**Results:** Yield of 30/37 (81%) with therapeutic success in 19/37 (51%) and 118 min (range 50–320). No complications were encountered. Polyps were detected and successfully removed in 10 patients with Peutz-Jeghers syndrome, 2 patients with Cowden’s syndrome, 2 patients with angiomatous malformations, and in a patient with tubulo-villous adenoma of the distal duodenum. A diagnosis was made in a patient with multiple angiomata not amenable to endotherapy, and in four with discrete angiomata treated with argon plasma coagulation. The source of bleeding was identified in a further patient with oesophageal varices. Two patients with protein-losing enteropathy were diagnosed to have isolated intestinal lymphangiectasia and 1 underwent laparoscopic assisted surgical resection with transmural transillumination by enteroscope. Diagnosis of Crohn’s disease was confirmed in 2 suspected patients and in 3 further patients to determine the extent of disease. One patient with feed intolerance found to have severe dysmotility. One patient with GI bleed had large ulcer at ileocolonic anastomosis site. DBE was normal or revealed minor mucosal friability in the remaining 7 patients. Hence a diagnostic yield of 30/37 (81%) with therapeutic success in 19/37 (51%) was achieved.

**Conclusion:** DBE appears to be a safe diagnostic and therapeutic tool with suspected small bowel disorders. Further larger studies are required to establish widespread application.

**Disclosure of Interest:** None declared.

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**PA-G-0048**

**Immunology**

**HISTO-BLOOD GROUP ANTIGEN RECEPTORS IN THE PROTECTION AGAINST NOROVIRUS INFECTIONS ELICITED BY BREAST MILK**

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**Objectives and Study:** Noroviruses (NoV) are a leading cause of viral epidemic gastroenteritis in childhood. Human histo-blood group antigens (HBGA) are the viral receptors necessary for NoV infection and spread.
The α1.2-fucosyltransferases (FUT-1,2) and the A and B glycosyltransferases are responsible for their expression and determine the ABO histo-blood group phenotype that may confer susceptibility or resistance to specific NoV. Human milk is rich in oligosaccharides, either in free forms or in conjugates of glycoproteins or glycolipids. These are encoded by the same genes that participate in the HBGAs synthesis, so they are structurally related and could act as decoy receptors avoiding NoV from the binding to mucosal epithelial cells HBGAs. There is growing evidence regarding the role of the non-specific immunity of breast milk in the protection against NoV infection. Our aim was to assess the implication of the HBGAs phenotype in the protection against NoV infection elicited by breast milk.

**Methods:** Colostrum, transitional, mature breast milk and serum samples from 112 volunteer mothers were analyzed by ELISA for antibodies to NoVs, and for their blocking activity on the binding to saliva of NoV GI4 VLPs produced in cells of Spodoptera frugiperda (SF 9) by recombinant baculovirus. The results were correlated with the secretor status analyzed by PCR.

**Results:** Two-hundred and ninety-three samples (106 colostrum, 102 transitional and 85 mature) of breast milk from 112 mothers and 74 samples of serum were obtained. Ninety-seven mothers were secretor (FUT2+) and 15 were non-secretor (FUT2-). The binding of NoV GI4 VLPs to human milk and saliva samples was confirmed by ELISA, even to samples from nonsecretor women. All the milk samples were able to block the binding of NoV VLPs to saliva, but samples from secretor mothers showed a stronger inhibition, suggesting the presence of free HBGAs that could act as decoy receptors competing with mucosal epithelial intestinal cell receptors. The binding of VLPs to high MW proteins (>150 kDa) in secretors’ skim milk, as well as to a ~65 kDa thermolabile protein and to a ~33 kDa protein was detected by Western blot.

**Conclusion:** Breast milk from secretor women shows a stronger protective property against NoV infection. The fact that nonsecretor women’s breast milk shares this feature, suggests that other components, different from FUT 2, could participate as NoV receptors. Further investigation regarding the identity of the breast milk proteins that bind NoV VLPs is required.

**Disclosure of Interest:** None declared.

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**Coeliac Disease and Enteropathies**

**PA-G-0049**

**IL-15 IN MONOCYTES AND DENDRITIC CELLS FROM PATIENTS WITH COELIAC DISEASE**

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**Objectives and Study:** Recent evidence indicates that innate immune response cells, including monocytes and dendritic cells (DCs) contribute to celiac disease pathogenesis. Interleukin (IL)-15 is mainly synthesized by these cells. IL-15 is a cytokine playing a pivotal role in the immune response. It has a membrane bound form that is biologically active (mbIL-15) which can be present on DCs or monocytes to promote T cell responses.

The aim of this study was to investigate the levels of IL-15 expression in monocytes and DC of CD patients and controls.

**Methods:** DCs, monocytes and CD8+ T cells were generated from blood PBMC from healthy donors (CTR), untreated celiac patients (CD) and gluten free diet patients (GFD). Analysis of cell surface markers was performed using flow cytometric analysis. In vitro proliferation assay was assessed.

**Results:** By FACS analysis we observed that the percentage of cells expressing mb-IL-15 on monocytes and DCs from CD patients (Mean ± SD 34.4 ± 18.5 and 13.7 ± 14.4, respectively) and from GFD patients (Mean ± SD 22.2 ± 7.25 and 83.2 ± 27.7, respectively) is statistically different (CD patients P < 0.0005 and P < 0.05, respectively, GFD patients P < 0.05 and P < 0.05) increased compared to CTR (Mean ± SD 16.7 ± 10.3 and 5.8 ± 5.4, respectively). Moreover, we observed a greater intensity of fluorescence of mb-IL-15 on monocytes and DCs from CD patients (MFI ± SD 139.7 ± 86.7 and 77.1 ± 49.3 respectively) (P < 0.0001 and P < 0.01) and from GFD patients (MFI ± SD 119.9 ± 79 and 83.3 ± 27.7 respectively) in comparison with CTR (MFI 11.9 ± 7.4 and 42.7 ± 22.6) (P < 0.0001 and P < 0.001). Monocytes and DCs of CD patients express more strongly the bright form of mb-IL-15 than CTR. This bright form is functionally involved in the activation of CD8+ T-cells. The massive increase of proinflammatory cytokine IL-15 in CD led us to investigate whether and how IL-15 on APC cells in CD patients might affect the CD8+ T-cell responses. Stimulation of CD8+ T cells with allogenic monocytes and DC cells from celiac patients strongly induces proliferative effects on these cells and this phenomenon is reverted by anti-IL-15 blocking antibodies in DC cells.

**Conclusion:** In CD patients more mb-IL-15 is expressed on monocytes and DCs. This form of IL-15 in CD is biologically active as it is able to induce allogeneic CD8+ T cells proliferation. These findings support the hypothesis of a central role for IL-15 in the pathogenesis of CD.

**Disclosure of Interest:** None declared.

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**Endoscopy: Diagnosis and Therapeutic Surgical Procedures**

**PA-G-0050**

**MEDICAL, ENDOSCOPIC AND SURGICAL MANAGEMENT OF GASTROINTESTINAL LESIONS IN BLUE RUBBER BLEB NAEVUS SYNDROME**

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www.jpgn.org
Objectives and Study: Blue rubber bleb naevus syndrome (BRBNS) is an unusual form of haemangioma, characterised by multiple cutaneous and visceral venous malformations. Intestinal lesions, often in the small bowel, are common and presents with haematemeses, haematochezia and transfusion-dependant haemorrhage. Assessment and treatment of BRBNS can be challenging, especially in the small bowel. Systemic agents such as corticosteroids, octreotide, vincristine, tranexamic acid and interferon have been tried with varying success. Reports of endoscopic interventions are few and limited surgical experience exists in this condition.

We proposed a review of the management of all patients presenting to our centre with BRBNS.

Methods: A review of all patients with BRBNS who presented to a paediatric tertiary gastrointestinal centre between 1999 and 2010, with particular emphasis on medical, endoscopic and surgical treatments.

Results:

Patient | Treatment
--- | ---
Patient 1 | Multiple colonoscopy and sclerotherapy. Laparotomy with resection of 80 lesions, resulting in symptom resolution for more than 1 year. Previous partially steroid-responsive. Recurrence of lesions now treated with a combination of medications, endoscopic and enteroscopic sclerotherapy with good result (2 blood transfusions in the last year).
Patient 2 | Multiple endoscopies and 7 single-balloon enteroscopies combined with sclerotherapy and Thalidomide, resulting in good bleeding control and 6-monthly blood transfusions. Thalidomide will be discontinued due to the onset of peripheral neuropathy. Previously steroid-responsive but suffered from side effects. An intra-operative enteroscopy with resection of lesions is planned.
Patient 3 | Endoscopic surveillance and resection of mesenteric lesion. All medical therapies had failed. Intestinal lesions have resolved but now suffer from large porto-systemic shunt with high-output congestive cardiac failure and pulmonary lesions.
Patient 4 | Currently asymptomatic despite evidence of lesions throughout the small bowel on Video Capsule Endoscopy. Annual monitoring is expected. Prophylactic tranexamic acid has been started.

Conclusion: Thalidomide, an inhibitor of angiogenesis by suppression of vascular endothelial growth factor, is very effective at controlling bleeding but appears to be more effective when used in combination with other therapies. Its use is limited by its side effects. Other medications, with the exception of corticosteroids, were ineffective in our group. Endoscopic and enteroscopic treatments perhaps limit the need for surgery, but possibly delay rather than avoid surgery. Endoscopic sclerotherapy and surgery are useful in the management of BRBNS but does not prevent recurrences. Despite these limitations, improved care and management have been achieved with these new developments.

Disclosure of Interest: None declared.

PA-H-0051

Hepatology

PREVALENCE AND SIGNIFICANCE OF RAISED IgG4 IN PAEDIATRIC AUTOIMMUNE LIVER DISEASE: A RETROSPECTIVE STUDY

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Objectives and Study: Immunoglobulin G subclass 4 (IgG4)-related liver disease is well described in adults and is associated with multisystemic symptoms and a particularly good response to corticosteroid treatment. The significance of raised serum IgG4 in paediatric liver disease is unclear. We have investigated prevalence and clinical significance of raised IgG4 levels in a large cohort of children with autoimmune liver disease.

Methods: Retrospective review of medical records, clinical, biochemical, radiological and histological data of 75 children with autoimmune liver disease (42 autoimmune hepatitis [AIH] and 33 autoimmune sclerosing cholangitis [ASC]) diagnosed from 2005–08. IgG4 levels were tested retrospectively in stored serum (−80 °C) collected at or close to diagnosis by immunoenzymatic assay (ELISA). Levels >1.35 g/L were considered abnormal, based on published data in autoimmune pancreatitis. Patients were divided into two groups: Group 1 (high IgG4) and Group 2 (normal IgG4). In 63 children with available liver biopsy at presentation, histological activity, staging, and number of IgG4+ plasma cells per high power field were also investigated.

Results: Group 1 comprised 25 children (33%), 12 with AIH and 13 with ASC; group 2 comprised 50 children, 30 with AIH and 20 with ASC. Inflammatory bowel disease was diagnosed in 28% of both groups. Group 1 had significantly higher IgG levels (median 29.4 g/L, range 14.5–63.8, P < 0.001) and IgG4/IgG ratio (9.9 [2.3–27.7], P < 0.001) compared to Group 2. Within patients with AIH, those in Group 1 had lower C3 levels (0.86 g/L, [0.41–1.35] P = 0.019) than those in Group 2. There was no difference in liver function tests and histological activity/staging at presentation between the 2 groups. IgG4+ cells (≥5 cells) within the liver tissue were more commonly seen in Group 1 than Group 2 (27% vs. 2%), but ≥10 IgG4 positive cells, as described in adult IgG4-related disease, were not identified. Granulocyte epithelial lesions were seen in only one case of ASC within Group 2. After a median follow up of 3 years [2–5] normal AST; immunoglobulins and negative auto-antibodies were recorded in 62% in Group 1 and 51% in Group 2.

Conclusion: 33% of children with autoimmune liver disease have high IgG4 levels at diagnosis with no difference between AIH and ASC. This proportion is higher than that reported in adults with AIH (3%) or primary sclerosing cholangitis (9%). No difference in disease severity or response to treatment was observed medium-term between patients with low or high IgG4, but longer follow up is necessary to determine whether children with high IgG4...
I. Goldschmidt 1, E29

ELASTOGRAPHY IN CHILDREN

Hepatology

LIMITATIONS OF TRANSIENT LIVER
ELASTOGRAPHY IN CHILDREN

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Objectives and Study: Transient elastography (Fibroscan) is increasingly recommended for the non-invasive diagnosis of liver fibrosis in children. This study aims at examining technical issues and limitations in transient elastography raised by the varying age and size of children.

Methods: Transient elastography was performed in 78 children aged 0.2–17 (median 5.9) years (33 f; 45 m; 38 liver patients, 40 healthy volunteers). Fibroscan results were accepted if the ratio of interquartile range and median of 10 successive readings was <30%. Fibroscan examinations were performed at 4 different sites in each patient. The position of the liver was determined by percussion, and transient elastography first attempted in the highest possible intercostal space (ICS) in the anterior axillary line (AAL1), then one ICS below the first (AAL2). A 3rd reading was attempted in the mid-clavicular line (MCL1), and 1 ICS below (MCL2). Success of the examination and diagnostic acceptability were recorded. After completion of the Fibroscan examination, position and thickness of the liver were verified by B-mode ultrasound. Quantitative variables are given as median (range). Results of Fibroscan examinations are compared by signed Wilcoxon rank test.

Results: Fibroscan examination was technically possible in AAL1/AAL2/MCL1/MCL2 in 92%/60%/53%/33% of cases and acceptable with IQR/Median <30% in 80%/45%/46%/27%, respectively. The number of acceptable readings correlated with age of the child (r = 0.62). Total failure rate (No reading obtainable at all) was 3.8%. In 14%, no reading was acceptable. Failure to obtain any acceptable reading was associated with low patient age. In children below 24 months of age (n = 28), total failure rate increased to 11%, and no acceptable reading was obtained in 39%. Examinations performed under general anaesthesia did not have significantly higher success rate in this age group. Liver stiffness values in MCL were significantly lower than in AAL of the same patients (5.3 (3.3–75) kPa vs. 6.5 (2.4–67.8) kPa, p < 0.05). The recommended cut-offs for S1 probe choice (chest circumference (CC) <45 cm/age <6 years) did not correspond in our patients (n = 15, age 0.2–6 (3.5) years, CC 31–60.5 (51) cm), S1 measurements were significantly higher than S2 at AAL1 in n = 8 patients aged 4.8–8.3 years (P < 0.05). In contrast, S2/M measurements did not differ significantly in n = 9 patients aged 10–17 years.

Conclusion: Transient elastography using the Fibroscan is feasible in children. Success rate is limited in children below 24 months of age both for anatomical and behavioural reasons. Site of examination and probe choice significantly influence results and should be taken into account when interpreting results.

Disclosure of Interest: None declared.

PA-H-0052

Hepatology

PA-H-0053

Hepatology

CLAUDIN-1, A TIGHT JUNCTION PROTEIN INVOLVED IN NISCH SYNDROME, PLAYS A ROLE IN HEPATIC PARACELLULAR PERMEABILITY: EVIDENCE IN HEPATOCELLULAR AND CHOLANGIOCELLULAR POLARIZED LINES

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Objectives and Study: Neonatal ichthyosis and sclerosing cholangitis (NISCH) syndrome is a rare recessive autosomal liver disease caused by truncating mutations of the CLDN1 gene encoding claudin-1, a tight junctions (TJ) protein. In this syndrome it is speculated that cholestasis is due to absence of claudin-1, leading to increased paracellular permeability and to hepatocyte and bile duct injuries secondary to paracellular bile regurgitation. The present work was performed to test the role of claudin-1 in the maintenance of hepatic paracellular permeability.

Methods: Two polarized rat cell lines, the hepatocellular Can 10 line and the cholangiocellular NRC line, were used. Both of them form TJ. However, in contrast to NRC, Can 10 cells do not express claudin-1. Therefore, Can 10 cells were first transfected with a plasmid encoding the normal gene encoding claudin-1, a tight junctions (TJ) protein. In this syndrome it is speculated that cholestasis is due to absence of claudin-1, leading to increased paracellular permeability and to hepatocyte and bile duct injuries secondary to paracellular bile regurgitation. The present work was performed to test the role of claudin-1 in the maintenance of hepatic paracellular permeability.

Results: Stable Can 10 clones expressing different levels of claudin-1 were isolated. In all of them, claudin-1 was colocalized with zonula occludens-1 at the TJ. Paracellular permeability was evaluated (qPCR, western blotting, immunolocalisation) in stable transfected clones and paracellular permeability of these clones was assessed by FITC-dextran passage. Then, in these transfected clones claudin-1 expression was inhibited by siRNA and the impact of this inhibition on paracellular permeability was evaluated. A similar approach of claudin-1 expression evaluation and inhibition was used for NRC cells. In this latter case, the effect on paracellular permeability was assessed, by measuring transepithelial resistance.

Conclusion: Transient elastography using the Fibroscan is feasible in children. Success rate is limited in children below 24 months of age both for anatomical and behavioural reasons. Site of examination and probe choice significantly influence results and should be taken into account when interpreting results.

Disclosure of Interest: None declared.
In NRC cells, claudin-1 silencing led to claudin-1 expression decrease and to a significant increase in paracellular permeability. 

**Conclusion:** Defect in claudin-1 expression increases paracellular passage in polarized hepatic cell lines, supporting the hypothesis that bile acid leakage through claudin-1 deficient TJ is involved in hepatocyte and bile duct injuries observed in NISCH syndrome.

**Disclosure of Interest:** None declared.

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**PA-H-0054**

**Hepatology**

**HUMAN ADULT-DERIVED LIVER STEM/PROGENITOR CELLS (hALDSC) CORRECT BILIRUBIN METABOLISM DEFECT IN GUNN RAT**

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**Objectives and Study:** Crigler-Najjar syndrome is a monogenic disease causing deficiency of UGT1A1 leading to unconjugated bilirubin (UCB) accumulation. Orthotopic liver transplantation remains the only curative treatment but the procedure does not guarantee lifelong complication free survival. Hepatocyte transplantation has established that metabolic control trough allogeneic cell infusion is possible but the procedure remains limited by organ shortage and poor resistance of hepatocytes to cryopreservation. Our team is developing a cell based product using human adult derived liver stem/progenitor cells (hALDSC) restoring enzymatic function in inborn errors of liver metabolism. We previously demonstrated their ability to differentiate and to exhibit key specific hepatic functions. We evaluated the potential of hALDSC to conjugate bilirubin in vitro and reduce UCB concentration blood level after injection in Gunn rats.

**Methods:** In vitro, hALDSC were incubated with UCB. Percentage quantification of conjugated bilirubin in the culture supernatant was assayed by HPLC (conjugated bilirubin concentration/total bilirubin concentration) × 100. Expression of bilirubin metabolism key enzymes including multidrug resistance-associated protein 2 (MRP-2) and UGT1A1 was assayed by RT-PCR. Five Gunn rats were injected via the portal vein with 2.5 millions undifferentiated hALDSC. UCB blood level was monitored during 6 months. Three animals underwent the sham operation and 3 animals injected via the portal vein with 2.5 millions undifferentiated hALDSC. UCB blood level was monitored during 6 months.

**Results:** In vitro we demonstrated the potential of undifferentiated and differentiated hALDSC to conjugate bilirubin reaching after 24 h and 48 h 3%–8% and 5%–9% respectively. Potential of conjugation was evidenced though the expression MRP-2 and UGT1A1 involved in conjugated bilirubin excretion and bilirubin glucuronidation, respectively. 6 months post-transplantation, 2 Gunn rats exhibit poor or no UCB blood level concentration reduction and 3 rats exhibit significantly reduced concentration blood level (2.72 ± 0.41 mg/dL), in contrast to 6.07 ± 0.39 mg/dL for SHAM rats and 3.27 ± 0.37 mg/dL in hepatocyte transplanted rats. As assayed by RT-qPCR, transplanted rat livers contain cells expressing hAlbumin/hGAPDH in contrast to SHAM rat livers who do not exhibit human mRNA positive cells. These results were confirmed after analyzing human albumin expression using IHC.

**Conclusion:** Our results demonstrate that hALDSC are able to specifically conjugate bilirubin in vitro to engraft in recipient rat liver and partially restore metabolic deficient function in Gunn rat model. Additional series are currently investigated in order to confirm these data.

**Disclosure of Interest:** None declared.

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**PA-N-0055**

**Clinical Nutrition**

**METHIONINE REQUIREMENT IN PRESENCE OF CYSTEINE IN THE ENTERALLY FED TERM NEONATE**

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**Objectives and Study:** Experimental evidence of essential amino acid requirement in neonates is scanty. Recently, the branched chain amino acid requirements in term neonates were successfully determined using the indicator amino acid oxidation method. Methionine, an essential amino acid, can be used for protein synthesis, but serves as a precursor for homocysteine and cysteine as well. Current recommended methionine intake of 80 mg/kg/d. The objective of this study is to quantify the requirement of methionine in presence of cysteine (91 mg/kg/d) in term neonates using the indicator amino acid oxidation method.

**Methods:** Fully enteral fed term infants received randomly graded intakes of methionine (3–59 mg/kg/d). Breath samples were collected for 13CO2 during L-[1–13C]phenylalanine (indicator amino acid) infusion, measured by isotope ratio mass spectrometry and analysed by applying a biphase regression crossover analysis.

**Results:** Twenty-five neonates (birth weight 3.23 ± 0.31 kg, gestational age 38.9 ± 1.1 wks) were studied at a mean postnatal age of 13 ± 7 d. Fractional L-[1–13C]phenylalanine oxidation rates were plotted against methionine intakes. With increasing methionine intake, L-[1–13C]phenylalanine oxidation rate decreased (r = −0.83, P < 0.01). The mean requirement is determined at 38 mg/kg/d.
Conclusion: Present recommendations for methionine requirement are too low, although current formulas provide sufficient methionine when 150 mL/kg/d is consumed.

Disclosure of Interest: L. Huang: None declared, F. Maingay-de Groof: None declared, J. Schoonenboom: None declared, M. van Dongen: None declared, G. Voortman: None declared, C. Chen: None declared, Y. Huang: None declared, H. van Goudoever Grant / Research Support from: Danone Research, Conflict with: Study Formulas produced by SHS.

PA-N-0056

Nutrition, Metabolism, and Experimental Approaches
COLOSTRUM AGAINST CHEMOTHERAPY-INDUCED GASTROINTESTINAL TOXICITY IN PIGLETS
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Objectives and Study: Allogeneic stem cell transplantation is initiated by myeloablative cytotoxic therapy that is associated with gastrointestinal (GI) complications and increased risk of infection. Bovine colostrum contains immunomodulating components that are known to prevent gut inflammatory lesions in sensitive newborns. We hypothesized that colostrum can protect against chemotherapy-induced GI toxicity. We used piglets as a model for myeloablative cytotoxic therapy prior to stem cell transplantation.

Methods: Three day-old piglets (n = 37) received chemotherapy (busulfan and cyclophosphamide) with enteral diets of bovine colostrum (chemo-C, n = 10) or infant formula (chemo-F, n = 10). Controls received the same diets without chemotherapy (ctrl-C, n = 8; ctrl-F, n = 9). Pigs were euthanized, clinical indices of GI toxicity were collected and organs were sampled on day 11 after start of chemotherapy treatment, or earlier if there were clinical signs of severe distress. Laboratory analyses included intestinal structure (villus height, crypt depth) and function (brush border enzyme activities)

Results: Chemotherapy treatment caused more pigs to be euthanized before the end of the protocol, relative to controls (12/20 versus 0/17, respectively, P<0.01). Out of 8 pigs euthanized before day 10, fewer pigs tended to be from the chemo-C group compared to the chemo-F group (2/10 vs. 6/10, P = 0.17). No clinical signs of mucositis were observed in any of the pigs euthanized before day 10. Among the remaining pigs, oral mucositis was significantly more frequent in the chemo pigs relative to controls (9/12 vs. 0/17, P<0.01). In chemo pigs, significant reductions were observed in villus height and crypt depth in the proximal but not distal intestine (−15 to −30%, P<0.05). Sucrase and maltase activities in the proximal intestine were higher in colostrum- vs. formula-fed pigs (+50–60%, P<0.01) and enzyme activities tended to be higher in chemo-C vs. chemo-F pigs (+70–120%, all P<0.13). Vomiting was observed less frequently for chemo-C vs. chemo-F pigs (1/10 vs.10/10, P<0.001).

Conclusion: Newborn pigs were surprisingly tolerant to high-dose cytotoxic chemotherapy with regards to intestinal mucositis, although villous structure and function were negatively affected in the proximal intestine. A slow intestinal enterocyte turnover may explain that chemotherapy induces less damage in the distal small intestine. In line with our results, chemotherapy generally results in fewer GI complications in children, relative to adults (having a higher intestinal enterocyte turnover). Colostrum may act to protect against chemotherapy-induced GI complications.

Disclosure of Interest: None declared.

PA-N-0057

Nutrition, Metabolism, and Experimental Approaches
A POSTNATAL DIET WITH A MORE BREAST MILK–LIKE LIPID MATRIX MARKEDLY REDUCES BODY FAT ACCUMULATION IN ADULT MICE
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Objectives and Study: We previously reported that lipid quality of postnatal nutrition, i.e. fatty acid composition, has a sustained effect on body composition and fat accumulation in adult mice. The current study investigates whether another aspect of dietary lipid quality in postnatal nutrition, i.e., the physical properties of lipid droplets, may also affect development of body composition. In contrast to lipid droplets in infant milk formula (IMF), fat globules in breast milk are up to ten times larger in size and coated with a phospholipid membrane. We developed an IMF with a complex lipid matrix (Nuturis) which more closely resembles those physical properties of fat globules in breast milk and evaluated the long term effects of Nuturis versus standard IMF on body composition in mice.

Methods: From day 16 to 42 after birth, male C57Bl/6j mice were subjected to a diet containing either Nuturis or standard IMF (CTR). Subsequently, the mice were challenged with a moderate Western style diet (20 w% lipid) during adolescence and adulthood until dissection at day 98. A reference group was included with mice raised on standard IMF switching to standard rodent chow instead of Western style diet from day 42 onward. Inguinal, retroperitoneal and epididymal fat depots were collected at dissection. Body composition was monitored by dual x-ray absorptiometry at 42, 70, and 98 days of age.

Results: Total body weight of mice at dissection was lower for Nuturis compared to the CTR group. This difference was entirely due to reduced fat accumulation, since lean body mass was similar in adult mice raised on Nuturis compared to CTR diet. In accordance, visceral and subcutaneous white
adipose tissue depots were smaller in mice raised on Nuturis compared to CTR diet. Moreover, total body weight and fat mass of the Nuturis group, but not CTR group, was similar to the reference group, which was not challenged by an unbalanced Western style diet in adulthood.

**Conclusion:** The markedly reduced body fat accumulation in mice raised on Nuturis in this study confirms our previous results showing a protective effect of complex lipid matrix on adult body composition. In conclusion, we have now repeatedly demonstrated that exposure to a diet early in life with a more breast milk-like lipid matrix (Nuturis) prevents excessive fat accumulation when challenged with a moderate Western style diet during adulthood.

**References:**


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**PA-N-0058**

**Pediatric Nutrition**

**THREONINE REQUIREMENT IN THE ENTERALLY FED TERM NEONATE IN THE FIRST MONTH OF LIFE**

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**Objectives and Study:** Essential amino acids are important for growth and development of neonates, and can have major influence on development later in life. Suboptimal protein intake will result in diminished growth and development, whereas an intake above requirement increases (e.g. the risk of obesity). Threonine (an essential amino acid) plays a crucial role in innate immunity, as it is a major constituent of mucins produced by goblet cells in the intestines. Current recommendation of threonine requirement for infants of 0–1 months (76 mg/kg/d) is based on average human milk composition, which varies widely in composition. Commercially available formula’s provide threonine ranging from 110 mg/kg/d to 180 mg/kg/d when infants receive 150 ml/kg/d. The aim of the study is to determine the threonine requirement in fully enterally fed term neonates by means of the indicator amino acid oxidation method, using phenylalanine as indicator amino acid.

**Methods:** After 24 h test diet adaptation of randomly assigned amounts of threonine (range 5–182 mg/kg/d) 27 fully enterally fed term neonates received a primed continuous infusion of respectively \[^{13}C\]bicarbonate and L-\[^{1-13}C\]phenylalanine. At baseline and during both infusions, breath samples were obtained for \(^{13}CO_2\), measured by mass spectrometry. Biphasic linear regression cross-over model was used to determine the breakpoint.

**Results:** Twenty-seven term neonates (gestational age of 39.3 ± 1.1 wks SD, birthweight 3.32 ± 0.28 kg SD, mean postnatal age 12 ± 5d SD) were studied. The mean requirement was 69 mg/kg/d, with a 95% CI upper limit of 114 mg/kg/d, representing the population safe intake.

**Conclusion:** The determined requirement of threonine is close to the current recommendation. However, most commercially available formulas provide more than necessary.

**Disclosure of Interest:** J. Hogewind-Schoonenboom Grant/Research Support from: The study formulas were manufactured by SHS UK and transportation towards Shanghai was facilitated by Dumex China. Financial support was received from Danone, L. Huang: None declared, F. Maingay-de Groof: None declared, G. Voortman: None declared, C. Chen: None declared, Y. Huang: None declared, J. van Goudoever: None declared.

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**PO-G-0020/PD-G-0059**

**Coeliac Disease and Enteropathies**

**IN CELIAC DISEASE ENTEROPATHIC T CELLS ARE INDUCED IN SITU BY GLIADIN, BUT IL-15 INTERFERES WITH THEIR SUPPRESSIVE ACTIVITY**

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**Conclusion:** The determined requirement of threonine is close to the current recommendation. However, most commercially available formulas provide more than necessary.

**Disclosure of Interest:** J. Hogewind-Schoonenboom Grant/Research Support from: The study formulas were manufactured by SHS UK and transportation towards Shanghai was facilitated by Dumex China. Financial support was received from Danone, L. Huang: None declared, F. Maingay-de Groof: None declared, G. Voortman: None declared, C. Chen: None declared, Y. Huang: None declared, J. van Goudoever: None declared.
Objectives and Study: Celiac disease (CD) is a condition where the regulation of the mucosal immune response to dietary gliadin might be altered. The transcription factor Foxp3 has been identified as a marker of a subset of regulatory T cells (Treg). In this study we have investigated the inducibility by gliadin in an organ culture system as well as their suppressive function. Moreover, we attempted to define whether interleukin 15 (IL-15), overexpressed in CD, could influence the regulatory activity of such cells.

Methods: The expression of Foxp3, was analysed by immunohistochemistry and flow cytometry in duodenal biopsies cultured in vitro with gliadin, taken from treated CD patients and from controls. Furthermore, we analyzed the suppressive function of intestinal CD4+CD25+ T cells of CD patients, on autologous responder CD4+CD25- T cells (Tresp), with or without IL-15. Such suppressive activity was tested measuring proliferation and IFN-γ secretion by Tresp cells.

Results: Lamina propria Foxp3+ cells in biopsies from treated CD, cultured with gliadin (mean ± SD: 16 ± 9) were significantly higher (P < 0.01) than in biopsies cultured with medium alone (6 ± 4). By contrast, no statistically significant differences were noted in the number of Foxp3+ cells when biopsies obtained from controls were cultured in the presence of PT-gliadin (5 ± 1) compared with those cultured in medium alone (5 ± 2). The FACS analysis confirmed the immunohistochemical data. Specifically, there was an significantly (P < 0.01) higher frequency of Foxp3+CD25+ T cells in treated CD biopsy samples cultured with PT-gliadin (21.4 ± 17.9 %) than in those cultured in medium alone (10.8 ± 7.9 %). No significant differences were noted in the percentage of Foxp3+CD25+CD4+ cells in biopsies of controls cultured with PT-gliadin (4.2 ± 0.87 %), in comparison to biopsies cultured with medium alone (3.7 ± 2.1 %). In co-culture studies, intestinal CD4+CD25+CD4- T cells of CD patients, significantly suppressed the proliferation of Tresp cells (P < 0.005) and induced a significant decrease of IFN-γ production (P < 0.001). Finally, IL-15 was effective in counteracting intestinal Treg cell-mediated suppression of anti-CD3-activated Tresp cells in terms of proliferation and IFN-γ production.

Conclusion: In conclusion, our results suggest that in CD intestinal Treg cells are induced in situ by gliadin. In CD intestinal Tregs are functionally competent. However, they can be impaired in vivo in their suppressor capacity by IL-15, this phenomenon contributing to maintain and expand the local inflammatory response in CD.

Disclosure of Interest: None declared.

PO-G-0008/PD-G-0060
Coeliac Disease and Enteropathies
ASSOCIATION BETWEEN GENOTYPE AND CLINICAL PRESENTATION OF CELIAC DISEASE IN CHILDREN
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Objectives and Study: The presentation of classic celiac disease (CD) changed during the last decade to a more atypical or silent form. CD is a complex disease, in which the genetic background plays an important role. The most important and best-understood genetic risk factors are HLA-DQ2 and HLA-DQ8 molecules. Recently, genomewide association studies (GWAS) revealed 26 non-HLA loci associated with CD and suggested 13 more loci predisposing to CD. For the first time we correlate the genetic background to the clinical presentation of CD. The aim of the study was to investigate whether the genetic background of atypical or silent presentation of CD differs from the genetic background in classical CD.

Methods: Using the GWAS results for a Polish population of children with CD and their clinical data, we performed association analysis of 447 patients with CD (257 with classical CD, 160 with atypical CD, 30 with silent CD). 39 single nucleotide polymorphisms (SNPs), previously found to be associated with CD, were compared separately for silent and atypical presentation with classical CD. The analysis was performed using a chi² test in PLINK (v.1.07, 2009).

Results: All together 447 Polish children with CD were included in the analysis. Significant association was found for the atypical presentation of CD and SNP rs3748816, located on chromosome 1 close to the gene MMEL1/TNFRSF14 (P = 0.03; OR = 1.38). We also identified 2 other SNPs that almost reached statistical significance: rs12928822 on chromosome 1 close to the gene MMEL1/TNFRSF14 (P = 0.05; OR = 1.38). Using the GWAS results for a Polish population of children with CD and SNP rs3748816, located on chromosome 1 close to the gene MMEL1/TNFRSF14 (P = 0.03; OR = 1.38). We also identified 2 other SNPs that almost reached statistical significance: rs12928822 on chromosome 1 close to the gene MMEL1/TNFRSF14 (P = 0.05; OR = 1.38). The FACS analysis confirmed the immunohistochemical data. Specifically, there was an significantly (P < 0.01) higher frequency of Foxp3+CD25+ T cells in treated CD biopsy samples cultured with PT-gliadin (21.4 ± 17.9 %) than in those cultured in medium alone (10.8 ± 7.9 %). No significant differences were noted in the percentage of Foxp3+CD25+CD4+ cells in biopsies of controls cultured with PT-gliadin (4.2 ± 0.87 %), in comparison to biopsies cultured with medium alone (3.7 ± 2.1 %). In co-culture studies, intestinal CD4+CD25+CD4- T cells of CD patients, significantly suppressed the proliferation of Tresp cells (p < 0.005) and induced a significant decrease of IFN-γ production (P < 0.001). Finally, IL-15 was effective in counteracting intestinal Treg cell-mediated suppression of anti-CD3-activated Tresp cells in terms of proliferation and IFN-γ production.

Conclusion: The differences in clinical presentation and the complexity of CD indicate that the genetic background might vary between atypical, silent and classical CD. We have shown a correlation of the MMEL1/TNFRSF14 locus with atypical presentation of CD; both genes seem to take part in T-cell maturation and differentiation. These results await replication in an independent cohort. There was no correlation found for silent CD, however the power was low because of the small sample size of this group.

Disclosure of Interest: None declared.

PO-G-0021/PD-G-0061
Coeliac Disease and Enteropathies
BIOLOGICAL ACTIVITY AND IMMUNOGENICITY OF 2 TRITICUM MONOCOCUM ACCESSIONS IN RELATION TO THEIR SAFETY FOR CELIAC PATIENTS
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Objectives and Study: Celiac disease (CD) is defined as a permanent intolerance to gliadin and related prolamin from rye and barley, in genetically susceptible individuals. Research is intense to find wheat varieties with absent or low toxicity to be implemented in new strategies for treatment and prevention of CD. Among candidates there are diploid wheat species. Aim of this study was to investigate in vitro biological and immunological properties of 2 accessions of ancient wheat *Triticum monococcum*, Monlis and ID331, in view of their possible use in CD patients. 

**Methods**: Peptic-tryptic (PT) digests of gliadin from the 2 *T monococcum* varieties and from hexaploid wheat gliadin (PTG) were tested in CaCo2 cells for their ability to induce phosphorylation of ERK (ERKp) and electric trans epithelial (PTG) were tested in CaCo2 cells for their ability to induce phosphorylation of ERK (ERKp) and electric trans epithelial resistance (TEER) changes. γIFN production induced by ID331 and monlis was measured as evidence of immune activation in PTG-specific intestinal T-cell lines from 8 CD patients, and in DQ2-α-I epitope specific T-cell clones. Finally, organ cultures of jejunal biopsies from 9 CD patients were set up to assess the effect of the different PT-gliadin digests on crypt proliferation, expression of IL15, density of lamina propria CD25 T cells and intraepithelial CD3 T cells.

**Results**: PT-Monlis or PT-ID331, as well as PTG, induced significant increase of ERKp and decrease of TEER in Caco2 cells. The 2 accessions of *T monococcum*, induced γIFN production in all intestinal gliadin-specific T-cell lines and clones. In organ culture studies Monlis and ID331 PT-digests were able to induce, like PTG, significant increase of IL-15 expression, increase of CD25 T cells and intraepithelial infiltration by T cells. Furthermore, Monlis and PTG, induce significant increase of crypt enterocytes proliferation, while ID331 had no effect.

**Conclusion**: In conclusion, our data show that both *T monococcum* accessions, Monlis and ID-331, are immunogenic in celiac patients and most likely they are not tolerated.

**Disclosure of Interest**: None declared.

PO-G-0037/PD-G-0062

**Coeliac Disease and Enteropathies**

**ALTERATIONS OF THE ENDOCYTIC TRAFFICKING, ACTIVATION OF SIGNALLING MOLECULES, AND INCREASED ENTEROCYTE CRYPTS PROLIFERATION ARE PRESENT IN CELIAC DISEASE (CD) INDEPENDENTLY FROM GLUTEN CONTENT OF THE DIET**

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**Objectives and Study**: Recent observations, from our and other laboratories point toward an effect of certain gliadin peptides (i.e. P31-43) on the maturation and function of early endocytic vesicles. Endocytosis has many effects on signaling affecting several cell functions that range from proliferative to cell motility. Why these effects of gliadin peptides on the endocytic compartment are so disruptive on the CD mucosa is not clear yet. Our aim has been to test the hypothesis that in CD mucosa a constitutive alteration of the endocytic compartment exists that may represent a predisposing condition to the gliadin damaging effects.

**Methods**: The endocytic compartment of biopsies from CD patients at gluten containing diet (GCD) or gluten free diet (GFD), potential CD and controls and the endocytic compartment of fibroblasts from skin grafts of CD patients on GFD and controls has been analyzed both morphologically and functionally. Phosphorylated proteins, including EGFR and ERK, have been analyzed by WB and by immunostaining. Proliferation of enterocytes has been evaluated by BrdU incorporation.

**Results**: We have found in CD enterocytes of the crypts (fluorescence intensity rose from 960 ± 60 of the controls to 1490 ± 26 in GFD, to 1110 ± 48 in GCD and 1320 ± 59 in potential CD patients) and fibroblasts (fluorescence intensity rose from 13,000 ± 150 of the controls to 13,000 ± 170 in GFD) an increase of EEA1 positive early endosomes with a delay of EGF endocytic trafficking, increase of total phosphorylated proteins, including EGFR and the downstream signaling molecule ERK, increase of EGF mRNA and, as a consequence of all this, increased proliferation of the enterocytes in the crypts (BrdU incorporation increased from 9% ± 4% of the controls to 18% ± 3.6% of the GCD, to 17% ± 8% in GFD and to 13% ± 2.5% in potential CD patients). These alterations are independent both from the diet, as they are present also in patients on GFD, and from the inflammation site, as they can be described also in skin fibroblasts from CD patients.

**Conclusion**: A constitutive alteration of the endocytic pathway is present in CD enterocytes and fibroblasts and may be a predisposing condition to gliadin damaging effects.

**Disclosure of Interest**: None declared.
Objectives and Study: The diagnosis of coeliac disease (CD) can be difficult in young, prospectively followed family members as initial changes might not be conclusive. In this study we prospectively evaluated a specific question whether being a first degree relative could add strength to the diagnosis instead of having symptoms.

Methods: The PreventCD birth cohort was recruited between 1/2007 and 7/2010 in 10 centers in 8 countries. HLA DQ2 and/or DQ8 carrier newborns with at least one first degree CD relative were followed for clinical symptoms and antibodies against transglutaminase 2 (anti-TG2) and gliadin (AGA) at 6, 9, 12, 18, 24 and 36 months of age after the double-blind introduction of gluten at 4 or 6 months. Duodenal biopsies were offered if CD symptoms and/or lasting anti-TG2 or AGA positivity developed. Children with seroconversion were also tested for deamidated gliadin peptide antibodies (DGP). A SAGE score grading symptoms (S), antibody results (A), HLA genotype (G) and endoscopic histology lesions (E) was calculated assigning for S either 0 or 1 depending on the presence of symptoms (I) or applying uniformly 1 for all babies as first degree family members (II). SAGE ≥4 was regarded as indicative of CD.

Results: From the 1344 enrolled newborns 941 had DQ2 or DQ8 and were followed. Up to 31.12.2010, 44 children developed anti-TG2. 48 biopsies were performed in 47 children and 31 CD cases were diagnosed having Marsh III lesions (all anti-TG2 positives, 29 with high serum levels). The other 16 children had at present preserved villous architecture (two having anti-TG2). One patient with IgA deficiency, AGA and DGP IgG but without anti-TG2 had first Marsh II then Marsh 0. 11 children with low or transient anti-TG2 were not yet biopsied. By approach I, SAGE score ≥4 was present in all 31 CD children, but none of the nonCD or transient seropositive cases. With approach II, 31 CD patients plus the Marsh II patient scored ≥4, but none of the subjects with transient anti-TG2. Score values ≥4 could be obtained with approach I in 18/31 CD children without biopsy but with approach II in 29/31 CD, while in none of the other children by either methods.

Conclusion: An item value of 1 can be taken into account in the SAGE score for being a first degree relative without substantial loss in the diagnostic accuracy. This approach would enable to make the diagnosis of CD in risk family members with HLADQ2 or DQ8 and high serum anti-TG2 also without the need of biopsy.

Disclosure of Interest: None declared.

PO-G-0024/PD-G-0064

Coeliac Disease and Enteropathies

DEVELOPMENT AND VALIDATION OF A SIMPLE DIAGNOSTIC SCORE FOR COELIAC DISEASE (SAGE) BASED ON SYMPTOMS, ANTIBODIES, HLA GENOTYPES, AND BIOPSY RESULTS

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Objectives and Study: The spectrum of coeliac disease (CD) is broader than classical villous atrophy and histology interpretation also may have technical pitfalls. Testing for CD specific endomysial (EMA) and transglutaminase 2 antibodies (anti-TG2) and for HLA-DQ is increasingly important, but anti-TG2 can occur also without CD. Further, even severe symptoms are not specific. We tested the hypothesis that combining the main information into a single score would help distinguish between real coeliac-type disease and nonspecific findings.

Methods: Data of 4295 consecutively registered EMA and/or anti-TG2 positive patients (median age 9 years) and of 1061 non-coeliac patients with biopsy results were analysed who entered clinical evaluation between 1988 and 2010 in our center. Symptoms (C), antibody results (A), HLA genotype (G) and endoscopic histology (E) were graded on a scale from 2 to −1, assigning higher values to findings that support more CD diagnosis and negative values to contradicting findings. The score results were statistically analysed to find the highest correlation with the clinically established diagnosis. The score was validated in an independent cohort of 70 consecutive problem patients coming to second opinion. The majority of these patients underwent diagnostic gluten challenge with new biopsies.

Results: Histology results were available in 3517 (81%) of all EMA or anti-TG2+ patients. The ≥4 result of the SAGE correlated with the clinical outcome in 99.3% of the patients eventually diagnosed with CD by presence of at least Marsh III lesions (n = 3389). The highest score value was 7 (malabsorption, high serum EMA/anti-TG2, Marsh IIIB-C and DQ2 or DQ8), present in 9.2% of CD. SAGE scores 6, 5 and 4 were found in 33%, 40% and 17% of the CD patients. The score enabled to establish the diagnosis of gluten sensitivity in 124 EMA+ patients who had normal villous structure initially, and of whom Marsh III lesion developed during follow up in 49. In the validation group SAGE showed correctly CD/nonCD already at presentation in 46 of the 47 CD and in all 23 nonCD patients, and 27 gluten challenge procedures could have been avoided.

Conclusion: The simple diagnostic score described here was useful to interpret clinical findings and correlated well with the traditional diagnosis. Most CD patients with high SAGE scores would qualify for the ≥4 value also without histology result, thus SAGE would help to establish CD diagnosis in subjects with clear symptoms, high serum CD specific antibodies and compatible HLA-DQ. The score also functioned to protect against overdiagnosis in cases with not convincing clinical results.


Disclosure of Interest: None declared.
PO-G-0010/PD-G-0065

Coeliac Disease and Enteropathies
RISK SCORE PREDICTS CHILDHOOD COELIAC DISEASE
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Objectives and Study: Coeliac disease (CD) is characterized by histological alterations in small-bowel biopsy specimens. However, the contribution of symptoms and sensitive noninvasive diagnostic tools, such as specific coeliac antibodies (tissue-transglutaminase-type 2 (anti-TG2) and endomyosium (EMA)) and HLA-DQ2/8 genotyping, play an important role in the diagnosis of CD. Recently several risk scores have been developed to assess if combinations of these parameters are predictive for intestinal alterations in CD. However, the assessments of the proposed scores may be the result of self-fulfilling prophecy, because the included children underwent an endoscopy due to elevated specific CD antibodies. The aim of the study was to create a risk score that predicts intestinal lesions consistent for childhood CD.

Methods: Retrospective multicentre study in the Netherlands between 2001–2009, including children ≥18 years who underwent an upper gastro-intestinal endoscopy and not following a gluten-free diet. In all biopsied children small-bowel specimens were taken from duodenum and since 2005 ≥1 from the bulb, irrespectively if they had symptoms or elevated antibodies consistent for CD. The distribution of symptoms, anti-TG2 and EMA (scored negative/strong positive), HLA-genotypes (scored HLA-DQA2/DQ8 positive), family history for CD and CD-associated diseases were compared among the biopsied patients who were diagnosed for CD and without CD (Non-CD). Anti-TG2 values were scored “positive” between upper limit of normal (ULN) and 10×ULN, and “strong positive” >10×ULN. Using logistic multivariate regression analysis, a predictive model was constructed for having CD and converted into a usable risk-score model.

Results: Included were 633 children (99%; median age 8 years SD ±5.3) of whom 337 girls (1:1 ratio boys/girls). CD was diagnosed in 169 children (27%). In our cohort, CD children had significantly more abdominal distension, anorexia, failure to thrive, (strong) positive anti-TG2 and EMA titres, and HLA-DQA2/8 positivity compared to the children without CD. Risk points for CD are: anorexia=1; failure to thrive=1; abdominal distension=2; positive anti-TG2=2; positive or strong positive EMA=4; strong positive anti-TG2=5 and HLA-DQA2/8 positivity=5 risk points. The assessment of the risk score for de diagnosis of CD in our cohort is presented in the table.

<table>
<thead>
<tr>
<th>Total points</th>
<th>Non-CD %</th>
<th>CD %</th>
<th>Risk score for CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 6</td>
<td>99</td>
<td>1</td>
<td>Low</td>
</tr>
<tr>
<td>7–10</td>
<td>44</td>
<td>56</td>
<td>Mediate</td>
</tr>
<tr>
<td>≥11</td>
<td>1</td>
<td>99</td>
<td>High</td>
</tr>
</tbody>
</table>

Conclusion: An easy-to-use risk score based on noninvasive parameters can predict in which children CD may be diagnosed without further investigations. In children with a high risk score small bowel biopsies may be avoided to diagnose CD. Histological investigations of small-bowel biopsies are necessary for the diagnosis of CD in the low and mediate risk score groups.

Disclosure of Interest: None declared.

PO-G-0017/PD-G-0066

Coeliac Disease and Enteropathies
MEASURING SERUM ANTIBODIES AGAINST TRANSGlutaminase 2 IS USEFUL FOR DIAGNOSING COELIAC DISEASE IN CHILDREN YOUNGER THAN 3 YEARS OF AGE
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Objectives and Study: It has been postulated that measuring transglutaminase-2 specific IgA antibodies (tTG-IgA) is less sensitive compared to anti-gliadin IgA antibodies (AGA-IgA) or IgG antibodies against deamidated gliadin peptides (DGP-IgG) to detect celiac disease (CD) in young children. We tested this hypothesis in young children with high risk for CD.

Methods: Patients from the PreventCD cohort, recruited from 10 centers in 8 countries. PreventCD is an European, multicentre, double-blind, randomized study, funded by a grant from the European Commission (www.preventcd.com). PreventCD prospectively follows from birth 1344 newborns with a first degree relative with CD for the development of the disease using clinical data and serology tests. In DQ2-DQ8 positive children, randomization takes place to receive either gluten or placebo at 4 months. The specific CD antibodies AGA-IgA and tTG-IgA are measured in the randomized children at months 4, 6, 9, 12, 18, 24, and 36. Total serum IgA is also measured. In case of IgA deficiency additionally AGA IgG and tTG-IgG are analysed. If any of these markers were positive, we also measured DGP IgG and IgA. Small bowel biopsies (SBB) are performed when there is clinical and/or serological suspicion for CD. Children are considered as CD if they have characteristic histological alterations in the SBB.

Results: Between 2007 and 2010, 48 biopsies have been performed in 47 children (5.3% from 905 randomized children). The mean age of biopsied children was 20 mo ± 0.63SD.
(7–39 mo) and we diagnosed CD in 31 children; mean age at diagnosis 23 mo ± 0.6 SD (11–39 mo). 14 Children; mean age 16 mo ± 0.57 SD (8–31 mo) were considered not to have CD and were considered potential CD in three, where despite positive (TG-IgA, SBB was not consistent with CD. All 16 non-CD children are followed on a normal diet and will be re-biopsied if titers increase or symptoms occur. One child with IgA deficiency underwent jejunal biopsy twice after AGA IgG persistently elevated and had Marsh 1 and 0.

<table>
<thead>
<tr>
<th>Antibody positivity in</th>
<th>CD %</th>
<th>Non-CD %</th>
</tr>
</thead>
<tbody>
<tr>
<td>the last sample before biopsy</td>
<td>(n = 31)</td>
<td>(n = 16)</td>
</tr>
<tr>
<td>tTG-IgA</td>
<td>100 (31)#</td>
<td>19 (3)</td>
</tr>
<tr>
<td>High tTG-IgA levels above 30 U/mL</td>
<td>94 (29)#</td>
<td>0</td>
</tr>
<tr>
<td>AGA IgA / AGA IgG</td>
<td>90 (28)</td>
<td>31 (5) / 6 (1)</td>
</tr>
<tr>
<td>DGP IgA / DGP IgG</td>
<td>80 (24)</td>
<td>19 (3) / 31 (5)</td>
</tr>
</tbody>
</table>

Chi-square + P < 0.001.

Conclusion: The preliminary results in this birth cohort of high-risk children do not confirm that testing for AGA-IgA or DGP-IgG in IgA competent young children below 3 years of age is superior for CD finding compared to tTG-IgA testing.


PO-G-0007/PD-G-0067

Coeliac Disease and Enteropathies

COMPLIANCE WITH GLUTEN-FREE DIET HAS A POSITIVE INFLUENCE ON GLYCEMIC CONTROL IN CHILDREN WITH CELIAC DISEASE AND TYPE 1 DIABETES

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Objectives and Study: Silent form of celiac disease (CD) is common in children with type 1 diabetes (T1D). The impact, however, of gluten-free diet on diabetes control is poorly defined. The aim of the study was to investigate whether compliance with gluten-free diet improves glycemic control in children and adolescents with T1D.

Methods: Thirty-three children (mean (SD) age 12.78 (5.17) years, 14 males; median (range) duration of diabetes 4.8 (0–16.2) years) of a total cohort of 450 patients (7.3%) with T1D had positive IgA class anti endomysium antibodies plus either IgA class anti-gliadin or IgA class anti-tissue transglutaminase antibodies, at their annual screening. Celiac disease was confirmed by jejunal biopsies in which histological abnormalities (Marsh 2/Marsh 3 lesions) were found in 27 (6%) patients. In 3 children jejunal biopsies were not conclusive, while biopsy was deferred by 2 children. Analysis was performed in 24 children and adolescents [mean (SD) age 10.4 (4.4) years; 10 boys; median disease duration 5.0 years], who were followed up after the diagnosis of CD [median (range) period 4.0 (1–9.67) years]. HbA1c, weight and height were measured every 3 months, while daily insulin requirements were recorded at each visit. BMI z scores were calculated at all time points. HbA1c, weight and height measurements as well as insulin requirements within 12 months prior to the positive screening with antibodies were compared to those carried out within 12 months prior to the negative screening. Seven children had concurrently positive anti thyroid antibodies and 3 of them received treatment with thyroxin.

Results: In total 113 anti CD antibodies measurements were carried out in 24 patients with T1DM and CD and 70 (61.9%) were positive. Patients who had negative antibodies (compliant), had significantly lower HbA1c levels within 1 year before the screening, compared to those who had positive antibodies (noncompliant): mean (SD) HbA1c (%) 7.2 (0.9) vs 8.2 (1.8), respectively; P < 0.0005. Furthermore, daily insulin requirements (U/kg) within 12 months before the screening were significantly lower in compliant patients compared to noncompliant: mean (SD) 0.96 (0.15) vs 1.03 (0.33); P = 0.028. Moreover, a positive correlation was found between HbA1c levels and both the presence of positive anti-celiac and anti-thyroid antibodies (R: 0.27, B: 0.99, SE(B): 0.33 p: 0.004 and B 0.89 SE(B) 0.38, P = 0.024). No differences were found between compliant and noncompliant patients with respect to BMI z scores.

Conclusion: Compliance with gluten free diet in children and adolescents with CD and T1D diabetes is associated with better glycemic control.

Disclosure of Interest: None declared.

PO-G-0015/PD-G-0068

Coelic Disease and Enteropathies

CELIAC DISEASE AND TYPE 1 DIABETES MELLITUS ASSOCIATION: A RETROSPECTIVE ANALYSIS OF PEDIATRIC CASES IN ROME

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Objectives and Study: A clinical association between celiac disease (CD) and type 1 diabetes mellitus (DM1) has largely been recognized. Experimental and clinical data have recently addressed the possible role of gluten in promoting DM1. According to several studies, the mean onset of DM1 is 8–9 years, while the age of CD diagnosis may be highly variable. Therefore, we wanted to assess the epidemiological features of pediatric patients affected both by CD and DM1
referred to 2 university hospitals in Rome (Sapienza and Tor Vergata Universities), from 1980 to 2010.

**Methods:** We retrospectively collected data (age at diagnosis, gender) from 1894 CD patients on a gluten-free diet and 971 DM1 patients referred to these hospitals, in order to elucidate specific patterns of disease onset. Data from CD patients with known poor compliance were not considered in the study design. On purpose, we analyzed data from the CD-DM1 cohort through logistic regression models.

**Results:** Among these 1894 CD patients (mean age at CD diagnosis: 7.9; SD: ±4.8), only 3, diagnosed with CD in the adolescence, afterward developed DM1; nevertheless, 80% of CD cohort (1515 patients) had been diagnosed with CD before ten years of age. Contrarily, among 971 DM1 children, CD had been then identified in 99 (10.1%) (mean age at CD diagnosis: 14.1; DS: ±6.9); only 28 of them, anyhow, had been diagnosed under ten years, while 49 and 22 received the diagnosis of CD between 10 and 20 and after 20 years of age, respectively. In a cohort of 99 children affected both by DM1 and CD, the male/female ratio was mostly 1:1 (51 F/48 M). Patients whose DM1 onset registered under two years of age had a risk of developing CD 5.7-fold increased (IC 95%: 1.16 – 28.79; \( P = 0.032 \)) compared to the overall DM1 group. The median interval of diagnosing CD following the onset of DM1 was 1.6 years (interquartile range: 3.1 years).

**Conclusion:** As previously reported, we described a CD prevalence of 10% among DM1 patients, with a male/female ratio of 1:1. On the other hand, we only detected 3 cases of DM1 among 1894 children previously diagnosed with CD; moreover, the age at CD diagnosis in non-DM1 children was significant lower than in the group of CD-DM1 children \( (P < 0.001) \). Thus, according to these two data, we could speculate that an early gluten avoidance in celiac patients might prevent the onset of DM1. Furthermore, as the early presentation of DM1 seems to increase the risk of CD, we strongly recommend a strict follow up for CD in this subpopulation and we call for upcoming studies investigating this specific issue.

**Disclosure of Interest:** None declared.

**PO-G-0041/PD-G-0069**

**Coeliac Disease and Enteropathies**

**SPONTANEOUS DISAPPEARANCE OF TISSUE TRANSGLUTAMINASE ANTIBODIES IS COMMON IN CHILDREN DIAGNOSED WITH DIABETES MELLITUS TYPE 1**

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**Objectives and Study:** The prevalence of celiac disease (CD) among type 1 diabetes mellitus (T1DM) patients is 5–10 times higher than in the general population. Thus, evaluating celiac serology is indicated at diagnosis of T1DM and on follow up. The aim of our study was to investigate the prevalence of spontaneous disappearance of tissue transglutaminase antibodies (TTG) in patients diagnosed with T1DM.

**Methods:** We performed a retrospective analysis of 815 children diagnosed with T1DM in our institution during a 6 years period and identified patients with elevated serum TTG. Patients were analyzed in 2 groups: Group 1 consisted of patients diagnosed with CD and Group 2: patients with spontaneous disappearance of TTG on a gluten containing diet. Group 1 was further divided to Group 1a–patients adherent to GFD and Group 1b those not adherent. Comparison between the groups was done at first positive TTG finding and one year later, for hemoglobin A1C (Hba1C) and anthropometric measurements.

**Results:** After excluding 77 patients due to incomplete data, we identified 48/738 (6.5%) patients with elevated TTG blood levels. Of these, CD was diagnosed in 31 (Group 1). TTG antibodies disappeared on gluten containing diet in 17/48 (35.4%) children (Group 2, median follow up time: 4.1 years, range 1.7–5.7 years). In Group 1, TTG blood levels were significantly higher than in Group 2 \( (P = 0.001) \) and in all cases in Group 2, TTG levels were < X 3 the upper limit of normal (ULN). A year after diagnosis, there was no significant difference between Group 1a, Group 1b and Group 2 neither in the HbA1c levels (7.1% vs. 8.2% vs. 7.37%, \( P = 0.24 \)), nor in the anthropometric measurements (median z score difference for weight 0.26 vs. –0.09 vs. 0.01, \( P = 0.91 \); median z score difference for height 0.02 vs. 0 vs. –0.33, \( P = 0.47 \); and z score difference for BMI 0.2 vs. –0.2 vs. 0.39, \( P = 0.47 \)).

**Conclusion:** TTG spontaneously disappear in one-third of children diagnosed with T1DM. We suggest that physicians treating T1DM patients should consider 12 months serologic follow-up on gluten-containing diet rather than immediate duodenal biopsy at least in children with mildly elevated TTG.

**Disclosure of Interest:** None declared.

**PO-G-0016/PD-G-0070**

**Coeliac Disease and Enteropathies**

**MAPPING HISTOLOGIC PATCHINESS OF CELIAC DISEASE BY PUSH ENTEROSCOPY**

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**E38**

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Objectives and Study: Despite the great improvement of serologic tests, diagnosing celiac disease (CD) still requires both a duodenal biopsy coupled with a positive response to a gluten-free diet. Nevertheless, the histological pattern of CD is often patchy, with the risk of missing diagnoses. To evaluate the patchiness of the histological lesions along the small bowel, push enteroscopy was performed instead of conventional upper GI endoscopy.

Methods: 20 consecutive pediatric patients on the suspicion of CD (positive anti-transglutaminase and anti-endomysial antibodies) were enrolled in the study. Written informed consent was obtained from all parents. Standard biopsies were taken from 5 different sites (1 biopsy per site): bulb, 2nd duodenal region, 4th duodenal region, proximal jejunum (30 cm from the ligament of Treitz) and distal jejunum (60 cm from the ligament of Treitz); all samples were oriented and sent to the same, non-blinded, expert GI tract pathologist. Specimens were graded according to the Marsh-Oberhuber criteria. Patchiness was defined as the presence, in the same patient, of lesion in one site and its absence in another.

Results: A homogeneous pattern of histological damage was found in 10 patients (50%); contrarily, 5 patients (25%) had a patchy pattern of lesions, whereas minor lesion variability among different sites was shown in 9 (45%); in addition, biopsies from 3 patients (15%) displayed also a certain degree of variability within the same specimen. The 2nd and the 4th duodenal regions were involved in 18 (90%) and 19 (95%) children, respectively. Bulb samples were positive in 18 cases (90%), but never exclusively, with a major frequency of severe grading (3c+ 3b+ 3a= 85%). On the other hand, both distal and proximal jejunal samples showed histological lesions in 18 children (90%), as well with a prevalent severe degree (3c+ 3b+ 3a= 85%) in the distal and 75% in the proximal jejunum; in one patient, lacking lesions in the bulb and duodenum, the diagnosis of CD could only be confirmed by proximal and jejunal biopsies (3b and 3c, respectively). Overall, no significant difference of severity was disclosed among the studied regions (p>0.05).

Conclusion: According to previous studies, push enteroscopy approach has confirmed that CD histological lesions has often a discontinuous distribution, more frequently showing only minor lesion variability along the small bowel. During this still ongoing study, 1 diagnosis of overt CD, otherwise classified as latent, could be achieved only when we obtained biopsies in the jejunum. Further investigation is needed to assess the role of push enteroscopy in detecting distal hidden lesions in CD beyond the duodenum.

Disclosure of Interest: None declared.

PO-G-0045/PD-G-0071

Coeliac Disease and Enteropathies

PRIMARY SCHOOL CHILDREN DETECTED BY RIA SALIVARY ANTI-TRANSGlutaminASE Antibodies: THE COELIAC ICEBERG IN THE CITY OF ROME

www.jpgn.org

R. Nenna1, R. Tiberti2, L. Petrarca1, M. Meninni1, G. Mastrogiorgio1, F. Panimolle2, M. Montuori1, F. M. Magliocca3, M. Bonamico1. 1Department of Pediatrics, 2Clinical Science, 3Experimental Medicine and Pathology, “Sapienza” University of Rome, Rome, Italy.

Objectives and Study: Coeliac disease (CD) is characterized by a wide spread of heterogeneous clinical expressions and may appear in a typical or atypical form, or remains still under-diagnosed because of the presence of many patients with a silent form. Unfortunately, the CD association with life-threatening conditions is well documented and it is irrespective of the clinical form. Our aim was to identify CD in children in order to perform a timely diagnosis that might permit a proper growth and prevent CD-related complications; characterize CD in school-age children of Rome and develop an “optimal package” intended for large-scale CD screenings.

Methods: 7377 children, attending the first and the second classes of the primary school were invited to participate in the study in 11 out of 20 municipalities of Rome. 5733 salivary samples were collected and tested for anti-transglutaminase antibodies (tTGab) using a radioimmunoprecipitation assay (RIA). Subjects’ salivary tTGab-positive were subsequently tested for serum CD-specific antibodies (RIA tTGab, ELISA tTGab and EMA). Confirmed positive children underwent endoscopy with multiple duodenal biopsies, and at CD diagnosis, started a gluten-free diet (GFD). After a follow-up lasting three years, compliance to the diet, Ab titers reduction, weight/stature increase and well-being were evaluated.

Results: Parents’ compliance to the screening was 83.4% and 93.2% of children collected an adequate salivary sample. 46 children were salivary tTGab positive and 16 had border-line levels. 45/46 and 5/15 of these subjects were also serum Ab positive, respectively. 42/43 children, who underwent intestinal biopsy, showed villous atrophy and three children started GFD without performing the endoscopy. The overall CD prevalence in the population investigated (including 25 previously diagnosed CD children) was 1.3%. A typical form was found in 33%, an atypical in 6% and a silent in 61%. At the follow-up, all patients showed a strict adherence to the GFD, a weight and stature increase and an improvement of the wellbeing. The 15% of children had parents of foreign nationalities and two screening-detected CD children had parents from Venezuela or Sri Lanka.

Conclusion: The salivary test is a well accepted screening tool, as shown by the high parents’ compliance. CD prevalence is growing in Italy, with a modified clinical spectrum. Until now, the compliance to the GFD in screened CD children has been optimal with a significant increase of the anthropometric parameters and no complications appeared. Salivary tTGab detection is a powerful, non-invasive, simple, reproducible and sensitive screening method.

Disclosure of Interest: None declared.
PO-G-0050/PD-G-0072

**Coeliac Disease and Enteropathies**

**MICROARRAY-BASED ANALYSIS OF THE GENETIC RISK FACTORS HLA-DQ2/DQ8—A NOVEL TEST SYSTEM FOR THE DIAGNOSTIC EXCLUSION OF CELIAC DISEASE**

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**Objectives and Study:** Celiac disease is a chronic indisposition predominantly of the small intestine. It is caused by the ingestion of gliadin containing wheat, rye, and barley. In genetically predisposed persons, deamidated (DQA1*0102/DQB1*0201) and DQA1*0101/DQB1*0301 combined with HLA-DQA1*05 and HLA-DQB1*02 alleles whereas HLA-DQA1*0102/DQB1*0301 combined with HLA-DQB1*0302 correspond to the DQ8 genotype. To provide a means for the effortless and accurate determination of the HLA-DQ2/DQ8 genotypes a microarray test was developed and validated.

**Methods:** In order to identify the disease associated DQ2 and DQ8 subunits unambiguously the test was designed to detect 7 different alleles or allele families respectively (in detail: DQA1*02, DQA1*02/DQB1, HLA-DQ2 is encoded by the HLA-DQA1*05 and HLA-DQB1*02 alleles whereas HLA-DQA1*0301 combined with HLA-DQB1*0302 correspond to the DQ8 genotype. To provide a means for the effortless and accurate determination of the HLA-DQ2/DQ8 genotypes a microarray test was developed and validated.

**Results:** The newly developed test system is easy and fast to perform, accurate and reliable. HLA-DQ2 and -DQ8 markers can be unambiguously determined based on the sophisticated array design including also non disease associated alleles. The fully automated output of the results and diagnostic findings will help to generate reliable results in the diagnostic laboratory.

**Disclosure of Interest:** None declared.

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**PO-G-0038/PD-G-0073**

**Coeliac Disease and Enteropathies**

**GLIADIN IS ACTIVE IN HEALTHY CONTROLS**

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**Objectives and Study:** Gliadin peptide P31–43 and P56–68 enter the cells by endocytosis. But only P31–43 localises at the early endocytic compartment and delays vesicle trafficking by interfering with Hrs-mediated maturation to late endosomes in cells and intestinal biopsies from CD patients. Consequently, in P31–43-treated cells, several pathways activate including EGFR and IL15 signalling. The aims of this study were to investigate the effects of P31–43 on the endocytic compartments in healthy controls (HC).

**Methods:** Confocal analysis of immunofluorescent staining consented to evaluate EEA1 staining. Trafficking of EGF-Alexa-488 in pulse and chase experiments allowed to test the functionality of the endocytic compartment. Phosphorylation of proteins including ERK and EGFR was evaluated by Western blot analysis.

**Results:** Small intestinal biopsies and skin derived fibroblasts from HC were treated with gliadin peptides. The endocytic compartment was investigated both morphologically and functionally. EEA1 staining of small intestinal biopsies (fluorescence intensity increased from 258 ± 32 to 512 ± 47) showed that P31–43 induced alterations of the endocytic compartment in HC. The interference of EGF-Alexa-488 is transiently delayed. As a consequence of the P31–43 induced alterations of the endocytic compartment EEA1 protein is increased and ERK signalling molecule is activated in HC cells.

**Conclusion:** In this study, we have shown that P31–43 induces, transiently, morphological and functional alterations of the endocytic compartment in HC. The interference with trafficking of vesicular compartments can activate signalling pathways in the HC cells.

**Disclosure of Interest:** None declared.
PO-G-0009/PD-G-0074

Coeliac Disease and Enteropathies
Differences in Gluten Consumption Between First-Degree Relatives of Celiac Disease Subjects and the General Child Population
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Objectives and Study: To evaluate daily gluten consumption in infants of CD families as compared to non-CD families.
Methods: A prospective study using a previously validated food frequency questionnaire specifically developed to assess gluten intake in 12 to 36 month’s old infants. Three groups of subjects aged between 12–36 months: siblings of coeliac children (Gs), sons or daughters of CD subjects (Gp) and infants of non-CD families (controls). All of them are healthy children on a normal non restricted diet.
Results: The survey was performed in N = 455 children; 104 in the Gs group, 111 in the Gp group and 228 controls.
Average daily gluten consumption

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Group</th>
<th>N</th>
<th>Mean ± SD (g/day)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>12–18 months (G1)</td>
<td>NON-CD CD</td>
<td>78</td>
<td>5.8 ± 2.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>19–24 months (G2)</td>
<td>NON-CD CD</td>
<td>66</td>
<td>4.9 ± 2.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>25–36 months (G3)</td>
<td>NON-CD CD</td>
<td>87</td>
<td>4.8 ± 2.58</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

When comparing the mean daily intake in children pertaining to CD families’ cfr non-CD families by applying the Student t test, the difference was statistically significant for each age group, t being respectively 7.46, 5.98 and 5.06 (P < 0.001, as shown in the table). Overall gluten intake increases with age in both CD-Families and Controls; however in Non-CD families the difference is only statistical significant between G1 and G3. Conversely in the CD families statistically significant differences were obtained between the G1 and G3 BUT ALSO between G1 and G2. No statistically significant differences were observed when comparing mean daily gluten intake between groups Gs and Gp for any of the 3 age groups.

Conclusion: Index cases do not influence gluten intake in infants pertaining to CD families, at least in the age range considered in this study. Introduction of a series of new foods, such as bread or pasta, accounts for an overall higher gluten intake after 18 months of age, both in CD and non-CD families. Statistically significant increase in gluten intake in between 19 and 24 months of age as compared to the younger group in the CD families but not in controls suggest CD families are cautious with gluten introduction in their infant’s diet before the age of 18 months. This view is also supported by the fact that at any age infants of CD families have a statistically significant lower gluten intake than infants of the general population. The lower daily gluten consumption, specially at 2–3 years of age, may account for lower positivity of serological markers and/or milder histological lesions in first degree relatives of CD subjects and has to be considered when screening for CD in this at risk group.

Disclosure of Interest: None declared.

PO-G-0040/PD-G-0075

Coeliac Disease and Enteropathies
Serum Intestinal Fatty Acid Binding Protein (I-FABP) Accurately Predicts Villous Atrophy and Mucosal Healing in Celiac Disease in Adults and Children
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Objectives and Study: Noninvasive tools for evaluating villous atrophy are needed to improve the diagnosis and follow up of celiac disease (CD). CD antibodies are sensitive screening tools, but so far duodenal biopsies are needed to confirm the diagnosis, and no sensitive tools are available for monitoring the effect of gluten-free diet (GFD). Intestinal fatty acid binding protein (I-FABP) is potentially useful since this small cytosolic enterocyte protein is released rapidly into the systemic circulation after intestinal damage. This study evaluated the usefulness of serum I-FABP in diagnosing CD in children and adults with positive screening and for monitoring mucosal healing after GFD.

Methods: Serum I-FABP levels at diagnosis and follow-up were analysed retrospectively in 49 children with biopsy proven CD. 19 children with elevated IgA-tTG and/or IgA-EMA levels but normal duodenal histology served as controls. Also, 58 adults were analysed at diagnosis and at time of normalised antibody levels (mean GFD time 26 months). 125 healthy adults served as controls.

Results: Initial I-FABP levels in CD children (median 458 pg/mL, range 0–2990 pg/mL) were significantly elevated compared to controls (median 0 pg/mL range 0–485 pg/mL, P<0.001). In 40/49 patients I-FABP levels were above the cutoff point, compared to 2/19 controls. I-FABP decreased rapidly after GFD; after 7, 12 and 26 weeks I-FABP was normalised in 45%, 80% and 92%, respectively. In adult patients initial I-FABP levels (median 755 pg/mL range 0–2362 pg/mL) were significantly elevated compared to controls (median 250 pg/mL range 0–2024 pg/mL, P<0.001) and decreased on GFD (median 391 pg/mL range 30–2136 pg/mL, P<0.001). Interestingly, in 20/53 patients on GFD I-FABP remained elevated despite normal antibody titers. Biopsies were available in 12 patients; 6 showed Marsh 2–3A, 3 showed Marsh 1, and 3 showed Marsh 0.
Biopsies of 23 adults on GFD with normalised I-FABP levels showed Marsh 3A, Marsh 1, and Marsh 0 in 4%, 17% and 78%, respectively.

**Conclusion:** Serum I-FABP accurately predicts villous atrophy in patients with positive CD screening and might be a useful additional marker in diagnosing CD. Moreover, the results suggest that I-FABP is useful for monitoring mucosal healing, and is more sensitive for ongoing intestinal damage at follow-up than CD antibodies. Prospective studies are being performed to assess whether increased I-FABP levels in patients with positive CD antibodies justify a diagnosis of CD without biopsy and to assess the value in monitoring CD activity.

**Disclosure of Interest:** None declared.

**PO-G-0012/PD-G-0076**

**Coeliac Disease and Enteropathies**

**MICROVILLUS INCLUSION DISEASE IS A DISORDER OF DISRUPTED EPITHELIAL CELL POLARITY**


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**Objectives and Study:** Microvillus inclusion disease (MVID) is a congenital enteropathy characterized by a loss of microvilli, the appearance of microvillus inclusions and the cytoplasmic accumulation of periodic acid-Schiff (PAS)-positive vesicles in enterocytes. Our recent studies have identified mutations in MYO5B, encoding the unconventional type Vb myosin motor protein, to be causally involved in the pathogenesis of MVID. Myosin Vb is implicated in maintaining cell surface polarity in epithelial cells. The aim of this study was to explore the impact on RNAi mediated myosin Vb depletion on the polarized organization of human intestinal CaCo-2-cells, as a model of polarized intestinal epithelium in vivo.

**Methods:** Myosin Vb knock down was performed in polarized, brush border possessing CaCo-2-cells. For polarization, CaCo-2-cells were grown to confluency on a Costar Transwell filter system for 10 days. For myosin Vb knock down studies, CaCo-2-cells were stably transfected with two different lentiviral shRNA constructs for designing an intestinal epithelial cell line with an inducible myosin Vb knock down. The polarized organization of myosin Vb depleted CaCo-2-cells was determined by fluorescence microscopy, Western blotting and electron microscopy.

**Results:** Myosin Vb depleted CaCo-2-cells displayed several features of impaired cell polarity including a loss in microvilli, formation of microvillus inclusions, disorganization of the actin cytoskeleton, a reduction of the transepithelial resistance, loss of adherent junctions and mislocalization of the basolateral transporters GLUT1 and the Na^+/K^+ ATPase, respectively. Finally, disruption of intracellular trafficking was shown by cytoplasmic mislocalization of effector proteins such as the small GTPases Rab11 and Rab8.

**Conclusion:** Our findings point to a critical role of myosin Vb in the polarized organization of human intestinal cells thereby defining MVID as a disorder of disrupted epithelial cell polarity.

**Disclosure of Interest:** None declared.

**PO-G-0067/PD-G-0094**

**Endoscopy: Diagnosis and Therapeutic Surgical Procedures**

**USEFULNESS OF SINGLE-BALLOON ENTEROSCOPY IN PEDIATRIC CROHN’S DISEASE**

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**Objectives and Study:** Endoscopic visualization of the small bowel (SB) in Crohn’s disease (CD), either with wireless capsule endoscopy (WCE) or with balloon assisted techniques is indicated in case of diagnostic uncertainty and when traditional upper gastrointestinal (GI) endoscopy does not account for the clinical activity of the disease. We describe the use of single-balloon enteroscopy (SBE) in consecutive pediatric patients with suspected or established CD. This investigative tool is rarely reported in children.

**Methods:** Thirty patients (age range: 7–18 years) were prospectively investigated: 16 (group A) with suspected CD and unspecific conventional upper and lower GI endoscopy; 14 (group B) with longstanding CD, 13 of which with previous surgery and showing signs unaccountable by conventional endoscopy. All underwent magnetic resonance (MR) and group A also WCE.

**Results:** In group A, SBE allowed diagnosis of CD in 12, eosinophilic enteropathy in 2, unspecific abnormalities in 2. Of 10 patients, WCE was diagnostic of CD only in 3, but was suggestive of CD or unspecific in the remaining subjects. In group B, SBE revealed a moderate-to-severe disease activity in most patients, leading to introduction or change in biological therapy, with subsequent marked decrease in the PCDAI. In 1 and 3 patients of group A and group B, respectively, SBE allowed successful dilation of SB strictures. No complications occurred in all investigated subjects.

**Conclusion:** SBE is a useful and safe endoscopic procedure to evaluate SB in pediatric subjects with suspected or established CD. It can allow a definite diagnosis of CD when the latter is uncertain and may redirect therapeutic choices in selected CD patients.

**Disclosure of Interest:** None declared.
Inflammatory Bowel Disease
MUCOSA-ASSOCIATED MICROBIOTA IN IBD PEDIATRIC PATIENTS AND METABOLIC PHENOTYPES
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Objectives and Study: Inflammatory bowel disease (IBD) patients, including Crohn’s disease (CD) and ulcerative colitis (UC), typically have higher numbers of mucosa-associated bacteria compared with normal subjects. The aim of this study is to analyze the composition of the dominant mucosa-associated microbiota in IBD pediatric patients, and to analyze human host urine metabolites in order to cross-correlate and to associate specific bacterial species presence to specific urinary metabolites induction.

Methods: Temporal temperature gradient gel electrophoresis (TTGE) was used in order to analyze the biodiversity of bacteria and to identify bacterial specie asociable to CD or UC disease. Biopsies from ileum, colon and rectum of 17 years were collected. Total DNA was extracted, and amplified products (by reconditioning PCR) of 16S ribosomal DNA were compared by TTGE. Solid phase micro-extraction coupled to gas chromatography/mass spectrometry (SPME-GC/MS) was used to characterize urinary metabolites.

Results: First results obtained from TTGE profiles revealed a peculiar dominant microbiota in relation to the different IBD pathology (UC or CD), evidencing a strong relationship between the dominant intestinal microbiota and the type of pathology. Clustering by partial least square discriminant analysis (PLS-DA) showed a clear separation between TTGE profiles of CD patients and the other 2 (P < 0.0001). The median bands number in the 3 patients groups differed significantly between CD patients and controls (P = 0.00012), and UC and control patients (P = 0.012), with a higher bands number in IBD groups. Results obtained showed high intra-individual similarity in all patients, ranging between 80% and 95% (Dice similarity index). The similarity between patients within each group is around 50% for CD patients, 60% for UC patients and 30% between controls. A major abundance of Proteobacteriaceae bacterial members in IBD was found. PLS-DA analysis on SPME-GC/MS spectra revealed a distinct separation between CD patients and controls (Chi-square=11.827, P=0.0006) with a model predictability of 90.9% (Fisher P=0.00014), showing qualitative and quantitative differences among peaks.

Conclusion: The presence of a dominant microbiota and a peculiar urinary metabolites profile asociable to pediatric IBD supports the hypothesis that intestinal bacteria play a role in IBD pathogenesis. These results, even if at a preliminary stage, highlight the peculiarity of the microflora/host interplay in such disorders, and point new functional insights towards a reformulated “probiotic reconstitution” of dysbiotic IBD microflora.

Disclosure of Interest: None declared.

Inflammatory Bowel Disease
BACTERIAL DIVERSITY OF THE COLONIC MICROBIOTA IN DE NOVO EXTENSIVE PAEDIATRIC ULCERATIVE COLITIS BY NEXT-GENERATION SEQUENCING

Objectives and Study: Dysbiosis and reduced bacterial diversity may contribute to inflammatory bowel disease (IBD) pathogenesis. High-throughput, parallel sequencing technology (next-generation sequencing) provides the means of assessing microbial diversity in samples from diverse ecosystems such as the colonic mucosa. We aimed to examine bacterial diversity in mucosal biopsies of treatment naïve, de novo paediatric ulcerative colitis (UC) compared to controls.

Methods: Paediatric patients undergoing colonoscopy were recruited to 2 groups: those with a new diagnosis of IBD at first presentation and controls with a normal colon and no evidence of IBD on biopsy. All subjects were free from antibiotics, steroids and immunosuppression for >3 months. 5 UC patients, extensive (E3) by Montreal criteria, and 5 controls with macroscopically/microscopically normal colons were selected. The median ages were 11.5 and 10.7 years, respectively. All were male. Colonic mucosal biopsies were from the rectum/sigmoid. DNA extraction was performed by a modified Qiagen QiAMP mini-kit method. The presence of bacteria was confirmed by universal primers before PCR utilising V3 Forward/V6 Reverse fusion primers. Bacterial diversity was assessed by 454 Titanium sequencing. Sequencing data was filtered, chimera and error checked then denoised before rarefaction to 13,000 reads per sample. Statistical comparisons were by Mann-Whitney U (Sigma Plot 11).

Results: All biopsies were positive for bacterial DNA with universal primers. The most commonly identified phyla (comprising 95.4% of sequence reads) were Bacteroidetes (45.3%), Firmicutes (40.5%) and Proteobacteria (9.7%). Bacteroidetes were significantly more common in controls than UC (7641 median reads versus 4062, P = 0.032) whereas Firmicutes were significantly more common in UC than controls (5471 median reads versus 3892, P = 0.016). The difference between Proteobacteria was

Disclosure of Interest: None declared.
not significant ($P=0.421$). Bacterial diversity assessed by the Shannon index was similar in both groups (medians of 6.1 in UC and 6.5 in controls, $P=0.841$).

**Conclusion:** Colonic mucosal bacteria differ between paediatric patients with extensive UC at diagnosis and controls. UC microbiota was typified by a reduction in Bacteroidetes and an increase in Firmicutes. Surprisingly, a reduction in bacterial diversity is not present in extensive UC at diagnosis. This is contrary to findings from previous studies in established disease and warrants further investigation.

**Disclosure of Interest:** None declared.

PO-G-0137/PD-G-0079

**Inflammatory Bowel Disease**

**THE ROLE OF THE MICROAEROPHILIC COLONIC MICROBIOTA IN DE NOVO PAEDIATRIC INFLAMMATORY BOWEL DISEASE**


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**Objectives and Study:** Helicobacter species can initiate animal colitis similar to human ulcerative colitis (UC). Campylobacter concisus has been linked to paediatric Crohn’s disease (CD). We aimed to establish the prevalence of Helicobacter and Campylobacter in treatment naive, de novo paediatric inflammatory bowel disease (IBD) and controls.

**Methods:** Paediatric patients undergoing colonoscopy were recruited to two groups: those with a new diagnosis of IBD at first presentation and controls with a normal colon and no evidence of IBD on biopsy. All subjects were free from antibiotics, steroids and immunosuppression for 3 months. 24 IBD patients and 26 controls were studied. The IBD cohort comprised 12 (50%) CD, 8 (33.3%) UC and 4 (16.7%) IBD unspecified (IBD-U). 15 (62.5%) of the IBD and 20 (77.8%) of the controls were male with median ages of 12.4 and 11.0 years respectively. 5–6 colonic mucosal biopsies were taken: 3 biopsies were taken in controls largely from the sigmoid/rectum and 11.0 years respectively. 5–6 colonic mucosal biopsies were taken: in controls from the most distal inflamed site. 3 biopsies were taken in IBD from the most distal inflamed site. 3 biopsies were cultured. 3 Campylobacter species were cultured: C. concisus from a subject with CD, Campylobacter curvus and Campylobacter showae from controls. Sutterella wadsworthensis was isolated from 13 subjects: 8 controls and 5 IBD. Nested PCR for Helicobacter genus was positive in 5 (10%) subjects, comprising 3 (12.5%) IBD and 2 (7.7%) controls. PCR for Campylobacter genus was positive in 38 (76%) subjects, comprising 19 (79.2%) IBD and 19 (73.1%) controls. Nested PCR for C. concisus was positive in 25 (50%) subjects, comprising 14 (58.3%) IBD (8/12 CD, 3/8 UC, 3/4 IBD-U) and 11 (42.3%) controls. PCR for S. wadsworthensis was positive in 48 (96%) subjects, comprising 23 (95.8%) IBD and 25 (96.2%) controls.

**Conclusion:** Campylobacter spp. and Sutterella wadsworthensis are commonly identified in the paediatric colon. C. concisus is more prevalent in CD although not significantly. Helicobacter spp. are uncommon. We have revealed no significant distinction between the microaerophilic microbiota of paediatric IBD versus controls. It is unlikely that these organisms have a role in the initiation of paediatric IBD.

**Disclosure of Interest:** None declared.

PO-G-0121/PD-G-0080

**Inflammatory Bowel Disease**

**IMPROVEMENT IN BIOMARKERS OF BONE FORMATION DURING 54-WEEK INFLIXIMAB THERAPY IN PEDIATRIC PATIENTS WITH CROHN’S DISEASE**

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**Objectives and Study:** Treatment with infliximab (IFX) may improve growth and disturbed bone metabolism in pediatric patients with Crohn disease (CD), but the characteristics of bone formation and resorption factors under IFX treatment are not well known. This study examined changes in bone formation (osteocalcin/OC, bone-specific alkaline phosphatase/bALP) and resorption (beta-crosslaps/bCL) under IFX treatment. Moreover, associations between bone biomarkers, and CRP, vitamin D level, disease activity index (Pediatric Crohn’s Disease Activity Index, PCDAI), and dual energy x-ray absorptiometry (DEXA) after 54 weeks of IFX therapy were analyzed.

**Methods:** Twenty-eight subjects (male, 15, mean age, 15.4 years) with moderate to severe CD received IFX induction treatment. Mean OC concentrations were 31.3 ng/mL versus 51.7 ng/mL, 61.6 ng/mL, and 64.3 ng/mL at week 0, weeks 6, weeks 30, and 54 weeks. Serum levels of bone formation OC increased significantly after IFX induction treatment. Mean OC concentrations were 31.3 ng/mL versus 51.7 ng/mL, 61.6 ng/mL, and 64.3 ng/mL at week 0, weeks 6, weeks 30, and 54 weeks.
respective (P < 0.005). bALP increased significantly between baseline and weeks 6 (mean, 110U/L, 161U/L, respectively, P = 0.002). There were no significant differences concerning bCtL and vitamin D at different time points. Nevertheless, both z score of the lumbar spine and femoral neck improved after 54 weeks when compared with baseline (lumbar spine, −0.65 (−2.9–0.9), −2 (−3.5–1.7), femoral neck, −0.9 (−3.6–1.1), −1.6 (−3.5–2.1), respectively. Increment of bone forming OC correlated negatively with decrement of CRP and PCDAI (week 0 vs. weeks 6, week 30, and weeks 54).

Conclusion: Clinical response to IFX therapy was associated with an increased level of bone forming osteocalcin in pediatric patients with 54-week treatment of IFX. In contrast to a previous study, bone resorption marker (bCL) was not increased suggesting a bone forming effect of IFX treatment.


Disclosure of Interest: None declared.

PO-G-0141/PD-G-0081

Inflammatory Bowel Disease

CORRELATION OF INFlixIMAB LEVELS AND ANTIBODIES WITH CLINICAL OUTCOME IN CHILDREN WITH IBD

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Objectives and Study: The anti-TNF agent Infliximab (IFX) is used in patients with Crohn’s disease (CD) and ulcerative colitis (UC). Despite of the profound clinical response questions arise concerning loss of efficacy and immunogenicity. We correlated the clinical outcome of IFX treated patients at our institution with serum levels of IFX, antibodies to IFX (ATI), and the occurrence of autoantibodies (ANA, dsDNA).

Methods: IFX trough levels and ATI were retro- and prospectively measured in 221 sera from 50 children (40 CD, 8 UC and 2 CI, 29 males). Serum samples were obtained before the 2nd until the 38th IFX infusion. The presence of ANA and dsDNA was determined before and during IFX treatment. Patients were divided in groups with respect to clinical outcome: acute allergic reaction (n = 4), delayed immune reaction with arthritis/arthralgia within one week after infusion (n = 7, including 2/4 with acute reactions), no response during induction therapy (n = 3), sustained response (n = 14), loss of response (n = 16; 11 complete, 5 partial). In 8 patients follow-up was <6 months.

Results: ATI were detectable in 47 sera sampled at different time points from 19/50 (38%) patients. ATI were always present when IFX trough concentrations were below the detection level (<0.002 µg/mL) (38 samples), but also occurred at low IFX concentration up to 1.2 µg/mL. The earliest occurrence of ATI was before the 2nd infusion in a child considered as non-responder. The presence of ATI was associated with acute allergic reactions (4/4) and loss of response (10/16). If ATI occurred they persisted, except in 4 patients with low titers which turned negative after IFX dose escalation or reintroduction of azathioprine. Before IFX treatment ANA-titers were high positive (>1;240) in 8 (16%) children. Additional 19 patients (38%) developed high ANA (max 1;7680) during IFX treatment. 7/50 children developed dsDNA. Autoimmunity was common in patients with acute or late reactions (7/9). IFX was discontinued because of side effects or loss of response in 22 patients after a median of 9 infusions. Neither a laboratory nor a clinical marker before IFX treatment nor IFX levels at 14 weeks was predictive for immune reaction or loss of response.

Conclusion: Measurement of IFX levels and ATI helps to tailor the treatment. The presence of ATI is related to acute allergic reactions and treatment failure. Dose adjustment of IFX was effective to overcome ATI but only at low titers. Randomized controlled trials in pediatric patients are needed whether the co-medication of immunomodulators decrease the risk for ATI, allergic reactions or loss of response during IFX treatment.

Disclosure of Interest: None declared.

PO-G-0107/PD-G-0082

Inflammatory Bowel Disease

DEVELOPMENT OF CROHN’S DISEASE DURING ETANERCEPT THERAPY IN CHILDREN WITH RHEUMATIC DISEASE

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Objectives and Study: Tumor necrosis factor alpha (TNF) has broad effects in the immune system including lymphoid organ development as well as the growth, survival, and function of immune cells. The pathogenesis of several autoimmune diseases including rheumatoid arthritis, juvenile idiopathic arthritis (JIA), psoriatic arthritis and Crohn’s disease (CD) has been linked to an elevated TNF expression at the site of inflammation. However, due to the complex function of TNF, immunodysregulation and even proinflammation have been reported. Patients treated with TNF antagonists (Etanercept) can develop several diseases chronic inflammatory bowel disease (IBD). The purpose of this study is to analyse the association between Etanercept treatment and the development of Crohn’s disease (CD).

Methods: A retrospective study during 25 years has been made. Patients under 18 years old with a rheumatic disease and who had received treatment with Etanercept were included.

Results: A total of 34 patients were studied; 30 of which had JIA (88.2%). During the period of study, 3 patients (8.8%)
developed Crohn’s disease (2 had JIA and 1 had Bechet’s disease). The average period for the diagnosis of a rheumatic disease in the children under study with IBD was 5.00 ± 3.36 years vs control group (patients without IBD that received Etanercept treatment) 4.90 ± 3.07 years (P = 0.86). Rheumatoid factor was negative in all patients, and antinuclear antibody was positive in 33% of Crohn’s disease group and 32% in the control group (P = 0.87). Uveitis was present in 0% and 19% respectively (P = 0.038). The average treatment time with Etanercept was 12.00 ± 11.25 months in the Crohn’s disease group, and 37.45 ± 25.19 months in the control group (P = 0.28). It is important to note that 2 of the 3 patients who developed Crohn’s disease were receiving Etanercept at the time when diagnosis was made.

Conclusion: There are only a few reports on the development of chronic inflammatory intestinal disease associated with etanercept therapy. Patients with JIA are not predisposed to developing CD, except adolescents affected by enthesitis-related arthritis or psoriatic arthritis. The development of Crohn’s disease during Etanercept treatment has occurred in a high percentage of our population under study. No significant differences between these patients and the ones of the control group have been found, to present a predisposition to develop an inflammatory bowel disease. However, uveitis was present in the control group and absent in the Crohn’s disease group. The observed percentage cannot be defined as an infrequent event, and we believe it should be considered when starting treatment with this anti-TNF agent

Disclosure of Interest: None declared.

PO-G-0106/PD-G-0083

Inflammatory Bowel Disease

ULTRASOUND SCAN WITH BOWEL WALL THICKNESS EXAMINATION IN ACUTE SEVERE ULCERATIVE COLITIS IN CHILDREN

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Objectives and Study: Many features of pediatric ulcerative colitis (UC) are similar to adult-onset disease, but the rate of extensive disease is doubled in children (pancolitis) and it is more frequently severe. Therefore, the incidence of severe attack is greater in pediatric age (30–40%) than in the adulthood (15%). PUCAI is a valid, noninvasive index to assess disease activity in pediatric UC, a PUCAI score >65 indicates severe disease. Objective measures of extent and severity of illness are required during severe attacks of UC. Ultrasoundography has recently been proposed as an auxiliary tool for the assessment of extent and severity of disease. We aimed to assess the existence of a correlation between the clinical severity of severe UC in the pediatric age (estimated by PUCAI) and the bowel wall thickness (BWT) evaluated by grayscale transabdominal ultrasonography. To assess if PUCAI correlates with increased parietal blood flow using color and power Doppler and to assess whether, during the treatment, clinical improvement (PUCAI reduction over time) corresponds to an improvement of the ultrasound findings (BWT).

Methods: We retrospectively reviewed patients with severe UC hospitalized at the Meyer Children’s Hospital of Florence over a period of 10 years (2000–2010). A total of 42 severe attacks of UC (PUCAI >65) were recruited. Disease activity was evaluated using PUCAI, through the analysis of medical records. We collected data from abdominal ultrasounds performed during hospitalization: BWT in mm with grayscale and color and power Doppler vascularity. To assess the existence of a correlation between PUCAI and BWT we carried out a linear regression analysis and to evaluate the relationship between PUCAI and vascularization a test for the difference between the mean values of 2 paired samples has been done.

Results: 25 attacks out of 42 (59.5%) were successfully treated with corticosteroids i.v (1 line therapy), 17 out of 42 (40.5%) were corticosteroid resistant and required a second line therapy. Colecotomy was necessary in 5 attacks out of 42 (12%). A child died for toxic megacolon and multiple organ failure (MOF) (2.4% mortality, 1/42). Considering all the colonic segments together, a correlation between PUCAI and bowel wall thickness was found (r1 = 0.3176 using all ultrasounds (P1 < 0.000) r2 = 0.4809 (P2 < 0.000) using only the first available scan for each attack). Of all colonic segments, the descending colon showed the best significance (r1 = 0.3760/P1 = 0.5277, P1 < 0.000/P2 < 0.001). A correlation between PUCAI and bowel vascularization on Power and Color Doppler was also found (P < 0.0001).

Conclusion: Ultrasound scan is a useful, auxiliary, noninvasive, instrument to monitor short-term outcome of severe ulcerative colitis in children.

Disclosure of Interest: None declared.

PO-G-0127/PD-G-0084

Inflammatory Bowel Disease

SINGLE BALLOON ENTEROSCOPY, MR-ENTEROGRAPHY, AND ABDOMINAL ULTRASOUND FOR EVALUATION OF SMALL-BOWEL DISEASE IN CHILDREN WITH (SUSPECTED) CROHN’S DISEASE


Objectives and Study: Crohn’s disease (CD) and ulcerative colitis (UC) are the two main types of inflammatory bowel disease (IBD) and are frequently diagnosed in the pediatric age group, accounting for 25% of all IBD patients.
PO-G-0108/PD-G-0085

Inflammatory Bowel Disease

A POPULATION-BASED SURVEY OF SURGICAL MANAGEMENT OF CHILDREN WITH INFLAMMATORY BOWEL DISEASES

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Objectives and Study: Around 425,000 children below 18 years of age live in Stockholm County. The now ubiquitous increase in the incidence of paediatric IBD, especially Crohn’s disease, was reported already ten years ago from this county. The aim of the study was to perform a population based survey of abdominal surgical management (excluding perianal procedures) in all children and adolescents below 18 years of age with Crohn’s disease (CD) and ulcerative colitis (UC) in Stockholm County.

Methods: Hospital registries and patient files from the paediatric (< 15 years of age) and all six adult surgical departments in Stockholm County were studied after approval by the regional ethical review committee. Data on patients with ICD-10 diagnoses codes K50–509 or K51 and with procedure codes JFA-JFW from 2000–2008 were collated. Patient notes were studied after written approval by caregivers and/or patients (when adults). Census of IBD patients in our paediatric hospital (Sachs Children’s Hospital) were extrapolated by using official population census and county health statistics.

Results: 11 girls/26 boys were operated for CD, 9 girls/4 boys were operated for UC, ages at operation; up to 8 years n = 2, 8–12 years n = 6, 13–15 years n = 20, 16–18 years n = 22. 22 children underwent ileocolonic resection for CD, 5 had isolated small bowel resection, 5 had enterostomy and 4 underwent colectomy with ileostomy. In 9/13 children with UC a colectomy with ileostomy was performed, 19 children (11 girls), with IBD underwent resection or removal of the colon before 18 years of age, the youngest was 6 years. 13/19 resections/removal of the colon were performed in 15- to 17-year-old children (13/19), and 9/19 had CD.11/50 children, who had abdominal surgery for IBD had a second surgical procedure before 18 years of age, in most cases to establish intestinal continuity. The prevalence of CD and UC in Stockholm County was 38 and 26/100,000 children under 18 years of age, respectively. The incidence of abdominal surgery in children under 18 with IBD during 2000–2008 was 2.5/100 person-year for CD and 1.3/100 person-year for UC.

Conclusion: To the best of our knowledge this is the first population based study with an estimation of a yearly incidence of surgery in paediatric Crohn’s and Ulcerative colitis. Surgery for IBD in children is rarely performed in Stockholm. Our study show that a large proportion of surgical procedures for CD are performed in 16- to 17-year-old patients, who are not included in epidemiological studies on paediatric IBD.

Disclosure of Interest: None declared.

PO-G-0147/PD-G-0086

Inflammatory Bowel Disease

RISING INCIDENCE OF ULCERATIVE COLITIS IN CHILDREN IN VICTORIA, AUSTRALIA: 1950–2009

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Objectives and Study: Clinical experience suggests a substantial increase in the number of children being diagnosed with inflammatory bowel disease (IBD) in Victoria and particularly in those with Crohn’s disease. This has impacted
on paediatric health care resource practice and utilisation. In this study, we wished to examine whether there had been a similar increase in diagnostic rates for ulcerative colitis in Victorian children using a collaborative approach across multiple hospital campuses in Victoria.

Methods: We conducted a 60-year retrospective review (1950–2009) of children aged 16 years or less diagnosed with ulcerative colitis in the state’s major paediatric centres. Data included demographic, diagnostic and clinical details.

Results: 1310 children with IBD were identified, of whom 342 had ulcerative colitis (26%), male to female ratio of 1.25:1.0, median age 10.9 years, interquartile range 7.0–13.2). The overall median annual incidence of ulcerative colitis was 0.55/105 children ≤ 16 years of age (interquartile range 0.18–0.66). The number of reported cases increased by 11-fold during the study period (P < 0.001). This marked increase appeared to occur from the early 1990s and had yet to plateau. Children diagnosed during the last two decades were older at diagnosis (median 10 y vs 11.6, P < 0.0001), and had higher weight- and height-for-age z scores than those diagnosed during the first 40 years (mean weight-for-age (standard deviation) 1950–89: −0.80 (1.56) vs 1990–2009: −0.11 (1.17), P < 0.001. Mean height-for-age (standard deviation) 1950–89: −0.50 (1.15) vs 1990–2009: −0.13 (1.12), P < 0.05). More recently diagnosed children also had more extensive disease (1950–89: 52% vs 1990–2009: 71%, P < 0.01).

Conclusion: The incidence of ulcerative colitis has increased markedly in Victorian children since 1990. The recognition of this sharply-defined increase has internationally important implications for our understanding of the pathogenesis of this condition and will influence both management and health care planning.

Disclosure of Interest: None declared.

Inflammatory Bowel Disease
ENVIRONMENTAL FACTORS AND RISK OF DEVELOPING PAEDIATRIC INFLAMMATORY BOWEL DISEASE—A PROSPECTIVE POPULATION BASED STUDY 2007–2009

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Objectives and Study: To investigate environmental risk factors of developing inflammatory bowel disease (IBD) in children below the age of 15 years.

Methods: From a well-defined geographical area in Denmark (Eastern Denmark, Funen and Aarhus) we prospectively recruited newly diagnosed IBD patients in the period 1.1.2007–31.12.2009. Healthy controls were randomly selected from the same geographical area. Patients and controls were mailed a questionnaire created by the International organization of IBD (1). The questionnaire was slightly modified for use in paediatric patients. The questionnaire included markers of exposure in several areas: socioeconomic status, area of residence, living conditions, infections, and diet. Data were analysed using uni- and multivariate logistic regression. Only results from the multivariate analysis were included.

Results: A total of 118 IBD patients (64 Crohn’s disease (CD), 49 ulcerative colitis (UC) and 5 IBD unclassified (IBDU)) and 545 healthy controls filled out the questionnaire. The response rates were 91% and 44% in patients and controls, respectively. IBD in first degree relatives were associated with an increased risk of developing IBD (odds ratio (OR): 5.4 (95%CI: 2.3–13.9), CD (OR: 5.4 (1.9–15.3) and UC (OR: 3.7 (1.1–12.2). Bedroom sharing were associated with an increased risk of IBD (OR: 2.2 (1.1–4.4) and CD (OR: 3.4 (1.4–8.4) but not UC (OR: 1.5 (0.6–4.5). Dietary factors associated with a protective effect of IBD were daily vs. less than daily vegetable consumption (IBD: OR: 2.4 (0.2–9.9), CD: 0.5 (0.2–1.4), UC (0.3 (0.1–0.9)) and whole meal bread consumption (IBD: OR: 0.5 (0.3–0.9), CD (0.5 (0.3–0.9), UC (0.7 (0.3–1.4). High sugar intake was associated with an increased risk of IBD (OR: 2.5 (1.1–5.6) and UC (OR: 3.2 (1.1–10.3) but surprisingly not significantly of CD (2.1 (0.7–6.9). Frequent gastrointestinal infections were associated with an increased risk of IBD (OR: 2.4 (1.0–5.7) and UC (4.9 (1.8–13.4) but not CD (1.2 (0.3–3.9). Stressful events in the form of parents divorce were associated with an increased risk of IBD (1.7 (1.0–2.8) and CD (2.0 (1.1–3.8) but not UC (1.5 (0.8–3.1). We found no association between population density and the risk of IBD. However, a decreased risk of a UC diagnosis compared to CD was shown for patients living in urban areas (OR: 0.8 (0.7–0.9).

Conclusion: We found several protective factors and risk factors of developing IBD compared to healthy controls. Some are well known associations (family history of IBD, dietary factors) others such as stressful events, bedroom sharing, frequent gastrointestinal infections could provide new aetiological clues, as could our finding that CD was associated with a urban living environment compared to UC.


Disclosure of Interest: None declared.

Inflammatory Bowel Disease
AN INCREASING HOSPITAL PREVALENCE OF PEDIATRIC INFLAMMATORY BOWEL DISEASES IN FRANCE: A 5-YEAR NATIONAL SURVEY 2005–2009

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Objectives and Study: We used the French hospital discharge administrative data base for identifying patients suffering IBD to estimate the prevalence, in both ambulatory
and hospitalized populations, in multiple geographic regions and across different practice types. Permission for accessing this data base was obtained from the Commission Nationale de l’Informatique et des Libertés.

Methods: We extracted records of <19 years old patients containing codes for Crohn’s disease (CD) and ulcerative colitis (UC) for years 2005 to 2009. Based on date of birth, sex and id in health care system, an anonymous and non reversible number is assigned to each patient (patient ID), allowing to identify the different hospitalizations of a patient and to estimate the number of stays and hospital days by patient. The French population data by sex, age, region, given by National Institute of Statistics and Economic Research (INSEE) was used to calculate hospital prevalence (HP) as the number of patients of a group hospitalized with IBD on the population of this group. Comparisons between groups used chi2 for categorical data and analysis of variance for continuous data. Statistical analyses were performed with SAS 8.2 (SAS Institute, Cary, NC, USA).

Results: From 2005 to 2009, 41,126 stays for 12,342 different patients were recorded with 49% females and an increasing proportion of patients aged 15–19 years from 65.8% to 69.2% (P < 0.0001). CD was recorded for 75.9% of stays, 78.6% for one day hospitalizations (ODH) and 71.1% for >24 h stays (P < 0.0001). There was a 60% increase in the number of stays and a lower increase (29%) in the number of patients. Percentage of ODH increased from 58% to 66% (P < 0.0001) with stays and patients increase respectively of 84% and 44%, and a number of patients by stay and year rising from 2.06 to 2.68 (P < 0.0001). For >24 h, stays and patients increased respectively of 27% and 23%, with a number of stays by patient per year rising from 1.45 to 1.56 (P < 0.01). For ODH, CD does not vary according to year, but for >24 h hospitalization, it decreased from 73.7% to 68.5% (P < 0.001). The stays took place at 82.3% in the public sector with an increment from 80.3% to 83.3% (P < 0.0001). This part is higher for day hospitalizations than traditional stays (86.4% versus 80.0%, P < 0.0001). Regions with highest HP are Champagne-Ardennes, Alsace and Nord-Pas de Calais and regions with lowest are Auvergne, Limousin and Rhône-Alpes.

Conclusion: This is the unique currently available national data base for assessing the prevalence of IBD. This national study from 2005 to 2009 shows an increasing HP of pediatric IBD and evidences, for the first time in France, regional distribution and provides information about the mode of hospitalization reflecting changes in medical practices.

Disclosure of Interest: None declared.

Inflammatory Bowel Disease
INCREASING INCIDENCE OF PEDIATRIC INFLAMMATORY BOWEL DISEASE IN SPAIN: EPIDEMIOLOGIC DATA OF A 25-YEAR PERIOD
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Objectives and Study: A growing incidence of pediatric IBD in European southern countries has been recently reported. Although IBD diagnosis in children has increased in the last decades in Spain, there are not consistent epidemiologic data available. The aim of the study was to describe the changing pattern of pediatric IBD incidence in Spain in the last 25 years.

Methods: A retrospective survey of newly diagnosed patients below 18 years of age in the period 1985–2009 was performed. Patients’ data at diagnosis were obtained from the different hospitals’ own databases. Rates of incidence were calculated using population-based epidemiologic data, available in our country since 1996 for pediatric age. Sixty-eight reference IBD centres, both pediatric and general, participated in our survey.

Results: Data from 2,273 patients were obtained: 1,250 CD (55%), 863 UC (38%) and 160 IBD (7%). Sex distribution: 55.2% male, 44.8% female, with higher male predominance for CD (58.3%) compared to UC (50.9%) (P = 0.002). Mean age at diagnosis: 11.8 years (SD: 4.1); with statistic differences between diseases: CD: 12.6 vs UC: 11.3 vs IBD: 8.2 (p < 0.001). Disease localization at diagnosis was: 1) CD: ileocolonic (L3) 56.4%, ileal (L1) 26.1%, colonic (L2) 16.3%; exclusive upper involvement (L4) 2%; perianal disease (p): 18.2%, 2) UC: extensive colitis (E3) 63.8%, left-sided colitis (E2) 26%, proctitis (E1) 10.2%. A clear increase of newly diagnosed patients per year all along this period was observed: from 19 diagnosed cases in Spain in 1985 to 204 cases in 2009. Incidence rates were calculated and compared for the last 14 years (1996–2009). Global IBD incidence rate has increased from 0.8 (95% CI 0.7–1.1) to 2.5 cases (95% CI 2.2–2.9)/100,000 inhabitants <18 years/year. Although this increase is more evident for CD (from 0.5 to 1.6) UC has also considerably risen (0.3–0.8).

Conclusion: This is the first attempt to calculate the current incidence of pediatric IBD in Spain. Our data show the important increase of IBD incidence rates in the studied period (1985–2009). In the last 14 years global pediatric IBD incidence has tripled, with a more important increase in CD incidence (tripled) than in UC. The type of disease, localization, age and sex distribution observed in our patients were in accordance with previously reported data.

Disclosure of Interest: None declared.

Inflammatory Bowel Disease
INCREASED PROINFLAMMATORY CYTOKINES REPRESS HEAT SHOCK PROTEIN 70 IN EXPERIMENTAL COLITIS MODELS

PO-G-0125/PD-G-0089

PO-G-0133/PD-G-0090
**Objectives and Study:** Interleukin-10 knockout (Il10<sup>−/−</sup>), mucin Muc2 knockout (Muc2<sup>−/−</sup>) and interleukin-10/Muc2 double knockout (Il10<sup>−/−</sup>/Muc2<sup>−/−</sup>) mice are well described experimental colitis models. Heat shock protein 70 (Hsp70) is suggested to be essential for cellular protection from stress thereby maintaining intestinal homeostasis. The aim of this study was to investigate the relationship between the expression of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ), Hsp70 expression and colitis severity.

**Methods:** Wild type, IL-10<sup>−/−</sup>, Muc2<sup>−/−</sup> and IL-10<sup>−/−</sup>/Muc2<sup>−/−</sup> mice were sacrificed at 5 weeks of age, and distal colonic tissue was collected for histochemical and molecular analysis. To study the effect of proinflammatory cytokines on Hsp70 induction, cells were pre-treated overnight by TNF-α and IFN-γ and then heat shocked for 45 minutes followed by a 60-minute recovery. The Hsp70 protein expression was analyzed by Western blot and the mRNA levels of Hsp70, TNF-α and IFN-γ were quantified by quantitative polymerase chain reaction.

**Results:** IL-10<sup>−/−</sup> mice were indistinguishable from wild type litter mates and did not show clinical or morphological signs of colitis. In contrast, Muc2<sup>−/−</sup> and IL-10<sup>−/−</sup>/Muc2<sup>−/−</sup> mice showed significant growth retardation and clinical and morphological signs of colitis. Remarkably, IL-10<sup>−/−</sup>/Muc2<sup>−/−</sup> mice even displayed mortality and developed more severe colitis than Muc2<sup>−/−</sup> mice. The mRNA levels of both TNF-α and IFN-γ were comparable among IL-10<sup>−/−</sup>/Muc2<sup>−/−</sup> and wild type mice. However, in IL-10<sup>−/−</sup>/Muc2<sup>−/−</sup> mice, which showed the most severe colitis, the transcription of both TNF-α and IFN-γ was dramatically upregulated. Focusing on Hsp70 expression, Hsp70 mRNA levels were significantly decreased in both Muc2<sup>−/−</sup> and IL-10<sup>−/−</sup>/Muc2<sup>−/−</sup> mice. However, Hsp70 protein levels were unaffected in Muc2<sup>−/−</sup> mice, but almost undetectable in IL-10<sup>−/−</sup>/Muc2<sup>−/−</sup> mice. In vitro studies revealed that the induction of Hsp70 mRNA and protein by heat shock was not affected by IFN-γ pretreatment. TNF-α pretreatment downregulated Hsp70 mRNA and inhibited Hsp70 mRNA induction, but did not affect Hsp70 protein expression. However, co-pretreatment by TNF-α and IFN-γ inhibited the heat shock-induced Hsp70 mRNA and protein expression.

**Conclusion:** In conjunction, these data indicate that increased TNF-α and IFN-γ repress Hsp70 expression, which in turn leads to less cellular protection against stress and therefore most likely more severe colitis in the IL-10<sup>−/−</sup>/Muc2<sup>−/−</sup> mice compared to IL-10<sup>−/−</sup> and Muc2<sup>−/−</sup> mice. Furthermore, these data demonstrate an important role of Hsp70 in limiting colitis severity in experimental colitis models.

**Disclosure of Interest:** None declared.
interactions resulting in decreased antigen sampling, increased DC maturation and a more proinflammatory type of DC. Therefore, autophagy-related SNPs may contribute to CD pathogenesis through dysregulation of the interactions between DC and epithelial cells resulting in a lack of immune tolerance.

**Disclosure of Interest:** None declared.

**Objectives and Study:** Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in the neonatal intensive care, with high morbidity and mortality rates in preterm infants. The disease is characterized by severe intestinal inflammation and necrosis. Increased tumor necrosis factor-alpha (TNF-α) and severe endoplasmic reticulum (ER) stress has been suggested to be a common consequence of intestinal inflammation. The aim of the present study was to investigate the possible role of TNF-α and ER stress in NEC.

**Methods:** Intestinal tissue from NEC patients (n = 54) and preterm control infants (n = 19) was obtained during surgical resection or at stoma closure after recovery. ER stress responses to TNF-α were detected in human intestinal epithelial cell line HT29 cells. The protein expression of ER stress proteins were analyzed by Western-blot and immunohistochemistry, and the RNA levels of TNF-α and ER stress proteins were quantified by quantitative polymerase chain reaction (PCR). Splicing of X-box binding protein 1 (XBP1), used as parameter for severe ER stress, was determined by PCR followed with DNA electrophoresis.

**Results:** TNF-α induced the translation of ER stress proteins 78 kDa glucose-regulated protein (GRP78) and activating transcription factor 4 (ATF4) in HT29 cells. However, splicing of XBP1, which is a hallmark for the unfolded protein response (UPR), was not observed in NEC patients and preterm control infants. There were no significant differences in TNF-α mRNA levels among the patient groups. Detailed analysis revealed that TNF-α gene expression correlated with GRP78 and ATF4 expression in A-NEC and preterm control groups, but not in NEC-R group. Splicing of XBP1 was not observed in NEC patients and preterm control infants.

**Conclusion:** All together these data indicate that the ER stress response in NEC patients is mild and most likely caused by mis-folded proteins. As splicing of XBP1 did not occur in A-NEC or in NEC-R, it is highly likely that severe ER-stress/UPR is not a common consequence of NEC.

**Disclosure of Interest:** None declared.

**Objectives and Study:** Prohormone convertase 1/3 (PC1/3) is involved in the cleavage of different prohormones into their bioactive fragments, including proinsulin, proglucagon and proopiomelanocortin. A deficiency in PC1/3 leads to a diverse combination of symptoms ranging from hypoglycemic episodes and severe diarrhea in the neonatal period to obesity at an older age. To date, three cases have been described in the medical literature. We follow two families in our institution and report the phenotypic assessment of affected family members.

**Methods:** Retrospective review of four male patients (2 from each family) who are followed in our gastroenterology clinic and have a mutation in the human PC 1 gene.

**Results:** Four patients at ages 18 months, 22 months, 7 years, and 11 years are described. All patients had a normal birth weight (mean 3.4 kg) and presented with severe osmotic diarrhea beginning within the first days of life. Three children needed total parenteral nutrition (TPN) due to severe malabsorption during the first year of life. There were no signs of pancreatic insufficiency (fetal elastase >200 μg/g stool, n = 2). Duodenoscopy showed patchy normoplastic villus atrophy in one neonate and normal mucosal architecture in two others. Severe obesity was manifested after the 2nd year of life in the older patients (weight 33.9 kg, BMI 28.8, p > 99, z2.8 at 7 y and 38.4 kg, BMI 26.2, p > 99, z2.7 at 7 y). Proinsulin was significantly raised in all patients (range 57–1116 pmol/L (norm 6.4–9.4)).

**Conclusion:** Our data confirm that PC1/3 deficiency is characterized by neonatal enteropathy followed by
early-onset obesity and should be considered in the differential diagnosis of congenital diarrhea. In contrast to most other causes of congenital diarrhea, the severe diarrhea in PC1/3 deficiency is transient and discontinuation of TPN can be expected. Later in life excessive weight gain becomes the primary clinical problem. The phenotype in all cases followed a similar pattern, although there appears to be variable severity of symptoms. All patients had hyperproinsulinemia. Thus elevated proinsulin may be a useful unique diagnostic marker for PC 1/3 deficiency in the neonatal period.

**Disclosure of Interest:** None declared.

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**Hepatology**

THE USE OF BRUM1 RESEQUENCING MICROARRAY TO IDENTIFY MUTATIONS IN PATIENTS WITH NEONATAL CHOLESTASIS


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**Objectives and Study:** Neonatal cholestasis is the presenting clinical feature of serious and potentially life limiting liver diseases such as progressive familial intrahepatic cholestasis (PFIC), arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome and Niemann Pick type C (NPC) disease. A single rapid molecular test to confirm the diagnosis would reduce the delay from molecular genetic investigation at multiple diagnostic centres thus facilitating optimal clinical management and counselling. We have designed a resequencing microarray (BRUM1) capable of simultaneously sequencing multiple genes associated with neonatal cholestasis. The aim of the study was to assess the utility of multiple diagnostic centres thus facilitating optimal clinical management and counselling of families.

**Disclosure of Interest:** None declared.

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**PO-H-0267/PD-H-0096**

**Hepatology**

DOES ADJUVANT STEROID THERAPY POST KASAI PORTO-ENTEROSTOMY IMPROVE THE OUTCOME OF BILIARY ATRESIA? SYSTEMATIC REVIEW AND META-ANALYSIS

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**Objectives and Study:** To complete a systematic review of the literature and perform a meta-analysis to determine the efficacy of adjuvant steroid therapy post Kasai porto-enterostomy (KP) on the outcome for biliary atresia (BA) in regards to normalization of serum bilirubin level (SBL) at six months post KP and the need for liver transplantation within the first year post-KP.

**Methods:** Systematic review and meta-analysis of randomized trials and/or observational studies published between January 1969 and June 2010 that examined the role of steroids on BA outcomes. Studies identified through MEDLINE, PubMed, EMBASE and Cochrane database. The search was done systematically and reviewed by two independent reviewers, discrepancies were resolved by consensus. We statistically combined the studies for meta-analysis in the absence of significant statistical heterogeneity.

**Results:** The search yielded 16 observational studies and 1 randomized control trial. Of these, 4 observational studies with total number of 160 participants (111 patients in 3 case-control studies and 49 patients in 1 cohort study) and a single
RCT with 73 participants satisfied the inclusion and exclusion criteria. In general, the mean age at surgery was 3 months or less in the selected studies. 12 studies were excluded for any of 3 major reasons: no control group; no single standardized steroid therapy protocol for all the patients; or the outcomes of interest were not measured. There was no statistical significant differences in the effect of steroids neither on normalizing SBL at 6 months (pooled OR = 1.48 (95% CI = 0.67–3.28), nor in delaying the need for early liver transplantation (within the 1st year post-KP (pooled OR = 0.59 (95% CI = 0.21–1.72).

Conclusion: Our meta-analysis did not find a significant effect of steroid over standard therapy either in normalizing SBL at 6 months or at delaying the need for early liver transplantation post KP. RCT studies of sufficient size and comprehensive design using high-dose steroids are still needed to determine the effectiveness of steroids on the short and intermediate post KP outcomes for BA patients.

Disclosure of Interest: None declared.

PO-H-0306/PD-H-0097

**Hepatology**

**UK EXPERIENCE OF TREATMENT OF CHRONIC VIRAL HEPATITIS C IN CHILDREN AND ADOLESCENTS: PREDICTORS OF VIRAL RESPONSE AND QUALITY OF LIFE**


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**Objectives and Study:** The objective of this study was to review efficacy, tolerability and quality of life (QoL) in children with chronic hepatitis C (HCV) treated with pegylated interferon (PEG-IFN) alfa and ribavirin in 3 national referral centers in the UK.

**Methods:** Demographic, laboratory and clinical outcome data on children up to 18 years of age treated for HCV with PEG-IFN alfa –2a/2b and ribavirin were reviewed. Information gathered from QoL questionnaires (CHQ-PF28) completed by parents during their children’s treatment was also available for one of the centers. Sustained viral response (SVR) was defined as undetectable HCV RNA at 24 weeks following end of treatment.

**Results:** The study sample comprised 75 children of whom 38 were males. The median age at the start of the treatment was 10 years (3.0–17.2 years). The most common mode of infection (83%) was via vertical transmission. Thirty-four patients were Genotype 1(G1); 39 Genotype 2&3 (G2&3); 2 Genotype 4(G4). SVR was achieved in 75%; 53 G1; 89% G 2&3; 100% G 4. There was no significant difference between baseline ALT and/or AST levels in those who achieved SVR compared to the non responder group. However the first group had a least 30% lower ALT and/or AST levels at 24 weeks posttreatment compared to the latter group P = 0.003 and P = 0.000 respectively. Younger children had higher SVR compared to older age groups, however this was not statistically significant P = 0.5. Low viral load at the start of the treatment (≤500,000 IU/mL) did not have significant effect on viral response P = 0.5. Early viral response (EVR) at 12 weeks of treatment was achieved in 46 and sustained in 40 (87%). Data on rapid viral response (RVR) at 4 weeks of treatment were available in 25; 17/25 (68%) achieved (RVR) which was sustained in 16 (94%). There was no significant change in the z scores for weight and height from start of treatment compared to 24 weeks posttreatment follow-up (p 0.2 and 0.5, respectively). Data on QoL were available for 31 children and their families. Treatment had significant impact on QoL during the initial 12 weeks of treatment compared to overall treatment duration with values returning to baseline at the end of treatment and at follow-up. There were no serious side effects reported and none discontinued treatment due to side effects.

**Conclusion:** HCV treatment with (PEG-IFN) and ribavirin is well tolerated by children with minimal negative impact on the quality of life and no significant effect on growth. EVR and RVR are good predictors of treatment response.

Disclosure of Interest: None declared.

PO-H-0329/PD-H-0098

**Hepatology**

**OBSTRUCTIVE JAUNDICE INDUCES HIGH-MOBILITY GROUP BOX 1 EXPRESSION AND TOLL-LIKE RECEPTOR ACTIVATION**

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**Objectives and Study:** Obstructive jaundice is associated with bacterial translocation and inflammatory cytokine induction. It is unknown if the sensor and effector arms of the innate immunity including toll-like receptors (TLRs) and their upstream and downstream signaling molecules are involved in the pathogenetic mechanism.

**Methods:** A rat model of cholestasis by ligation of the extrahepatic bile duct (BDL) for 2 weeks was created. TLRs, interferon regulatory factors (IRFs), IL-6, IL-8, antimicrobial peptide β-defensin and cathelicidin, as well as high-mobility group box 1 (HMGB1) expressions were studied by using real-time quantitative reverse transcription–polymerase chain reaction, immunohistochemistry, Western blotting and enzyme-linked immunosorbent assay.

**Results:** Obstructive jaundice for 2 weeks was associated with significant up-regulation of TLR1, 2, 4, 6, and 9 mRNA expressions. There were significant increase of liver IRF5, IL-6 and β-defensin 1 mRNA levels in the BDL rats than in the sham and non-operative control rats, which were associated with significant increase of immunoreactive IRF5 protein staining in the nucleus of Kupffer cells and
neutrophils. Hepatic HMGB1 expression and release into serum were significantly elevated in the cholestatic rats than in the sham and control rats.

**Conclusion:** The results indicate that obstructive jaundice may induce hepatic HMGB1 expression with activation of TLR1, 2, 4, 6, 7 and 9, as well as IRF5, which is associated with increased IL-6 and β-defensin production.

**Disclosure of Interest:** None declared.

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**PO-H-0279/PD-H-0099**

**Hepatology**

**EFFICACY OF HIGH VERSUS LOW DOSE ADJUVANT CORTICOSTEROID TREATMENT IN CHILDREN WITH BILIARY ATRESIA: A SINGLE-CENTRE, CONTROLLED STUDY**

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**Objectives and Study:** Biliary atresia (BA) is the main cause of chronic liver disease leading to transplantation in infancy. Recent studies pointed to a pro-inflammatory commitment of lymphocytes in these subjects having a potential role in bile duct obliteration, therefore corticosteroid treatment after Kasai portoenterostomy (KP) has been considered. The aim of this study was to evaluate the efficacy of a high-dose adjuvant steroid protocol to restore bile flow in children with BA.

**Methods:** From January 2001 to May 2006 infants with type III BA, all diagnosed by liver biopsy and intraoperative cholangiogram, received consecutively two different steroid regimens: overall 35 mg/kg of prednisolone from day 7 after KP over 21 days followed by tetracosactide 0.5 mg subcutaneously (Group 1, G1); overall 130 mg/kg starting on day 1 and tapered in 3 months (Group 2, G2). All patients had KP performed by the same surgeon and received antibiotics and tapered in 3 months (Group 2, G2). Overall 130 mg/kg starting on day 1 and tapered in 3 months (Group 2, G2).

**Results:** More than 60% of children with type III biliary atresia operated within 90 days of life may achieve a restored biliary drainage. In this cohort of patients a high dose adjuvant steroid treatment was not superior to a low dose regimen to increase the rate of native liver, jaundice-free survival at 6 and 36 months after KP.

**Disclosure of Interest:** None declared.

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**PO-H-0286/PD-H-0100**

**Hepatology**

**NONINVASIVE ASSESSMENT OF FIBROSIS IN PAEDIATRIC LIVER DISEASE: A COMPARISON OF TRANSIENT ELASTOGRAPHY AND BLOOD BIOMARKERS**

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**Objectives and Study:** Outcome of liver disease in children is mainly determined by severity and progression of liver fibrosis. Liver biopsy is the accepted standard for evaluating fibrosis but is limited by the need for sedation in children, sampling error and risks including bleeding. The aim of this study was to compare tools for non-invasive assessment of liver fibrosis in a paediatric cohort.

**Methods:** Children undergoing liver biopsy for chronic liver disease were recruited and underwent transient elastography (TE) and serum collection on that day. Liver biopsies were scored by a hepato-histopathologist from F0 (no fibrosis) to F4 (cirrhosis). Serum samples were analysed for the Enhanced Liver Fibrosis (ELF) test; comprising hyaluronic acid, P3NP and TIMP1 (iQur, UK). CK18-M30 levels (caspase cleavage fragments) were measured using ELISA. Biomarkers were compared to biopsy score.

**Results:** During the study period 79 children (51 boys) were enrolled. Median age: 13.8 years (range 6–18 years). Diagnosis was autoimmune liver disease in 25; nonalcoholic fatty liver disease in 25; 13 children were post-transplant; 8 children had hepatitis B/C; 3 had Wilson disease and the remainder miscellaneous. Some degree of fibrosis was evident in 73 (93%) biopsies: 22 scored as F1, 20 as F2, 26 as F3 and 5 as F4. TE was successful in all but 5 patients and was a good discriminator of significant fibrosis (≥F2) (P < 0.001), severe fibrosis (≥F3) (P < 0.001) and cirrhosis (F4) (P = 0.003). The area under the receiver operating characteristic curve for the prediction of ≥F2, ≥F3 and F4 using TE were 0.78, 0.81 and 0.92, respectively. The diagnostic performance of the ELF score was better with increasing stages of fibrosis with an area under the ROC curve for cirrhosis of 0.86. CK18-M30 level was accurate in distinguishing...
significant fibrosis (≥F2) \( (P = 0.015) \) with an area under the ROC curve of 0.69.

**Conclusion:** In this, the largest paediatric series reported to date, TE was found to be a reliable tool in distinguishing different stages of liver fibrosis in paediatric patients. Serum biomarkers may be of use in combination with TE especially in the stratification of more severe disease. Routine use of these techniques may serve as a useful adjunct to liver biopsy for diagnostic purposes and provide a reliable method of non-invasively monitoring liver disease progression in children.

**Disclosure of Interest:** None declared.

**PO-H-0287/PD-H-0101**

**Hepatology**

**TRANSLATION OF CLINIC-TO-BENCH RESEARCH TO A SPINOFF COMPANY: AN ESSENTIAL STEP TO PROCURE UNLIMITED ACCESS TO CELL-BASED PAEDIATRIC LIVER REGENERATIVE MEDICINE**

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**Objectives and Study:** Portal infusion of liver cells has demonstrated the concept of restoring a missing metabolic function via allogenic cell transplantation. The technique is limited by organ shortage, poor resistance of hepatocytes to cryopreservation and need of an accredited GMP environment for cell isolation and quality control. Our objective was to develop an industrial based stem cell advanced therapy product to provide a much greater access to liver cells, allowing dispatch of cells to treating hospitals.

**Methods:** We used a liver progenitor cell that expresses the functional characteristics of the human hepatocyte and that can be expanded in vitro and stored cryopreserved (Najimi 2007, Khuu 2010). The cells engraft and differentiate in animal models. The cell is considered as a medicinal product according to EU regulation on advanced therapies. Development of this technology until market approval requires to fulfill all steps established by health authorities in term of potency, preclinical experiments, regulatory procedures, GMP large scale production and clinical development plans, which are far beyond the scope of an academic laboratory.

**Results:** Following extensive due diligence for scientific and business model evaluation, a private public consortium led by Vesalius Biocapial and University tech transfer Sopartec invested in this technology via a spinoff company, Promethera Biosciences. Promethera’s mission is to develop the product up to market authorization for the treatment of liver based inborn errors of metabolism. Orphan drug status was obtained for OCT deficiency and Crigler Najjar syndrome. As the cell is a comprehensive healthy metabolic system, other liver-based errors of metabolism can be treated. After two years of development, Promethera is about to start clinical trials.

**Conclusion:** The current bench-to-clinical and industry translation approach is the only way to develop such innovative therapeutic approach beyond academic research up to market approval and hence availability for all children. Academic spinout of Promethera illustrates the new societal interaction demanded to us, clinical and academic researchers.


**Disclosure of Interest:** E. Sokal, Shareholder with: Founder, Consultant for: Promethera (CSO), C. Monterrat, Shareholder with: Founder, Employee of: Promethera, E. Halioua, Shareholder with: Founder, Employee of: Promethera (CEO), X. Stephenne: None declared, F. Smets: None declared, M. Najimi, Shareholder with: Founder, Consultant for: Promethera (scientific advisor)

**PO-H-0272/PD-H-0103**

**Hepatology**

**FEASIBILITY AND EFFICACY OF TRANSJUGULAR INTRAHEPATIC PORTO-SYSTEMIC SHUNT IN CHILDREN**

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**Objectives and Study:** Transjugular intrahepatic portosystemic shunt (TIPS) is part of the armamentarium adopted in adults to treat complications of portal hypertension (PH). Few series have been reported in children, in which TIPS has been considered technically demanding and offering short-term benefits. The aim of this study is to report our experience on placement and efficacy of TIPS to control severe PH in children.

**Methods:** We performed a retrospective analysis of TIPS insertion carried out at our centre in the last 5 years. TIPS was considered in children with compensated liver disease and complications of PH unresponsive to medical and endoscopic management. An expanded polytetrafluoroethylene-covered stent was placed following the invasive measurement of pressures and gradients. Ultrasound scan follow-up was carried out 3 monthly. We reviewed the features of the
eligible patients, pressures and gradients before and after the procedure, the efficacy to control portal hypertension and the period of patency of the device.

Results: 12 patients were considered but one had low portohepatic gradient. We placed TIPS in 11 children (F/M = 7/4), median age 9.8 years (range 2.2–18), median weight 30.0 kg (11.5–96.0) affected by congenital hepatic fibrosis (n 2), portal vein thrombosis (n 2), sclerosing cholangitis, cystic fibrosis, intestinal failure associated liver disease, nonsyndromic bile duct paucity, Budd-Chiari syndrome, veno-occlusive disease, Alagille syndrome (1 each). The median portosystemic pressure gradient before and after TIPS was 23 (16–35) and 10 mmHg (5–15) respectively (P < 0.00001). Complications of PH disappeared completely in 8/11 (73%), partially in 2, persisted in 1. Median ammonia levels before and after the procedure were 42 (28–96) and 23 (16–35) respectively (P = 0.01); none developed overt encephalopathy. All patients maintained patent shunts, 3/11 required dilatation or re-stenting. After a median follow up of 1.2 years (0.2–5.7) 4 patients (36.3%) eventually underwent liver transplantation after a median of 6 months (1.5–33 months), whereas 7 still have patent TIPS.

Conclusion: TIPS is a safe and effective procedure in paediatric patients with portal hypertension refractory to medical and endoscopic treatment. Despite increase in blood ammonia the drop of porto-systemic gradient did not cause overt encephalopathy in this cohort of patients. With the use of covered devices and a regular radiological survey TIPS in children can be maintained patent and act both as a bridge to liver transplantation or as an effective porto-systemic shunt.

Disclosure of Interest: None declared.

PO-H-0325/PD-H-0104

Hepatology

BILIARY ATRESIA IN THE NETHERLANDS: OUTCOME OF 214 PATIENTS DIAGNOSED SINCE 1987

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Objectives and Study: Biliary atresia (BA) is a cholestatic disease of infancy with unknown cause. Initial treatment involves a surgical portoenterostomy (Kasai). When fibroses nevertheless progresses, liver transplantation (OLT) becomes the second treatment option. The aim of the study was to determine the outcome of BA and its evolution in time, and to identify prognostic factors for outcome of BA in the Netherlands, using a national database.

Methods: All children born between 1987 and 2008 who underwent surgical correction for BA were retrieved from the NeSBAR (Netherlands Study Group on Biliary Atresia Registry) database. The outcome in terms of clearance of jaundice (bilirubin<20 µmol/L within 6 months post-surgery); 4-year transplant-free survival; and 4-year overall survival were compared in 2 cohorts (A: born in 1987–1997, n = 110; and B, 1998–2008, n = 104). Survival rates were calculated using Kaplan Meier and prognostic factors were determined using log rank tests and Cox’s regression analysis.

Results: In cohort 1987–1997, 26% (28/110) of BA patients underwent OLT before the age of 4, compared with 40% (41/104) in cohort 1998–2008 (P < 0.01). All other outcome parameters were similar in the 2 cohorts. Since 1987, clearance of jaundice has occurred in 36% of the patients, 4-year transplant-free survival had been 46 ± 4%, and 4-year overall survival 73 ± 3%. Transplant-free survival rate was 56 ± 5% in patients with Kasai surgery ≤60 days of age and 34 ± 5% with surgery >60 days (P = 0.003). Patients who cleared jaundice had a 4-year transplant-free survival of 92 ± 3% compared to 18 ± 3% in patients who did not (P < 0.0001).

Conclusion: Outcome parameters in the Netherlands were equivalent to those reported from other Western countries. The transplantation rate increased in time, whereas other outcome parameters remained similar. Timely surgical correction (<60 days), clearance of jaundice and postoperative administration of antibiotics were individually associated with a higher transplant-free survival. Although the annual case-load per centre is rather small in our country, our results do not provide arguments for centralization of surgical correction.

Disclosure of Interest: None declared.

PO-H-0314/PD-H-0105

Hepatology

BILIRUBIN HAS ANTIBACTERIAL PROPERTIES AGAINST GRAM-POSITIVE BACTERIA: A POTENTIAL BENEFIT OF PHYSIOLOGICAL JAUNDICE?


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Objectives and Study: Hyperbilirubinemia is so common in newborns as to be termed physiological (1). It is also
known to rise in response to significant stresses including sepsis (2). Limited studies show that bilirubin has antioxidant properties and is beneficial in Gram-negative endotoxin shock (3–5). Little thought has been given to the possible beneficial role of hyperbilirubinemia. We aimed to examine whether hyperbilirubinemia in the early neonatal period may confer an advantage to the host by inhibiting bacterial replication.

Methods: Group B Streptococcus, Escherichia coli, and Coagulase negative Staphylococci were the organisms chosen for study because of their relevance to neonatal sepsis. Three isolates of each organism obtained from neonatal blood cultures were grown in triplicate on Columbia agar plates containing 5% horse blood and defined concentrations of bilirubin. The plates were incubated aerobiologically in the dark at 37°C for 12 hrs before being examined and the total number of colonies on each plate recorded. Statistical comparison was by paired t-test in PASW Statistics version 18.

Results: There was a mean reduction in Group B Streptococcus colony counts between 0 and 100 μmol/L of 19.6 (95% CI 12.1 to 27.2, P < 0.001). The mean reduction in coagulase negative Staphylococcus colony counts between 0 and 100 μmol/L bilirubin was 12.9 (95% CI 3.6 to 22.2, P = 0.013). The growth of Escherichia coli was not significantly altered as a result of bilirubin.

Conclusion: Hyperbilirubinemia has a detrimental effect on the growth of the Gram-positive organisms Group B Streptococcus and Coagulase negative Staphylococci suggesting that physiological jaundice may have an evolutionary role in protection against early-onset neonatal sepsis.


Disclosure of Interest: None declared.

PO-H-0328/PD-H-01077

Hepatology

LONGITUDINAL MONITORING ON T-REGULATORY CELL FREQUENCIES IN CHILDREN WITH CHRONIC HEPATITIS B INFECTION

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Objectives and Study: T regulatory (Treg) cells are hypothesized to play a dominant role in immune tolerant phase of chronic hepatitis B virus (HBV) infection to minimize host immune response and liver inflammation. We aimed to

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PO-H-0328/PD-H-0107

Hepatology

SCREENING FOR AUTOIMMUNE-RELATED LIVER DISEASE IN THE MEMBERS OF FAMILIES OF CHILDREN WITH AUTOIMMUNE HEPATITIS

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Objectives and Study: Autoimmune hepatitis (AIH) has a long asymptomatic period before clinical manifestation is present. AIH may have genetic background thus familial predisposition for the disease can be expected. The aim of the study was to screen members of AIH children families for the presence of liver function test abnormalities and assess the possible risk of autoimmune-mediated liver disease.

Methods: 233 subjects (mothers-68, fathers-58, sisters-59, brothers-48) from 69 families affected with AIH in a child were screened. AIH risk index rating the presence of autoimmune-related diseases in familial history, abnormalities in liver function tests, increased IgG or gammaglobulin concentration and presence of autoantibodies was calculated for each subject. No risk of AIH was defined as index=0, low AIH risk as index=1, moderate risk as index=2 or 3 and high as index=4.

Results: 41 (59.5%) of the families had autoimmune diseases in familial history (thyroid diseases – 22, diabetes mellitus – 14, rheumatoid arthritis – 12, other – 15). In parents group abnormal values of ALT was found in 14 (11.1%), gammaglobulin in 14 (11.1%) and IgG in 8 (6.3%) subjects. Fathers had higher than mothers rate of ALT and GGTP abnormalities (respectively 20.7% vs 2.9%, P < 0.01 and 24.1% vs 7.4%, P < 0.001). In siblings group abnormal ALT was present in 5 (4.7%) and abnormal IgG in 6 (5.6%) of subjects. No differences in laboratory parameters were found between brothers and sisters. ANA was positive in 97 (41.6%), SMA in 138 (59.2%) and LKM was positive in 3 (1.3%) of family members. No differences in the autoantibodies positivity rate between parents and siblings were noted. 43 (18.5%) of subjects had no AIH risk factors (parents-22, siblings 21). 147 (63.1%) subjects (parents-77, siblings-70) had low risk of AIH (index=1). Moderate AIH risk (index=2 or 3) was found in 43 (18.5%) subjects (parents-27, siblings-16). None of the subjects had high AIH risk defined as index=4.

Conclusion: Screening procedures applied in this study showed high rate of positivity for autoantibodies in the families of AIH children. None of the subjects had high risk index for AIH and moderate risk (index=2 or 3) was present in 43 (18.5%) subjects. Higher number of fathers than mothers with abnormal liver function tests (ALT and GGTP) may suggest that fathers are exposed for environmental hepatotoxic factor.

Disclosure of Interest: None declared.
investigate the temporal profiles of T regulatory (Treg) cells in chronic hepatitis B virus (HBV) infection in children.

**Methods:** Totally 190 children and young adults (M:F=102:88) with chronic HBV infection who were enrolled at <age 15 were followed-up for liver function profiles and HBV seromarkers every 6 months. The patients were generally asymptomatic and received a blood sampling on a routine base. A consecutive 3-year follow-up was conducted to study Treg cell frequency (Treg/CD4+), which were estimated in the peripheral blood mononuclear cells annually. The patients were grouped according to their HBeAg and ALT status.

**Results:** Treg frequency of HBeAg positive and abnormal ALT (>40 U/L) group was highest (5.9 ± 1.7%, n = 30), followed by HBeAg negative and abnormal ALT group (5.8 ± 3.1%, n = 12), HBeAg negative and normal ALT group (5.5 ± 1.9%, n = 77), and HBeAg positive and normal ALT group (4.7 ± 2.6%, n = 71). Treg cell frequency of HBeAg positive and normal ALT group was lowest among all of the other groups (P = 0.003). Among these 190 patients, 104 of them were followed up for 3 consecutive years (Table 1). The average Treg cell frequencies remain stationary in patients at the immune tolerant phase (with persistently normal ALT levels and positive HBeAg), while the Treg frequency changed year by year in those who entered inflammatory phase (ever having abnormal ALT levels with or without HBeAg seroconversion). A total of 190 children and young adults (M:F=102:88) with chronic HBV infection who were enrolled at <age 15 were followed-up for liver function profiles and HBV seromarkers every 6 months. The patients were generally asymptomatic and received a blood sampling on a routine base. A consecutive 3-year follow-up was conducted to study Treg cell frequency.

**Conclusion:** Treg frequency was relatively stable during the immune tolerant phase of chronic HBV infection. Treg cell frequency gradually changed once the child entered inflammatory phase with elevated ALT levels, which may drive the child to leave the immune tolerance phase.

**Disclosure of Interest:** None declared.

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**Table 1. The average Treg cell frequencies in patients with different course of chronic hepatitis B infection grouped by HBeAg and ALT status**

<table>
<thead>
<tr>
<th>Infection course</th>
<th>1st year</th>
<th>2nd year</th>
<th>3rd year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (n = 48)</td>
<td>Persistently positive HBeAg and normal ALT levels</td>
<td>4.9 ± 1.4%</td>
<td>4.5 ± 1.5%</td>
</tr>
<tr>
<td>Group 2 (n = 8)</td>
<td>HBeAg seroconversion</td>
<td>4.5 ± 0.9%</td>
<td>3.7 ± 0.8%</td>
</tr>
<tr>
<td>Group 3 (n = 24)</td>
<td>Already HBeAg seroconverted</td>
<td>5.2 ± 1.9%</td>
<td>4.9 ± 1.2%</td>
</tr>
<tr>
<td>Group 4 (n = 24)</td>
<td>Persistently positive HBeAg and at least one abnormal ALT episode</td>
<td>5.2 ± 2.1%</td>
<td>4.3 ± 1.5%</td>
</tr>
</tbody>
</table>

**PO-H-0313/PD-H-0108**

**Hepatology**

**BILIARY ATRESIA IN PRETERM INFANTS**

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**Objectives and Study:** Biliary atresia (BA) is the most common cause of death from liver disease in children. Early surgical intervention (the Kasai procedure) is needed for an improved outcome. This study was aimed to investigate the characteristics of BA in preterm infants.

**Methods:** Nationwide screening for BA in Taiwan using an infant stool color card was launched from 2004. By accessing the stool card registry center database, we investigated the characteristics of BA in preterm infants.

**Results:** We identified 202 BA cases during the period from January 2004 to June 2010. The overall incidence of BA was 1.55 cases per 10 000 live births (1.13–1.91 per 10 000). The annual incidence of biliary atresia per 10 000 live births in term and preterm infant was 1.48 and 2.28 (P = 0.05), respectively. The sensitivity of detecting biliary atresia using stool cards before 60 days of age was 92.6% in term, 96.2% in preterm. The national rate of the Kasai operation before 60 days of age 68.0% in term to 48.0% in preterm. The jaundice-free rate (<2 mg/dL) at 3 months after the Kasai operation among infants with biliary atresia in term/preterm was 60.9/40.0% (103 of 169/15 of 25).

**Conclusion:** This study illustrates the incidence of biliary atresia in preterm infant is more frequent than term infant. Diagnosis of biliary atresia in preterm infants is more difficult and requires a high index of suspicion and careful workup.

**Disclosure of Interest:** None declared.

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**PO-H-0295/PD-H-0109**

**Hepatology**

**SERONEGATIVE AUTOIMMUNE HEPATITIS: A DISTINCT ENTITY IN CHILDHOOD**

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**Objectives and Study:** Serum autoantibodies represent the conventional serologic repertoire of autoimmune hepatitis
(AIH) in childhood. However few reports describe patients with a cryptogenic inflammatory liver disease, responsive to immunosuppressive treatment, but lacking of all type of autoantibody. The aim of this study was to describe features and long term follow-up of a series of children with seronegative AIH collected among 3 collaborative pediatric liver centers.

**Methods:** Between 1989 and 2009, 374 patients were consecutively admitted in the 3 centers for a cryptogenic inflammatory liver disease; in 337 of them, serum autoantibodies allowed the diagnosis of AIH type 1 in 217 (58%) and type 2 in 120 (32%). In the remaining 37 (10%) histological features suggested AIH, in absence of conventional and non-conventional autoantibody repertoire. The charts of these patients were retrospectively reviewed.

**Results:** 17 were females, mean age was 106.5 months (range 17–240 mo), 9 had a family member affected by an autoimmune disorder. In 28 the onset was symptomatic with jaundice while in 9 the liver disease was serendipitously discovered through an elevation of liver enzymes. All had elevated aminotransferase activity (mean AST 42 × UNL; range 2.5–145; mean ALT 38 × UNL range 3–126); 25 had increased gamma glutamyl transferase activity (mean 3 × UNL, range 1.5–9). Immunoglobulins G were elevated for age in 19 (51%). Mean total bilirubin in jaundiced patients was 6.3 mg/dL, albumin was ≥ 3.5 g/dL in 21 and prothrombin activity ≤ 60% in 19. Extended serology’s and molecular biology studies for common and occasional hepatotropic virus were not diagnostic in all. Liver biopsy showed interface hepatitis in all, with lobular inflammation in 29 and evidence of bile duct damage in 10. An associated autoimmune disorder was diagnosed in 10 (3 celiac disease, 1 autoimmune thyroiditis, 1 type 1 insulin-dependent diabetes, 1 juvenile chronic arthritis, 4 autoimmune hematologic disorders). All patients were treated: 23 with prednisone and azathioprine, 7 with prednisone alone, 9 with cyclosporine, alone in 7 and associated with prednisone in 2 and obtained complete remission in a mean period 9.4 weeks. Nine patients relapsed during discontinuation of therapy, 25 are still treated, and 3 could stop any treatment with a mean follow-up of 67.6 mo.

**Conclusion:** Seronegative AIH represent a distinct group of AIH in childhood. Both sexes are equally represented, the onset is almost with the features of acute hepatitis and hypergammaglobulinemia can be absent. Histology is crucial for diagnosis and a few patients display evidence of inflammatory bile duct damage. All patients responded to immunosuppressive therapy with the possibility of full stopping in the long term in few.

**Disclosure of Interest:** None declared.

PO-H-0294/PD-H-0111

**Hepatology**

**ILEAL EXCLUSION IN CHILDREN WITH PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS - OWN EXPERIENCE**

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**Objectives and Study:** Different methods of surgical treatment have been proposed in children with PFIC after ineffective UDCA therapy: partial external biliary diversion (PEBD), ileal exclusion (IE) and finally liver transplantation (LTx). The aim of the study was to assess IE in PFIC children.

**Disclosure of Interest:** None declared.
Methods: In 9 children with PFIC (3 confirmed genetically PFIC type 2, all with low GGTP), 3 boys, 6 girls, aged 2–19 years, IE was performed. In one pts PEBD was not feasible due to cholecystectomy in the past; in 3 children parents refused permission for PEBD (aesthetic reasons); two patient originally underwent PEBD but due to the postoperative complications of stoma (dyselectroliemia) IE was performed after 2 months in both; 3 adolescent girls (8, 10, and 11.6 years after successful PEBD) were converted to IE due to bad quality of live with stoma. IE was performed according to the surgical technique described by Holland’s and co-workers (J Pediatr Surg. 1998, 33: 220–224).

Results: In 5 children (one after unsuccessful PEBD) after 7 days following IE a partial relief from pruritus (from 4 to 10) and decreased bilirubin and bile acid (b.a) concentration were observed. After 6 months due to repeated increase of bilirubin and b.a. concentration, UDCA was administered to all children. After the following 2 years only 2 children experienced improvement, with normal bilirubin and b.a. concentration. One pt was successfully converted to PEBD (initially parents refused permission for the procedure), in the second child LTx was performed. After 10 year 3 children remain in observation. All of them present with 2-4 pruritus and mild to severe elevation of b.a. concentration, in spite of UDCA treatment.

In the next 4 children IE was performed after successful PEBD, so before IE they had no pruritus and bilirubin and b.a. were in normal range. After 7 days b.a. increased in all and UDCA administration was necessary. In 3 adolescent girls after next 2 years excellent quality of live, no pruritus, normal bilirubin and b.a. concentration were noticed. One of them aged 21 y, 2 years after IE, during pregnancy had very severe pruritus, but after delivery improvement was observed. In 4th child, 10 years after IE, 2nd pruritus and mild elevation of b.a. in serum (59.8 µmol/L) persisted.

Conclusion: IE is not as effective as PEBD and therefore shouldn’t be the primary treatment in children with PFIC. In adolescent patients, after successful PEBD, it is possible to convert to IE, probably without any long-term negative effect, but further studies are needed.

Disclosure of Interest: None declared.

PO-H-0315/PD-H-0112

Hepatology
THE PROGNOSTIC VALUE OF DUCTAL PLATE MALFORMATION IN BILIARY ATRESIA
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Objectives and Study: The intrahepatic bile ducts develop from the fetal ductal plate through a process called ductal plate remodeling. Disturbances in this process give rise to ductal plate malformation (DPM) which has been observed in some cases of biliary atresia (BA). We have evaluated the presence of DPM as an indicator of prognosis, along with ductular proliferation, Ishak score and age at operation.

Methods: Between 1984 and 2008, 38 patients with BA underwent hepatopancreatoduodenectomy (HPE). Three patients were lost to follow-up. We analyzed 28 biopsy specimens for presence of DPM using cytokeratin 19 staining. DPM was present when a concentric cellular arrangement was detected. Outcome at 3 months and 2 years after HPE and SNL was calculated. Patients with onset of cholestasis with acholic stools in the first week of life and/or associated congenital anomalies were assigned in the fetal, and the others were in the perinatal group.

Results: Eight out of 28 patients (28.6%) had DPM and 20 were DPM-negative. Duration of follow up ranged from 5.7 to 220.3 months, with a mean age of 73.2 months. Twelve (49.2%) patients were male, and 16 (57.1%) were female. Even though DPM-negative patients had lesser grades of fibrosis, and lesser extent of ductular proliferation they have less favourable outcome at 3 months and 2 years after HPE (Fisher exact test) and shorter SNL (log rank test). There was difference in terms of fetal/perinatal ratio between the DPM-positive and negative group, but it didn’t reach statistical significance. None of our patients with fetal type BA had DPM, and all of them had poor outcome. There was no difference between the DPM-positive and negative patients in perinatal group regarding any outcomes.

Table. Characteristics of infants affected by BA with or without DPM

<table>
<thead>
<tr>
<th>Age at HPE (mean ± SD)</th>
<th>SNL (median mean ± SD)</th>
<th>Success at 3 mo post-HPE***</th>
<th>Success at 2 y post-HPE***</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPM-positive</td>
<td>67.5 ± 16 (n = 8)</td>
<td>58.2 ± 46.1*</td>
<td>5 / 8**</td>
</tr>
<tr>
<td>DPm-negative</td>
<td>73.5 ± 34.2 (n = 20)</td>
<td>51.2*</td>
<td>(62.5%, 106.8%)</td>
</tr>
<tr>
<td>DPm-negative</td>
<td>84 ± 35.9 (n = 12)</td>
<td>69 ± 64.8*</td>
<td>4 / 12</td>
</tr>
</tbody>
</table>

**χ² 1.992, p = 0.046, (log-rank test). **p = 0.042, OR 6.667 and p = 0.040, OR 7.000 respectively (Fisher exact test). ***total bilirubin <35 µmol/L, colored stools.

Conclusion: Our results do not support thesis that presence of DPM is correlated with unfavourable outcome in patients with BA. It is fetal type of BA atresia, which is associated with shorter SNL and early failure of HPE (p 0.001, log-rank test). In 2 subgroups of patients with perinatal type of BA regardless of DPM presence there was no difference in outcome. In patients with DPM, surgery was performed earlier, and that may have contributed to better outcome.

Disclosure of Interest: None declared.
**PO-N-0235/PD-N-0113**

**Pediatric Nutrition**

**INTAKE OF DHA/ARA VIA BREAST MILK OR FORMULA SUPPLEMENTATION DURING INFANCY CAN AFFECT THE INCIDENCE AND RECURRENCE OF ALLERGIC MANIFESTATIONS IN YOUNG CHILDREN**

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**Objectives and Study:** We have previously shown that infants fed DHA/ARA-supplemented formula throughout their first year experience reduced incidence of wheezing/asthma/atopic dermatitis (AD) through 3 years of life. Data from a breast-fed reference group are now available for inclusion in the analysis.

**Methods:** Infants from two cohorts who had completed randomized, double-blind studies of formula supplemented with preformed DHA and ARA (0.32% and 0.64% of total fatty acids, respectively, to match worldwide breast milk levels) or unsupplemented formula fed from <5 days through 12 months of age and a breast-fed reference group were followed. Blinded study nurses reviewed the infants’ medical charts for allergic manifestations (wheezing, asthma, AD, allergic rhinitis, allergic conjunctivitis, food allergy, and urticaria). Incidence and number of episodes were analyzed using a multiple logistic regression model and an ordinal model, respectively. Gender, family history of allergy, and smoking in the home were included as covariates.

**Results:** Parents of 36 infants who had received DHA/ARA-supplemented formula, 47 infants who had received unsupplemented formula, and 25 breast-fed infants, consented to participate. Compared to unsupplemented infants, the breastfed and supplemented infants had significantly lower odds of having at least one episode of wheezing/asthma/AD or any allergy (Table). In addition, the odds of having an increased number of episodes of wheezing/asthma/AD (OR (95% CI) DHA/ARA 0.38 (0.16–0.92), breast-fed 0.35 (0.13–0.95) or any allergy [DHA/ARA 0.40 (0.18–0.92), breast-fed 0.31 (0.12–0.80]) from 0 to 3 years of age was significantly reduced.

**Conclusion:** Children who received DHA/ARA in infancy via breast milk or supplemented formula demonstrated a similar pattern of lower incidence and recurrence of allergic manifestations in the first 3 years of life compared to infants fed unsupplemented formula.


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**PO-N-0249/PD-N-0114**

**Pediatric Nutrition**

**IGF-I AT 9 MONTHS, BREAST-FEEDING AND LATER OBESITY IN HEALTHY DANISH INFANTS FROM THE SKOT COHORT**

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**Objectives and Study:** High IGF-I concentrations in infancy has been associated with later obesity but the interactions between diet, IGF-I concentrations and growth in early life are complex and involve programming of the IGF-I axis. This paper examines how IGF-I and IGFBP-3 concentrations measured at 9 mo is related to diet and growth in infancy.

**Methods:** In the Danish SKOT cohort healthy term infants were included at age 9 mo with follow-up at age 18 mo. A total of 252 infants had a full data set and were included in the analysis. Measurements include weight, length, skinfold thickness, waist circumference, 7-d food records, and blood analysis of IGF-I, and IGFBP-3.

**Results:** Infants not being breastfed at 9 mo of age (46%) had higher median IGF-I concentration than breastfed infants (51.6 vs. 44.2 ng/mL, P=0.0006) and there was a negative dose response effect of daily numbers of breast-feedings on IGF-I concentration. Among those not being breast-fed at 9 mo IGF-I was positively associated with energy intake, but not with intake of milk or dairy protein. IGF-I concentration was negatively associated with birth weight and positively related to increase in weight, length and BMI between birth and 9 mo. Between 9 mo and 18 mo of age increase in length was positively and increase in BMI was negatively related to IGF-I concentration.

**Conclusion:** Breast-feeding has a strong negative dose-response effect on IGF-I concentrations in late infancy. Although IGF-I concentrations at 9 mo of age were negatively associated with change in BMI during the following 9 mo we speculate that this could reflect an early adiposity rebound and thereby an increased risk of obesity later in life.

**Disclosure of Interest:** None declared.
PO-N-0221/PD-N-0115

Pediatric Nutrition

TRANSCRIPTIONAL RESPONSES OF THE NEONATAL RHESUS INTESTINE TO OSTEOPONTIN

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Objectives and Study: Osteopontin (OPN) is a multifunctional protein involved in many physiologic processes, including immune activation, wound healing, angiogenesis, and bone remodeling. Human milk contains 130 mg OPN/L compared to 10 mg/L in infant formula. Herein, the impact of supplemental OPN on the neonatal intestinal transcriptome was assessed.

Methods: Rhesus monkeys were obtained at birth from the California Regional Primate Research Center (Davis, CA) and randomized to be breast-fed (BF; n = 4) or fed formula (FF; n = 6), or formula + 125 mg OPN/L (OPN; n = 6). At 3 months, jejunal samples were obtained and mRNA extracted and applied to the Affymetrix Rhesus Macaque arrays. Probe sets with <0.5 log2-fold difference from the highest to lowest GCRMA values across all arrays were removed, leaving 25,878 probe sets for analysis. Data were fit to a linear model using the linear models of microarray analysis package. Functional relationships were analyzed using the Metacore software program.

Results: Pairwise comparisons demonstrated 129 probe sets that were significantly differentially expressed between FF and OPN; 225 between BF and OPN and 1025 between FF and BF. The addition of OPN reduced the difference in gene expression relative to BF by over 5-fold from 1025 to 225 genes. The main canonical pathways differing between FF and OPN were related to development, galactose metabolism, cytoskeleton remodeling and immune response. The main canonical pathways differing between OPN and BF were arachidonic acid metabolism, immune response, G-protein signaling and leptin signaling through JAK/STAT and MAPK cascades. Pathways differing between FF and BF encompassed cytoskeletal remodeling, cell adhesion structure and cell adhesion. Differences in signaling pathways regulating stem cell proliferation (WNT), gastrointestinal patterning (HEDGEHOG) and cell fate between secretory and absorptive lineages (NOTCH) were differentially expressed among the groups.

Conclusion: Key differences in gene expression exist between BF and FF monkeys. OPN added to formula shifted overall gene expression differences towards a profile more similar to BF.

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PO-N-0192/PD-N-0116

Pediatric Nutrition

IN VIVO AND IN VITRO EVALUATION OF THE RESIDUAL ALLERGENICITY OF PARTIALLY HYDROLYSED INFANT FORMULAS

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Objectives and Study: Hypoallergenic infant formulas are commonly used for genetically predisposed children and infants diagnosed with cow’s-milk allergy. This study describes a new strategy for a comprehensive and detailed picture of the potential allergenicity of hydrolyzed infant formulas.

Methods: Whey protein, hydrolysed whey protein (hWhey) and a partial hydrolyzed whey formula (Hydrolysed formula) were compared. Residual trace amounts of whey protein and peptide profile were analyzed by a sandwich-type ELISA and dodecyl sulfate-polyacrylamide gel electrophoresis. To investigate residual allergenicity on the effector phase of the allergic response, the cross-linking capacity of whey and Hydrolysed formula on RBL cells transfected with the human FcRI receptor (RBL-hFcRI) sensitized with cow’s-milk–specific IgE from cow’s-milk–allergic patients was assessed. To investigate the sensitizing capacity, mice were sensitized orally to whey, hWhey, Hydrolysed formula or an amino acid–based (AA) formula for 5 weeks using cholera toxin as an adjuvant. The whey-specific antibodies, anaphylactic reactions, body temperature and acute allergic skin response were determined after intradermal whey challenge.

Results: Electrophoretic patterns indicated that β-lactoglobulin, one of the major allergens in whey, was completely degraded after hydrolysis. In a degranulation assay with RBL-hFcRI, a strong degranulation reaction was observed to whey. The hydrolysed formula was not able to induce cross-linking of cow’s-milk–specific IgE antibodies and
therefore did not provoke degranulation. In mice, sensitization with whey resulted in elevated levels of whey-specific IgE/IgG1 levels, anaphylactic symptoms and a significant drop in body temperature. In contrast, sensitization with the hWhey and Hydrolysed formula did not induce a whey-specific IgE response and strongly reduced whey-specific IgG1 levels. Moreover, no anaphylactic reactions or drop in body temperature were observed indicating that the whey hydrolysates lost their putative capacity to sensitize.

Conclusion: hWhey and the hydrolysed formula lost their putative sensitizing capacity in a mouse model using oral sensitization. In combination with the lost capacity of hydrolysates to cross-link human IgE antibodies on RBL-huFcRi in vitro, both the sensitization and the challenge phase of the allergic response were studied. This combination of assays is proposed as a strategy for the screening of new hypoallergenic formulas aimed at preventing sensitization in atopic children and avoiding clinical symptoms in infants suffering from cow’s-milk allergy.


PO-N-0260/PD-N-0117

Pediatric Nutrition
PROTON MAGNETIC RESONANCE SPECTROSCOPY IN PROTEIN ENERGY MALNUTRITION PATIENTS
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Objectives and Study: The present study was carried out to assess the diagnostic and prognostic role of proton MRS of the brain in protein energy malnutrition (PEM) patients.

Methods: The study included 16 PEM patients (8 edematous and 8 non edematous) with age range of 6–24 months. Nine clinically healthy age and sex matched infants served as the control group. All cases were subjected to full history taking laying stress on dietetic history, thorough anthropometric and clinical examination. Routine laboratory investigations had been done besides the application of Bayley scale of infant development. Additionally, the radiological study with proton MRS of the brain was performed for all cases using multivoxel study on three brain regions (frontal lobe, basal ganglia and thalamus) bilaterally and assessment of three brain metabolites namely N-acetylaspargate (NAA), choline (Cho) and creatine (Cr) was done and was interpreted in the form of metabolic ratios NAA/Cr, Cho/Cr, NAA/Cho and NAA/Cho+Cr. Nutritional rehabilitation was carried out for 2–4 months then the PEM patients were re-evaluated using the same methods mentioned before.

Results: The results showed statistically significant decrease of all ratios in both types of malnourished infants as compared to the controls. The basal ganglia and the thalami showed the least values in both groups of malnutrition while the frontal areas were less affected. Additionally, the edematous group showed lower values of all studied MRS ratios compared to the non-edematous one yet this finding was not of statistical significance. Regarding, neurodevelopmental assessment using BSID-II; the results showed that their mean values were significantly decreased in both groups of malnourished infants as compared to the controls. All the previous changes showed improvement after nutritional rehabilitation.

Conclusion: In conclusion, PEM patients show developmental and cognitive delay coupled by changes in their MRS finding of the brain denoting metabolic brain affection and some of these persist in spite of the apparent success of the nutritional rehabilitation program. This observation urges us to continue following up these patients for longer durations to make sure no permanent damage occurs due to the PEM insult to the growing brain.

Disclosure of Interest: None declared.

PO-N-0238/PD-N-0118

Pediatric Nutrition
A FOLLOW-ON FORMULA WITH THE PROBIOTIC LACTOBACILLUS FERMENTUM CECT5716 DECREASES THE INCIDENCE OF RESPIRATORY AND GASTROINTESTINAL INFECTIONS: A RANDOMIZED CONTROLLED TRIAL

Objectives and Study: The intestinal microbiota has been shown to play a key role in the development of the intestinal function and the immune system at an early age. We previously identified and selected Lactobacillus fermentum CECT5716 from human breast milk and characterized the safety and probiotic potential of the strain. The objective of the present study is to evaluate the effect of a follow-on formula containing L. fermentum CECT5716 on the incidence of infections in children.

Methods: A randomized controlled trial was carried out in 188 children who consumed from 6 months until 12 months of age a follow on formula with L. fermentum CECT5716 plus GOS (experimental group, EG) or the same formula with GOS but without probiotics (control group, CG).
PO-N-0240/PD-N-0119

Pediatric Nutrition

NEONATAL FATTY ACID STATUS AND PARAMETERS OF CARDIOVASCULAR HEALTH AT 9 YEARS

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Objectives and Study: Both long-chain polyunsaturated fatty acid (LCPUFA) status and trans fatty acid status are associated with risk of cardiovascular diseases in adulthood. Currently it is not clear whether LCPUFA or trans fatty acid status during early life is associated with cardiovascular health in later life. The present study aimed to evaluate associations between neonatal docosahexaenoic acid (DHA), arachidonic acid (AA) and trans fatty acid status and some parameters of cardiovascular health at 9 years.

Methods: 229 children (121 boys, 108 girls) took part in the study. The children participated in a double-blind randomised controlled trial on the effects of supplementation of formula with 0.30% DHA and 0.45% AA during the first two postnatal months. Neonatal fatty acids status was determined by measuring fatty acid composition in the wall of umbilical blood vessels; the present analysis focuses on DHA, AA and trans fatty acids. At 9 years of age, systolic and diastolic blood pressure, heart rate and body mass index (BMI) were assessed. Multivariate analyses were carried out to evaluate the effect of fatty acid status while adjusting for perinatal and social confounders.

Results: AA status in the umbilical artery wall was negatively associated with systolic (r = -0.19; P = 0.005) and diastolic blood pressure (Spearman’s r = -0.18; P = 0.010). AA status in the vein wall was negatively associated with diastolic blood pressure only (Spearman’s r = -0.16; P = 0.017). Multivariate analyses confirmed these associations. Moreover, multivariate analyses demonstrated a positive association between neonatal venous DHA status and systolic blood pressure in females (B = 2.32; 95% CI = 0.19, 4.45; P = 0.033). Neonatal AA and DHA status were not associated with heart rate and BMI at 9 years. Higher percentages of neonatal arterial trans fatty acid status was associated with a higher heart rate at 9 years (B = 5.70; 95% CI = 0.42; 10.99; P = 0.035) but not with blood pressure or BMI. Venous trans fatty acids were not associated with parameters of cardiovascular health.

Conclusion: 1. Higher AA status at birth may have a beneficial association to blood pressures later in life. 2. Higher DHA status at birth may be associated to higher systolic blood pressure in girls, but not in boys. 3. Higher trans fatty acid status at birth may be associated with increased heart rate at 9 years of age.

Disclosure of Interest: H. Kikkert: None declared, C. de Jong: None declared, T. Decsi: None declared, G. Boehm: None declared, M. Hadders-Algra: None declared.

PO-N-0241/PD-N-0120

Pediatric Nutrition

SIGNIFICANT REDUCTION IN CATHETER-RELATED BLOODSTREAM INFECTIONS WITH TAUROLIDINE LOCK IN CHILDREN TREATED WITH INTRAVENOUS NUTRITION FOR 16,000 CATHETER DAYS

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Objectives and Study: The aim of this study was to review the incidence and type of catheter related bloodstream infection (CRBI) in children on treatment with long-term
intravenous/parenteral nutrition (PN) before and after the introduction of taurolidine. CRBI and associated morbidity, mortality and financial burden are a major complication for patients on PN treatment. Taurolidine is a catheter lock solution which prevents biofilm formation and has broad spectrum bactericidal and anti-fungal action. It is widely used in adult patients on haemodialysis and oncology patients with recognized benefits. The use of taurolidine in paediatric patients on PN has only been reported in case studies.

Methods: Nineteen children treated with long-term, home PN and using taurolidine line lock were reviewed. They all were on cyclical overnight PN treatment with care by parents who had undergone a formal training programme. No other aspect of care was changed when taurolidine was introduced. Diagnoses were: short gut (7 cases), enteropathy (8 cases) and gastrointestinal dysmotility (4 cases). Mean age on commencing taurolidine was 69 (range 8–238) months. Incidence and type (Gram negative or positive bacteria or fungal infection) of sepsis was reviewed for 7–12 (mean 10) months with heparin catheter lock and 2–32 (mean 17) months with taurolidine catheter lock. This accounted for 6570 catheter days with heparin and 9640 days with taurolidine.

Results: There were 7.5 episodes CRBI per 1000 catheter days with heparin lock and 0.8 episodes per 1000 catheter days with taurolidine lock (P = 0.001). Fourteen or 74% of patients had no infections for up to 32 months after changing to taurolidine. There were 49 infectious episodes with taurolidine. The use of taurolidine has significantly decreased the incidence of CRBSI without the risk of induction of multi-resistant organisms or adverse effects with taurolidine.

Conclusion: To our knowledge, this is the largest reported series of paediatric patients on long-term/home PN using taurolidine. The use of taurolidine has significantly decreased the incidence of CRBSI without the risk of induction of multi-resistant organisms or adverse effects. This finding definitely supports the use of taurolidine in patients with a proven susceptibility to infection on cyclical PN administration. Larger studies preferably randomized controlled trials to further establish the benefits of taurolidine lock are warranted.

Disclosure of Interest: None declared.

PO-N-0193/PD-N-0121

Pediatric Nutrition
FIRST RESULTS OF A RING TRIAL
AIMED TO VALIDATE A MOUSE MODEL FOR COW’S-MILK ALLERGY TO ASSESS THE POTENTIAL ALLERGENICITY OF HYDROLYSED COW’S-MILK–BASED INFANT FORMULAS

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Objective and Study: The EC-directive 2006/141/E on infant formulae requires that objective and scientifically verified data are available to the claim hypoallergenicity. For safety reasons, hypoallergenic formulas should not be able to sensitize animals. No validated animal models are currently available to assess the potential residual sensitizing capacity, but guinea pig assays are frequently used for this purpose. This study is part of a multiphase project which aims to validate a novel mouse model for cow’s milk allergy to assess the potential allergenicity of hydrolysed cow’s milk–based infant formulas. In this first phase of a multicenter ring trial the feasibility to introduce a recently developed mouse model for cow’s milk allergy in 4 independent research laboratories was evaluated.

Methods: C3H/HeOUJ mice were sensitized by oral administration of whey (2 and 20 mg) at weekly intervals for 5 weeks. One week after the last sensitization the acute allergic skin response (ear swelling at 1 hour) and anaphylactic symptoms were determined upon intradermal whey injection into the ear. Subsequently, mice were challenged orally with 50 mg whey and blood samples were taken after 30 minutes. Serum was analyzed for whey-specific immunoglobulins and mMCP-1. All protocols, test substances, and procedures were standardized and animals were from the same age and obtained from the same breeder.

Results: All participating research laboratories were able to sensitize mice to whey as shown by elevated levels of whey-specific IgE/IgG1/IgG2a and serum mMCP-1 as a reflection of mast cell degranulation. An acute allergic skin response after intradermal whey challenge compared to nonsensitized mice was observed in 3 out of 4 research centers. Anaphylactic symptoms were present at all 4 research centers although minor responses were measured at 1 of the facilities.

Conclusion: In this ring trial a mouse model for cow’s milk allergy was simultaneously introduced at 4 independent research facilities in the Netherlands. The first results indicated that it is possible to transfer this model with low interlaboratory variation. In the next phase of the validation process whey hydrolysates will be included and these results will indicate whether the proposed mouse model, using oral sensitization, will potentially be suitable as a new sensitization model for hypoallergenicity testing of hydrolysed cow’s milk formulas.

**PO-N-0233/PD-N-0122**

**Pediatric Nutrition**

**HIGH OMEGA-6/OMEGA-3 RATIO IN EARLY PLASMA AND BREAST MILK HAS NEGATIVE EFFECTS ON MENTAL AND EMOTIONAL DEVELOPMENT IN PREMATURE INFANTS DURING INFANCY**

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**Objectives and Study:** The requirement of essential fatty acids for the development of the brain is well documented. However, most of these studies are on full-term infants. We have reported earlier that an imbalance of omega-6 to omega-3 fatty acids and Mead acid were negatively associated with early development of prematures (40 and 44 weeks of gestational age) (1). The aim of this study was to investigate whether the early motor, mental and behavioral development from 3 to 18 months in this group of preterm infants was associated with the fatty acid concentrations in breast milk at 1 postnatal week and infants’ plasma phospholipids at 44 weeks’ gestational age.

**Methods:** Fifty-one premature infants with a median gestational age of 34 weeks (interquartile range, 32–35) were included. The quality of general movements (GMs) was assessed at 3 months corrected age and motor, mental and behavioural development was assessed with Bayley Scales of Infant Development-Second Edition (BSID-II) at 3, 6, 10 and 18 months corrected age. The behaviour rating scale includes attention arousal, orientation, engagement, emotional regulation and motor quality. The association between the infants’ development and fatty acid concentrations as well as background factors was analyzed by stepwise multiple linear regressions.

**Results:** Linoleic acid (18:2w6, LA) concentration in early breast milk showed negative associations with mental and emotional regulation development at 6 months (β = 0.40, p< 0.002 and β = 0.41, p = 0.001, respectively) and the ratio LA/alpha-linolenic acid (18:3w3) in early breast milk with mental development at 3 months (β = 0.36, P = 0.006) and with orientation development at 10 months (β = 0.32, P = 0.026). DHA in infants’ plasma at 44 weeks showed positive associations with orientation and emotional regulation development at 10 and 18 months (β = 0.39, P = 0.007 and β = 0.33, P = 0.028, respectively). The ratio of arachidonic acid (20:4w6, AA) to docosahexaenoic acid (22:6w3, DHA) in infants’ plasma at 44 weeks was negatively associated with mental development at 10 months (β = 0.32, P = 0.021) and with mental and orientation development at 18 months (β = 0.37, P = 0.008 and β = 0.46, p = 0.002, respectively). Of the background factors, mother’s education had the highest impact.

**Conclusion:** Our study indicates that early dominance of omega-6 fatty acids is negative associated with premature infants’ development, that early concentrations of DHA is positive associated and that the early omega-6 to omega-3 ratio has an influence on premature infants’ development during infancy.


**Disclosure of Interest:** None declared.

**PO-N-0261/PD-N-0123**

**Pediatric Nutrition**

**NEUTRAL PREBIOTIC OLIGOSACCHARIDE SUPPLEMENTATION EARLY IN LIFE AND ALLERGY ASSOCIATED SYMPTOMS LATER ON: A 5-YEAR FOLLOW-UP**

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**Objectives and Study:** A mixture of neutral prebiotic oligosaccharides has been shown to reduce the incidence of atopic dermatitis and allergy associated symptoms during the first 2 years of life (1,2). Objective of this study was to evaluate if this protective effect against allergy lasted beyond the intervention period until 5 years of age.

**Methods:** In a prospective, double-blind, placebo-controlled fashion, healthy term infants at risk of atopy were fed either a prebiotic-supplemented (0.8 g/100 mL scGOS/lcFOS) or placebo-supplemented (0.8 g/100 mL maltodextrin) hypoallergenic formula during the first 6 mo of life. Following this intervention period, follow-up continued until 5 y of life. At 5 years, we evaluated the cumulative incidence of allergic symptoms (atopic dermatitis, allergic rhinitis, and recurrent wheezing).

**Results:** Eighty-nine children (49 in placebo group, 40 in intervention group) completed the 5-year follow-up study. During this period, children in the scGOS/lcFOS group had significantly lower incidence of atopic dermatitis (AD), and allergic rhinitis, and any allergic symptom. Cumulative incidences for AD, allergic rhinitis and any allergic symptom were 20, 2.5, and 30 %, respectively in the scGOS/lcFOS group, 38.8, 16.3, and 63.3 % in the control group (P< 0.05 for all). There was no difference in the cumulative incidence of recurrent wheezing at 5 years.

**Conclusion:** Oligosaccharide prebiotics (scGOS/lcFOS), when started early in life have a protective effect against allergy. The protection lasts beyond infancy until 5 years of life, particularly for atopic dermatitis and allergic rhinitis.

2. Arslanoglu S, Moro GE, Schmitt J, Tandoi L, Rizzardi S, Boehm G. Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic

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PO-0208/PD-N-0124

Pediatric Nutrition
HUMAN MILK OLIGOSACCHARIDES PREVENT NECROTIZING ENTEROCOLITIS IN NEONATAL RATS
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Objectives and Study: Necrotizing enterocolitis (NEC) is one of the most common and often fatal intestinal disorders in preterm infants. Almost 10% of all very-low-birth-weight infants develop NEC, more than a quarter of them die. Breast-fed infants are at much lower risk to develop NEC than formula-fed infants. However, the protective compounds in breast milk are vastly unknown. The objective of our study is to assess whether human milk oligosaccharides (HMO), complex glycans highly abundant in breast milk but not in formula, contribute to the protective effects of breast-feeding in the context of NEC.

Methods: We used an established in vivo NEC model with neonatal Sprague-Dawley rats. Pups were either left with the dam to serve as breast-fed control or orally gavaged with formula without and with HMO. All pups were fed 2x/day, exposed to hypoxia 3x/day, and sacrificed on day-of-life 4. Their intestines were analyzed for macroscopic and microscopic signs of NEC. Histology sections of the terminal ileum were scored blindly from 0 (healthy) to 4 (complete destruction).

Results: While all breast-fed pups survived until day-of-life 4, the survival rate dropped to 72% in formula-fed pups. In parallel, NEC pathology scores increased significantly from 0.34 in breast-fed pups to 1.98 < 0.001). We then isolated HMO from pooled human milk and added them to the formula. Survival rates improved to 95% and pathology scores improved to 0.44 < 0.30, which was significantly lower compared to the group receiving formula without HMO (<0.001) but not different from the breast-fed control. Galactooligosaccharides (GOS), currently added to infant formula, had no protective effect in the rat model. Since more than 150 different HMO have been identified so far, we used 2-dimensional glycan chromatography to determine which ones are most protective from NEC. We identified one distinct HMO that significantly improved survival and reduced NEC pathology scores in our rat model.

Conclusion: Our results show that HMO in general and 1 specific HMO in particular improve survival and reduce pathology scores in a rat model of NEC. Whether these results translate to the human preterm infant is currently unknown, and so are the underlying cellular and molecular mechanisms for the protective effects.


PO-0239/PD-N-0125

Pediatric Nutrition
INFANT'S NEONATAL FATTY ACID STATUS AND LATER PSYCHOPATHOLOGICAL OUTCOME
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Objectives and Study: High docosahexaenoic acid (DHA) levels during pregnancy influence early postnatal neurodevelopment of the child, but less is known about their effect on long-term psychopathology outcome. In our study we assess the impact of fatty acids at birth on children’s behavior problems at the age of 10 years.

Methods: The ongoing population-based birth cohort study LISA provided data of 416 children which were analyzed here. After birth, venous cord blood was collected and individual umbilical fatty acid status in blood (%) per interquartile range (IQR) was calculated. Data on children’s behavior was collected by parent-reported SDQ at age 10 years, and fatty acid intake was determined by food questionnaire at age 10 years. Zero inflated poisson regression models were applied adjusted for parental education, breastfeeding, smoking, and drinking alcohol during pregnancy and social and psychological strain during pregnancy.

Results: We found a significant association for an increase of umbilical DHA and lower total behavioral problems. This association remained significant when adjusted for confounding factors (exp(b)=0.93, SE=0.02, P<0.0001. An increase of umbilical DHA was related with lower scores on the hyperactivity/inattention scale and remained significant when adjusted for confounding factors (exp(b)=0.94, SE=0.03, P<0.04. The associations between umbilical DHA and emotional symptoms were not significant.

Conclusion: This study provides data that highlights the importance of adequate DHA requirement for mothers.
PO-N-0228/PD-N-0126

Pediatric Nutrition

GLUCAGON-LIKE PEPTIDE 2 (GLP-2) STIMULATES INTESTINAL FUNCTION DURING WEANING

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Objectives and Study: Transition from milk to solid food can be associated with intestinal atrophy and malabsorption. The gut hormone, glucagon-like peptide 2 (GLP-2) stimulates gut adaptation. We hypothesized that post-weaning gut adaptation is improved by GLP-2 administration, and that GLP-2 effects vary according to differences in disease sensitivities (e.g., sanitary environment). We used piglets as models for infants.

Methods: In Exp. 1, 3-week-old pigs were weaned in a high-sanitary environment and injected with native GLP-2 (80 μg/kg/12 h, n = 8) or saline (control, n = 8) and compared with preweaning pigs (n = 6). In Exp. 2, pigs were weaned in a low-sanitary environment and injected with native GLP-2 (150 μg/kg/12 h, n = 11) or saline (control, n = 11) and compared with preweaning pigs (n = 8). In Exp. 3, pigs were weaned in a low sanitary environment and injected with a stabilized acylated GLP-2 analogue (25 μg/kg/12 h, n = 8) or saline (control, n = 8).

Results: Pigs injected with acylated GLP-2 (Exp. 3) showed a lower diarrhea score (2.3 vs. 3.5, P < 0.01), higher intestinal weight, villi and crypts in the proximal intestine (+20–30%, P < 0.01) and marked increases in 6 digestive enzymes (+50–100%, all P < 0.05). Native GLP-2 used in low-sanitary conditions (Exp. 2) increased the density of goblet cells (P < 0.05), reduced colonic short-chain fatty acid levels (P < 0.01) but did not prevent weaning diarrhea. In the absence of diarrhea in high sanitary conditions (Exp. 1), native GLP-2 injections did not improve gut function. Relative to suckling pigs, intestinal atrophy was similar between controls and GLP-2 treated pigs in both Exp. 1 and 2. In the high-sanitary environment (Exp. 1), the activities of 5 brush border enzymes were markedly increased in both GLP-2 and controls with suckling pigs (>300%, P < 0.05). In contrast, activities of 3 peptidases were markedly reduced in both GLP-2 and controls in the low-sanitary environment (Exp. 2, ~50%, P < 0.001) compared with suckling pigs. Plasma GLP-2 levels were highest in Exp. 3 (constant levels of ~20 nmol/L), lower in Exp. 2 (daily cycles of 5–30 nmol/L) and lowest in Exp. 1 (daily cycles <1.0 nmol/L).

Conclusion: Exogenous GLP-2 treatment improves gut digestive function during the dietary transition from milk to solid food, but the effects are most pronounced with high-dose GLP-2 and high disease sensitivity. Improved digestive function toward adult type diets may play an important role in the prevention of diarrhea, which is a common clinical response to the weaning transition in both infants and animals.

Disclosure of Interest: None declared.

PO-N-0237/PD-N-0127

Pediatric Nutrition

SYSTEMATIC REVIEW OF FATTY ACID STATUS IN OBESITY

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Objectives and Study: N-6 polyunsaturated fatty acids (PUFAs) have been recently related to the pathogenesis of obesity (Ailhaud et al, 2006). We systematically reviewed data on n-6 PUFA status in obese as compared to normal weight subjects.

Methods: The Ovid MEDLINE, Scopus and Cochrane Library CENTRAL databases were searched from inception to September 2010 for trials, without restriction in study design, which included observational and intervention studies on obesity. We used formal inclusion/exclusion criteria and applied standard operation procedures for data extraction, validity assessment and meta-analysis.

Results: We found 10 relevant studies (1 randomised controlled trial and 9 case-control studies) comparing fatty acid composition of plasma phospholipids (PL, 5) and total plasma fatty acids (tFA, 5). Five of the studies were carried out in children (n = 429), 4 in adults (n = 978) and 1 in adolescents (n = 60).

Values of the principal n-6 PUFA, linoleic acid (C18:2n-6) were significantly lower in the obese group compared to normal weight controls in 3 studies. Gamma-linolenic acid (C18:3n-6) values were higher in tFA in obese compared to controls in two studies. In contrast the weighted mean difference of C18:3n-6 in PL was significantly lower in obese compared to normal weight controls in 3 studies. Gamma-linolenic acid values were significantly higher in tFA in obese compared to normal weight controls in 3 studies. In contrast the weighted mean difference of C18:3n-6 in PL was significantly lower in obese compared to normal weight controls (Table). Three studies reported significantly higher values of dihomo-gamma-linolenic acid (C20:3n-6) in obese than in normal weight children. Both significantly higher and significantly lower contributions of arachidonic acid (C20:4n-6) were reported in obese as compared to normal weight subjects.

Disclosure of Interest: None declared.
Table. Weighted mean differences in major n-6 polyunsaturated fatty acids in plasma phospholipids and total plasma-lipids in obese compared to normal weight subjects

<table>
<thead>
<tr>
<th></th>
<th>LA</th>
<th>GLA</th>
<th>DHGLA</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL</td>
<td>−0.18</td>
<td>−1.02</td>
<td>0.22</td>
<td>−0.34</td>
</tr>
<tr>
<td></td>
<td>[−0.76, 0.40]</td>
<td>[−1.61, −0.43]</td>
<td>[−0.47, 0.90]</td>
<td>[−2.66, 1.98]</td>
</tr>
<tr>
<td>tFA</td>
<td>−1.86</td>
<td>0.13</td>
<td>0.15</td>
<td>−0.09</td>
</tr>
<tr>
<td></td>
<td>[−3.73, 0.01]</td>
<td>[−0.22, 0.49]</td>
<td>[−0.04, 0.33]</td>
<td>[−0.31, 0.13]</td>
</tr>
</tbody>
</table>

AA, arachidonic acid; DHGLA, dihomo-gamma-linolenic acid; GLA, gamma-linolenic acid; LA, linoleic acid; PL, plasma phospholipids; tFA, total fatty acids; data are % wt/wt, mean (95% CI).

Conclusion: Systematic review of fatty acid compositional data in obese as compared to normal weight subjects does not appear to support the concept of the pathogenic role of n-6 PUFA in obesity.

Funding: Supported by the European Communities 7th Framework Programme (NUTRIMENTHE Grant agreement number: 212652).

Disclosure of Interest: None declared.

PO-N-0257/PD-N-0128

Pediatric Nutrition

LAPAROSCOPIC ADJUSTABLE GASTRIC BANDING “LAGB” FOR MORBIDLY OBESE ADOLESCENTS: THE FIRST EXPERIENCE IN FRANCE


Objectives and Study: An increase in the incidence of obesity-related co morbidities is now seen in the pediatric French population. A significant majority of adolescents already affected by severe obesity will continue to carry this disease and its complications into adulthood. The success rate of multidisciplinary interventions is modest in severely obese adolescents. Bariatric surgery is the only viable option for providing durable and significant weight loss and health improvement for morbidly obese adults. LAGB represents an attractive treatment with minimal morbidity. We studied the place of bariatric surgery in the management of severely obese adolescents when lifestyle change is not enough.

Methods: Since July 2008, weight loss surgery is considered for adolescents (≥14 years) with severe obesity (BMI≥40 kg/m²) enrolled in Necker’s multidisciplinary program after 1 year lifestyle intervention at least. Laboratory evaluations, comorbide conditions, anthropometric measures and body composition were assessed pre- and postoperatively. Patients and their families have given a fully informed consent.

Results: On the 70 eligible patients, 20 were excluded after psychosocial and familial evaluation. 15 adolescents (age: 16.56 ± 0.95 years) have undergone LAGB. The mean BMI was (46.09 ± 5.03 kg/m²).

All participants had central obesity and insulin resistance (HOMA IR = 6.03 ± 3.8). Fatty liver was revealed by ultrasound examination on 13 patients. The mean length of hospital stay for surgery was 48 hours. 13 patients completed 6 months follow-up and 9 one-year follow up. The group had a mean of 10 visits (8–12) during the first year follow-up. At 1 year follow-up body weight (−18.11 ± 15.15 kg), total fat mass (−10 kg) and trunk FM (−4 kg) were decreased. This corresponded to a mean percent excess weight losses of 30.52 ± 23.73 (1–80). BMI and HOMA IR were reduced to 40.11 ± 7.88 and 3.3 ± 1.5 respectively. No LAGB-related nutritional complications were detected. Two reoperations were required on 1 patient for cholecystectomy and for removal of the band after proximal pouch dilatation.

Conclusion: Our initial results, demonstrated an average 39.3% excess weight loss (20–88) after a mean follow up of 15 months (6–30) and resolution or improvement in the majority of comorbidities. Because of its relative safety, LAGB offers an effective treatment option in selected adolescents. More short- and long-term evaluation is needed for safety and efficiency of LAGB as a surgical adjunct to a multidisciplinary obesity program.

Disclosure of Interest: None declared.

PO-N-0258/PD-N-0129

Pediatric Nutrition

POSTDISCHARGE FORMULA IN PRETERM INFANTS: EFFECTS ON LATER GROWTH, BLOOD PRESSURE, AND BODY COMPOSITION


Objectives and Study: Growth failure at discharge is common in preterm infants and a nutrient-enriched post-discharge formula (PDF) is often used to promote catch-up growth. Promotion of faster postnatal growth by an increased plane of postnatal nutrition may increase long-term cardiovascular risk in both term and preterm infants. Some studies suggest that PDF has beneficial effects on infant growth but there is a paucity of data on long-term risks versus benefits. This study investigated the impact of PDF on growth, body composition and blood pressure (BP) later in childhood.

Methods: BP was measured at 5–8 years in 127 of 229 (55%) of a cohort of infants born <37 weeks gestation and randomized in the week before discharge to receive a protein, energy, and micronutrient-enriched PDF (n = 73) or a standard term formula (n = 54) until 9 months post-term. A reference group breastfed (BF) until at least 6 weeks post-term were also followed up (n = 40). Fat mass (FM) was calculated using bioelectric impedance analysis (BIA) and
the sum of four skinfold thicknesses and normalized for height (fat mass index (FMI) = fat mass/height²).

**Results:** Weight, height and BMI z score did not differ between randomized groups. FM, FMI and BP were similar in both groups: (unadjusted mean difference in FMI from BIA = −0.3%, 95% CI: −9.5% to 8.8%; P = 0.9; and from skinfolds = 7.3%, 95% CI: −4.0% to 18.5%; P = 0.2); (unadjusted mean difference for systolic BP = 0.2 mmHg, 95% CI: −3.0 to 3.5; P = 0.9; for diastolic BP = −0.3 mmHg, 95% CI: −2.9 to 2.3; P = 0.8). BF infants had similar weight, height, BMI z score and FMI compared to formula-fed groups: (unadjusted mean difference in FMI from BIA = −7.5%, 95% CI: −15.1% to 16.6%; P = 0.1; and from skinfolds = 8.8%, 95% CI: −2.6% to 20.2% P = 0.1). There was no significant difference in BP between BF and formula fed groups: (unadjusted mean difference for systolic BP = 0.2 mmHg, 95% CI: −3.0 to 3.5; P = 0.9; for diastolic BP = 0.4 mmHg, 95% CI: −2.4 to 3.2; P = 0.8).

**Conclusion:** In this prospective follow-up of a randomized trial, use of PDF in preterm infants had no effect on anthropometry, fat mass or blood pressure later in childhood. One explanation for the discrepancy between these data and studies of nutritional interventions earlier in the postnatal period is that the critical window for programming effects of faster infant weight gain is very early in postnatal life. Promoting growth without change in preterm infants may not therefore have adverse programming effects on later cardiovascular risk. However, the lack of effect on later height suggests that beneficial effects of PDF may be confined to infancy. Despite its association with slower growth in infancy, breastfeeding after discharge in this study was not associated with later deficits in height.

**Disclosure of Interest:** N. Onyeador Conflict with: The original study was part funded from a charitable grant from Farley Health Products which also supplied the infant formula. The author has previously received industry funding for clinical trials. K. Kennedy Conflict with: The original study was part funded from a charitable grant from Farley Health Products which also supplied the infant formula. The author has previously received industry funding for clinical trials. M. Fewtrell Conflict with: The original study was part funded from a charitable grant from Farley Health Products which also supplied the infant formula. The author has previously received industry funding for clinical trials. A. Lucas Conflict with: The original study was part funded from a charitable grant from Farley Health Products which also supplied the infant formula. The author has previously received industry funding for clinical trials. A. Singhal Conflict with: The original study was part funded from a charitable grant from Farley Health Products which also supplied the infant formula. The author has previously received industry funding for clinical trials.

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**PO-N-0230/PD-N-0130**

**Pediatric Nutrition**

**THE VARIATION OF HUMAN MILK ADIPONECTIN AND THE CORRELATION WITH INFANT GROWTH IN CHINESE COHORT**

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**Objectives and Study:** To investigate the variation of human milk adiponectin (APN) concentration during lactation, analyze the relationship of APN concentrations in human breast milk with APN in infant serum, determine the association between maternal milk APN and infant body proportionality in the first year of life, the period of greatest human milk exposure.

**Methods:** Subjects included 78 mother-infant pairs from one hospital of Shanghai. Human milk was collected at 2, 4, 13, 26 weeks and infant serum was drawn at 26 weeks. The concentration of APN in maternal milk and infant serum were measured with commercially available ELISAs (R&D Systems). Weight-for-age z scores (WAZ), length-for-age z scores (LAZ), weight-for-length z scores (WLZ) and body mass index z scores (zBMI) of infants up to 1 year of age were calculated using World Health Organization (WHO) standards. Then we estimated the variation of maternal milk APN, analyzed the correlation between maternal milk APN and infant serum APN, meanwhile determined the association between maternal milk APN and infant anthropometrics.

**Results:** The concentration of APN in maternal milk at 2, 4, 13, and 26 weeks was respectively 14.55 μg/L (5.93–140.4 μg/L), 7.26 μg/L (2.04–29.35 μg/L), 6.84 μg (2.72–15.65 μg/L) and 4.9 μg/L (1.12–13.38 μg/L). Higher milk APN in postpartum 13 weeks was inversely associated with infant WAZ at 13, 26, and 52 weeks (P < 0.05). Milk APN in postpartum 4 weeks was inversely related to infant WAZ, WLZ, zBMI at 13, 26, and 52 weeks (all P < 0.05). Meanwhile, milk APN in postpartum 13 weeks has significantly negative correlation with infant WAZ at 13, 26, and 52 weeks (P < 0.01). Milk APN in postpartum 26 weeks also was inversely associated with infant WAZ, WLZ and zBMI at 52 weeks (P < 0.05). After adjusting covariates like birth weight using longitudinal models, the effect of milk APN on body proportionality also exists. There is a positive relationship between maternal milk APN and infant serum APN at 13 and 26 weeks.

**Conclusion:** Milk APN concentration declines throughout lactation. Maternal milk APN may play a vital role in the growth and development of breastfed infants, particularly closely associated with infant weight. Infant serum APN clearly related to maternal milk APN.

**Disclosure of Interest:** None declared.
PO-G-0061/PD-G-0131

Endoscopy: Diagnosis and Therapeutic Surgical Procedures

EVALUATION OF INTESTINAL TRANSPLANTATION: MULTIVISCERAL TRANSPLANTATION

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Objectives and Study: Intestinal failure ± associated liver disease may lead to isolated intestinal transplantation or combined liver and intestine transplantation. However, these techniques may remain insufficient in case of diffuse motility disorders, and/or associated renal failure. We report our experience of multivisceral transplantation in such cases.

Methods: Three children aged 4 to 5 years underwent multivisceral transplantation in our unit. The indication was: Hirschsprung’s disease extended to the duodenum or stomach and cirrhosis in 2 patients, with pre-terminal renal failure secondary to congenital renal dysplasia in 1 case; severe intestinal pseudo-obstruction without cirrhosis in the 3rd patient. The graft included stomach, duodeno-pancreas, small bowel and right colon, with liver (2 cases) and 2 kidneys (1 case). In 2 patients, the abdominal wound was closed progressively in the first post-operative week, using a silo. Baseline immunosuppression relied on tacrolimus, basiliximab and steroids.

Results: With a follow-up of 12, 11, and 5 months, the 3 children are alive, at home, off parenteral nutrition, with satisfactory hepatic, pancreatic and renal functions. Two children still have enterostomies. The main complications were infectious (bacterial, CMV, EBV, HSV, mucormycosis), immunological (cellular ± humoral rejection, autoimmune haemolytic anaemia), tumoral (EBV related lymphoproliferation), toxic (hypertension, impairment of renal function), and dermatologic (severe generalised dermatosis, probably multifactorial: GVH, HSV6, medication toxicity).

Conclusion: Multivisceral transplantation provides children who were previously beyond therapeutic possibilities with a new chance of life. However, this technique remains difficult, and long-term outcomes are still uncertain.

Disclosure of Interest: None declared.

PO-G-0068/PD-G-0133

Endoscopy: Diagnosis and Therapeutic Surgical Procedures

A PROSPECTIVE RANDOMIZED CLINICAL TRIAL OF SCLEROTHERAPY VERSUS BAND LIGATION IN THE TREATMENT OF ESOPHAGEAL VARICES IN CHILDREN WITH EXTRAHEPATIC PORTAL HYPERTENSION

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Objectives and Study: Endoscopic retrograde cholangiopancreatography (ERCP) is not widely used in infants. Performing an ERCP before an intraoperative cholangiogram may lead to other diagnoses apart from extrahepatic biliary atresia (EHBA) thus avoiding surgery.

Methods: Infants less than 3 months of age who underwent ERCP from 2000–2010 were reviewed.

Results: 27 infants, (14 males) were examined; median age was 55 days (range, 33–89). Ultrasound was normal in 16 infants, and others showed small gallbladder (5), biliary stones (4) and dilated bile ducts (2). Ten underwent prior liver biopsy which was inconclusive. ERCP lead to the diagnosis of biliary atresia in 12 infants who had subsequent surgery. In others, ERCP showed choledocal cyst (2), biliary stones (1), dilated bile ducts (1) normal exam (6), failures (5). The final diagnoses in our cohort were EHBA (14), biliary stones (5), neonatal hepatitis (3) choledocal cyst (2) paucity of intrahepatic bile duct and congenital hepatic fibrosis (1). Diagnoses in the failures group included: biliary atresia (2), bile duct paucity (1) and biliary stones (1); lost to follow-up (1). In 6 (22%) infants with clinical suspicion of EHBA, a normal ERCP ruled out the diagnosis and avoided an intraoperative cholangiogram. No complications including pancreatitis were reported.

Conclusion: ERCP is feasible and safe. It may serve as an additional diagnostic tool in neonatal cholestasis in unclear cases and may prevent more invasive procedures. ERCP may be part of the algorithm of neonatal cholestasis, when it is available and other investigations fail to confirm a diagnosis.

Disclosure of Interest: None declared.

PO-G-0064/PD-G-0132

Endoscopy: Diagnosis and Therapeutic Surgical Procedures

ENDOSCOPIC RETROGRADE CHOLANGIPANCREATOGRAPHY IN INFANTS: A 10-YEAR SINGLE-CENTER EXPERIENCE

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Objectives and Study: Endoscopic band ligation (EVL) and endoscopic sclerotherapy (EIS) are both effective measures in the treatment of bleeding esophageal varices, but the efficacy and result of these techniques have not been clearly established in children. In the present study we performed a prospective randomized study to compare: 1) the efficacy of both treatments in eradicating esophageal varices, and 2) the complications of these treatments.

Methods: Thirty-two children (aged between 5 to 15 years old) with extrahepatic portal hypertension and esophageal varices (grade II or more) that had referred to the Children’s
Medical Center in a period of 18 months (from January 2007 to June 2008) were studied. Based on the endoscopic therapeutic method, they were divided randomly into: sclerotherapy (EVS) and band ligation (EVL) groups. During the follow up period, both groups were compared in regard to number and grade of varices, number of therapeutic endoscopic sessions, and complications of these procedures.

**Results:** There were 18 patients in the EVS group (male 72.2%) with mean age = 9.2 ± 3.1 y and 14 patients in the EVL group (male 50%) with mean age = 9 ± 3.1 y. The mean number of endoscopic series in the EVS group during the study was 7.2 ± 2.4, while in the EVL group it was 4.8 ± 1.2 with a significant difference (P = 0.001). The number of endoscopic sessions to decrease the varicose score (varices grade × number of varices) in EVL group (2.1 ± 1.1) was less than in the EVS group (5.8 ± 3.1) (P < 0.001). During a period of 1–3 months follow-up, reduction of varicose score in EVL group was more than EVS group (3.7 ± 1.5 and 7.1 ± 2.6, respectively) (P = 0.001). None of the patients had infection, esophageal stricture, perforation or death. There was no significant difference between the 2 groups in regards to age, sex, appearance of re-bleeding and complications.

**Conclusion:** EVL might be a preferable procedure as compared to EVS because of faster eradication of varices. No differences were observed in case of recurrence.

**Disclosure of Interest:** None declared.

**PO-H-0300/PD-H-0134**

**Hepatology**

**INTEROBSERVER AGREEMENT ON ENDOSCOPIC CLASSIFICATION OF OESOPHAGEAL VARICES IN CHILDREN: A MULTICENTER STUDY**

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Objectives and Study: The investigation and management of portal hypertension in children is compromised by the lack of evidence based guidelines. The major reason for this is that there have been no large multicentre studies. Ensuring concordance in the diagnosis and grading of oesophageal varices is crucial to the design of future multicentre trials in this field. The aim of this study was to evaluate the agreement among paediatric endoscopists from different European centres to classify oesophageal varices in children with portal hypertension.

**Methods:** Endoscopic pictures of 100 children with a clinical diagnosis of portal hypertension were collected and distributed to 10 paediatric endoscopists. Good quality in patient selection, picture selection, observation conditions was ensured. Observers provided classifications in 4 degrees (no varices, small, medium, large varices, Class A), one in 3 degrees (no varices, small and large varices, Class B), 1 for red wales (presence or absence, Class C). Cohen’s kappa test was used to evaluate observers agreement, which was considered good if kappa was > 0.41, excellent if > 0.61, perfect if > 0.81.

**Results:** Frequencies of the grading variables were distributed evenly in Class A (P = 0.17), unevenly in Class B (P = 0.0003) and Class C (P = 0.0003, Chi-square test). Agreement between observers was good for Class A (kappa = 0.52) and Class B (kappa = 0.60) as a whole, and excellent for the presence of red wales (kappa = 0.77). The agreement within Class A was perfect for absence of varices (kappa = 0.85) and excellent for small (kappa = 0.74), medium size (kappa = 0.67) and large varices (kappa = 0.78). The agreement within Class B was perfect for absence of varices (kappa = 0.83) and excellent for small (kappa = 0.63) and large varices (kappa = 0.75). Class A lead to a diagnosis of large varices in 26% of children versus 36% in Class B (P = 0.08).

**Conclusion:** Paediatric endoscopists working in different centres are in excellent agreement to diagnose and grade oesophageal varices in children. The best concordance is recorded for absence of varices and presence of large varices. A 3-degrees classification would lead to treat varices in a larger percentage of patients. The excellent interobserver agreement of this study supports the feasibility of reliable multicenter trials in children with portal hypertension and oesophageal varices.

**Disclosure of Interest:** None declared.

**PO-H-0271/PD-H-0135**

**Hepatology**

**BEYOND PLASMA BILIRUBIN CONCENTRATIONS: EFFECTS OF PHOTOTHERAPY AND ALBUMIN ON BILIRUBIN LEVELS IN THE BRAINS OF GUNN RATS**

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Objectives and Study: Unconjugated hyperbilirubinemia carries the risk of neurotoxicity. Only free bilirubin, the fraction of unconjugated bilirubin (UCB) not bound to plasma proteins, can translocate across the blood-brain barrier. Brain UCB levels cannot be measured in patients. Therefore, strategies to prevent neurotoxicity, such as phototherapy (PT) with or without albumin administration, have relied on total plasma bilirubin concentrations. It has remained unclear, however, if bilirubin accumulation in the brain can be decreased (during chronic hyperbilirubinemia) or prevented (during acute hyperbilirubinemia) by PT or by PT + albumin. The aim of the study was to determine the effects of PT with or without albumin administration on UCB accumulation in the brain in a chronic and an acute model for hyperbilirubinemia.

Methods: Gunn rats have a spontaneous, long-term unconjugated hyperbilirubinemia, due to a genetic deficiency of the bilirubin conjugating enzyme UDPGT1A1. As a chronic model, resembling patients with Crigler-Najjar disease, we treated adult Gunn rats with PT for 2 weeks, and then with either human serum albumin (HSA; 2.5 g/kg) or saline. To determine the effects on acute hemolytic jaundice, such as occurs in neonatal jaundice, we induced hemolysis in adult Gunn rats by acetyl-phenyl-hydrazine, and then treated the rats for 48 hrs with PT, HSA, or the combination PT + HSA. Brain UCB levels were compared to plasma UCB concentrations.

Results: In the chronic hyperbilirubinemic model, PT and PT+HSA decreased brain UCB levels by 38% and 77% (Figure), and plasma UCB concentrations by 35% and 68% respectively, compared with controls (each \( P < 0.01 \)). In the acute (hemolytic) hyperbilirubinemic model, PT alone failed to prevent the accumulation of UCB in the brain as well as in the blood. However, PT+HSA completely prevented an increase in UCB levels in the brain compared with no treatment (Figure). Compared with no treatment, the combination therapy decreased the rise in plasma UCB concentrations only by 26%, and in brain UCB level by 56% (\( P < 0.01 \)).

Conclusion: During chronic hyperbilirubinemia, plasma UCB concentration closely correlates with brain UCB levels. During acute hyperbilirubinemia, however, plasma UCB does not correlate with brain UCB levels. Both under chronic and acute hyperbilirubinemia, albumin administration profoundly increases the therapeutic effect of PT, by decreasing brain UCB levels.

Disclosure of Interest: None declared.

PO-H-0327/PD-H-0136

Hepatology

ASSESSMENT OF SKELETAL MATURATION IN CHILDREN WITH NONALCOHOLIC FATTY LIVER DISEASE

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Objectives and Study: Nonalcoholic fatty liver disease (NAFLD) is considered as a hepatic manifestation of metabolic syndrome (MetS). Patients with NAFLD usually present with overweight or obesity and are exposed to complications of MetS in the early adulthood. On the other hand it is known that obese patients have accelerated biological maturation and growth. In the previous study it was shown that children with primary hypertension had increased rate of bone maturation compared to healthy subjects.\(^1\) This study aimed to investigate whether NAFLD is linked to aberrations of growth and maturation processes.

Methods: To evaluate the possible relation between the rate of biological maturity and NAFLD, bone age (BA) assessments on the basis of dual x-ray absorptiometry (DXA) left hand-wrist scans were performed in 23 children with NAFLD aged 13.2 yrs (9.5–17.5) and 23 healthy overweight/obese controls matched for the absolute BMI (weight/obese controls matched for the absolute BMI, height, and sex). In addition to hand scans, total body bone mineral density (TBBMD, g/cm\(^2\)) and content (TBBMC, g), lean mass (LBM, g) and the TBBMC/LBM ratio were evaluated by DXA. Reference values were used to calculate respective z scores (age- and sex-matched), SD scores (height- and sex-matched), and SDS scores (weight- and sex-matched).

Results: Healthy obese controls had a mean BA of 14.8 ± 2.3 y that was significantly higher than their chronological age (CA) of 13.8 ± 2 y. In children with NAFLD, the BA of 13.7 ± 2 y was also markedly higher than their CA of 13.2 ± 2.2 y. Interestingly, the BA was significantly lower in NAFLD patients when compared to healthy controls (13.7 ± 2 vs 14.8 ± 2.3 y, \( P = 0.01 \)). Similarly the magnitude of acceleration of skeletal maturation expressed as (BA-CA) that was significantly higher in overweight/obese healthy controls compared with age and BMI-matched NAFLD patients (1.0 ± 1.6 vs 0.46 ± 1.1). Bone mineral density (TBBMD) did not differ between the groups (1.07 ± 0.12 vs 1.04 ± 0.1).
respectively), as well as BMD z score and BMD-SD score. There were no differences in bone mineral content (TBBMC) but TBBMC/LBM index was significantly decreased in NAFLD (0.05 ± 0.007) in comparison to healthy controls (0.06 ± 0.01).

**Conclusion:** Even if skeletal maturation accelerates in a fatty liver disease compared to chronological age it is delayed when compared to matched overweight/obese healthy children. Thus, fatty liver disease presents with distinct features of bone maturation when compared to other presentations of metabolic syndrome.


**Disclosure of Interest:** None declared.

**PO-H-0277/PD-H-0137**

**Hepatology**

**SERUM LEVELS AND TISSUE EXPRESSIONS OF CELLULAR MARKERS FOR THE ASSESSMENT OF LIVER FIBROSIS IN PATIENTS WITH BETA THALASSEMIA MAJOR TREATED WITH DEFERASIROX**

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**Objectives and Study:** Iron overload enhances oxidative stress within the liver, which is associated with the development of liver fibrosis by interactions between various resident hepatic cell populations.

**Methods:** This study was run in Turkey following the completion of the ICL670A0107E extension. Beta-thalassaemia major patients who completed core phase and continued with deferasirox or switched to deferasirox during 4-year extension were included after consenting for participation in this study. Frozen serum samples and liver biopsy specimens which were collected at baseline, 1st year and end of study of those patients was used. Liver iron concentrations (LIC), liver fibrosis scores according to Ishak and iron stages according to Sciot were obtained. Serum tenascin, collagen-4, tissue inhibitors of metalloproteinase (TIMP-1), and matrix metalloproteinase (MMP) levels were measured with ELISA kits. Liver tissues were immunostained with antibodies against alfa-SMA, collagen-4, TIMP-1 and MMP-1. The intensity of immunostaining in entire representative slides was semiquantitatively graded from 0 to 4.

**Results:** 66 patients who received deferasirox (n = 41) since the core study and switched to deferasirox (n = 25) after 1 year and completed 4-year extension study were included. LIC significantly decreased from baseline (21.2 ± 1.6) at 1sty (14.6 ± 1.2) and the EOS (9.4 ± 1.0) (P < 0.001) while mean fibrosis score did not differ significantly. However, fibrosis scores decreased significantly by decrease in LIC (P = 0.018). Decrease in hepatocyte iron rather than kupffer or portal iron was significantly related with decrease in fibrosis during Deferasirox therapy up to 5 years (P = 0.012). During treatment all serum fibrosis markers decreased significantly compared to baseline (P = 0.000). However, only decrease in TIMP-1 was significantly correlated with decrease in portal iron at tissue level (P = 0.04). In respect of tissue expressions of fibrosis markers, changes in portal aSMA showed a significant correlation with changes of fibrosis from baseline at 5 years (P = 0.04). Further, portal aSMA showed correlation with the hepatocytes iron (P = 0.03) which was also found in correlation with fibrosis.

**Conclusion:** This study revealed that hepatic stellate cell (HSC) activation markers are well correlated with hepatocyte iron and progression of fibrosis. Deferasirox chelation.

**Disclosure of Interest:** A. Yesim Grant/Research Support from: Novartis Pharma AG, ISTANBUL. D. Nart Grant/Research Support from: Novartis Pharma AG, Istanbul. I. Sasma: None declared, D. Canatan: None declared, L. Agaoglu: None declared, Y. Kilinc: None declared, A. Canatar: None declared, O. Arikan: None declared, E. Alnegenis: None declared, C. Arikan Grant/Research Support from: Novartis Pharma AG, Istanbul.

**PO-H-0266/PD-H-0138**

**Hepatology**

**HEPATOCYTE ULTRASTRUCTURE IN CHILDREN WITH ALPHA-1-ANTITRYPsin DEFICIENCY-PROGNOSTIC FACTORS**

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**Objectives and Study:** α-1-antitrypsin (α-1-AT) deficiency is a disease with variable prognosis ranging from hepatitis to liver failure which can not be explained solely by the genetic background of the disease. Two pathways, an autophagia or via proteasome, has been suggested as responsible for disposal of α-1-AT, still the mechanisms of hepatocellular injury remain not clear. The aim of the study was to determine features of morphological expression in patients with α-1-AT corresponding with good or unfavorable prognosis which was not addressed in earlier studies.

**Methods:** We reviewed 14 liver tissue samples obtained from homozygous patients (PiZZ), divided into 2 groups: with unfavorable prognosis (I group, N = 6): with liver failure (1), death in course of cirrhosis (1) or transplanted (4) and with good prognosis- no evidence of liver cirrhosis (II group, N = 8). The age of biopsy sampling did not differ significantly.
between the groups: it was 0.6 (0.3–3.5) y in the I group vs. 0.25 (0.2–0.8) y in the II group [median (min-max)]. Liver biopsy samples were investigated using electron microscopy and morphometric methods. The follow up of the patients in II group was 14.75 (10–19) y, while in the I group the patients were followed until LTx or death for 14.6 (6.58–18) y [median (min-max)].

**Results:** Electron microscopical investigations revealed presence of abundant profiles of SER (smooth ER) in 6/6 pts, dilated RER (rough ER) in 4/6 patients, myelin structures in mitochondria matrix or formed in contact with external mitochondrial membrane in 1/6 patients in the I group, and in the II group these abnormalities were observed in 1/8, 0/8, 4/8 patients, respectively. 2 populations of mitochondria were found in the I group: oval- with the cross sectional area of 0.51 μm² [mean ± SD] and long mitochondria with a higher area of 0.99 ± 0.18 μm². In the II group only oval or round mitochondria were observed (0.46 ± 0.2 μm²). The renin-angiotensin system (RAS) plays a major role in the regulation of water-electrolyte system and the peripheral resistance arteries, and participates in the pathogenesis of hypertension, atherosclerosis and tissue fibrosis. Polymorphism of ACE gene due to the insertion (I) or deletion (D) of 278 base pairs resulting in three genotypes II, ID, DD. The D allele is associated with higher circulating and tissue ACE levels. The aim of the study was to assess polymorphism of ACE (I/D), eNOS (Glu298Asp), MTHFR (C677T), LDL-R, LPA, CRP (1059G>C), and PPARγ (Pro12Ala, Pro115Gln, C161T) in children with nonalcoholic fatty liver disease (NAFLD) compared to obese children and healthy subjects.

**Methods:** Materials and methods: We used a case-control study design comparing a series of unrelated 72 NAFLD (15 girls, 57 boys), 249 obese (53 girls, 196 boys) and 182 healthy children (92 girls, 90 boys). DNA was extracted from blood leukocytes using the phenol method. Polymorphism of ACE gene was evaluated by polymerase chain reaction. All samples found to be DD were confirmed by nested PCR. Other polymorphisms were evaluated by RFLP-PCR. We assessed differences in the distribution of all genotypes and allelic frequencies.

**Results:** In NAFLD group we found significantly higher numbers (P < 0.05) of children homozygous for the D allele (DD - 28/72–38.9%) when compared to obese children (64/249–25.7%) or healthy controls (43/182–23.6%). The analysis of other haplotypes (C677T MTHFR, LDL-R, LPA, 1059G>C CRP, and PPARγ (Pro12Ala, Pro115Gln, C161T) revealed no differences in their frequencies between patients and controls, and they were not associated with NAFLD.

**Conclusion:** We concluded that there is association between the I/D ACE gene polymorphism and NAFLD in a Polish population of children. It may indicate the role of increased ACE activity in children with liver steatosis.

**Disclosure of Interest:** None declared.

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**PO-H-0269/PD-H-0139**

**Hepatology**

**COMMON VARIANT OF THE ACE, BUT NOT ENOS, MTHFR, LDL-R, LPA, CRP, AND PPARγ GENE POLYMORPHISM IS ASSOCIATED WITH NONALCOHOLIC FATTY LIVER DISEASE IN CHILDREN**

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**Objectives and Study:** The renin-angiotensin system (RAS) plays a major role in the regulation of water-electrolyte system and the peripheral resistance arteries, and participates in the pathogenesis of hypertension, atherosclerosis and tissue fibrosis. Polymorphism of ACE gene due to the insertion (I) or deletion (D) of 278 base pairs resulting in three genotypes II, ID, DD. The D allele is associated with higher circulating and tissue ACE levels. The aim of the study was to assess polymorphism of ACE (I/D), eNOS (Glu298Asp), MTHFR (C677T), LDL-R, LPA, CRP (1059G>C), and PPARγ (Pro12Ala, Pro115Gln, C161T) in children with nonalcoholic fatty liver disease (NAFLD) compared to obese children and healthy subjects.

**Methods:** Materials and methods: We used a case-control study design comparing a series of unrelated 72 NAFLD (15 girls, 57 boys), 249 obese (53 girls, 196 boys) and 182 healthy children (92 girls, 90 boys). DNA was extracted from blood leukocytes using the phenol method. Polymorphism of ACE gene was evaluated by polymerase chain reaction. All samples found to be DD were confirmed by nested PCR. Other polymorphisms were evaluated by RFLP-PCR. We assessed differences in the distribution of all genotypes and allelic frequencies.

**Results:** In NAFLD group we found significantly higher numbers (P < 0.05) of children homozygous for the D allele (DD - 28/72–38.9%) when compared to obese children (64/249–25.7%) or healthy controls (43/182–23.6%). The analysis of other haplotypes (C677T MTHFR, LDL-R, LPA, 1059G>C CRP, and PPARγ (Pro12Ala, Pro115Gln, C161T) revealed no differences in their frequencies between patients and controls, and they were not associated with NAFLD.

**Conclusion:** We concluded that there is association between the I/D ACE gene polymorphism and NAFLD in a Polish population of children. It may indicate the role of increased ACE activity in children with liver steatosis.

**Disclosure of Interest:** None declared.

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**PO-H-0270/PD-H-0140**

**Hepatology**

**IGF1 AND HIGH-MOLECULAR-WEIGHT ADIPONECTIN IN CHILDREN WITH OBESITY-RELATED FATTY LIVER DISEASE**

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**Objectives and Study:** In adults, a lower IGF-1 concentration is associated with an increased risk of diabetes also a few reports on decreased IGF-1 concentrations in adults with non-alcoholic fatty liver disease. The new marker high-molecular-weight (HMW) adiponectin was investigated in adiposity and plays a crucial and causal role in obesity-linked insulin resistance and metabolic syndrome. We aimed in our study to investigate IGF-1, HMW adiponectin as well as other cytokines, lipids, and antioxidants in children with obesity-related fatty liver disease compared to healthy controls.

**Methods:** We investigated 30 patients with NAFLD (diagnosis based on ultrasound accompanied with increased transaminase activity) aged 13.52 (11.7–16.01) y and
Results: There were significant differences among NAFLD and healthy children in serum/plasma IGF 1-total and IGF 1-free levels as low as 8 μg/mL also had a favourable outcome. Nevertheless, a fraction of the patients lied in the “grey zone” where CIT was incompletely predictive.

Conclusion: CIT can help in the evaluation of a patient with SBS. Plasma CIT measured at age 2 years provides a good predictive value for clinical outcome. However, this marker cannot drive therapeutic decisions such as PN weaning or intestinal transplantation as its level is not always strictly correlated with the clinical outcome. Thus, there was a tendency for CIT to increase during adaptation in patients with a favourable course toward PN weaning, and a trend for CIT to decrease in patients with a bad outcome. Nevertheless, a fraction of the patients lied in the “grey zone” where CIT was incompletely predictive.

Disclosure of Interest: None declared.
born to cesarean section are more likely to develop obesity in adulthood.

Methods: We carried out a newborn cohort study in Ribeirão Preto, Brazil, started in 1978. A randomized sample of 2057 subjects from the original cohort (6827 individuals) was reassessed in 2002 in order to take anthropometric measurements and other co-variables at 24 years of age. The following co-variables were collected after birth: type of delivery, birth weight, maternal smoking and maternal schooling. The following subjects’ data were obtained at the time of their birth weight, maternal smoking and maternal schooling. The following subjects’ data were obtained at the time of their birth weight, maternal smoking and maternal schooling. Obesity was considered when BMI ≥ 30. A Poisson multivariable model was performed aiming to determine the impact of cesarean section on BMI in offspring at adulthood, considering gender distribution. The model was adjusted for participant’s (birth weight, income, smoking, schooling and physical activity) and maternal factors (schooling and smoking).

Results: The rate of obesity in young adults born by cesarean section was 15.2% vs 10.4% in those born by vaginal delivery (P=0.002). Subjects who were born by cesarean section had an increased risk of obesity in adulthood, after controlling by co-variables from the mothers and from the subjects. The Poisson regression model pointed out the positive impact of cesarean section on BMI in adulthood as the effect remained significant after adjustment for socio economic status co-variables. The subjects born by cesarean section had 50% higher probability for obesity in adulthood compared with those born by vaginal delivery.

Conclusion: Subjects who were born by cesarean section had an increased risk for obesity at adulthood. We may hypothesize that the differences in intestinal flora related to type of delivery section may have a role on the epidemic obesity worldwide.

Disclosure of Interest: None declared.

PO-N-0253/PD-N-0143

Nutrition, Metabolism, and Experimental Approaches

ADIPOSITY IN PRETERM AND FULL-TERM INFANTS

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Objectives and Study: Excess of intraabdominal adipose tissue plays a significant role in the development of unfavorable metabolic and cardiovascular risk. Imaging methods for intraabdominal adipose tissue quantization have become a focus of attention, particularly in clinical research setting. Among those, magnetic resonance imaging (MRI) is fairly well established. Preterm infants may be at risk for altered adiposity. We conducted an observational study to evaluate total body fat and intraabdominal adipose tissue in preterm infants at term corrected age.

Methods: 23 preterm and 9 full-term infants entered the study at 0–1 month of corrected and postnatal age, respectively. Inclusion criteria were birth weight <1500 g, singleton pregnancy. Exclusion criteria were: presence of congenital diseases, chromosomal abnormalities, chronic lung disease, severe brain, metabolic, cardiac or gastrointestinal diseases. The total body fat mass was assessed by means of an air displacement plethysmography system (Pea Pod LMI, USA) and the intraabdominal adipose tissue by means of MRI (software program SliceOMatic, Version 4.3, Tomovision, Canada). The intraabdominal adipose tissue was defined as the internal adipose tissue contained in the slices ranging from the top of the liver to the heads of the femurs inside the fascial plane.

Results: Mean gestational age (weeks) was 30±2 and 38±0.8 in the preterm and term infants, respectively. Birth weight (g) was 1165±252 and 3383±505 in the preterm and term infants, respectively. Preterm infants showed a mean weight at MRI assessment equal to 3155±548 g and a mean corrected age of 42.5±1.5 weeks whereas full-term infants weighed 3326±141 g and were 41.6 weeks old. Total body fat mass was 633±183 g (19.8±3.8 %) and 538±203 (17.5±5 %) in the preterm and term infants, respectively. The intraabdominal adipose tissue (g) was 14.18±4.9 and 19.9±11.4 in the preterm and term infants, respectively.

Conclusion: These preliminary data suggest that the preterm infants studied, although exhibiting a total body fat mass higher than the full-term infants, do not show an increased intraabdominal adipose tissue.

Disclosure of Interest: None declared.
disturbances in gut bacterial colonisation shortly after birth affect offspring gut IAP development.

Methods: This hypothesis was tested in the swine model by treating pregnant sows (11 antibiotic treated-ATBQ, vs. 12 untreated controls-C) with the large spectrum antibiotic amoxicillin (40 mg/kg BW/d) orally around parturition (day –10 to day 21) in order to disturb sows’ microbiota and offspring bacterial gut colonisation. Offspring (1/sow/ time) were slaughtered at day 14, 28 (weaning) and 42 and gut tissues and caecal and rectal contents were collected for IAP activity measurements and ileal tissue for mRNA determination. Data were analysed by SAS with a MIXED model for testing effects of treatment, age and interaction.

Results: IAP activity in offspring jejunal and ileal mucosa decreased with age (P<0.01). Ileal tissue IAP activity was threefold lower in ATBQ than in C group at d14, with no differences at d28 and d42 (treatment by time interaction, P<0.01). Antibiotic treatment of sows did not influence offspring jejunal and colonic tissue IAP activities which were positively and linearly correlated (P<0.01). Ileal tissue IAP mRNA levels tended to be lower at d28 than at d14 or d42 (P=0.06) with no significant effect of ATBQ treatment on ileal IAP activity. Ileal tissue IAP mRNA levels and IAP activity were positively and linearly correlated (P<0.01).

Conclusion: Maternal antibiotic treatment of the mother affects the development of gut IAP in her offspring, with more pronounced effects in the ileum than in the jejunum or the colon. The drastic reduction in IAP tissue activity in offspring ileum at d14 with antibiotic treatment of the mother suggests differential patterns of gut microbial colonisation between groups. Reduced IAP activity may lead to reduced bacterial lipopolysaccharide detoxification and increased risk of gut inflammation in offspring. Future work will investigate the involvement of the microbiota in these IAP changes and long-term effects on offspring gut physiology.


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Disclosure of Interest: None declared.

PO-N-0223/PD-N-0145

Nutrition, Metabolism, and Experimental Approaches

GHRELIN IN BREAST-FED AND FORMULA-FED INFANTS IN THE FIRST 6 MONTHS OF LIFE, IN THEIR LACTATING MOTHERS, AND IN BREAST MILK

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Objectives and Study: Ghrelin, a protein hormone produced primarily in the stomach, acts as a short-term regulator energy balance with orexigenic and lipogenic activities and it may play a role in the regulation of growth and development of feeding behaviour. The aim of the study was to evaluate serum ghrelin concentration in infants in the first six months of life, in their lactating mothers and in breast milk (BM). Moreover we evaluated the relation between serum ghrelin levels in infants in the first six months of life and the kind of feeding. Finally to investigate the relationship between ghrelin in infants’ and lactating mothers’ serum and in BM.

Methods: We enrolled 79 AGA healthy infants less than 6 months of age, admitted to our Department, and 36 lactating mothers. We evaluated ghrelin presence in 40 BM samples. Serum and milk ghrelin concentration has been determined using commercial kit Ghrelin (total) RIA-3967 DRG Diagnostics. Mann-Whitney test and Spearman correlation were applied. Statistical significance was set at P<0.05.

Results: Median (IR—interquartile range) serum ghrelin concentration in infants was 922.11 (868.44) pg/mL; higher serum ghrelin concentrations were found in BF infants (n=56) 705.82 (827.32) pg/mL than in FF ones (n=23) 1069 (436.9) pg/mL, with a statistically significant difference between the 2 kinds of feeding (P=0.021). The median (IR) of serum ghrelin of nurses (n=36) was 667.88 (942.78) pg/mL, while the median value (IR) of ghrelin in BM (n=40) was 526.4 (439.86) pg/mL. Positive correlations emerged between serum ghrelin values in lactating mothers and in BM samples (P=0.021, r=0.450), between serum ghrelin in BF infants and hormone values nurses’ serum (P<0.001, r=0.789) and finally between serum ghrelin in BF infants and in BM samples (P<0.001, r=0.581).

Conclusion: In our study we observed ghrelin serum levels significantly higher in FF infants than in BF infants, confirming the results of studies previously carried out in our department (1). It could be argued that higher levels of ghrelin observed in FF infants may create a greater stimulus to food intake with important implications in regulating appetite during early infancy. Highlighting the presence of ghrelin in breast milk, its levels were positively correlated with those found in infants’ and lactating mothers’ serum. These findings could raise questions about the possibility of a direct passage of ghrelin from the mothers to the infants through breast milk, even though further investigation are needed to clarify uptake, absorption and metabolic effects of this feeding stimulus that is present in breast milk.

References

Disclosure of Interest: None declared.

PO-N-0234/PD-N-0146

Nutrition, Metabolism, and Experimental Approaches

INCREASED ENERGY INTAKE DIRECTLY FOLLOWING BIRTH DOES NOT INCREASE GSH SYNTHESIS RATES IN PRETERM INFANTS

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Objectives and Study: Preterm neonates are subjected to increased oxidative stress due to excessive formation of reactive oxygen species and reduced antioxidant defenses following birth. Oxidative stress is strongly related to major neonatal morbidities such as bronchopulmonary dysplasia, necrotizing enterocolitis, retinopathy of prematurity and periventricular leucomalacia. Early amino acid administration (AA) is known to increase glutathione (GSH) concentration, the main intracellular antioxidant, in preterm infants. We hypothesized that additional energy (provided via intravenous lipids), results in a further upregulation of anti-oxidant defense mechanisms via increased GSH synthesis rates.

Methods: Preterm infants (gestational age < 32 weeks) were randomly assigned to receiving AAs (2.4 g/kg/d) or AAs and lipids (2 g/kg/d) from birth onwards. On day 2, infants received a primed, continuous infusion of [U-13C]glycine, a precursor for GSH synthesis, to determine fractional synthesis rate (FSR) using mass spectrometry. Absolute synthesis rate was calculated from the FSR and erythrocyte GSH concentration. Data of this ongoing trial are presented as median (min-max).

Results: Birth weight (800 (585–1140) g) and birth weight-SD (−2.0 (−4.3–0.2)) were not significantly different between groups. Gestational age was lower in infants receiving AA + lipids (25.7 (24.7–29.9) weeks) compared to infants receiving only AAs (28.3 (26–31.3) weeks), p < 0.05. Protein intake was not different between the groups: 2.5 (1.9–2.8) on day 1 and 2.5 (1.9–3.1) g/kg/d on day 2. Protocol was the non-protein energy significantly higher in the infants receiving lipids; 50 (35–62) vs 30 (26–36) kcal/kg/d on day 1 (P = 0.001) and 53 (45–74) vs 33 (27–37) kcal/kg/d on day 2 (p < 0.001). GSH concentration and synthesis rate were not different between the groups (Table). Table.

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<thead>
<tr>
<th></th>
<th>AA (n = 6)</th>
<th>AA + lipids (n = 9)</th>
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<tbody>
<tr>
<td>Concentration (mmol/L)</td>
<td>1.7 (0.5–2.1)</td>
<td>1.7 (0.9–2.1)</td>
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<tr>
<td>Fractional synthesis rate (%/d)</td>
<td>43 (32–59)</td>
<td>39 (34–59)</td>
</tr>
<tr>
<td>Absolute synthesis rate (mg/kg/d)</td>
<td>6.3 (1.6–10.7)</td>
<td>6.9 (3.2–10.4)</td>
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GSH synthesis rate was not correlated with gestational age (P = 0.9).

Conclusion: Increased energy intake in preterm infants does not result in increased GSH synthesis rates.

References

Disclosure of Interest: None declared.

PO-N-0265/PD-N-0147

Nutrition, Metabolism, and Experimental Approaches

Pancreatic Fat Fraction is Increased in Obese Adolescents and Related to Metabolic Syndrome

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Objectives and Study: Visceral fat has been associated with the development of metabolic syndrome in obese patients. There has been much focus on hepatic fat fraction, but very little is known about pancreatic fat accumulation and its possible associations with metabolic syndrome (MetS) and glucose metabolism. To date, pancreatic fat fraction (PFF) has not been explored in lean and obese adolescents. The aim of this study was to quantify PFF in lean and obese adolescents and explore its relationship with metabolic parameters.

Methods: We recruited 25 lean and 24 obese adolescents (mean age 13.6 ± 1.5 y; mean BMI 18.9 ± 30.3 kg/m²). Pancreatic fat fraction (PFF) and visceral fat were determined using MRI. We measured fasting glucose, insulin, liver enzymes, leptin and lipid levels, as well as blood pressure. Obese subjects underwent an oral glucose tolerance test.

Results: PFF was significantly different between lean and obese groups (3.6 ± 0.9 vs. 4.8 ± 1.2; p < 0.001) and was associated with visceral fat, gamma-GT, triglycerides, HDL-cholesterol, leptin concentrations, and with the presence of metabolic syndrome (P < 0.05 for all). Obese subjects had higher insulin levels, but none of them had glucose intolerance. When adjusted for visceral fat, the following 3 parameters correlated negatively with PFF: fasting, 30 and 120-minute insulin levels. Using the International Diabetes Federation definition of MetS, we divided subjects into 3 groups: 1) lean without MetS; 2) obese without MetS and 3) obese with MetS. Pancreatic fat fraction increased gradually among groups (Group 1, n = 25, 3.56 ± 0.88; Group 2, n = 19, 4.70 ± 1.06; Group 3, n = 5, 5.34 ± 1.49%; F = 10.36, p < 0.001). There was no correlation with hepatic fat fraction (HFF).

Conclusion: Obese adolescents accumulate fat in the pancreas. PFF correlates with the presence of MetS. Even in absence of glucose intolerance, pancreatic fat deposition is associated with impaired insulin response to glucose overload. This suggests that beta-cell dysfunction is already present in non-diabetic obese adolescents, mirroring what has been shown in adults. It also suggests that pancreatic fat accumulation may participate in obesity-associated pancreatic endocrine dysfunction.

Disclosure of Interest: None declared.

PO-N-0216/PD-N-0148

Nutrition, Metabolism, and Experimental Approaches

Therapeutic Effects of the New Synthetic Butyrate Derivative N-(1-Carbamoyl-2-Phenyl-Ethyl) Butyramide on Diarrhea and Visceral Pain in Mice


Objectives and Study: Pancreatic fat has been associated with the development of metabolic syndrome in obese patients. There has been much focus on hepatic fat fraction, but very little is known about pancreatic fat accumulation and its possible associations with metabolic syndrome (MetS) and glucose metabolism. To date, pancreatic fat fraction (PFF) has not been explored in lean and obese adolescents. The aim of this study was to quantify PFF in lean and obese adolescents and explore its relationship with metabolic parameters.

Methods: We recruited 25 lean and 24 obese adolescents (mean age 13.6 ± 1.5 y; mean BMI 18.9 ± 30.3 kg/m²). Pancreatic fat fraction (PFF) and visceral fat were determined using MRI. We measured fasting glucose, insulin, liver enzymes, leptin and lipid levels, as well as blood pressure. Obese subjects underwent an oral glucose tolerance test.

Results: PFF was significantly different between lean and obese groups (3.6 ± 0.9 vs. 4.8 ± 1.2; p < 0.001) and was associated with visceral fat, gamma-GT, triglycerides, HDL-cholesterol, leptin concentrations, and with the presence of metabolic syndrome (P < 0.05 for all). Obese subjects had higher insulin levels, but none of them had glucose intolerance. When adjusted for visceral fat, the following 3 parameters correlated negatively with PFF: fasting, 30 and 120-minute insulin levels. Using the International Diabetes Federation definition of MetS, we divided subjects into 3 groups: 1) lean without MetS; 2) obese without MetS and 3) obese with MetS. Pancreatic fat fraction increased gradually among groups (Group 1, n = 25, 3.56 ± 0.88; Group 2, n = 19, 4.70 ± 1.06; Group 3, n = 5, 5.34 ± 1.49%; F = 10.36, p < 0.001). There was no correlation with hepatic fat fraction (HFF).

Conclusion: Obese adolescents accumulate fat in the pancreas. PFF correlates with the presence of MetS. Even in absence of glucose intolerance, pancreatic fat deposition is associated with impaired insulin response to glucose overload. This suggests that beta-cell dysfunction is already present in non-diabetic obese adolescents, mirroring what has been shown in adults. It also suggests that pancreatic fat accumulation may participate in obesity-associated pancreatic endocrine dysfunction.

Disclosure of Interest: None declared.
Calignano1, R. Berni Canani2,* 1Department of Experimental Pharmacology, 2Department of Pediatrics, University of Naples, Naples, Italy.

Objectives and Study: The short chain fatty acid butyrate has a pivotal role in the intestinal physiology. The low palatability and stability limit a wide therapeutic use of this substance. We have recently obtained a high palatable and stable synthetic butyrate derivate, N-(1-carbamoyl-2-phenyl-ethyl)butyramide (BuBull). In this study we investigated the effects of this new compound in two established animal models of diarrhea (castor oil-induced diarrhea) and visceral pain (acetic acid–induced writhings).

Methods: Young male ICR mice were used in all experiments. Equimolar maximal effective doses of sodium-butyrate (NaBu) (100 mg/kg) or BuBull (212 mg/kg) administered 1 h (acute treatment), or once daily for 4 d (chronic treatment), before 0.2 ml/animal of castor oil given by oral route, or 0.5 mL of 0.5% of acetic acid/animal given through intraperitoneal injection. Occurrence of diarrhea (evaluated through an apposite score: 0–loose; 1–liquid stools) and number of writhings evaluated for 1 h and 30 min after stimulation with castor oil or with acetic acid.

Results: Acute or chronic pre-treatment with BuBull resulted in a significant higher inhibition of castor oil-induced diarrhea compared to NaBu (median score, acute: 0.3; chronic: 0.9; NaBu 2.4; BuBull 1.5; NS, n = 8; p < .001, n = 8). Chronic but not acute treatment with BuBull resulted in a higher inhibition of viscer al pain elicited by acetic acid (median number of writhings, acute: control 1.8 ± 0.7; NaBu 1.0 ± 0.3; BuBull 0.6 ± 0.3; p < .001, n = 8; chronic, control 1.8 ± 6; NaBu 1.4 ± 9; BuBull 0.3 ± 0.2; p < .001, n = 8). Chronic but not acute treatment with BuBull resulted in a higher inhibition of visceral pain elicited by acetic acid (median number of writhings, acute: control 40 ± 1.8; NaBu 22 ± 2.4; BuBull 15 ± 1.5; NS, n = 8; chronic, control 41 ± 2.0; NaBu 26 ± 2.4; BuBull 12 ± 1.4; p < .001, n = 8).

Conclusion: The new synthetic butyrate derivate, N-(1-carbamoyl-2-phenyl-ethyl)butyramide is more effective than the corresponding natural compound in inhibiting diarrhea and visceral pain in the animal model. The effect on visceral pain, not previously described, opens new therapeutic perspectives for this compound.


SP-G-0149

Inflammatory Bowel Disease

ANTI-INFLAMMATORY ROLE OF OBESTATIN AND GHERLIN IN DSS-INDUCED ACUTE AND CHRONIC COLITIS IN RATS

E80

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Objectives and Study: Obestatin and ghrelin are 2 hormones derived from the same gene, having opposite effects in many physiological processes. Ghrelin is a novel growth hormone-releasing, orexigenic peptide and has an anti-inflammatory activity which has been shown in ischemic reperfusion injuries and colitis. However, likelihood anti-inflammatory effect of obestatin has not been studied yet. The aim of the study was to analyze the anti-inflammatory effect of exogenous obestatin and ghrelin on dextran sulfate sodium (DSS) induced acute and chronic colitis in rats.

Methods: The anti-inflammatory action of ghrelin and obestatin was investigated in acute and chronic colitis, induced in Sprague-Dawley rats by administration of 3% DSS into the drinking water for 5 and 10 days, respectively. Control group received plain water for drinking. Intraperitoneal pretreatment with ghrelin (20 µg/kg) or obestatin (50 µg/kg) was started 12 h before the induction of colitis, and continued for 5 (acute colitis) and 10 (chronic colitis) days. Clinical signs of the disease (weight loss, diarrhea, disease activity index) and histopathology were evaluated. The mechanisms involved in the potential therapeutic effects of ghrelin and obestatin were investigated by measuring byproducts of lipid peroxidation, neutrophil activation [malondialdehyde (MDA), myeloperoxidase (MPO), glutathion peroxidase (GSH) activities] and inflammatory cytokines (IL-1β, IFN-γ, TNF-α), anti-inflammatory cytokines (TGFβ, IL-10) in colonic tissue.

Results: Obestatin significantly ameliorated clinical and histopathologic severity of DSS-induced acute and chronic colitis; abrogating body weight loss, diarrhea, and inflammation. However, ghrelin was effective only in the acute form of colitis. Therapeutic effect of ghrelin and obestatin in acute colitis was associated with down regulation of MDA and Th1 induced inflammatory response (IL-1β, IFN-γ, TNF-α). Potential therapeutic role of obestatin in chronic colitis, on the other hand, involved the suppression of PMNL infiltration (MPO) and enhancement of GSH synthesis as well as down regulation of MDA and Th1 induced inflammatory response (IL-1β, TNFα, IFN-γ). In this study, another potential therapeutic effect of obestatin was attributed to the increased secretion of anti-inflammatory cytokines in chronic model of colitis.

Conclusion: This study demonstrated novel anti-inflammatory effect of obestatin and ghrelin in acute and chronic colitis. While obestatin exerted anti-inflammatory effect in both acute and chronic colitis, ghrelin was effective only in acute model. Consequently, ghrelin and obestatin administration may represent a promising therapeutic approach for inflammatory bowel diseases in the future.

Disclosure of Interest: None declared.
Inflammatory Bowel Disease

IMMUNO-PROTEASOME SUBUNIT LMP7 AND AUTOPHAGOSOMAL MARKER LC3 ARE UPREGULATED AND COLOCALIZED IN MONONUCLEAR CELLS IN COLONIC TISSUE OF CHILDREN WITH ULCERATIVE COLITIS

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Objectives and Study: The pathogenic mechanism of UC involves dysregulation of the intestinal immune response to intestinal environmental antigens, such as intestinal microflora. Autophagy represents a lysosomal pathway involved in the degradation of cellular proteins and organelles as well as microbes into the peptide antigens presented to MHC class II molecules. Defective autophagy is now recognized as an important factor in the development of inflammatory bowel diseases (IBD). LC3, the protein present in the membrane of autophagosomes, is the only credible marker of the autophagosome in mammalian cells. It has also been shown that inflamed mucosa of IBD patients contains increased amounts of cytokine inducible proteasome LMP subunits and that LMP7 is directly involved in IBD development, presumably as the result of regulation of cytokine production in inflammatory cells. The proteasomes containing LMP subunits, the so-called immunoproteasomes, constitute the major nonsylosomal pathway of the degradation of cellular proteins into the peptide antigens presented into the MHC class I system. The aim of the study was to analyze the expression of immunoproteasome subunit LMP7 and autophagosomal marker LC3 in colonic tissue of children with untreated ulcerative colitis (UC).

Methods: We study 9 children (6 girls; mean age 12.4 years; range 3–17) with UC and 6 children with constipation and rectal bleeding (4 boys, mean age 10.5 years, range 4–15) as the control group. In all patients biopsy samples were taken from the sigmoid. The paraffined sections of tissue were immunolabeled by anti-LMP7 and anti-LC3 antibodies followed by incubation with secondary IgG antibodies conjugated to AlexaFluor 594 (red) or AlexaFluor488(green), respectively. Sections were also stained with DAPI to visualize nuclei.

Results: A higher levels of LMP7 and LC3 were found in colon of the children with CU as compared to the control group. Both proteins were solely detectable in the mononuclear cells of lamina propria but were not present in epithelial cells. The most important finding was that LMP7 colocalizes with LC3 in perinuclear area of this cells both in inflammed tissues and innormal mucosa.

Conclusion: Our study demonstrates for the first time that in the colon of the children with ulcerative colitis, the immunoproteasome subunit LMP7 and autophagosomal marker LC3 are up-regulated and co-localized exclusively in mononuclear cells. This finding suggests the cross-talk between immunoproteasomes and autophagy pathways in inflammatory cells.

Disclosure of Interest: None declared.
Cystic Fibrosis
GASTROINTESTINAL PROBLEMS IN CHILDREN WITH CYSTIC FIBROSIS
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Objectives and Study: Cystic fibrosis (CF) is characterised by progressive lung disease and exocrine pancreatic insufficiency (EPI). A number of patients continue to have severe gastrointestinal (GI) symptoms despite pancreatic enzyme replacement therapy (PERT) for EPI. Underlying gut inflammation (GIN) may be a contributing factor leading to impaired secondary pancreatic exocrine function making supplementation ineffective. Early recognition of GIN is important as it is a treatable cause of morbidity. However, little is known about the types of gut inflammation in CF. The aim of our study was to evaluate the histopathological features of gut mucosa in CF children with severe GI symptoms and response to treatment.

Methods: Case notes of all CF children referred to the GI clinic from our regional CF unit between 2001 and 2010 and who underwent a diagnostic endoscopy were reviewed. Histology findings, demographics, lung function, genetics, GI symptoms, exocrine pancreatic function and treatment were recorded.

Results: A total of 180 children with CF were identified of which 30 (15 F; mean age 8.1 ± 5.5 y) were referred. 21/30 underwent gastroscopy and colonoscopy, 1/30 had gastroscopy alone. Indications include persistent abdominal pain (6/22), loose stools (12/22), faltering growth (6/22), vomiting (5/22), constipation (3/22), rectal bleeding (2/22). Abnormal mucosal histology occurred in 16/22 (72%). 10/22 (45%) had small bowel inflammation (4/22 chronic inflammation (CI), 3/22 active inflammation (AI) with villous atrophy and crypt hyperplasia, 3/22 eosinophilic enteritis). 9/21 (41%) had colonic involvement (6 CI, 1/21 eosinophilic enterocolitis, 2/21 AI). 7/22 had gastric inflammation (1/22 AI, 6/22 CI) and 2/22 (9%) had CI of oesophagus. 21/22 had EPI requiring PERT. 30% were homozygous delF508. Mean FEV1: 76% predicted. Treatment include dietary exclusion, cromoglicate, antihistamines, mesalazines, immunomodulators (prednisolone, azathioprine) and parenteral nutrition (1 patient). 15/16 children were treated in our hospital. 3 with mild GIN required only symptomatic treatment with antireflux or anti-motility agents. 4/15 responded to diet exclusion, cromoglicate, antihistamine and/or ketotifen. 5/15 responded to immunomodulators whilst 2/15 have persistent symptoms.

Conclusion: To our knowledge, this is the largest paediatric series describing the histopathological features of gut mucosa in CF children with significant GI symptoms. In our cohort of 180 CF children, severe GI symptoms occurred in 16% with underlying GIN found in 72% of those who underwent endoscopy. The majority responded well to treatment with some requiring immunomodulators. In summary, GIN may be more prevalent in CF children than previously thought and recognition of this entity is important amongst CF paediatricians for early diagnosis and treatment.

Disclosure of Interest: None declared.

Hepatology
GALLBLADDER LENGTH ON ULTRSONOGRAPHY AS A SCREENING TOOL IN IDENTIFICATION OF AUTOIMMUNE SCLEROSING CHOLANGITIS
A. Batra1, K. Au-Yong2, D. A. Kelly1, H. Alton2, P. McKiernan1. 1Hepatology, 2Radiology, Birmingham Children's Hospital NHS Trust, Birmingham, United Kingdom.

Objectives and Study: Autoimmune liver disease (AILD) in children encompasses autoimmune hepatitis (AIH), autoimmune sclerosing cholangitis (ASC) or, where there are features of both, overlap syndrome (OS). Differentiating between these entities requires cholangiography. The gold standard for the diagnosis of ASC is endoscopic retrograde cholangiopancreatography which is invasive. Magnetic resonance cholangiopancreatography can be used as an alternative but is not universally available and may require anaesthesia in young children. Anecdotally, we had found increased gallbladder (GB) length on ultrasound to be a useful marker of bile duct involvement in AILD. Our aim was to study the role of ultrasound measurement of gallbladder length as a screening tool for identifying ASC in children with AILD.

Methods: Children, under the age of 18 years at the time of presentation, diagnosed with AILD were identified from the departmental database. Patients for whom a fasting ultrasound at the time of presentation, was available on our radiology department’s digital imaging system were included. Cases were categorised using established criteria into 2 groups; those with AIH and those with ASC with or without OS. A retrospective case notes review was performed on all eligible children, their ultrasound scans were reviewed by a single radiologist and GB length measured.

Results: 50 cases were included. 32/50 (64%) had AIH type1 and 6/50 (12%) had AIH type2. 12/50 (24%) had ASC and 6/50 (12%) of these had OS. The average age at presentation was 10.18 years (range 1.3 – 16 years). The age at presentation was similar for both groups (AIH 10.1 y; ASC 10.2 y). Overall there were 22 males and 28 females but among the group with ASC there were 8 males and 4 females. The duration of symptoms before diagnosis was 5 months and was similar in both groups. The median (25th, 75th centile) GB length in children with ASC was 9.75 cm (7.2 cm, 10.6 cm) and in children with AIH was 6.8 cm (5.5 cm, 7.9 cm) [p value 0.003]. The normal GB length in children older than 1 year is up to 7 cm. GB length ≥ 7 cm was seen in 9/12 (75%) children with ASC compared to 15/38 (39.5%) of children with AIH (P = 0.04). GB length ≥ 9 cm was seen in 7/12 (58.3%) children with ASC and 4/38 (10.5%) children with AIH (P = 0.005).

Disclosure of Interest: None declared.
SP-H-0154

**Hepatology**

**MANAGEMENT OF PRURITIS IN PAEDIATRIC PATIENTS WITH ALAGILLE SYNDROME: A REVIEW OF 15 YEARS’ EXPERIENCE**

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**Objectives and Study:** Pruritis due to cholestasis is common in Alagille syndrome and can severely affect quality of life. No management guidelines are available for children. Our aim was to review the current management of pruritis in children with this condition to inform appropriate guidelines.

**Methods:** An analysis was performed of the clinical records of children who were diagnosed with Alagille syndrome at King’s College Hospital between 1995 and 2010.

**Results:** Sixty-two children were included (34 male). 52 presented as neonates, median time to follow-up was 7.17 years. Of these, 51 (82.3%) had pruritis; this was severe in 23 and moderately severe in 20. UDCA was the most prescribed anti-pruritic (n = 40). Others included rifampicin (n = 39), cholestyramine (n = 18), naltrexone (n = 14), sedative anti-histamines (n = 13), nonsedating anti-histamines (n = 7), ondansetron (n = 5) and phenobarbitone (n = 1). Combination therapy was required in 35 (70%). UDCA had some or a good effect in 34 (85.0%). Side effects in 3 patients related to gastrointestinal intolerance. Rifampicin had some, good, or very good effect in 37 (95%) with side effects in 1. Cholestyramine had some effect in 7 (38.9%) and a very good effect in 3 (16.7%) with poor compliance in 5. Naltrexone had some or a good effect in 11 (78%), 4 had side effects. Alimemazine had provided some benefit in 9, nonsedating anti-histamine agents provided some improvement in 3. Ondansetron had a good effect in 3. Phenobarbitone was prescribed to 1 patient in whom a good effect was observed. No side effects were reported in patients receiving anti-histamines, ondansetron or phenobarbitone. MARS was used in 1 patient with a reported good effect. Surgical management (PEBD or ileal exclusion) was not used in this cohort. 16 patients were listed for liver transplantation, 11 patients have been transplanted to date. Intractable pruritus was the sole indication for transplantation in 2 (12.5%) and was a contributory factor in all listed. Overall patient survival was 95% (n = 59). Pruritus resolved in 20 (39.2%); 1 without any treatment. 9 patients were able to discontinue medication and 11 were transplanted. In the others; pruritus was a significant problem in 5 and intractable in 5 patients all whom are currently listed for transplantation.

**Conclusion:** Pruritis is a significant and severe problem in the majority of children with Alagille attending a tertiary paediatric liver centre. Most required multiple medications and a significant number required liver transplantation.

**Disclosure of Interest:** None declared.

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<th>GB Length, cm</th>
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**Conclusion:** In AILD GB length is significantly increased in children with ASC. GB length ≥ 9 cm has 90% specificity for the diagnosis of ASC. GB length < 7 cm is 88% exclusive of ASC. GB length measurement is useful screening test for ASC in children with AILD. We would recommend a further study with greater number of patients.

**Disclosure of Interest:** None declared.

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**SP-N-0155**

**Pediatric Nutrition**

**NEONATAL FATTY ACID STATUS AND NEURODEVELOPMENTAL OUTCOME AT 9 YEARS**

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**Objectives and Study:** The importance of long-chain polyunsaturated fatty acids (LCPUFA) for prenatal brain development is generally recognized. Higher neonatal docosahexaenoic acid (DHA) and arachidonic acid (AA) status have been associated with better developmental outcome in early infancy. In contrast, neonatal trans fatty acid status has been associated with worse neurodevelopment at 18 months. The present study is the first on the relationship between fatty acid status at birth and neurodevelopmental outcome at 9 years.

**Methods:** 229 children (121 boys, 108 girls) took part in this double-blind randomized controlled trial on the effects of supplementation of formula with 0.30% DHA and 0.45% AA during the first two postnatal months. Fatty acids status at birth was determined in the wall of umbilical vessels; the present data analysis focuses on DHA, AA and trans fatty acids. Neurodevelopmental assessment consisted of detailed neurological assessment (Touwen), cognitive assessment (Wechsler Abbreviated Scale of Intelligence, developmental neuropsychological assessment NEPSY, Test of Everyday Attention for Children) and behaviour assessment via questionnaires. Multivariate analyses were carried out to evaluate the effect of fatty acid status at birth while adjusting for perinatal and social confounders.

**Results:** 74% of the original study group was assessed at 9 years. Fatty acid status at birth, obstetrical and social characteristics of the children, who were and who were not assessed at 9 years, were largely comparable. Children with the complex form of minor neurological dysfunction had significantly lower DHA levels in umbilical vesselwall lipids than children with a better neurological condition. This was multivariately confirmed, arterial DHA: both genders (OR=0.314, 95% CI=0.145–0.681, P = 0.003); venous
DHA: boys only (OR = 0.220, 95% CI = 0.0179 – 0.614, \( P = 0.004 \)). Two types of neurologically dysfunction were associated with lower venous DHA: dysfunctional posture and tone regulation (OR = 0.415, 95% CI = 0.218 – 0.790, \( P = 0.007 \)) and dyskinesia (OR = 0.108, 95% CI = 0.018 – 0.660, \( P = 0.016 \)). Neonatal AA was not associated with neurological outcome. Neonatal DHA and AA were not associated with cognition or behaviour at 9. Venous and arterial trans fatty acid levels showed a positive association with selective attention (multivariate analyses: venous \( \rho = 0.164, P = 0.002 \); arterial \( \rho = 0.276, P = 0.001 \)).

Conclusion: DHA status at birth showed a significant positive association with neurodevelopment at 9, but it was not associated with cognition and behaviour. AA status at birth was not associated with neurodevelopment at 9. Lower trans fatty acid levels were associated with better selective attention at 9.

Disclosure of Interest: C. De Jong: None declared, H. Kikkert: None declared, G. Boehm Industry of: Employee of Danone research, T. Decsi: None declared, M. Hadders-Algra: None declared.

SP-N-0156

Nutrition, Metabolism and Experimental Approaches

CONSUMPTION OF A DAIRY PRODUCT ENRICHED WITH FISH OIL DURING PREGNANCY MAINTAINS THE DHA STATUS OF THE MOTHER AND INCREASE DHA CONCENTRATION IN CORD BLOOD


Objectives and Study: Docosahexaenoic acid (DHA, 22:6 n-3) is the most abundant fatty acid in the human brain and retina. During the last trimester of gestation there is an increase accretion of DHA by the fetus through placenta which could lead to a decrease in the mother’s DHA deposits. The objective of this work is to evaluate the role of a dairy product enriched with fish oil on DHA levels in mother’s serum and cord blood.

Methods: A double-blind randomized and controlled trial was carried out with 95 pregnant women who were divided in 2 groups, women in the control group received 400 mL/day (in 2 doses of 200 mL) of a dairy product without DHA, whereas women in the fish oil group (FO) received the same product enriched with fish oil (100 mg of DHA/100 mL). Women consumed those products daily from week 28thof gestation. During all this time women from both groups received a controlled diet under the supervision of a dietician.

Results: The product was well tolerated for mothers and no adverse effects were reported. There were no differences between groups in the height, weight and head circumference of children at birth. APGAR test values were also in the normal range in both groups. There were statistically significant differences neither in gestational age at birth nor in the mode of delivery. At the moment of labor, women in the control group have experienced a statistically significant decrease of serum concentration of DHA. In contrast, serum DHA levels of women in the DHA group did not change from week 28 to labor. In addition eicosapentaenoic acid (EPA, 20: n-3) and DHA concentration in cord blood was significantly higher in the FO than in the control group.

Conclusion: Consumption of a dairy product enriched with fish oil during the last three months of gestation does not affect somatometric values of children during the first year of life, helps to avoid the decrease of DHA levels during gestation and increased DHA content in cord blood.

Disclosure of Interest: None declared.

PL-N-0157

Nutrition, Metabolism, and Experimental Approaches

BIFIDOBACTERIA SECRETED INTO BREAST MILK; TRUE OR FALSE? THE FACT IS CONTAMINATION BUT NOT SECRETION

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Objectives and Study: To investigate the origins of Bifidobacterium and Lactobacillus predominantly detected in the neonatal microflora, we have studied the population levels of those microorganisms in maternal feces, vaginal fluid, breast milk and babies’ feces by a micromolecular method.

Methods: We have performed quantitative analysis of Bifidobacterium and Lactobacillus on the samples of maternal feces, vaginal fluids, breast milk, skin regions around nipples and those of babies’ feces by quantitative RT-PCR (RT-qPCR) targeting 16S rRNA. The vaginal fluids and maternal feces were sampled at 56 days before and at the delivery, respectively. The babies’ feces were obtained on 0, 4, and 28 days after birth, and breast milk accompanied with skin swabs were sampled on day 4 and 28.

Results: 43 healthy mothers and their babies born by transvaginal delivery were enrolled in the study. At the delivery period, both Bifidobacterium and Lactobacillus were detected in 4 and 22 neonates’ feces out of 41 samples tested, respectively. Both bacterial populations were detected at the frequency of 12% and 91% in the vaginal fluids of the mothers before birth: sequence analysis figured out that 3 out of the Bifidobacterial species detected in the vagina were the same as those detected in the corresponding neonates’ feces just after birth. With regards to Lactobacilli, L. gasseri subgroup was detected predominantly in most of the vaginal fluids and the neonatal feces. In contrast,
Bifidobacterium the environment surrounding the mother but not from breast milk may be due to the contamination from infants or vaginal fluid but not secreting from breast milk.

Disclosure of Interest: None declared.

**Conclusion:** Bifidobacterium and Lactobacillus in the neonatal feces may originate in both of maternal feces and skin fluid but not secreting from breast milk.

Disclosure of Interest: None declared.

**PL-N-0158**

**Pediatric Nutrition**

**IMMUNE RESPONSE TO 100-MG GLUTEN INTRODUCED AT 4 MONTHS OF AGE IN CHILDREN WITH GENETIC RISK FOR COELIAC DISEASE**


Methods: The PreventCD birth cohort was recruited from 1/2007 to 7/2010 in 10 centers in 8 countries. Included were newborns with at least 1 first-degree CD relative. Children with DQ2 or DQ8 were randomized 1:1 to receive gluten from the age of 4 or 6 months.

Results: From the 1344 enrolled newborns 905 were randomised. At least 5-fold AGA increase over baseline was found in 129 infants at 6 months. This early AGA-IgA elevation exceeded the 95th centile of normal in 108 and the diagnostic cutoff of the kit in 72 infants, median value 36.2 U/ml (range 17–100), and declined with a median of 182 days (range 57–574). These frequencies correspond to 29%, 24% and 16% of children randomised to gluten. Up to 31.12.2010, 44 children developed anti-TG2 in the whole cohort of whom CD was confirmed by biopsy in 31, but only 4 of them had early AGA response. Children biopsied in reason of AGA without anti-TG2 (n = 5) had normal villi. The current frequency of anti-TG2/CD is 3.1% in the early CD group whereas 5.1% in the rest of children (P = 0.028).

Conclusion: Infants aged 4–6 months react often with AGA to small amounts of gluten introduced as the first oral antigen besides breast-feeding. This transient early AGA response does not seem to be disease inducing. Its possible role to delay, attenuate or prevent CD can only be established after a longer follow-up. The early AGA/DGP response should be further investigated at the level of T cells.

Disclosure of Interest: I. Korponay-Szabo: None declared, J. Gyimesi: None declared, S. Koletzko: None declared, K. Werkstetter: None declared, C. Hogen Esch: None declared, G. Castillejo: None declared, E. Mummert Employee of: Phadia GmbH, R. Troncone: None declared, F. Koning: None declared, M. L. Mearin: None declared.

**PL-N-0159**

**Nutrition, Metabolism, and Experimental Approaches**

**BILE SALT-STIMULATED LIPASE: A KEY ENZYME IN INFLAMMATION BESIDES IN NEONATAL FAT DIGESTION?**

1. S. Lindquist, 2. E.-L. Andersson, 3. L. Lundberg, 4. O. Hernell

Methods: The PreventCD birth cohort was recruited from 1/2007 to 7/2010 in 10 centers in 8 countries. Included were newborns with at least 1 first-degree CD relative. Children with DQ2 or DQ8 were randomized 1:1 to receive gluten from the age of 4 or 6 months.

Results: From the 1344 enrolled newborns 905 were randomised. At least 5-fold AGA increase over baseline was found in 129 infants at 6 months. This early AGA-IgA elevation exceeded the 95th centile of normal in 108 and the diagnostic cutoff of the kit in 72 infants, median value 36.2 U/ml (range 17–100), and declined with a median of 182 days (range 57–574). These frequencies correspond to 29%, 24% and 16% of children randomised to gluten. Up to 31.12.2010, 44 children developed anti-TG2 in the whole cohort of whom CD was confirmed by biopsy in 31, but only 4 of them had early AGA response. Children biopsied in reason of AGA without anti-TG2 (n = 5) had normal villi. The current frequency of anti-TG2/CD is 3.1% in the early CD group whereas 5.1% in the rest of children (P = 0.028).

Conclusion: Infants aged 4–6 months react often with AGA to small amounts of gluten introduced as the first oral antigen besides breast-feeding. This transient early AGA response does not seem to be disease inducing. Its possible role to delay, attenuate or prevent CD can only be established after a longer follow-up. The early AGA/DGP response should be further investigated at the level of T cells.

Disclosure of Interest: I. Korponay-Szabo: None declared, J. Gyimesi: None declared, S. Koletzko: None declared, K. Werkstetter: None declared, C. Hogen Esch: None declared, G. Castillejo: None declared, E. Mummert Employee of: Phadia GmbH, R. Troncone: None declared, F. Koning: None declared, M. L. Mearin: None declared.

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and in circulating blood collected from healthy volunteers. It was also found to be stored in platelets and released upon their activation. These novel findings encouraged us to define the hypothesis that BSSL is a player in the inflammatory process.

**Methods:** Collagen-induced arthritis (CIA) in mice is a commonly used experimental model with inflammation as a key component. CIA reproduces many of the pathogenic mechanisms of human rheumatoid arthritis (RA), i.e. increased cellular infiltration, synovial hyperplasia, pannus formation and erosion of cartilage and bone in the distal joints. We made use of the CIA model and compared the response in BSSL-deficient knock-out (BSSL-KO) mice and BSSL wild-type (BSSL-WT) mice. CIA was initiated by intradermal injection of collagen type II (CII) and 21 days later a boost immunization. Arthritis development (defined by swelling and redness of the joints) was followed by clinical scoring 2–3 times a week. Blood samples were taken at the end of the study.

**Results:** The most striking, BSSL-KO mice were protected from arthritis; only 1 out of 17 BSSL-KO mice developed disease (swollen joints) compared to 13 out of 16 BSSL-WT mice. Serum levels of anti-CII antibody response and cartilage oligomeric matrix protein (COMP-a marker for cartilage degradation) were determined. There was no difference in antibody response against CII, but significantly lower COMP levels were found in BSSL-KO compared to BSSL-WT mice, which correlates with the arthritis development. A follow-up study confirmed these results and further showed that BSSL-heterozygote mice are less prone to develop disease as compared to BSSL-WT mice but not as resistant as BSSL-KO mice.

**Conclusion:** These results strongly support that BSSL is a key player in the inflammatory process and open an intriguing question whether a BSSL-neutralizing agent could serve as a therapeutic model to reduce inflammatory activity.

**Disclosure of Interest:** None declared.

**PL-N-0160**

**Clinical Nutrition**

**ADDITIONAL EARLY VITAMIN A SUPPLEMENTATION IN INFANTS AT RISK OF RETINOPATHY OF PREMATURITY (ROP): EFFECT UPON BODY STORES OF VITAMIN A AND RETINAL FUNCTION AT 36 WEEKS’ POSTMENSTRUAL AGE (PMA)**

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**Objectives and Study:** Supplementing preterm infants with vitamin A improves respiratory function at 36 weeks PMA and may reduce the incidence of ROP. Intramuscular (IM) vitamin A reduces biochemical evidence of vitamin A deficiency but is not widely used: there are few data to support the current intravenous (IV) dose of vitamin A. Retinal sensitivity in infants at risk of ROP is reduced at term corrected age compared to newborn term infants, consistent with reduced retinal stores of vitamin A, and may better reflect vitamin A status than plasma retinol concentration. This study tested the hypothesis that retinal function in infants at risk of ROP would be improved by additional early vitamin A supplementation.

**Methods:** Double-blind randomised controlled study. 89 infants at risk of ROP were enrolled within 72 hours of birth and randomised to receive either standard nutritional support (including Vitlipid N at manufacturer’s recommended dose, delivering 920 IU vitamin A/kg/day) or standard nutrition plus 10,000 IU IM vitamin A thrice weekly from day 2 continued until routine oral supplementation at 14 days (or for a maximum of 12 doses in infants poorly tolerant of enteral feeds). Hepatic stores of vitamin A were assessed by relative dose response (RDR) at 36 weeks’ PMA. The primary outcome measure was dark-adapted retinal sensitivity, measured by electroretinogram at 36 weeks’ PMA.

**Results:** Median gestational age was 29.3 (range 24–33) weeks and median birthweight was 1130 (range 580–1800) g. Six babies died, 3 in each group. Additional vitamin A increased plasma concentration of retinol at 7 and 28 days (median 0.95 vs 0.5 μmol/L (P < 0.001) and 0.7 vs 0.6 μmol/L (P < 0.03), respectively), but did not affect plasma retinol at 36 weeks’ PMA. 58% of supplemented and 67% of control infants had evidence of reduced hepatic stores of vitamin A (RDR >10%); this difference was not significant. Retinal sensitivity was greater in supplemented infants (mean difference −0.19 log units, P < 0.03) and correlated with total vitamin A intake/kg (Pearson R = −0.27, P < 0.05), but was not related to RDR.

**Conclusion:** Early IM vitamin A supplementation in preterm infants increases plasma concentrations of retinol at 7 and 28 days and improves retinal function at 36 weeks’ PMA. The current IV dose requires revision.


**Disclosure of Interest:** None declared.

**PL-N-0161**

**Clinical Nutrition**

**BRANCHED CHAIN AMINO ACID REQUIREMENT IN THE TERM NEONATE**

F. Maingay-De Groof1,2, L. Huang3, G. Voortman1, C. Chen1,2, Y. Huang3, H. van Goudoever4, 1Neonatology, Erasmus MC Sophia, Rotterdam, Netherlands, 2Neonatology, 3Pediatrics, Fudan Children’s Hospital, Shanghai, China, 4Pediatrics, Academic Medical Centre/VU Medical Centre Amsterdam, Amsterdam, Netherlands.
Objectives and Study: Dietary intake should meet the requirement to obtain an optimal growth and neurodevelopment in the neonate. The essential branched chain amino acids (BCAAs), leucine, isoleucine, and valine, are mainly used for incorporation into body protein. The BCAA show interaction due to their common catabolic enzymes and the leucine:isoleucine:valine (Leu:Ile:Val) ratio influences protein synthesis. Current recommended BCAA requirements for infants 0–1 month (respectively 165, 95 and 95 mg·kg⁻¹·d⁻¹, ratio 1.8:1:1) are based on the amino acid content of human milk. Questions remain on the validity to use mean amino acid composition of human milk to determine requirements since human milk varies greatly in composition and intake. In adults and children > 6 months, a factorial approach is used that is based on the fact that maintenance requirement is the same in adults and children and the requirement needed for growth is added by using fetal protein and accretion data. By using this factorial approach in term neonates 0–1 month, the leucine, isoleucine and valine requirements are estimated to be lower than current recommendations (respectively 109, 59, and 72 mg·kg⁻¹·d⁻¹). The objective of this study is to quantify the requirement of leucine, isoleucine and valine in term neonates using the indicator amino acid oxidation method.

Methods: Enterally fed term infants received randomly graded intakes of leucine (15–500 mg·kg⁻¹·d⁻¹), isoleucine (5–216 mg·kg⁻¹·d⁻¹) and valine (5–236 mg·kg⁻¹·d⁻¹). Breath samples containing 13CO₂ were collected during L-[1-13C]phenylalanine (indicator amino acid) administration, measured by isotope ratio mass spectrometry and analysed using a biphasic regression crossover analysis.

Results: 83 term Asian neonates (birth weight: 3.29 ± 0.4 kg, gestational age: 39.4 ± 1.3 wks, postnatal age: 12.6 ± 5.1 d) were included. The mean requirement (at breakpoint) for leucine, isoleucine and valine was respectively 140, 105 and 110 mg·kg⁻¹·d⁻¹, which indicates an optimal Leu:Ile:Val ratio of 1.3:1:1.

Conclusion: Our study shows that current recommendations based on the content of amino acids in breast milk are closer to the actual needs than the estimations based on the factorial approach. The Leu:Ile:Val ratio should be 1.3:1:1 in term infant formula.

Disclosure of Interest: F. Maingay-De Groof: None declared, L. Huang: None declared, G. Voortman: None declared, C. Chen: None declared, Y. Huang: None declared, H. van Goudoever Grant/Research Support from: Danone Research, study formulas produced by SHS.

PO-G-0076/PD-G-0163

Food Allergy

COST-EFFECTIVENESS OF USING AN EXTENSIVELY HYDROLYSED FORMULA COMPARED TO AN AMINO ACID FORMULA AS FIRST-LINE TREATMENT FOR COW’S-MILK ALLERGY IN THE UNITED KINGDOM

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Objectives and Study: We have previously found decreased plasma levels of arginine (Arg) and citrulline (Cit) in critically ill children, with a strong inverse relation to C-reactive protein (CRP) (1). Arg de novo synthesis in the body comes from the precursor Cit only. We hypothesized that Arg becomes an essential amino acid as a result of reduced Cit availability during inflammation. Therefore we studied Cit and Arg production, using stable isotope technology, in relation to the severity of inflammation in critically ill children.

Methods: 22 critically-ill children (age 0.89 ± 0.04 years) with different levels of inflammation were studied on day 3 post-admission. They were subdivided in 3 groups: viral bronchiolitis (group 1, n = 9), infectious disease without shock (group 2, n = 6) and septic shock (group 3, n = 7). A 2-hour stable isotope tracer protocol was performed after at least 4 hours fasting to determine Arg and Cit kinetics. Data as mean ± SE. Statistics by ANOVA, Pearson’s correlation.

Results: CRP was significantly different between groups (group 1, 21.7 ± 8.6 mg/L; group 2, 151.5 ± 32.8 mg/L; group 3, 288.0 ± 33.6; group 1 vs 2 and 1 vs 3, P < 0.001; group 2 vs 3 P = 0.015). Cit production was significantly lower in the group with highest inflammation compared with the group with lowest inflammation (group 1, 10.00 ± 1.56 μmol/kg/h; group 2, 6.35 ± 0.75 μmol/kg/h; group 3, 4.67 ± 0.69 μmol/kg/h; group 1 vs 3, P < 0.05). Cit production was inversely correlated with plasma CRP (r = −0.58, P < 0.001).

Conclusion: With increasing rate of inflammation the production of Arg’s precursor Cit is severely depressed. Previously we found that de novo Arg production is almost equal to Cit production (2). As a consequence Arg availability becomes fully dependent on tissue protein breakdown and nutrition. Arginine becomes an essential amino acid during critical illness in children, depending on the rate of inflammation.

References

Disclosure of Interest: None declared.
Objectives and Study: To estimate the cost-effectiveness of using an extensively hydrolysed formula (eHF; Nutramigen) compared to an amino acid formula (AAF; Neocate) as first-line treatment for cow’s-milk allergy (CMA) in the UK, from the perspective of the National Health Service (NHS).

Methods: A decision model was constructed depicting treatment paths and associated resource use attributable to first-line management of CMA with the 2 formulae. The model was based on the case records of 145 AAF-treated patients and 150 matched eHF-treated patients from the Health Improvement Network (THIN) database (a nationally representative database of patients registered with general practitioners (GPs) in the UK). The model estimated the costs and consequences of patient management over 12 months following their initial GP visit for CMA.

Results: Patients with a combination of gastrointestinal (GI) symptoms and eczema accounted for 44% of all patients. Those with GI symptoms alone and eczema alone accounted for a further 39% and 13%, respectively. Those with urticaaria and failure to thrive accounted for <5% and <7% of all patients, respectively. Patients’ age at presentation was a mean 2.6 months. It took a mean 2.1 months for a formula to be prescribed after the initial GP visit. Time to symptom resolution after starting treatment with eHF and AAF was a mean 1.1 and 0.9 months, respectively. eHF-treated patients had a mean 13.1 GP visits over the 12 months compared to 17.5 visits made by AAF-treated patients (P < 0.001). The estimated NHS cost of managing a CMA infant over the first 12 months following the initial GP visit was £1853 and £3161 for an eHF-treated and AAF-treated patient, respectively. Clinical nutrition preparations accounted for 37% and 53% of the cost in the eHF and AAF groups, respectively. GP visits, outpatient visits and hospital admissions accounted for up to a further 25%, 16% and 12% of the cost, respectively. The incremental cost for each additional symptom-free week following use of AAF instead of eHF was estimated to be £1500, hence AAF was not considered a cost-effective treatment. The model was robust to changes in all model inputs and the only scenario considered a cost-effective treatment. The model was estimated the costs and consequences of patient management over 12 months following their initial GP visit for CMA.

Conclusion: First-line treatment of newly diagnosed infants with CMA with eHF (Nutramigen) instead of AAF (Neocate) affords a cost-effective use of NHS resources. Moreover, in the absence of published evidence showing superiority of one formula over the other, the eHF (Nutramigen) is the preferred first-line treatment in newly diagnosed infants receiving their first clinical nutrition preparation.


PO-G-0077/PD-G-0164

Food Allergy

CAN HYDROLYZED SOY PROTEIN FORMULA AFFECT THE DEVELOPMENT OF REPRODUCTIVE TISSUE: A PILOT STUDY WITH ULTRASONOGRAPHIC PATTERNS OF REPRODUCTIVE ORGANS IN INFANTS

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Objectives and Study: Phytoestrogens are compounds in plants that have estrogenic activity, being the soy the most concentrated source of isoflavones. Concerns have been raised about potential adverse consequences of isoflavones associated with soy protein isolate on development of reproductive tissues, including the mammary gland and uterus. However, little is known about the effect of hydrolyzed soy protein formula (HSF) on development of reproductive tissues. HSF, which contains 40% soy protein, has been used in Brazil for more than 12 years as a good option in replacing milk formula in cow’s-milk allergy (CMA). Until now no study has been performed to ensure whether the exposure to phytoestrogens HSF is safe.

Methods: The volumes of reproductive tissues, breast buds, uterus, ovaries, prostate, and testicles of infants, girls (G) and boys (B) (n = 15–5 G and 10 B) with CMA who received HSF (Alergomed, ComidaMed from Germany) for at least 6 months, were evaluated by ultrasonography. The birth weight and length, HSF intake period and age at ultrasonography were shown in Table 1.

Results:

<table>
<thead>
<tr>
<th></th>
<th>Girls</th>
<th>Boys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight, kg</td>
<td>3217±47.26</td>
<td>3238±200</td>
</tr>
<tr>
<td>Age at ultrasound, mo</td>
<td>18.67±0.04</td>
<td>12.40±3.20</td>
</tr>
<tr>
<td>HSF intake period, mo</td>
<td>8.0±0.00</td>
<td>6.6±1.15</td>
</tr>
</tbody>
</table>

The data were expressed as mean ± SD. The HSF intakes average (mL) were 623.30 ± 46.31 to G and 730.00 ± 83.67 to B. The phytoestrogens intake average (µg/L) were 12.47 ± 1.6 (G) and 14.60 ± 1.6 (B). No alteration was observed on the breast buds (G 0.15 ± 0.06; B 0.11 ± 0.05), ovarian (0.42 ± 0.08), uterus (0.87 ± 0.25) and prostate (0.70 ± 0.20) volumes in infants evaluated in relation to reference values for healthy normal infants.

Conclusion: Our preliminary data shows that the HSF ingestion for at least 6 months does not alter the reproductive organs size.


E88
FAECAL MICROBIOTA AND GUT METABOLIC PROFILES IN INFANTS WITH COW’S MILK PROTEIN ALLERGY BEFORE AND AFTER LACTOSE-CONTAINING DIET

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Objectives and Study: Cow’s milk protein allergy (CMA) has become a common disease in early childhood. The treatment of CMA is the elimination of CM protein by the use of extensively hydrolyzed formulas (eHF). However, thid elimination diet may lead to alteration of gut microbiota. The present study is aimed to investigate the gut microbiota and metabolic profiles in infants with CMA before and after lactose containing diet.

Methods: Infants with CMA were enrolled in a prospective trial. All infants received an eHF (whey) with no lactose (CMA-NL: Alfare`, Nestle`) for 2 months followed by an identical eHF containing 3.8% of lactose (CMA-L; Althera, Nestle`) for 2 months. Healthy infants with no history of CMPA had matched for age/gender were used as controls (HC). All infants provided faeces at the end of the lactose restricted and containing diet. The following determinations were performed: enumeration of cells present in faeces by FISH using probes targeting the main bacterial groups of human gut, counts of viable bacterial cell as described by Macfarlane (1); gas-chromatography mass spectrometry/solid-phase microextraction (GC-MS/SPME) analysis for the metabolic studies.

Results: 21 infants with CMA and 15 HC were recruited. Based on FISH and counts of viable bacteria the total cell numbers did not differ between the groups; however, the number of Bifidobacteria was higher in HC and CMA-L vs. CMA-NL (P<0.05) while the number of Bacteroides/Clostridia were significantly lower in HC and CMA-L vs. CMA-NL (P<0.05). Median values of lactic acid bacteria were similar HC and CMA-L while the lowest count was found in CMA-NL (P<0.01). The median concentration of total short chain fatty acids (SCFA) was significantly higher in faecal samples of CMA-L (SCFA) as compared to CMA-NL (P<0.05). Major differences were found for acetate and butyrate (P<0.05). Total median values of aminoacids (threonine, uridine, histidine, tyrosine, methionine, arginine) were significantly higher in CMA-L and HC as compared to CMA-NL (P<0.05).

Conclusion: The addition of lactose to an eHF is able to significantly increase the total fecal counts of Lactobacillus/Bifidobacteria and to decrease the counts of Bacteroides/Clostridia. This modification of the gut microbiota determines a significant modification of the metabolic profile with the increase of median concentration of SCFA (mainly acetate and butyrate acids) and aminoacids. The modulation of the gut microbiota with the increase of LAB/Bifidobacteria correlates with protection against atopy (2).

References

Conclusion: CMPA is usually transient (90%). However, CMPA is associated with an increased risk of other food allergies (34%) and asthma (38%). Baseline hypersensitivity to multiple foods is associated with both greater severity of CMPA (longer need for a cow’s-milk–free diet and higher rate of development of other food allergies) and asthma.

Disclosure of Interest: None declared.

PO-G-0075/PD-G-0167

Food Allergy

EXPLORATIVE EVALUATION OF MICROBIAL AND IMMUNE BIOMARKERS IN INFANTS AT RISK FOR ALLERGIES FED AN INTACT COW’S-MILK FORMULA–CONTAINING SPECIFIC NONDIGESTIBLE CARBOHYDRATES


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Objectives and Study: Oligosaccharides may alter postnatal immune development by influencing the composition of gastrointestinal microbiota. This study investigated the effect of a specific prebiotic mixture of short-chain galactooligosaccharides (scGOS) and long-chain fructooligosaccharides (lcFOS) on microbiota and immune biomarkers during the first 6 months of life in high-risk infants for allergies fed a formula based on intact cow’s-milk protein. The study was a prospective, double-blind, randomised, placebo controlled trial. If formula feeding was started, the infant was randomly assigned to 1 of 2 cow’s-milk formula groups (0.8 g/100 mL scGOS/lcFOS or maltodextrine as control). A reference group consisted of 90 exclusively breast-fed infants up to six months of age.

Methods: Faecal microbiota was analysed by use of selective media. In a subgroup blood was collected at 6 months of age for analysis of serum biomarkers for atopic dermatitis. Total and β-lactoglobulin-specific IgE and IgG4 was measured using immunoCAP. The functional degranulation capacity of IgE was tested with RBL-hEla-2B12 cells. Total kappa and lambda IgG-free light chain concentrations were determined by ELISA.

Results: 380 infants at risk for atopy were enrolled in the study. 51 infants in the prebiotic group and 51 infants in the control group completed the study. The scGOS/lcFOS supplementation showed a significantly higher number of faecal bifidobacteria and lactobacilli counts compared to controls, accompanied by significantly lower pH values in the faeces. In serum the scGOS/lcFOS group showed a trend towards a decrease in total IgE levels (8.4 kU/L vs 17.2 kU/L, P = 0.06) as well as a trend towards a decrease in the percentage of children with elevated (>15 kU/L) IgE (13.6% vs 28.6%, P = 0.1). There were no differences found in the amount of kappa and lambda IgG-LC between the two groups.

Disclosure of Interest: From this clinical study it can be concluded that the scGOS/lcFOS administration significantly influences the composition of intestinal microbiota. There were some reactions with respect to the immune parameters which need further investigation.

Disclosure of Interest: None declared.

PO-G-0078/PD-G-0168

Food Allergy

DIETARY N-3 PUFA SUPPRESS THE ALLERGIC EFFECTOR RESPONSE AND ENHANCE REGULATORY T-CELL NUMBERS IN A MOUSE MODEL FOR COW’S-MILK ALLERGY

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Objectives and Study: Cow’s-milk allergy is the most common food allergy in children and no effective treatment is available to prevent or cure the disease. Dietary components such as lipids can modulate the immune system. This might be beneficial in the prevention of (cow’s-milk) allergy. Aim of this study was to assess the effects of dietary supplementation with n-3 polyunsaturated fatty acids (n-3 PUFA) on the prevention of food allergy.

Methods: C3H/HeOuJ mice were fed a 4% soybean oil/6% tuna oil diet rich in n-3 PUFA (mostly docosahexaenoic acid (DHA)) or a control diet (10% soybean oil, high in n-6 PUFA) before and during oral sensitization with whey, using cholera toxin as an adjuvant. One week after the last sensitization the mice were challenged intradermally (in the ear pinnae) and orally with whey, using cholerat as an adjuvant. One week after the last sensitization the mice were challenged intradermally (in the ear pinnae) and orally with whey, using cholerat as an adjuvant. One week after the last sensitization the mice were challenged intradermally (in the ear pinnae) and orally with whey, using cholerat as an adjuvant. One week after the last sensitization the mice were challenged intradermally (in the ear pinnae) and orally with whey, using cholerat as an adjuvant. One week after the last sensitization the mice were challenged intradermally (in the ear pinnae) and orally with whey, using cholerat as an adjuvant. One week after the last sensitization the mice were challenged intradermally (in the ear pinnae) and orally with whey, using cholerat as an adjuvant.

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Disclosure of Interest: None declared.
concentrations in serum were strongly suppressed in the n-3 PUFA diet group ($P < 0.05$), and IgE showed the same tendency. The n-3 PUFA diet was found to enhance the percentage Treg in spleen after sensitization as compared to sham sensitized mice ($P < 0.05$). In addition, both the percentages of Th1 and Th2 cells in the spleen were reduced; this effect was most pronounced in sham mice fed the n-3 diet and resulted in a decreased Th1/Th2 ratio ($P < 0.01$).

**Conclusion:** Dietary n-3 PUFA largely prevented allergic sensitization in a mouse model for food allergy by increasing the percentage of Treg and suppression of a Th2 type B cell response, resulting in a strong reduction of the allergic effector response.

**Disclosure of Interest:** None declared.

**PO-G-0081/PD-G-0169**

**Food Allergy**

**DAILY COSTS OF DIET IN 12- AND 24-MONTH-OLD INFANTS WITH AND WITHOUT FOOD ALLERGY**

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**Objectives and Study:** Well constructed diet is essential in management of food allergy. Knowledge about dietary costs would assist health care professionals in improving the treatment and support of the patients and their families. The objectives here were to evaluate the daily cost of diet and factors contributing to the costs in infants with and without food allergy.

**Methods:** Children (N=80, 60.3% boys) with (n = 23) and without (n = 57) food allergy were evaluated at the ages of 12 and 24 months. The diet-related costs were calculated from 3-day diet records. The food prices were obtained from local supermarkets and prices of vitamin, mineral and food preparations from the University Pharmacy. Data on reimbursed part of cost due to use of hydrolysed infant formulas was a mean of €8.67 (SD 7.78) and 4.86 (SD 5.15) at the ages of 12 and 24 months. The daily cost of diet at 12 months correlated negatively to length of breast-feeding in both infants with ($r = -0.58$, $P = 0.004$) and without ($r = -0.37$, $P = 0.006$) food allergy and positively with the use of infant formula ($r = 0.86$, $P = 0.0001$) in allergic infants.

**Table 1. Average daily costs of diets**

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<thead>
<tr>
<th>With food allergy</th>
<th>Without food allergy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Average daily cost, €</strong></td>
<td><strong>n</strong></td>
</tr>
<tr>
<td>12 mo</td>
<td>23</td>
</tr>
<tr>
<td>24 mo</td>
<td>20</td>
</tr>
<tr>
<td>Infants using hydrolysed formula</td>
<td>12 mo</td>
</tr>
</tbody>
</table>

**Conclusion:** Management of infant’s food allergy increased only modestly the daily costs of diet to the family. The costs of diet due to use of hydrolysed infant formulas would be doubled to family without reimbursement. Longer breast-feeding was related to lower dietary costs. These results can be used to support families by health care professionals.

**Disclosure of Interest:** None declared.

**PO-G-0074/PD-G-0170**

**Food Allergy**

**REDUCTION OF THE ALLERGIC EFFECTOR RESPONSE IN MICE FED DIETARY PREBIOTICS DURING ORAL SENSITIZATION WITH WHEY**

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**Objectives and Study:** Cow’s-milk allergy is one of the most common food allergies in children. So far, no effective treatment is available to prevent or cure food allergy. The purpose of this study was to analyze the effects of dietary supplementation with specific prebiotic mixtures on the outcome of the allergic response when provided 2 weeks before and during oral sensitization with whey in mice.

**Methods:** Three-week-old female C3H/HeOuJ mice were fed diets containing different combinations and ratios of scGOS (short-chain galacto oligosaccharides), lcFOS (long chain fructose oligosaccharides) and/or pAOS (pectin- derived acidic oligosaccharides). Mice were orally sensitized to whey for 5 consecutive times during weekly intervals. The acute allergic skin response was determined by measuring ear swelling. Antigen-induced anaphylaxis was scored. Whey-specific serum immunoglobulin’s and mouse mast cell protease-1 were determined in serum. Mesenteric lymph node dendritic cells were characterized and T cell proliferation was measured using flow cytometry.
Results: In mice fed with the combination of scGOS/lcFOS/pAOS, both the allergic skin response and the anaphylactoid reaction were strongly reduced compared to whey-sensitized mice fed the control diet. The mouse mast cell protease-1, whey-specific IgE and IgG2a responses were decreased by dietary interventions with some combinations of probiotics but not all.

Conclusion: Dietary supplementation with scGOS/lcFOS/pAOS, provided before and during sensitization, reduces the cow’s-milk allergic effector response in a murine model of IgE-mediated hypersensitivity that mimics the human route of sensitization.


PO-G-0092/PD-G-0171

Gut Infection
IDENTIFICATION OF VIRULENCE-ASSOCIATED GENES OF SALMONELLA TYPHIMURIUM RESPONSIBLE FOR BACTERIAL ADHESION, INVASION, AND INTRACELLULAR REPLICATION IN HUMAN EPITHELIAL CELLS USING TRANSPONSON DIRECTED-INSERTION SITE SEQUENCING

Objectives and Study: To identify virulence-associated genes responsible for Salmonella adhesion, invasion, and intracellular replication in human epithelial cells using a high throughput screening model based on Transposon Directed Insertion-site Sequencing (TraDIS).

Methods: HEp-2 cells were infected (MOI = 50) with 1,440 Tn5 transposon mutants of Salmonella Typhimurium SL1344 (Input pool) for 2 hours, then the cells were: (i) treated with plain medium for 1 hour and washed to remove non-adherent bacteria (Output pool A; cell-associated mutants which adhere to and invade cells), (ii) gentamicin-treated for 1 hour to kill extracellular bacteria (Output pool B; cell-invading mutants), or (iii) infected for a further 7 hours (Output pool C; mutants which invade and proliferate within cells). Genomic DNA was then extracted from the Input/Output pools. TraDIS was used to determine Tn5 integration sites in the genomes of individual mutants from Input/Output pools. Individual integrations among the 4 pools were pairwise compared in their relative abundance expressed as a log2 fold change. P < 0.01 was considered statistically significant from 2 independent biological replicates.

Results: TraDIS identified 1,371 transposon mutants with 47 mutants involved in bacterial adhesion. The 4 genomic loci sucD-cybA, g1yA, yqiC, w+w, and rfaI were significant for adhesion and invasion. The 31 genes/intergenic loci (parB, rfaG, glnG, rfaP, araO, decS, acrB, yheO, SLP3_0003, udhA, rfaI, sucC, SLP3_0004, yrbH, pyrD, rfaQ, purH, fliJ, purD, metL, fimZ, yihW, gor, fimF, trxB, SLP3_0007/8, SL1344_4095, imp-djlA, ntpA-aspS, and vacT-ompH) were involved in bacterial cell-association, with fimZ and fimF only transiently responsible for adhesion. rbl, hiiD, and invA were required for invasion but not adhesion, whilst speG was required for intracellular replication but not adhesion or invasion. Interestingly, SPI-2 mutants were not impaired in adhesion, invasion, or intracellular replication in this system.

Conclusion: We identified novel Salmonella virulence-associated genomic loci responsible for adhesion, invasion, and intracellular replication. The “adhesive but noninvasive” or “invasive but nonproliferative” attenuated mutants are potential candidates as targeted vectors or oral vaccines.

Disclosure of Interest: None declared.

PO-G-0086/PD-G-0172

Gut Infection
GENES ASSOCIATED WITH IN VIVO ROTAVIRUS INHIBITORY ACTIVITY OF BIFIDOBACTERIUM LONGUM
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Objectives and Study: Molecular study to identify Bifidobacteria genes associated with in vivo rotavirus inhibitory activity. The method was a combination of bacterial genome sequencing and microarray analysis with protection tests in an infant mouse rotavirus disease model.

Results: B. longum strains were tested for their protective activity in an infant mouse rotavirus (RV) diarrhea model. B. longum strain NCC2705 significantly reduced diarrhea duration and diarrhea severity score. This RV-inhibitory strain was sequenced and protective and nonprotective strains were investigated for their gene content on NCC2705-specific microarrays by hybridization. According to this combined biological and hybridization screening, 11 NCC2705 genes remained associated with antirotavirus activity. To reduce the number of candidate genes of B. longum for anti-RV activity further, we reasoned that only NCC2705 genes transcribed in the intestine of mice are likely to affect the in vivo RV-inhibitory activity in the mouse model. Therefore we investigated the intestinal gene expression of NCC2705. About 900, 400 and 150 of the
about 2000 B. longum genes were expressed in the colon, cecum and small intestine, respectively. When asking for genes that were both in vivo expressed and specific to RV-inhibitory B. longum strains only two NCC2705 genes remained associated with the anti-RV phenotype: BL774 and BL1762. Gene BL774 is annotated as a nucleotide pyrophosphohydrolase, while BL1762 lacks any bioinformatic links. As knock-out mutants cannot be produced at will in B. longum, we opted for a knock-in approach of the candidate genes from the protective NCC2705 into the non-protective NCC3001 strain. Mice that received NCC3001 containing a plasmid with BL1762 showed a significantly reduced diarrhea duration and score compared to the infection control. This was not the case for mice receiving NCC3001 with a plasmid containing BL774 or a control gene from NCC2705 (BL884).

**Conclusion:** A combination of comparative genomics and transcriptome approaches led to the tentative association of a single albeit anonymous gene in B. longum with anti-RV activity as assessed in a mouse RV diarrhea model. RV-protective and non-protective strains were also found in B. breve. A close homologue of BL1762 was also found in B. breve, but its presence was not limited to the protective strains. Apparently, different species of bifidobacteria mediate anti-RV activity with different genes.


PO-G-0090/PD-G-0173

**Gut Infection**

**PROPHYLACTIC ANTIBIOTIC TREATMENT IMPROVES INTESTINAL FUNCTION AND RESISTANCE AGAINST NECROTIZING ENTEROCOLITIS IN PRETERM PIGS**

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**Objectives and Study:** Preterm birth, formula-feeding and inappropriate bacterial colonization predispose to necrotizing enterocolitis (NEC). However, the exact association between NEC and gut colonization remains unknown, and it remains unclear how prophylactic antibiotics alters the short- and long-term risk of NEC. We hypothesized that prophylactic broad-spectrum antibiotics would improve NEC resistance and intestinal function in the immediate postnatal period of NEC-sensitive preterm pigs.

**Methods:** Caesarean delivered preterm pigs (92% of gestation) received 2 d parenteral nutrition and minimal enteral nutrition prior to 2 d full enteral formula-feeding. From birth, one group (ANTI, n = 11) received oral and systemic antibiotics (gentamycin: 5 mg/kg/d; ampicillin: 200 mg/kg/d; metronidazole: 40 mg/kg/d) and a control group received saline (SALINE, n = 13). Sugar absorptive capacity was tested 24 h after introduction to full enteral feeding, as the blood increments of galactose following an oral test bolus. On day 5, pigs were euthanized and the gastrointestinal organs were weighed and evaluated for NEC. Intestinal samples were collected for analyses of digestive enzyme activity, villus height, goblet cell density and microbiology. Intestinal permeability was estimated as the post mortem urine lactulose/mannitol ratios following oral administration 4 h before euthanasia.

**Results:** The NEC incidence was 85% for SALINE and 0% for ANTI pigs (P < 0.0001). Although 9/11 ANTI pigs showed mild hyperaemia. Compared with SALINE, ANTI pigs had a higher relative intestinal weight (47%, P < 0.001), higher intestinal villi (44% P < 0.01), higher activity of aminopeptidases A and N, lactase and sucrase, (30–85%, P < 0.01), and more goblet cells in colon (100%, P < 0.0001). ANTI pigs tended to have improved sugar absorption (50%, P = 0.18) and intestinal integrity (–50% for lactulose/mannitol ratio, P = 0.21). Antibiotics reduced the total concentrations of short-chain fatty acids in stomach contents (~50%, P < 0.05) and colon contents (~95%, P < 0.01) and bacterial numbers in cecum contents (~3×10^5fold, P < 0.0001). Culture independent analysis (T-RFLP) supported lower density of total bacteria (~50% intensity, P < 0.01) and Enterococcus faecium (~70%, P < 0.001) in ANTI versus SALINE pigs.

**Conclusion:** Prophylactic antibiotics during the first days of life prevent NEC and improve gut structure and function, probably via reduced bacteria mediated inflammation. Possible side effects of broad-spectrum prophylactic antibiotics as well as more long-term effects on intestinal health and gut colonization remain to be determined.

**Disclosure of Interest:** None declared.

PO-G-0055/PD-G-0174

**Cystic Fibrosis**

**AGE-RELATED PATTERN OF INTESTINAL MICROFLORA IN CHILDREN WITH CYSTIC FIBROSIS**

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**Objectives and Study:** Modifications of gut microecology have been recently described in chronic inflammatory states, such as IBD and obesity and may play an active role in their
Pathogenesis. Intestinal inflammation is frequent in cystic fibrosis (CF) and improves in parallel with respiratory function following Lactobacillus GG (LGG) supplementation. We investigated the composition of intestinal microflora in CF children groups by age and its relationship with intestinal inflammation.

**Methods:** Intestinal inflammation was measured by fecal calprotectin. DGGE (denaturing gradient gel electrophoresis) analysis, RT-PCR (real-time polymerase chain reaction) and FISH (fluorescent in situ hybridisation) were used to study intestinal microecology.

**Results:** Forty-one CF patients were enrolled, including 6 infants (median age: 4 months), 19 children below 10 years (median: 7 years) and 16 children older than 10 years (median: 12.5 years). Calprotectin concentration was similar in CF infants and controls, but it was significantly increased in older CF children than in controls (184 ± 146 μg/g vs 52 ± 46 μg/g and 151 ± 147 μg/g vs 41 ± 54 μg/g, respectively, in both those < and > 10 years). Intestinal microflora was characterized by a reduced richness and a major variability in CF than in controls, with an age-dependent pattern. DGGE showed a decrease in Bacteroides species in infants. In CF < 10 years, there was a significant reduction of Eubacterium rectale \((1 \times 10^{10} \pm 2 \times 10^{10} \text{CFU/g of stools; } P < 0.01)\) and Bacteroides species \((1.28 \times 10^{9} \pm 3 \times 10^{9} \text{CFU/g of stools; } P < 0.01)\) and Bacteroides uniformis \((8.5 \times 10^{8} \pm 5.74 \times 10^{9} \text{CFU/g of stools; } P < 0.01)\) and Bacteroides uniformis \((1.23 \times 10^{9} \pm 4.7 \times 10^{7} \text{CFU/g of stools; } P < 0.01)\). Moreover, a modest reduction in Bifidobacterium pseudocatenulatum was shown by RT-PCR. In CF >10 years, DGGE profile showed more evident bands corresponding to Escherichia coli and Dialister invisus. FISH showed decreased levels of Eubacterium rectale, Bacteroides and the absence of Faecalibacterium prausnitzii CF patients. No apparent correlation between specific bacterial species and fecal calprotectin was observed.

**Conclusion:** Intestinal microecology in CF children shows an age-related abnormal composition. These findings may explain the clinical beneficial effects observed with LGG in CF. Targeting intestinal microecology through probiotic administration may be a novel strategy in the approach to CF.

**Funding:** Supported by Fondazione per la ricerca sulla fibrosi cistica (grant FFC#23/2009) and by Lega Italiana Fibrosi Cistica Onlus-Associazione Toscana Onlus e For me S.r.l.

**Disclosure of Interest:** None declared.

**PO-G-0070/PD-G-0175**

**Endoscopy: Diagnosis and Therapeutic Surgical Procedures**

**FREQUENCY AND FACTORS ASSOCIATED WITH GASTRIC GLANDULAR ATROPHY AND GASTRIC INTESTINAL METAPLASIA IN PEDIATRIC PATIENTS**

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**Objectives and Study:** Gastric glandular atrophy (GA) or gastric intestinal metaplasia (IM) are most frequently associated with _H. pylori_ (HP) infection in adults and may progress to dysplasia. On the contrary they are rarely observed in children and data concerning etiologic factors are scarce. The aim of the study is: to evaluate the frequency and factors associated with GA and IM in children.

**Methods:** Cross-sectional, retrospective, monocentric review, according to the updated Sidney system, of histological findings of all antral and fundic biopsies systematically performed in 2006–2009, followed by an ulterior review of the charts of the patients (P) presenting with GA or IM.

**Results:** Out of 2829 upper endoscopies, 30 were found positive for GA or IM in 23 P (16 M/7 F; median age 11 y, range 6 months to 20 y) presenting with either isolated IM in 21 or GA in 2 and an association of both in 1, localized in the antrum 22/23 and in the fundus only in 1/23. The main indications of endoscopy were: epigastric pain (8), suspicion of IBD (2) cyclic vomiting (1), failure to thrive (1) and follow-up after surgery (11) comprising oesophageal atresia (3), oesophageal agenesis with gastric transposition (3), fundoplication for GOR (5) with subsequent pyloroplasty in 3/5. None was diabetic and 1 presented with spondilarthritis and thyroiditis. Endoscopic abnormalities observed: antral erythema or erosion in 5/23, pangastritis in 2/23, excessive presence of bile in the stomach in 3/23 P. Giardia lamblia was isolated in 1 and HP infection known in 4/23 P (still active in 2 and successfully previously eradicated in 2). HP was absent in the remaining 19 even in those undergoing repetitive endoscopies. During a follow up period of 2 to 16 years (median 4 y) in 13/23 P, multiple endoscopies (2 to 5, median 4) with successive biopsies showed persistence GA or IM in 8/13 P (inconstantly in 1 of them) and absence in the remaining 5/13.

**Conclusion:** IM is more often observed than GA, but they are both infrequent. The low HP infection rate corresponds to the one observed in our population and is not the main aetiologic factor for GA or IM in our paediatric patients whereas a past history of surgery seems to play a key role perhaps due to an increased risk of biliary reflux. Patchy distribution of IM as well as usual random sampling of biopsies could yield false negative results. New endoscopic techniques such as NBI targeting more accurately biopsy sampling could allow a better evaluation of the true incidence, natural history and aetiology of GA and IM in children.

**Disclosure of Interest:** None declared.

**PO-G-0066/PD-G-0176**

**Endoscopy: Diagnosis and Therapeutic Surgical Procedures**

**CLINICAL CHARACTERISTICS AND MANAGEMENT OF CONGENITAL ESOPHAGEAL STENOSIS**

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**E94**

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Objectives and Study: Congenital esophageal stenosis (CES) is a rare clinical condition in childhood and is frequently associated esophageal atresia (EA). The purpose of this study was to present the French experience of CES in terms of diagnosis, management and outcome. Medical records of all the patients with CES from the French network on congenital and malformative esophageal diseases were reviewed retrospectively with regard to diagnostic method, therapy and outcome.

Results: During the last 18 years, 61 patients (30 boys) were found to have CES. CES was associated with esophageal atresia (EA) in 29 patients. The average age at diagnosis was 24 months (from 1 day to 14 years). Patients with associated EA were younger at the time of diagnosis than patients with isolated CES (7 vs. 126 months, P < 0.05). 24 of 61 CES had no clinical symptom: 6 were found fortuitously, 18 (of 29 with associated EA) were diagnosed at the time of surgical repair of EA or during post operative systematic esophageal x-ray opacification. For the 37 remainders initial symptoms were dysphagia (54%), vomiting (43%), and food impaction (54%). 15/37 patients presented with respiratory distress and/or dyspnea. 14/37 presented with impaired growth at diagnosis. Diagnosis of CES was confirmed with barium esophagram (56/61) and/or esophageal endoscopy (50/61) in all patients. Of the 61 patients, 16 had tracheobronchial remnants (TBR), 40 had a fibromuscular stenosis (FMS) and 5 had a membrane stenosis (MS). None had multiple stenosis; MS was never found in patients with EA (0/29, P < 0.05). 34 patients were treated by dilation only (13/34 were asymptomatic a follow-up), 15 patients were treated by dilation first but required surgery (4/15 remained asymptomatic at follow up), whereas 9 patients had immediate surgical intervention (4/9 were asymptomatic at follow-up). Dilations were complicated by esophageal perforation in 2 patients. At follow-up, dysphagia was present in 28% of patients (not different in EA group compared to isolated CS: 10/29 vs. 7/32, P = 0.27). All patients with TBR underwent operative repair (resection and anastomosis).

Conclusion: Dilation may be effective for treating patients with FMS and MS while surgical repair is the preferred treatment for TBR. CS associated with EA can be missed at the time of initial esophageal surgical repair.

Disclosure of Interest: None declared.

PO-G-0059/PD-G-0177

Endoscopy: Diagnosis and Therapeutic Surgical Procedures

STEPWISE DIAGNOSTIC APPROACH TO CHRONIC DIARRHEA COMPARED WITH THE ALGORITHM PROPOSED IN THE NEW NELSON TEXTBOOK OF PEDIATRICS

Objective and Study: Chronic diarrhea (CD) has a broad etiology and often requires an invasive, cumbersome and expensive diagnostic approach. Endoscopy is considered a key step for diagnosis. We evaluated the feasibility and efficacy of a multistep diagnostic approach to CD which is included in the new (19th edition) of the Nelson Textbook of Pediatrics.

Methods: The clinical records of patients with CD admitted to a tertiary care centre for gastrointestinal diseases were analyzed. The type and time sequence of diagnostic steps were recorded and compared with the algorithm proposed in the Nelson textbook. Children were divided in three groups according to the diagnostic procedure needed for final diagnosis: group 1 (G1) in which endoscopy is required for diagnosis (eg, inflammatory bowel diseases-and celiac disease); group 2 (G2) in which diagnosis is clinical (eg, toddler’s diarrhea) and group 3 (G3) including heterogeneous conditions whose diagnosis is reached with laboratory investigations (eg, infectious diarrhea).

Results: We analyzed 50 children (mean age 6.4 years, range 1m-18 y). G1 included 17 patients (11 IBD and 5 celiac disease, 1 with indeterminate colitis, mean age 8 y), G2 included 14 patients (6 with irritable bowel and 8 with functional diarrhea according to Rome III criteria, mean age 7 y) and G3 consisted of 15 patients (8 with infectious diarrhea, 7 with food allergy, mean age 3 y). In 4 patients the etiology was not detected. In the vast majority of patients, intestinal microbiology (45/50), fecal calprotectin (43/50) and serology for celiac disease (43/50) were obtained at first evaluation, in agreement with the Nelson stepwise approach. Other non invasive tests were less frequently performed, with a variable time lag after the first evaluation, such as the steatocrit and/or prick/patch tests, fecal elastase, abdominal ultrasound. Nutrient absorption tests were performed in a minority of patients (13/50). Endoscopy was performed in 22/50 children (44%), on average 8.5 days after initial evaluation. All G1 patients, 2 from G2 and 3/4 children without a final diagnosis underwent endoscopy, with a positive diagnostic yield of 77% (17/22).

Conclusion: A stepwise approach allows detection of the etiology in the vast majority of CD patients. In our series of CD children seen at a reference gastroenterology center, less than 50% underwent endoscopy and diagnosis was often based on combined evaluation of clinical data and specific non invasive tests. Endoscopy should be driven by noninvasive tests to limit unnecessary procedures and reduce costs, although this may result in a longer time to reach diagnosis.

Disclosure of Interest: None declared.
PO-G-0060/PD-G-0178

Endoscopy: Diagnosis and Therapeutic Surgical Procedures

ENDOSCOPICAL INJURIES IN A PEDIATRIC POPULATION: PERSONAL DECENNAL EXPERIENCE

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Objectives and Study: Gastrointestinal (GI) endoscopic procedure has become an essential modality for evaluation and treatment of GI diseases. Few studies are reported in literature especially in pediatric age. The aim of this study is to report a decennial experience of endoscopic complications in paediatric patients.

Methods: a review of our data (between 2000 and December 2010) identified 9874 patients who underwent upper or lower endoscopy. The range of age varied from 1 days of life and 18 years old, with a mean age of 8.4 years. 46% were female. Diagnostic examination in patients younger than 6 months were executed without sedation, but in patients younger than 20 days or with associated disease a neonatologist/anaesthetist was present at the endoscopy. In patients over 6 months who underwent a diagnostic endoscopy (upper endoscopy, rectalsigmoidoscopy) midazolam was administered by the gastroenterologist. In patients who underwent ileocolonoscopy sedation was performed by an anaesthetist. In patients who underwent operative endoscopy (polypectomy, dilatation, foreign bodies extraction, variceal ligation, endoscopic placement of videocapsule, placement of percutaneous endoscopic gastrostomy (PEG)) a sedation or a general anaesthesia in surgery room was administered by the anaesthetist.

Results: 3422 lower endoscopy (2348 complete colonoscopy, 1074 rectalsigmoidoscopy) and 6452 upper endoscopy were performed. 12.4% of the endoscopy were operative: dilatation 4.3%, foreign body extraction 3.2%, polypectomy 3.1%, variceal ligation 0.7%, PEG 0.8%, endoscopic placement of videocapsule 0.3%. No complications occurred during diagnostic examination. Two injuries (0.02%) happened during operative endoscopy; 1 perforation during a duodenal polypectomy, 1 bleeding 7 days after a rectal polypectomy. The duodenal perforation was immediately recognized and the child underwent surgery without any other complications. The rectal bleeding was stopped by placing 2 metallic clips and blood transfusion was administered. Minor complications such as sublingual hematoma happened in 1 patient (0.01%). No major complication associated with the sedation happened.

Conclusion: Endoscopic injuries are uncommon, especially during diagnostic endoscopies, although gastroenterologists with paediatric experience are necessary.

Disclosure of Interest: None declared.

PO-G-0097/PD-G-0179

Immunology

THE ROLE OF MANNOSE-BINDING LECTIN GENE POLYMORPHISM IN THE PROGRESSION OF CHRONIC HEPATITIS B INFECTION IN CHILDREN

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Objectives and Study: Although chronic hepatitis B is a preventable infectious disease, it is still an important health problem. Vertical and horizontal ways are the main transmission routes for children and adolescents. The progression of hepatitis B infection depends on transmission route, virulence factors of the virus and immune system of the host. Mannose-binding lectin is a member of innate immune system and activates complement system through lectin pathway. Mannose-binding lectin deficiency is considered to be associated with infectious and autoimmune diseases. In this study the relation of mannose-binding lectin gene polymorphism and serum levels with the progression of chronic hepatitis B infection in children is evaluated.

Methods: The study included 67 patients aged between 2 and 18 years with the diagnosis of chronic hepatitis B. The patients divided into three groups according to the disease status: immuntolerant, chronic inactive and treatment group. Mannose-binding lectin gene polymorphism and serum levels were measured in all patients. The associations of the clinical, laboratory and histopathological findings with the serum levels of mannose-binding lectin and the existence of mannose-binding lectin gene polymorphism were evaluated.

Results: Mannose-binding lectin gene polymorphism rates were found to be higher in our patient group than general population; homozygous codon 54 mutation was found in 8.9% and heterozygous mutation was found in 11.9% of our patients. Serum mannose-binding lectin levels were inversely correlated with gene polymorphism. The rate of mutation was similar in all groups and moreover it was not different in responsive and nonresponsive patients in the treatment group. Lower levels of serum mannose-binding lectin are found to be related with higher histological activity in liver biopsy specimens, without a statistical significance.

Conclusion: Codon 54 mutation of mannose-binding lectin gene is seen commonly in children with chronic hepatitis B infection. This mutation is considered to be a risk factor for the persistence of disease, but it did not have an influence on the progression of chronic hepatitis B infection in children.

Disclosure of Interest: None declared.

PO-G-0156/PD-G-0180

Intestinal Motility

99 PATIENTS AND COUNTING: WHAT HAVE WE LEARNED FROM A 10-YEAR EXPERIENCE USING ANTEGRADE ENEMAS IN CHILDREN?
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Objectives and Study: Administration of antegrade enemas through a cecostomy is an increasingly popular therapeutic option in children with constipation and/or fecal incontinence after failure of maximal conventional therapy. The aim of this study was to describe a single-center 10-year experience with the administration of antegrade enemas.

Methods: Retrospective analysis of 99 patients (57 boys) receiving a cecostomy between 2000 and 2010 at Nationwide Children’s Hospital. Medical history, symptoms, irrigation regime, complications and outcome were reviewed.

Results: Patients (median age 8 years, range 2–22 years at the time of the procedure) were followed for a mean time of 46 months (range 2–125 months) after the cecostomy placement. 71 patients had the cecostomy placed percutaneously by interventional radiology and 28 by surgery. Coexistent diagnoses included spinal abnormality (n=34), cerebral palsy (n=8), imperforated anus (n=14), Hirschsprung disease (n=8), urological disorders (n=43), and behavioral problems (n=18). Before cecostomy placement, 88.9% of the children had constipation, 74.7% fecal incontinence and 54.5% had been hospitalized for disimpaction. 65 subjects had lifelong symptoms, in the other 34 subjects the mean duration of symptoms was 5.9 years. Major complications, which required hospital admission or surgical intervention, occurred in 12 patients; minor complications in 47 and 40 patients had no complications. After using daily antegrade enemas 71% became symptom-free, in 20 subjects symptoms improved, in 2 patients symptoms did not change and in 7 subjects symptoms worsened. Poor outcome was associated with surgical placement of the cecostomy (P<0.001), history of Hirschsprung disease (P=0.05), cerebral palsy (P=0.03), previous abdominal surgery (P=0.01), younger age (P=0.02) and shorter duration of symptoms (P=0.01). Children with a previous abnormal colonic manometry had a significant worse outcome vs patients with normal colonic motility (P=0.004). In 88% the most successful irrigation solution included use of a stimulant laxative and those patients did significant better (P<0.001) than subjects who started without a stimulant. In 13 patients the cecostomy was removed a mean time of 49.7 months after its placement because of resolution of symptoms.

Conclusion: Antegrade enemas are a successful therapeutic option in children with severe constipation and/or fecal incontinence. We have identified factors associated with successful outcome. A subgroup of patients may be weaned from the daily use of antegrade enemas without recurrence of symptoms, possibly related to improved function of the previously dilated rectum or to overcoming withholding behavior.

Disclosure of Interest: None declared.

Intestinal Motility

PO-G-0148/PD-G-0181

Objectives and Study: Autism spectrum disorders (ASDs) are commonly diagnosed in children with an estimated prevalence of 0.6 to 1%. Several studies have shown a higher prevalence of functional defecation disorders in children with an ASD when compared to the general population. However, no data are available about the prevalence of ASDs and autism spectrum features in children with functional defecation disorders. The aim of this study was to describe the co-occurrence of autism spectrum disorders and autism spectrum symptoms in children presenting with functional defecation disorders.

Methods: Children (age 4–12 yrs) presenting at a specialized outpatient clinic with functional constipation or functional nonretentive fecal incontinence according to the Rome III criteria were included in this study. Parents or caregivers were asked to complete 2 validated questionnaires about their child; the Social Responsiveness Scale (SRS) and Social Communication Questionnaire Lifetime (SCQ-L).

Results: A total of 144 patients (74 male) with a mean age of 7.8 yrs were included in this study. Of these, 129 children fulfilled the Rome III criteria for functional constipation and 15 were diagnosed with functional nonretentive fecal incontinence. Eight children (5.5%) had been previously diagnosed with an ASD. Fourteen out of 144 children (9.7%) had both SRS and SCQ-L scores above cutoff points, strongly suggestive for the presence of an ASD. Solely high SRS scores were present in 8 children (5.5%), whereas 4 children (2.8%) only scored above the cutoff point of the SCQ-L, being both suggestive for the presence of an ASD.

Conclusion: These results show that a substantial amount (18%) of children presenting with functional defecation disorders at a tertiary hospital have concomitant symptoms of autism spectrum disorders. This percentage is much higher than the prevalence of ASDs in the general population.
and deserves further investigation. Screening for ASDs in children with functional defecation disorders seems feasible as ASDs in these children are frequently overlooked by clinicians.

Disclosure of Interest: None declared.

PO-G-0167/PD-G-0182

Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori

WHICH IS THE MOST RELIABLE DIAGNOSTIC TEST FOR CLARITHROMYCIN RESISTANCE OF HELICOBACTER PYLORI?

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Objectives and Study: A reliable diagnostic test for clarithromycin resistance of Helicobacter pylori (Hp) is important as it is a key factor of Hp eradication failure in adults and children. Resistance to clarithromycin in clinical Hp isolates is caused predominantly by distinct point mutations within the peptidyl transferase centre of 23S rRNA. There are some tests to determine resistance such as restriction fragment length polymorphism (PFLP), fluorescence in situ hybridisation (FISH), polymerase chain reaction (PCR) and agar dilution. The aim of this study was to compare FISH, RFLP and agar dilution for determination clarithromycin resistance in Hp-positive patients.

Methods: A total 100 Hp-positive gastric biopsy samples obtained from adults and children according to histopathological examination were included to study. Samples were examined for the presence of clarithromycin resistance of Hp by FISH, RFLP and agar dilution methods. Fluorescent-labeled oligonucleotide probes binding to Hp 23S rRNA sequences were used for FISH analysis. The 23S rRNA gene of Hp was amplified by PCR and the mutations responsible for clarithromycin resistance were detected for RFLP with Bsa1 and Bbs1 restriction endonucleases. Phenotypic antibiotic susceptibilities of the isolates were tested with agar dilution.

Results: FISH and RFLP tests were applied to all samples, but agar dilution test was used only in 52 cultured samples. Clarithromycin resistance rates according to FISH, RFLP and agar dilution results were 26%, 16% and 7%, respectively. The results reveal that there was strong positive correlation between the results of FISH and RFLP. However there were not such strong correlations between RFLP and culture and FISH and culture.

Conclusion: The agar dilution test has low positive results because of the number of cultured samples were almost half of the whole samples. RFLP results have an acceptable rate, but this result only shows A2144G and A2143G mutations in 23S rRNA. FISH is cheaper, detects more mutations with less effort, and had highest results in the study. Therefore, we suggest that FISH is an appropriate method for determination of Hp clarithromycin resistance, even when live bacteria are no longer available.

Disclosure of Interest: None declared.

PO-G-0162/PD-G-0183

Oesophagus, GDR, Ulcer Disease, andHelicobacter pylori

A RANDOMIZED SHAM-CONTROLLED TRIAL OF LEFT LATERAL BODY POSITIONING VS. ACID SUPPRESSION FOR INFANTILE GASTROESOPHAGEAL REFUX

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Objectives and Study: Treatments for symptoms of infantile gastroesophageal reflux disease (GERD) include proton pump inhibitors (PPI) but lack evidence of efficacy. Left lateral body positioning (LLP) reduces GER episodes and may be a useful adjunct therapy. However, LLP also delays gastric emptying (GE). We assessed the effect of LLP in infants with symptoms of GERD.

Methods: Fifty-nine infants (0–6 mo) with symptoms of GERD were investigated by 8 hr pH-impedance with symptoms marked at the time of occurrence, GE breath test and the IGERQ parental questionnaire (Kleinman et al, 2006 Clin Gastroenterol Hepatol). Using a parallel group design, infants with a positive symptom association probability for GER and symptoms (SAP >95%) were randomized to 1 of 4 therapies for 2 weeks; LLP + PPI (1 mg/kg omeprazole o.d.), head of cot elevation (HE) + PPI (1 mg/kg omeprazole o.d.), HE + AA. HE and AA were considered sham therapies. PPI and AA were given double blind. LLP and HE were performed for 2 hr after feeding with infants supervised to prevent accidental prone positioning. After 14 days studies were repeated on therapy. Analyses of GER and GE were performed blind. For all variables, the change from baseline to D14 was compared across the four treatment arms using a 2-way analysis of variance general linear model and pairwise multiple comparison procedures.

Results: 35 patients (16 male, mean age 11 wks (range 0–24 wks)) were included for analysis (10 patients had a negative SAP, 8 patients had a normal IGERQ and 6 patients withdrew). The average difference in the number of symptoms recorded on D14 compared to baseline was reduced during LLP and was increased during PPI treatments. The effect of LLP reached statistical significance (Table). A reduction in the number of GER episodes (liquid + mixed) was more likely with LLP than PPI but was not significant (P=0.136 vs P = 0.954, respectively). LLP significantly

Disclosure of Interest: None declared.
slowed GE while PPI significantly reduced esophageal acid exposure (Table).

<table>
<thead>
<tr>
<th>No. symptoms</th>
<th>LLP (20.2)</th>
<th>HE (18.7)</th>
<th>PPI (11.5)</th>
<th>AA (5.4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. GER</td>
<td>18.7 (4.7)</td>
<td>–8.3 (4.8)</td>
<td>–13.7 (4.7)</td>
<td>–13.3 (4.8)</td>
</tr>
<tr>
<td>Acid exposure (% time pH&lt;4)</td>
<td>–2.7 (1.8)</td>
<td>–4.8 (1.8)</td>
<td>–6.8 (1.8)*</td>
<td>–0.6 (1.8)</td>
</tr>
</tbody>
</table>

**Conclusion:** LLP significantly improves GERD symptoms. PPI on its own or in combination with LLP produces no symptomatic benefit.

**Disclosure of Interest:** None declared.

**PO-G-0168/PD-G-0184**

**Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori**

**CHANGES IN THE FATTY ACID COMPOSITION OF BLOOD CELL MEMBRANES IN CHILDREN WITH INFLAMMATORY DISEASES**

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**Objectives and Study:** Inflammatory diseases of the gastrointestinal tract (IDGIT) represent an important problem because of their high proportion in children morbidity and rather frequent severe courses of these diseases resistant to traditional therapy. Precursors of inflammation regulators - pro- and anti-inflammatory eicosanoids are metabolites of various fatty acid families: omega 6 and omega 3, respectively. Our aim was to study fatty acid composition of erythrocyte membranes in children with inflammatory diseases of the gastrointestinal tract (with normal body mass and obesity) in comparison with basically healthy children and fatty acid composition of leukocyte membranes in children with bronchial asthma.

**Methods:** Fifty seven children aged from 7 to 14 years were examined: 13 with IDGIT (eosophagitis, gastroduodenitis, stomach ulcer), 25 with IDGIT complicated by obesity (stages I–II), 9 with bronchial asthma and 10 basically healthy children. Composition of cell membranes fatty acid methyl esters was studied by gas-liquid chromatography. Results were expressed as % of the sum of cell membrane fatty acids. Statistical treatment was performed using the Student t test, the nonparametric Mann-Whitney test, and the SPSS 14 program. Differences were considered as statistically significant at P < 0.05.

**Results:** The study revealed for the first time that both IDGIT and bronchial asthma caused significant and similar changes in fatty acid composition of cell membranes as compared to healthy children. These included accumulation of omega-3 eicosapentaenoic acid (EPA) and the decrease of docosahexaenoic acid (DHA); this phenomenon observed in both erythrocyte and leukocyte membranes suggests a common feature of the detected changes in fatty acid composition of cell membranes at 2 different types of inflammation. There was a significant decrease in the level of membrane omega 6 polyunsaturated fatty acids (PUFA), first of all arachidonic acid and total omega-6 PUFA. EPA accumulation in membranes may be a compensatory response to low dietary omega-6 PUFA supply and/or their increased loss for the synthesis of pro-inflammatory eicosanoids (prostaglandins, leukotriens, thromboxans) during inflammatory process.

**Conclusion:** Significant changes of the fatty acid composition of erythrocyte membranes in children with IDGIT were found for the first time. These changes were typical for different types of inflammation and various types of cells. It may be proposed that changes in fatty acid composition of cell membranes can significantly influence their functional properties. This may be a pathogenically important characteristic property of these inflammatory diseases, which requires specific therapeutic approaches including those aimed at fatty acid membrane composition correction by diet therapy.

**Disclosure of Interest:** None declared.

**PO-G-0165/PD-G-0185**

**Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori**

**ROLE OF SYMPTOM INDEX AND SYMPTOM ASSOCIATION PROBABILITY AS PREDICTING FACTOR OF RESPONSE TO ANTI-SECRETORY TREATMENT**

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**Objectives and Study:** Several studies evaluate the contribution of the symptom index (SI) and symptom association probability (SAP) in the diagnosis of GERD. Watson et al. (Gut 1997;40:587–90) showed a correlation between a positive SI and the outcome of treatment with proton pump inhibitors (PPIs). Our aim is to evaluate the correlation of SI and SAP and the response to PPIs in children.

**Methods:** Eighteen children with reflux symptoms, recorded with a validated questionnaire, and with an abnormal Multichannel Intraluminal Impedance/pH-monitoring (MII/pH), were treated with PPIs (1.4 mg/kg/day) for 3 months. At the end of the treatment, all patients were called to report symptoms using the same questionnaire. For each patient we calculated the SI and the SAP and correlated them to the outcome of treatment. Statistical evaluation was done with the Fisher exact test (P < 0.05 was considered statistically significant).

**Results:** 9/18 (50%) children had positive SI (>50%); of them 5 (55.5%) patients became symptom free. 5/9 children...
(55.5%) with a negative SI were symptom free and 4 were not cured (P: 0.36). The same evaluation was done for the SAP. 8/18 patients (44.4%) had positive SAP (>95%); of them 5 children (62.5%) were healed and 3 were not. This outcome was compared with the 10 patients who did not have a positive SAP; of them 6 patients were healed and 4 were not (P: 0.64). Five patients had positive SI and SAP; of them 3 patients were symptom free and 2 were not. This result was compared with the 10 children who had a negative SI and SAP; of them 6 patients were healed and 4 were not (P: 0.71).

**Conclusion:** Our study did not show a relation between a positive SI, SAP, or both and the response to the PPI treatment. We cannot confirm in children the data reported by Watson and co-workers in adults. SI and SAP evaluate the relation between reflux and symptoms but they cannot be considered as predicting factors for the outcome of PPI treatment in children. Cut-offs of SI and SAP have been considered as predicting factors for the outcome of PPI treatment in children. The same cut-offs in children.

**Disclosure of Interest:** None declared.

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**PO-G-0177/PD-G-0186**

Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori

**GASTROESOPHAGEAL DISEASE AND EXERCISE-INDUCED BRONCHIAL HYPERREACTIVITY IN ASTHMATIC CHILDREN IN 48-H ESOPHAGEAL PH-IMPE DANCE MONITORING**

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**Objectives and Study:** Bronchial asthma and gastroesophageal reflux disease (GERD) may coexist together. Exercise induced cough and bronchospasm are results of uncontrolled asthma but also they may be caused by GERD. The aim of the study was to detect the relationship between exercise and simultaneous reflux episodes (RE) and their implication in developing of cough and/or bronchospasm in asthmatic children.

**Methods:** 30 children with uncontrolled bronchial asthma and/or coexisting cough were enrolled into the study (16 boys, mean age 14.2 ± 2.3 yrs). 48-hour esophageal pH-impedance was performed in all children. The exercise test was done during pH-impedance monitoring. The records before and after exercise test were analysed, seperately for pH-metry and pH-impedance. Asthma treatment was continued during the investigation in all children.

**Results:** Acidic GER was diagnosed in 8 (26.6%) children based on pH-metry. Both, acidic and nonacidic GER were found in 14 (46.6%) children based on pH-impedance monitoring. The exercise test did not increase reflux episodes in 6 children with positive exercise test (decreased FEV1>10%) nor in 24 children with negative exercise test.

Total impedance all reflux percent time (TIARPT) was significantly higher in children treated with longacting b-2 agonists (1.82% ± 0.59 vs 1.22% ± 0.51 (P < 0.016)).

**Conclusion:** Gastroesophageal reflux episodes were not relevant to exercise induced bronchospasm and/or cough in studied group of asthmatic children. Short-lasting intensive exercise did not induce reflux episodes. In asthmatic children nonacidic GER is more common than acidic.

**Disclosure of Interest:** None declared.

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**PO-G-0028/PD-G-0187**

Coeliac Disease and Enteropathies

**CELIAC PATIENT TRANSGLUTAMINASE 2-TARGETED AUTOANTIBODIES INHIBIT ANGIogenesis IN VIVO LIKELY THROUGH OVEREXPRESSION OF RHOB**

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**Objectives and Study:** Celiac patient-derived anti-transglutaminase 2 (TG2) antibodies disturb several steps in angiogenesis at least in vitro, but it is not known whether this also occurs in vivo and what is the detailed molecular mechanism. Therefore, we performed in vivo angiogenesis “matrigel plug” assays in mice to solve whether the presence of celiac autoantibodies inhibit in vivo angiogenesis. In addition, we analyzed by microarray technology the expression of a set of genes related to angiogenesis and endothelial cell biology in order to identify factors which could explain the anti-angiogenic effects of celiac patient autoantibodies.

**Methods:** In vivo angiogenesis was assayed in mice by matrigel plug assays in the presence of anti-TG2-targeted miniantibody derived from a celiac patient or relevant control antibodies. Human umbilical vein endothelial cells (HUVECs) were treated with either celiac patient or non-celiac control total IgA and the expression of 116 genes were analyzed by microarray technology. The importance of the identified gene products in the anti-angiogenic effects exerted by celiac patient autoantibodies was verified by small interfering RNA (siRNA).

**Results:** Celiac patient autoantibodies inhibited angiogenesis in vivo. In addition, celiac patient IgA induced a consistent up- or down-regulation of 10 genes including ras homolog gene family member B (RhoB). RhoB expression was found to be up-regulated at both mRNA and protein level in response to celiac patient total IgA as well as anti-TG2-targeted miniantibody derived from a celiac patient. Down-regulation of RhoB by specific siRNA treatment
could rescue the anti-angiogenic effects caused by celiac disease anti-TG2 antibodies.

**Conclusion:** We conclude that antibodies against TG2 can inhibit angiogenesis in vivo and they modulate both the expression of genes and protein synthesis related to angiogenesis. In addition, RhoB plays a key role in the regulation of the vasculature in the context of celiac disease.

**Disclosure of Interest:** None declared.

**PO-G-0035/PD-G-0188**

**Coeliac Disease and Enteropathies**

**INDICATIONS FOR REDUCED ABSORPTION OF A FAT BOLUS DURING METHOTREXATE-INDUCED GASTROINTESTINAL MUCOSITIS IN A RAT MODEL**

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**Objectives and Study:** Gastrointestinal mucositis is a severe and debilitating side effect of chemotherapy, especially in children. Patients with mucositis often suffer from weight loss and malnutrition. We developed a methotrexate (MTX)-induced mucositis rat model to study nutrient digestion and absorption. We previously showed that during mucositis, glucose absorption is still intact, when supplied in trace amounts, in spite of decreased mRNA and protein expression of glucose transporters. Since others found fatty acid transporters I-FABP and L-FABP to be less affected than glucose transporters during mucositis, fat absorption might be preserved during mucositis. Here, we studied plasma appearance of stable isotope labeled saturated and unsaturated fatty acids during mucositis, as an indicator of fat absorption.

**Methods:** Young Wistar rats (6 wk old) were i.v. injected with MTX (60 mg/kg) or NaCl 0.9% (controls). Four days later, during MTX-induced mucositis, we orally administered an [U-13C]palmitic acid- and [U-13C]linolic acid-enriched, meal size fat bolus (25% olive oil - 75% medium chain tricaprylglycerol oil mixture, 400 ul/rat) and quantified appearance of labeled fatty acids in the plasma for 6 hours (by gas chromatography-mass spectrometry). The capacity to absorb fatty acids in MTX- and NaCl-treated rats was estimated by calculation of the area under the concentration curves (AUC) of labeled fatty acids during the experimental period (time 0–6 hour). Finally, we collected the small intestine to assess histology and mucosal myeloperoxidase (MPO) levels, and determined plasma citrulline levels.

**Results:** MTX-treated rats suffered from severe mucositis, as shown by profound villus atrophy and epithelial damage, increased MPO levels (34-fold) and decreased citrulline levels (7-fold), as compared to controls (both \( P < 0.01 \)). From 1 hour after bolus administration on, plasma concentrations of [U-13C]palmitic acid and [U-13C]linolic acid were significantly decreased in MTX-treated rats, as compared to controls (\( P < 0.01 \)). During the experimental period, the AUC of both [U-13C]palmitic acid and [U-13C]linolic acid was 5.5-fold respectively 6.0-fold lower in MTX-treated rats, as compared to controls (\( P < 0.01 \)).

**Conclusion:** We conclude that plasma appearance of saturated and unsaturated fatty acids is severely decreased during MTX-induced mucositis, when orally administered as a bolus. Our data are indicative of reduced absorption of a fat bolus during mucositis. Therefore, bolus feeding seems not an adequate method to administer fat to patients with chemotherapy-induced mucositis.

**Disclosure of Interest:** None declared.

**PO-G-0039/PD-G-0189**

**Coeliac Disease and Enteropathies**

**DUODENAL MICROBIOTA AND EXPRESSION OF TOLL-LIKE RECEPTORS AND THEIR REGULATORS IN CHILDREN WITH COELIAC DISEASE**

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**Objectives and Study:** Coeliac disease is a common autoimmune disease triggered in small intestine by gluten proteins in individuals expressing HLA-DQ2 or HLA-DQ8 genes. However, less than one tenth of the carriers of these risk genes develop the disease indicating that also other genetic and environmental factors are important in the pathogenesis of coeliac disease. Role of gut microbiota has been addressed in a few studies with inconsistent findings. The aim of our study was to evaluate microbiota, its receptors (Toll-like receptors, TLRs) and regulators of the TLRs in the duodenum of children with coeliac disease.

**Methods:** Local microbiota and expression of TLRs, their regulators and cytokines in duodenal biopsies were analysed by quantitative PCR (21 microbial group- and species-specific primers) and rtPCR (15 gene-specific primers) in 10 children with coeliac disease (untreated coeliacs), 9 children with normal duodenal mucosa (controls) and 6 adult coeliacs with normal duodenal mucosa who had followed gluten-free diet (treated coeliacs).

**Results:** Differences in the characterised duodenal microbiota components were small between controls, untreated coeliacs and treated coeliacs. Expression of interleukin 8, a marker of intestinal inflammation, was significantly increased in untreated coeliacs when comparable with treated coeliacs and controls (\( P = 0.001 \)). Expression of TLR-9 was significantly increased whereas expression of TLR-2 was significantly decreased in untreated and treated coeliacs when compared with controls (\( P = 0.03 \) and \( P = 0.003 \), respectively). Expression of Toll-interacting protein (Tollip), an inhibitor of TLR-signalling, tended to be upregulated in controls when compared with untreated and treated coeliacs (\( P = 0.06 \)).
Conclusion: Different duodenal expression of TLRs and their inhibitor in treated and untreated coeliacs when compared with controls suggests that microbiota-associated factors may be important in the development of coeliac disease.

Disclosure of Interest: None declared.

PO-G-0025/PD-G-0190

Coeliac Disease and Enteropathies

ANTI-TISSUE TRANSGLUTAMINASE ANTIBODIES ACTIVATE CYTOSOLIC TISSUE TRANSGLUTAMINASE BY MOBILIZING CALCIUM IONS FROM INTRACELLULAR STORES

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Objectives and Study: Active celiac disease is accompanied by the presence of serum antibodies against tissue transglutaminase (tTG), a ubiquitously expressed multifunctional protein. Beside its presence in the extracellular matrix and at the cell surface, tTG is mainly localized in the cytosol where its catalytic activity is regulated by the concentration of intracellular calcium ions. Since we preliminarily observed that acute exposure to anti-tTG antibodies activated normally inactive cytosolic tTG, we aimed to investigate whether anti-tTG antibodies could modulate intracellular calcium ions level.

Methods: We performed functional studies on calcium homeostasis by Fura-2 AM single cell microfluorimetry. We also used specific store depotry to establish the involvement of each organelle. We performed in situ tTG activity assay by using the substrate 5-(biotinamido)-pentylamine. We revealed activity by conventional microscopy and quantify 5-(biotinamido)pentylamine incorporation inside the cells by spectrophotometric analysis.

Results: Rapid administration of anti-tTG antibodies (both commercial CUB 7402 and recombinant minibody from celiac patient, clone 2.8) determined an increase of intracellular calcium concentration in Caco-2 cells, both in the presence and in the absence of extracellular calcium ions. This effect was partially prevented by both the mitochondrial uncoupler FCCP and by thapsigargin, an inhibitor of SERCA ATPase located on the endoplasmic reticulum. We found that antibodies-induced calcium release from intracellular stores was able to activate cytosolic tTG. In fact, in situ activity of intracellular tTG increased of about 55% respect to basal value of untreated cells. Microscopic observation revealed that tTG activity was increased both in cytosolic and nuclear compartments. On the contrary, non specific IgG did not modify either basal tTG activity or intracellular calcium level.

Conclusion: By inducing rapid calcium mobilization, anti-tTG antibodies can potentially trigger several intracellular signalling, as well as activate calcium-dependent enzymes. In particular, activation of cytosolic tTG may have an important and still poorly unknown role in the context of celiac disease pathogenesis.

Disclosure of Interest: None declared.

PO-G-0052/PD-G-0191

Coeliac Disease and Enteropathies

NOVEL MUTATIONS UNDERLYING MICROVILLOUS INCLUSION DISEASE


Objectives and Study: Microvillous inclusion disease (MVID) is a congenital disorder of the intestinal epithelial cells that cause an intractable watery diarrhea with usual onset near birth. MVID is characterized by lack of microvilli on the surface of enterocytes with the occurrence of intracellular vacuolar structures containing microvilli. MVID is a rare disorder inherited as autosomal recessive trait. Recently, mutations of MYO5B were identified as the underlying lesion resulting in MVID.

Methods: Three Saudi families with 3 children with clinical diagnosis of MVID were investigated. Available unaffected individuals were subjected to genome-wide homozygosity scans using the Affymetrix 250K SNP array. Analysis with the copy number tool CNAG identified shared homozygous regions unique to the affected subjects.

Results: Of the 3 families with MVID, homozygosity was observed in 2 families at a locus on chromosome 18 which included MYO5B. Sequencing of MYO5B in individuals from these families identified 2 novel nonsense mutations in exons 24 and 36 (Q1047X and E1589X). In the third family, homozygosity was absent at the MYO5B locus. However, a locus on chromosome 2 which included EpCAM was found to be homozygous in this family. Sequencing of EpCAM identified a 1 bp insertion (c.499insC) in exon 5 resulting in premature truncation of the mature protein. This was consistent with congenital tufting enteropathy rather than MVID.

Conclusion: The present study has identified novel nonsense mutations in MYO5B and EpCAM associated with autosomal recessive enteropathies in Saudi families. Our findings expand the limited spectrum of MYO5B and EpCAM mutations associated with gastrointestinal genetic disorders and provide an opportunity to investigate phenotype/genotype correlations.

Disclosure of Interest: None declared.
PO-G-0027/PD-G-0192

Coeliac Disease and Enteropathies
TREATMENT OF SCREEN-DETECTED AND ASYMPTOMATIC COELIAC DISEASE PATIENTS: A RANDOMIZED CLINICAL TRIAL

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Objectives and Study: Due to the continuously increasing serological screening of coeliac disease, asymptomatic patients are frequently detected. At present the long-term outcome and possible benefits of a gluten-free diet in these individuals remains obscure. The aim of this randomized trial was to evaluate whether screen-detected and asymptomatic adult coeliac patients would benefit from an early diagnosis and dietary treatment.

Methods: The serum endomysial antibodies were screened from a total of 3031 voluntary relatives of coeliac disease patients. Those who were under 18 years of age, had earlier coeliac disease diagnosis, refused to participate or had significant comorbidities were excluded. Altogether 40 asymptomatic and endomysial antibody-positive subjects continued on trial and were randomized either to start a gluten-free diet or continue with their normal diet. An upper gastrointestinal endoscopy, extensive serological and clinical evaluations and bone mineral density measurement were carried out both at baseline and after 1 year in all. Furthermore, in each visit the study subjects fulfilled validated Gastrointestinal Symptom Rating Scale (GSRS) and Psychological General Well-Being (PGWB) questionnaires. After the first year of study also those who had been on a gluten-containing diet could start the treatment and undergo reevaluations after another year.

Results: The small-bowel mucosal villous damage ameliorated and celiac antibodies decreased in subjects on a gluten-free diet, whereas no significant change was observed in those who remained on gluten. Also, although the participants were apparently asymptomatic, both the total GSRS and PGWB scores improved significantly in the gluten-free diet intervention group but not in the gluten group. Among the laboratory parameters the folic acid and vitamin B12 levels increased significantly in the gluten-free diet group. There were no significant differences between or changes within the study groups in bone mineral density. After the trial was completed, 85% of the patients were willing to continue on strict gluten-free diet and 58% experienced their serological screening either as “positive” or “very positive.” None experienced it as “negative.”

Conclusion: Our results indicate that an early diagnosis and treatment of coeliac disease is beneficial in most of the screen-detected and apparently asymptomatic individuals. In addition, a relatively good adherence to gluten-free diet can be achieved also in this patient group.

Disclosure of Interest: None declared.

PO-G-0100/PD-G-0193

Immunology
TOLL-LIKE RECEPTOR 2/6 STIMULATION IN COMBINATION WITH INTESTINAL INFLAMMATION LEADS TO TH17 RESPONSES AGAINST ORALLY ADMINISTERED ANTIGENS

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Objectives and Study: Interest in T helper cells during the initiation and progression of inflammatory bowel disease (IBD) has increased as a result of the probable role of Th17 cells in IBD pathogenesis. The dextran sodium sulfate (DSS) model of colitis recruits many T lymphocytes to the inflamed colon, however, very little is known about the subtype (Th1, Th2, Th17 and Treg) and what factors steer their development. Research on gut antigen-primed T cells in DSS colitis is hampered by a lack of knowledge about the antigens presented in the gut. Thus, an oral tracker antigen (ovalbumin) was employed to follow gut antigen-primed T cells in mice under the influence of gastrointestinal Toll-like receptor (TLR) triggering during DSS-induced colitis.

Methods: DSS-colitis was induced by administering DSS (1.5%) in the drink water over a period of 6 days. Ovalbumin and the TLR ligands were given orally during the DSS treatment and mice were sacrificed 1 week later. Adaptive immune responses were measured by examining T cell responses and numbers with flow cytometry before and after ex vivo stimulation with ovalbumin.

Results: Ovalbumin-specific CD4+ T cells were detected in the spleens and mesenteric lymph nodes of mice after the resolution of inflammation (14 days after the start of DSS administration). These responses were found in mice that were treated orally with bacteria or with ligands for the TLR2/6 heterodimer during colitis and not in mice that were treated orally with ligands for TLR1/2 and TLR4. Using antibodies specific for transcription factors, it was determined that the ovalbumin-specific CD4+ T cells were Th17 or Treg, expressing either RORγT or Foxp3 respectively.

Conclusion: These results demonstrate that breaking tolerance against gut antigens requires a combination of local inflammatory signals to develop gut antigen-specific Th17 and Treg cells that may be found systemically after the resolution of inflammation. These insights will ultimately help elucidate how the gut environment and pathogen-associated molecular patterns steer the development of adaptive immune responses during the initiation of colitis and how pathogen-recognition receptors can be used to manipulate
the development or resolution of inflammation to treat pediatric gastrointestinal disease.

Disclosure of Interest: None declared.

PO-G-0099/PD-G-0194

Immunology

IMMUNE DYSFUNCTION IN PATIENTS WITH PTEN HAMARTOMA TUMOR SYNDROME

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Objectives and Study: The PTEN/P13K/Akt signalling pathway is critically involved in cell proliferation, migration and apoptosis. Mice with defects in this pathway develop multiple alterations in T and B lymphocyte homeostasis leading to thymus hyperplasia, lymphadenopathy, autoimmunity and lymphomas. Development and maintenance of Foxp3+ regulatory T cells have been linked to this pathway. The immunological consequences of PTEN deficiency in humans with Bannayan-Riley-Ruvalcaba syndrome and Cowden syndrome are not understood.

Methods: We investigated gastrointestinal immune activation in 12 unrelated patients with PTEN hamartoma tumor syndrome (PHTS) by multicolor-immunofluorescence microscopy. These studies were complemented by FACS and in vitro proliferation and apoptosis assays.

Results: Immune dysregulation in patients with Bannayan-Riley-Ruvalcaba syndrome and Cowden syndrome included thymus hyperplasia, tonsil hypertrophy and extensive intestinal lymphoid hyperplasia in stomach, small intestine and colon. Large numbers of preferentially naïve lymphocytes circulate and accumulate in lymphoid organs. There was decreased apoptosis as well as increased proliferation and mTOR signalling within CD20+CD10+ germinal centre B cells. B1 lymphocytes accumulate. The intestinal lymphoid hyperplasia was associated with normal proliferation within T cell areas and normal numbers of CD4+FoxP3+ T cells. However, FOXP3+ T cells showed increased proliferation and activation of the mTOR pathway in situ suggesting a threshold effect of PTEN activity. Except for inflammatory intestinal polyps there was no further intestinal inflammation or signs of autoimmunity.

Conclusion: These data show that functional loss of the central cell cycle regulator PTEN is associated with defects B cell homeostasis and T cell subsets and mucosal immune dysregulation in humans.

Disclosure of Interest: None declared.

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Immunology

INTESTINAL SENSITIVITY TO BACTERIAL LIGANDS AROUND PRETERM AND TERM BIRTH

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Objectives and Study: Preterm neonates show enhanced sensitivity to nutrient malabsorption and bacteria-mediated gut inflammatory disorders, such as necrotizing enterocolitis (NEC). Toll-like receptors (TLRs) recognizing Gram-negative bacterial ligands, eg, TLR4, are hypothesized to play a pivotal role in NEC, but it is unclear whether increased TLR4 expression is a predisposing factor or an effect of NEC. We hypothesized that intestinal sensitivity to bacterial endotoxins is increased after preterm birth and reduced in the first days after birth. Hence, we investigated the immediate postnatal development in nutrient absorption and inflammatory factors in the preterm and term pig intestine.

Methods: Pigs were delivered by caesarean section at preterm (92% gestation, n = 20) or term (n = 17) gestation. Small intestinal sections were collected at birth or after two days of colostrum feeding, followed by stimulation with lipopolysaccharide and mixed bacterial cultures, previously collected from preterm pigs with NEC. Brush-border enzyme activity and nutrient absorption in tissues were determined ex vivo, and expression of the inflammation related genes IL-6, TNFα, TLR1, 2, 4, 5, and 9 was measured by quantitative real-time PCR.

Results: Brush border enzyme activities were reduced in newborn preterm vs. term pigs (39–45%, P < 0.05) but increased to similar levels after 2 days of feeding. Leucine and glucose absorption increased with gestational age before birth, and decreased following feeding after birth. Bacterial stimulation reduced the nutrient uptake similarly at birth and after 2 days in preterm and term pigs (23–41%, P < 0.05), whereas IL-6 and TNFα expression increased only at birth. At birth, no difference was seen in TLR1, 2, 4, 5, and 9 in preterm vs. term pigs. The expressions of all TLRs were markedly higher in term 2-d-old pigs, compared with all other groups (23–44-fold, P < 0.001).

Conclusion: Digestive and absorptive functions increase in the prenatal period and they are affected by postnatal feeding and bacterial exposure, but to a similar extent in preterm and term pigs. NEC in preterm neonates can therefore not be explained by an abnormal response of the immature intestine to feeding and bacterial toxins. Since TLRs were markedly upregulated only in 2-d-old term pigs (normally showing no signs of NEC), overexpression of TLRs are unlikely to be a key predisposing factor to NEC in preterm neonates.

Disclosure of Interest: None declared.
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Immunology

INTESTINAL PROTEOME CHANGES AS BIOMARKERS OF INFANT NECROTIZING ENTEROCOLITIS

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Objectives and Study: Necrotizing enterocolitis ( NEC ) is a serious gut inflammatory condition that often requires surgical resection of parts of the infant small intestine and colon. Preterm birth, inappropriate enteral feeding and bacterial colonization are three main predisposing factors to NEC, but the disease etiology is unknown. We hypothesized that a global proteome analysis of all NEC-related protein changes in the small intestine and colon would help to identify biomarkers of disease progression and markers to differentiate between serious and more mild tissue inflammation (requiring surgical or medical treatment, respectively). Corresponding proteome analyses using a preterm pig model of NEC (1) can be used to validate proteome expression changes in infants with NEC.

Methods: Gel-based proteomic analysis was performed for 0.5 cm tissue sections removed from human infants with surgical NEC intervention of the small intestine (n = 6) and/or colon (n = 4). The proteomes of necrotic sections and the adjacent healthier tissue sections from the same intestine or colon were compared.

Results: Up to 30 proteins could be identified that showed differential expression between NEC and healthy sections for each gut section (small intestine, colon). Histamine receptor subunit, cytoskeletal proteins and immunoglobulins were found both in small intestine and colon. Heat shock proteins (HSPA5 and HSP27) that had been identified as sensitive markers in our porcine NEC model (Jiang et al. 2008, 2009) were identified also in human small intestine. Western blot analyses showed that the effects were limited to the small intestine and immunohistochemistry indicated that the response may be related to intense epithelial contact with commensal bacteria, as supported also from our pig studies (1). Novel possible biomarkers of NEC progression were related to angiogenesis, antioxidation systems and secretory proteins.

Conclusion: Numerous intestinal proteome changes occurred during the progression of infant NEC. If biomarkers of these tissue proteome changes can be identified in urine or plasma, such markers may help to evaluate NEC severity in individual patients, and thereby the need for surgical and/or medical interventions in preterm infants suffering from this devastating intestinal disease.

References:

Disclosure of Interest: None declared.

PO-G-0095/PD-G-0197

Immunology

THE EFFECT OF ENTERAL SUPPLEMENTATION OF A PREBIOTIC MIXTURE OF NEUTRAL AND ACIDIC OLIGOSACCHARIDES ON IMMUNOGLOBULIN FREE LIGHT CHAINS IN PRETERM INFANTS

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Objectives and Study: Preterm infants have immature immune system. Prebiotic oligosaccharides may influence the intestinal microbiota and may positively modulate postnatal development of the immune system. Previous studies show that prebiotic oligosaccharides decrease the incidence of atopic dermatitis in term infants. Immunoglobulin free light chains (Igflc) may play a role in the pathogenesis of atopy. We hypothesise that a prebiotic mixture consisting of neutral and acidic oligosaccharides (scGOS/lcFOS/AOS) may decrease the serum concentration of Igflc. Therefore, we aimed to determine the effect of enteral supplementation of scGOS/lcFOS/AOS on Igflc in preterm infants.

Methods: In a randomised controlled trial, preterm infants with a gestational age <32 weeks and/or birth weight <1500 g received enteral supplementation of scGOS/lcFOS/AOS or placebo (maltodextrin) between days 3 and 30 of life in a maximum dose of 1.5 g/kg/day. Serum samples were taken at 1 year of age. Igflc consist of 2 identical light chains; κ-Igflc and λ-Igflc. Total serum concentrations of κ-Igflc and λ-Igflc were analysed using ELISA. Data were expressed as median (range) and analysed by linear regression analysis.

Results: In total, 42 infants in the prebiotics and 41 infants in the placebo group were included. Baseline patient and nutritional characteristics were not different between both groups. In the prebiotics group, κ-Igflc serum concentration (μg/mL) was 13.2 (4.9–61.3) and λ-Igflc serum concentration (μg/mL) was 13.2 (4.4–71.3). In the placebo group, κ-Igflc serum concentration (μg/mL) was 11.7 (4.3–43.7) and λ-Igflc serum concentration (μg/mL) was 14.9 (3.1–81.0). There was no difference in both the κ-Igflc and λ-Igflc between the prebiotics and placebo group (Beta 0.09, 95% CI −0.16–0.40; P = 0.41 and Beta 0.04, 95% CI −0.22–0.30, P = 0.75, respectively). Correction for possible confounding factors did not change the results.

Conclusion: Enteral supplementation with a prebiotic mixture consisting of neutral and acidic oligosaccharides does not influence the κ-Igflc and λ-Igflc serum concentration in...
Disclosure of Interest: E. Westerbeek: None declared, B. C. van Esch: None declared, J. Garssen Employee of: Also partly employee of Danone Research, Danone Research provided the preterm formula (Nenatal Start) and post-discharge formula (Nenatal 1), neutral and acidic oligosaccharides and placebo supplementation. The funding source had no role in the study design, data collection, data analysis and interpretation of the study results, R. van Elburg: None declared.

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Immunology
MODULATION OF RSV SPECIFIC T-CELL RESPONSES BY SPECIFIC ORALLY APPLIED NONDIGESTIBLE CARBOHYDRATE
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Objectives and Study: Prebiotic nondigestible carbohydrates (NDC) are known to modulate intestinal microbiota composition. However, the exact mechanisms of associated immune-modulating effects are still poorly understood. Therefore, we set out to investigate the role of TLR-signaling and immune modulating capacity of specific dietary prebiotic oligosaccharides scGOS/lcFOS/pAOS for Respiratory Syncytial Virus (RSV) infected mice.

Methods: Female C57BL/6 mice were fed semi synthetic AIN-93G diet with or without scGOS/lcFOS/pAOS for a period of 5 weeks. To assay primary RSV specific immune responses, mice were intranasally (i.n.) infected with 2×10⁶ PFU RSV-A2 and sacrificed at time points indicated. Secondary RSV specific immune responses after vaccination with formalin inactivated alum adjuvanted vaccine (FI-RSV) were measured after i.n. live virus challenge. Time dependent development of lung antigen specific T-cell responses against 2 RSV epitopes and complete virus was examined by MHC/tetramer and intracellular cytokine staining. Lung single cell suspensions were stimulated for 6 h with: I. pulsed MHC/tetramer and intracellular cytokine staining. Lung against 2 RSV epitopes and complete virus was examined by flow cytometry development of lung antigen specific T-cell responses were measured after i.n. live virus challenge. Time dependent RSV specific immune responses after vaccination with formalin inactivated alum adjuvanted vaccine (FI-RSV) were measured after i.n. live virus challenge. Time dependent development of lung antigen specific T-cell responses against 2 RSV epitopes and complete virus was examined by MHC/tetramer and intracellular cytokine staining. Lung single cell suspensions were stimulated for 6 h with: I. pulsed MHC/tetramer and intracellular cytokine staining. Lung against 2 RSV epitopes and complete virus was examined by flow cytometry.

Results: The primary immune response is dominated by IFN-γ producing CD4⁺ and CD8⁺ T cells that can be visualized from day 6 after (i.n.) RSV infection. Dietary intervention with scGOS/lcFOS/pAOS resulted in a significant (P<0.05) increase in lung CD4⁺ IFN-γ production 8 days postinfection. In addition a significant (P<0.05) decreased CD4⁺ IL-4, −5 and −13 cytokine production 6 days postinfection in the more severe “enhanced disease” RSV infection. At day 8 after challenge the diet GFA was no longer significantly different for Th2 cytokine responses as compared to control diet. However, at this time IFN-γ production by CD4⁺ T cells was significantly enhanced in mice receiving GFA as compared to control diet.

Conclusion: These results indicate that orally applied specific oligosaccharides can modulate immunity locally in the lungs of RSV infected mice. The role of microbiota changes as well as TLR involvement will be the subject of further investigation.


PO-H-0321/PD-H-0199

Hepatology
NONINVASIVE BIOMARKERS AND TRANSIENT ELASTOGRAPHY IN MONITORING LONG-TERM GRAFT FUNCTION IN PAEDIATRIC LIVER TRANSPLANT RECIPIENTS
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Objectives and Study: Chronic graft fibrosis and hepatitis have been increasingly reported in long term liver transplant recipients. In a significant number of children post-liver transplantation, fibrosis may develop silently over the years leading to graft loss. Liver biopsy is currently the accepted method of assessing fibrosis. However, this is a static, invasive measure and repeat biopsy carries a risk of morbidity and mortality. The aim of this study was to evaluate the use of noninvasive markers of liver fibrosis in post-transplant patients.

Methods: Children underwent a protocol liver biopsy at 10 years post-transplant (only children who had biochemically normal liver function were included). Blood was taken on the day of biopsy. ELISA was used to assay plasma for CK18M30 fragments. Serum was analysed for the enhanced liver fibrosis test (ELF) using an immune-1 analyser. A smaller cohort also underwent transient elastography (TE). Biopsies were scored for fibrosis by a hepato-histopathologist using a standard score from F0 (no fibrosis) to F4 (cirrhosis).
Results: Twenty children (11 male); median age 14 years, were recruited. Initial diagnosis was biliary atresia in 8, Alagille in 3, acute liver failure in 3 and miscellaneous in the remainder. Fibrosis stage: F1 in 10, F2 in 7, F3 in 3, none had F0 or F4. Median ELF scores for F1, F2 and F3 were 9.83, 10.2 and 10.99ng/ml. AUROC for severe fibrosis (≥F3) for ELF was 0.74, CK18M30 and APRI had AUROC of 0.667 and 0.627 respectively. TE had an AUROC of 0.875 for ≥F3.

Conclusion: Liver fibrosis was universal in children even with normal LFTs at 10 years post-transplantation. The use of blood biomarkers in combination with TE proved an effective mode of monitoring fibrosis progression in liver allografts.

Disclosure of Interest: None declared.

PO-H-0281/PD-H-0200

Hepatology

THE RETROSPECTIVE VALIDATION OF SELECTED SCORES PREDICTING OUTCOME IN CHILDREN POISONED WITH AMANITA PHALLOIDES

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Objectives and Study: Amanita phalloides poisoning is common reason of acute liver or multiorgan failure in paediatric patients in summer and autumn every year in Poland. Mortality is still high (12% to even 50%) and liver transplantation (LTX) is necessary in many cases. It is still crucial to predict which patients require LTx to prevent death. The aim of the study was retrospective validation of selected (Ganzert’s, Escudie’s, Kleine’s and King’s College Hospital) scores predicting outcome in children with acute liver failure due to Amanita phalloides poisoning.

Methods: We retrospectively estimated data of 78 children with acute liver failure (INR > 2.0 or INR > 1.5 and encephalopathy) due to Amanita phalloides poisoning hospitalized in our center from 1983 to 1990 (before LTx and extracorporeal liver support therapy in children were available in Poland). 35 (aged 8.2 ± 3.5) died, 43 (aged 8.9 ± 3.6) remained alive. The sensitivities and specificities of selected scores in this group of patients were assessed.

Results: The results of selected scores were as follows: [sensitivity (95% CI); specificity (95% CI)], Ganzert’s criteria: 0.48 (0.29 to 0.67); 0.97 (0.85 to 0.99), Escudie’s criteria: 0.37 (0.21 to 0.55); 0.97619 (0.87 to 0.99), Kleine’s criteria: 0.97 (0.85 to 0.99); 0.581395 (0.42 to 0.72), King’s College Hospital criteria: 0.45 (0.28 to 0.63); 0.90 (0.77 to 0.97).

Conclusion: As it is crucial to establish which patients require LTx, the Klein’s scores is the most sensitive to select them, despite it is the lowest specificity.

Disclosure of Interest: None declared.

PO-H-0336/PD-H-0201

Transplantation

LONG-TERM IN VITRO CULTURED ADULT-DERIVED HUMAN LIVER PROGENITOR CELLS MAINTAIN APPROPRIATE GATEKEEPERS, PREVENTING CELL TRANSFORMATION

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Objectives and Study: Liver cell transplantation is a promising treatment for human liver inborn errors of metabolism diseases. Therapeutic use of adult derived human liver progenitor cells (ADHLPC) rely on the demonstration of their stabilized properties during long-term culture. We therefore investigated in vitro and in vivo genetic stability of these cells cultured up to senescence.

Methods: ADHLPC were isolated from 12 adult cadaveric donors. Cells were characterized by measuring cell cytoplasmic and surface markers expression by flow cytometry, immunofluorescence and qPCR. We followed the growth, cell morphology and anchorage dependance at each passage. Hepatic differentiation potential was assessed by performing karyotype, telomere length, measure of telomerase activity and gene expression related to tumorogenesis. Tumorigenic potential was investigated after injection of 1 × 107 cells in a xenograft model.

Results: Proliferative capacity was variable between cell cultures. Cells maintained their original phenotype and could acquire mature hepatic metabolic functions after differentiation. ADHLPC (n = 8/12) that grew fast (mean doubling time 6.35 days) were cytogenetically and reached senescence after a culture period of 142 ± 47 days. Four cell cultures demonstrated early growth slowdown (mean doubling time 28.6 days) correlated to premature senescence as shown by positive senescence associated beta galactosidase staining. In those, random karyotype instability was detected from 6th-8th culture passage. Cytogenetic anomalies were different for all cell populations. There was a significant relationship between longer time in culture and the occurrence of aneuploidy (P < 0.01). Chromosomal instability was not correlated with a tumorogenic potential in vivo or in vitro. The cells did not express telomerase activity or alternative telomere lengthening mechanisms. Human telomerase reverse transcriptase expression was not detected. Function and/or expression of cell cycle related genes such as p53, p16, pRb were normal. Nude mice subcutaneously injected with ADHLPC did not develop tumors.

Conclusion: ADHLPC can be expanded in vitro while maintaining a stable phenotype and differentiation capacity. A few cell cultures displayed random karyotype instability after long-term culture, leading to early senescence. Despite these observations, all ADHLPC cultures progressively enter

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Disclosure of Interest: None declared.

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Transplantation
PORTAL PRESSURE IN CHILDREN WITH INTESTINAL FAILURE ASSOCIATED LIVER DISEASE
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Objectives and Study: Approximately 40%–60% of children on long term parenteral nutrition develop intestinal failure associated liver disease (IFALD). A careful assessment of the severity of IFALD is important to guide therapy, and in transplant candidates to determine whether isolated intestinal or combined liver-intestinal transplant is indicated. Liver biopsy has a limited role as appearances can be patchy. Splenomegaly detected on ultrasound scan is not always a useful indicator of portal hypertension in IFALD and oesophageal varices are a late finding. Noninvasive markers of liver disease have not been well validated in children with IFALD. Portal pressure (PP) can be measured by Hepatic venous pressure gradient (HVPG) or splenic pulp pressure (SPP). In adult patients with cirrhosis, HVPG has been shown to be the reproducible and the best predictor of complications of portal hypertension (1). There is little experience with the clinical use of PP measurements in IFALD. We audited the use of HVPG and SPP in assessment of children with intestinal failure associated liver disease in our unit.

Methods: A retrospective audit of all patients who had portal pressure measured during assessment for intestinal transplant during the period between 1990 and 2000 was undertaken. HVPG or SPP and laboratory measures including AST to platelet ratio index (APRI >1 predicts liver disease) were reviewed. Standardised data proforma was used and data entered onto an Excel spreadsheet.

Results: Portal pressure measurements were performed in 16 patients (age 9 months–15 years) HVPG was performed in 11 patients, SPP in 5, in whom HVPG was not possible because of loss of vascular access. Nine out the 16 patients had elevated PP. Seven patients with elevated PP had also elevated APRI. Of the 7 patients who had normal PP, 3 patients had APRI >1. Non invasive marker (APRI) agrees with PP in 11 out of the 16 patients. Of the 9 patients with elevated PP, 5 patients had combined liver-intestinal transplant, 4 patients are listed for combined liver-intestinal transplant. Of the 7 patients with normal PP, 2 had combined liver-intestinal transplant, 1 had intestinal transplant, 3 are listed for intestinal transplant and 1 patient is currently on nontransplant management.

Conclusion: Portal pressure measurement is feasible and safe in children with IFALD. Portal pressure measurement provides important clinically relevant information in children with IFALD.

References:

Disclosure of Interest: None declared.

PO-H-0337/PD-H-0203

Transplantation
INDOCYANINE GREEN CLEARENCE AS A TOOL TO PREDICT THE NEED FOR LIVER TRANSPLANTATION IN PEDIATRIC ACUTE LIVER FAILURE
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Objectives and Study: Pediatric acute liver failure (PALF) is a rare disease that results in death or the need for liver transplantation (LT) in nearly 50% of cases. Distinguishing the patients with PALF who require LT from those patients who will survive with medical care alone remains unclear. The scoring systems available for the prognosis evaluation in adults are not able to predict survival without LT in pediatric patients. The aim of the study was to assess the use of indocyanine green plasma disappearance rate (ICG-PDR) as a tool to predict the evolution of patients affected of PALF and compare it with King’s College (KHC) and Clichy’s criteria.

Methods: All patients were younger than 18 years without chronic liver disease, and presented acute liver failure (hepatitis with a prothrombine time (PT) > 15 sec or INR > 1.5 in the presence of hepatic encephalopathy (HE) or a PT > 20 sec or INR > 2 regardless of the HE). ICG-PDR were taken on diagnosis and repeated every 24 hours until ALF resolution, death or LT. For each measurement, 0.25 mg/Kg of ICG (ICG-Pulsion Medical Systems, AG, Munchen, Germany) was given intravenously and its blood concentration was detected over time with a non-invasive method, the LiMON monitor (Pulsion Medical System AG, Munich, Germany). We calculated the sensitivity (S), specificity (E), positive predictive value (PPV) and negative predictive value (NPV) of ICG-PDR, KHC and Clichy’s criteria. All the ICG-PDR measurements were performed under hemodynamic stability (Sat,Hb > 70%, MAP > 60 mmHg, Diff, CO2 < 8) without splanchic vasoconstrictive drugs and with intradominal pressure < 8 mmHg.

Results: From January 2003 to July 2010 68 patients were diagnosed with PALF. The most frequent identifiable etiology was ischemic hepatitis (33%) followed by drug-induced liver injury (13.2%). A total of 217 ICG-PDR were performed with a median ICG-PDR of 12.6%/min.
TO MILD HYPERPHENYLALANINEMIA

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Objectives and Study: In phenylketonuria (PKU) patients, the intellectual quotient outcome is directly related to the levels of phenylalanine during infancy. Best prognosis is associated with maintenance of average phenylalanine levels below 400 µmol/L (6.6 mg/dL) in children less than 10 years of age. There is currently no specific treatment beside protein restriction and phenylalanine free products. The metabolism of phenylalanine is exclusively liver based. Hence, liver cell transplantation is an attractive and logical option to help controlling phenylalanine levels in the most severely affected patients. Best results of liver cell transplantation are achieved with good quality cells, freshly isolated, from young donors and short ischemic time. This is made extremely infrequent due to lack of organs offered for liver cell transplantation.

Methods: The candidate patient was a 6-year-old male with severe PKU. He was poorly equilibrated despite a close medical, dietary and psychosocial follow up. Genotype P281L / IVS10–11G>A confirmed the diagnosis of severe PKU with no residual activity of PAH, and no improvement was obtained under tetrahydrobiopterin treatment. The child had frequent elevated levels of phenylalanine above the safe limit of 400 µmol/L for the last three years. Tolerance to phenylalanine was low, and normal levels could only be reached when the child was hospitalized. In this context, we performed liver cell transplantation, using 1.7 billion fresh cells from a 14 months old girl with type 1b glycogen storage. The child received a second infusion of 0.8 billion fresh cells 7.5 months later from a healthy donor.

Results: Following this, mean phenylalanine level 3 months before transplantation, 11.1 ± 3.8 mg/dL (n = 11), decreased

adverse events, 12% are diabetic, 4% require antihypertensive drugs and 24% have creatinine levels higher than 1.2 mg/dL. One patient had required renal transplantation. From the social point of view, 84% refer to have good quality of life and 80% are studying or working. 3 have had offspring.

Conclusion: Most of the patients transplanted at a paediatric age more than 20 years ago have normal functioning grafts, an excellent social adaptation and few immunosuppression-related adverse events when compared to adult series.

Disclosure of Interest: None declared.

PO-H-0333/PD-H-0205

Transplantation
HEPATOCYTE TRANSPLANTATION TRANSFORMS SEVERE PHENYLKETONURIA TO MILD HYPERPHENYLALANINEMIA

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Objectives and Study: In phenylketonuria (PKU) patients, the intellectual quotient outcome is directly related to the levels of phenylalanine during infancy. Best prognosis is associated with maintenance of average phenylalanine levels below 400 µmol/L (6.6 mg/dL) in children less than 10 years of age. There is currently no specific treatment beside protein restriction and phenylalanine free products. The metabolism of phenylalanine is exclusively liver based. Hence, liver cell transplantation is an attractive and logical option to help controlling phenylalanine levels in the most severely affected patients. Best results of liver cell transplantation are achieved with good quality cells, freshly isolated, from young donors and short ischemic time. This is made extremely infrequent due to lack of organs offered for liver cell transplantation.

Methods: The candidate patient was a 6-year-old male with severe PKU. He was poorly equilibrated despite a close medical, dietary and psychosocial follow up. Genotype P281L / IVS10–11G>A confirmed the diagnosis of severe PKU with no residual activity of PAH, and no improvement was obtained under tetrahydrobiopterin treatment. The child had frequent elevated levels of phenylalanine above the safe limit of 400 µmol/L for the last three years. Tolerance to phenylalanine was low, and normal levels could only be reached when the child was hospitalized. In this context, we performed liver cell transplantation, using 1.7 billion fresh cells from a 14 months old girl with type 1b glycogen storage. The child received a second infusion of 0.8 billion fresh cells 7.5 months later from a healthy donor.

Results: Following this, mean phenylalanine level 3 months before transplantation, 11.1 ± 3.8 mg/dL (n = 11), decreased

adverse events, 12% are diabetic, 4% require antihypertensive drugs and 24% have creatinine levels higher than 1.2 mg/dL. One patient had required renal transplantation. From the social point of view, 84% refer to have good quality of life and 80% are studying or working. 3 have had offspring.

Conclusion: Most of the patients transplanted at a paediatric age more than 20 years ago have normal functioning grafts, an excellent social adaptation and few immunosuppression-related adverse events when compared to adult series.

Disclosure of Interest: None declared.

PO-H-0333/PD-H-0205

Transplantation
HEPATOCYTE TRANSPLANTATION TRANSFORMS SEVERE PHENYLKETONURIA TO MILD HYPERPHENYLALANINEMIA

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adverse events, 12% are diabetic, 4% require antihypertensive drugs and 24% have creatinine levels higher than 1.2 mg/dL. One patient had required renal transplantation. From the social point of view, 84% refer to have good quality of life and 80% are studying or working. 3 have had offspring.

Conclusion: Most of the patients transplanted at a paediatric age more than 20 years ago have normal functioning grafts, an excellent social adaptation and few immunosuppression-related adverse events when compared to adult series.

Disclosure of Interest: None declared.
Liver cell therapy can significantly improve phenylketonuria. 

Conclusion: This is the first demonstration in humans that liver cell therapy can significantly improve phenylketonuria in severely affected patients.

Disclosure of Interest: None declared.

PO-H-0342/PD-H-0206

Transplantation

DEVELOPING A TRANSITION PROGRAMME FOR ADOLESCENTS POSTLIVER TRANSPLANTATION: OUR SINGLE-CENTRE EXPERIENCE WITH AN INITIATING SURVEY

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Objectives and Study: Increased rates of rejection and graft loss in adolescents and young adults have led to the development of transition programmes for young people in a number of paediatric transplant centres. Before initiating a programme in our centre we evaluated the requirements of our patients and their parents and asked for their views towards the outline of an established programme elsewhere.

Methods: Doctors and social workers from our centre developed a 9-item questionnaire asking for individual needs of young people to support the development of self-management skills. We also asked for views as to how well these needs were met by the layout of an established transition programme elsewhere. One copy of the questionnaire was sent to each patient post orthotopic liver transplantation aged 12 years (y) and above and another copy to their parents. Results were entered into an ACCESs based platform and analyzed using the statistical software SAS/Enterprise Guide 9.2.

Results: 56 liver transplant recipients aged 12–18 y (33 male, mean age 14.8 y; 23 female, mean age 13.9 y) were identified of whom 27 (45.5%, 17 male, mean age 15.2 y; 10 female, mean age 13.4 y) replied. 24 parents returned the questionnaire. The majority of adolescent patients (63%) and an even higher percentage of parents (92%) were in favour of the implementation of additional education programmes for their children than on their own, only 42% of parents would object to this. In contrast to published data which suggests 12 y as the appropriate age for beginning a transition programme, our patients and parents prefer to start with 14.5 y and 14.6 y.

Conclusion: Our findings suggest significant differences in the perception of needs for transitional care between parents and young people with parents suggesting closer support of young people by medical professionals. Our survey also suggests that transition programmes cannot simply be exchanged between centres with different cultural background. A new transition programme will need to be developed with active participation by young people, their parents and health professionals.

Disclosure of Interest: None declared.

PO-H-0332/PD-H-0207

Transplantation

WAITING LIST RISK OF MORTALITY IN LIVER TRANSPLANTATION: PAEDIATRIC END-STAGE LIVER DISEASE VERSUS A NEW PAEDIATRIC HEPATOLOGY DEPENDENCY SCORE

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Objectives and Study: We developed the Paediatric Hepatology Dependency Score (PHD) in a national paediatric unit specialising in liver disease, and combined liver/intestinal transplantation. The objective was to compare the PHD score with the PELD score, the latter originally developed in the USA and now established as a tool to identify children at higher risk of death while awaiting liver transplantation.

Methods: 67 consecutive children were listed for transplant: 42 for liver graft of which 11 were fulminant cases; 3 were listed for liver/kidney grafts; 20 were listed for liver/bowel grafts and 2 for isolated bowel graft. The PHD score was developed from parameters relating to liver biochemistry (AST, albumin, bilirubin); hepatic decompensation (prothrombin time and presence of ascites) and nursing dependency (requirement for nutritional support; blood product support; additional organ dysfunction, sepsis, type of intravenous access). Each PHD parameter scored 0–4 (maximum theoretical score 40). 1 PELD was calculated using the published formula. Analysis included linear regression. Waiting list mortality was studied by receiver operating curves (ROC), proportional hazards regression and cross classification aspects using Fisher exact test.

Results: The median time spent on the waiting list was 45 days (0–463). Seven patients died without receiving a transplant (5 who were awaiting liver/bowel transplant; 1 with cystic fibrosis, 1 from fulminant liver failure). The two scores correlated well (r = 0.71, P = 0.001), but for patients with co-morbidity such as intestinal failure, the PHD score discriminated better than PELD in predicting waiting list mortality. ROC analysis showed that a PHD score greater
than 15.5 was associated ($P < 0.001$) with waiting list mortality with a sensitivity of 86% and specificity of 85%. The threshold PELD greater than 8 was also associated with a sensitivity of 86%, but had a specificity of 40%. Cox proportional hazard regression of time spent on the waiting list prior to either death or transplant/delisting showed a significant association with both PHD ($P = 0.006$) and PELD ($P = 0.008$). There was no noteworthy correlation between either PELD or PHD score with posttransplant mortality.

**Conclusion:** The sample number in this study is relatively small and the observation that the PHD score was able to discriminate waiting list deaths at least as well as the PELD score needs further evaluation. However, the PHD score is convenient and, as it does not require access to logarithmic transformation, is simple to apply at the bedside.

**References:**

**Disclosure of Interest:** None declared.

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**PO-H-0346/PD-H-0208**

**Transplantation**

**BILIARY STRICTURES AFTER LIVER TRANSPLANTATION IN CHILDREN**

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**Objectives and Study:** Biliary complications are common after liver transplantation and in severe cases may lead to graft loss. The aim of the study was to evaluate efficiency of nonsurgical methods in the management of biliary strictures after paediatric liver transplantation.

**Methods:** Between 1990 and 2010 466 LTx were performed in our institution. We retrospectively analyse charts of patients (23 M/17 F) after liver transplantation (25 CAD/15 LR) with biliary complications. The patients were referred to endoscopic retrograde cholangiopancreatography (ERCP), percutaneous transhepatic cholangiography-PTC with bile duct balloon dilatation and biliary stent/catheter placement or surgical revision.

**Results:** In 40 children after LTx presenting with anastomotic biliary structure we performed 56 PTC and 41 ERCP. At the moment of first intervention 23 patients had Roux-en-Y loop and 17 had duct-to-duct anastomosis. The mean age at the first intervention was 9.75 years (SD 4.1) and time from LTx was 1.92 years (SD 2.4). After LTx the total mean follow up without re-transplantation/death was 2.6 (SD 2.2) and after biliary intervention 1.74(SD1.4) years. Early biliary complications <30 days after LTx occurred in 16 patients (40%): bile leakage in 8, fistulas in 5, stenosis in 5 cases. 11 children (27.5%) underwent surgical reconstruction of biliary anastomosis after unsuccessful endoscopy/PTC, 7 underwent ReLtx and 2 were deceased due to post-transplant infections. The overall good outcome of non-surgical interventions was achieved in 29 patients (72.5%).

**Conclusion:** Nonsurgical approach is effective and safe in biliary complications after liver transplantation. The majority of patients require repeatedly performed interventions. Surgical approach should be considered in selected cases with poor response to primary treatment.

**Disclosure of Interest:** None declared.

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**PO-H-0331/PD-H-0209**

**Transplantation**

**AUTOPHAGY IN ISCHEMIA AND REPERFUSION INJURY**

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**Objectives and Study:** Currently the short-term outcomes after solid organ transplantation are excellent. However, the long-term outcomes for patients who require transplantation have not changed over the past decade. This is of particular concern for children where excellent long-term outcomes are needed. There is convincing evidence that ischemia reperfusion injury shapes the later development of long-term kidney and liver graft damage. Recent studies indicate that a cellular recycling pathway called autophagy may help to remove the toxic reactive oxygen species that kill cells during ischemia reperfusion injury. Our objective was to explore the role of autophagy in mammalian cells in the setting of ischemia reperfusion (IR).

**Methods:** In vitro simulated ischemia (sI) was achieved by placing primary human endothelial cells transfected with LC3-GFP in a hypoxic chamber (1% O2 for 2 h) and IR by resupplying nutrients by replacing media and providing oxygen (for 2 h). Cells were treated with rapamycin to enhance and 3-methyladenine (3MA) to block autophagy. Autophagy was assessed by confocal microscopy of LC3–GFP puncta and immunoblotting for LC3-II flux in the presence or absence of bafilomycin. Cell survival was determined using the MTT assay.

**Results:** Our initial results showed that autophagy was increased following starvation, sI and IR for 4 hours as assessed by increased LC3 puncta and LC3-II conversion. Treatment with 3MA blocked the induction of autophagy while rapamycin increased autophagy under each of the conditions. Cell survival was reduced after sI in comparison with control cells. However, under conditions of sI cells treated with rapamycin showed a trend towards enhanced cell survival (increase by 25%, n = 2).

**Conclusion:** Our results show that autophagy impacts cell survival during IR injury. Our findings suggest that manipulation of autophagy may be a novel treatment option to
PO-H-0345/PD-H-0210

Transplantation
THE IMPACT OF NITISINONE TREATMENT ON THE NEED FOR AND OUTCOME OF ORTHOTOPIC LIVER TRANSPLANTATION IN CHILDREN WITH TYROSINAEMIA TYPE 1
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Objectives and Study: Tyrosinaemia Type 1 (TT1) is a disorder of tyrosine metabolism which may lead to liver failure and a high risk of hepatocellular carcinoma (HCC). Treatment previously consisted of dietary restriction and orthotopic liver transplantation (OLT) but was transformed by the introduction of nitisinone in 1992. Here we report how nitisinone has altered the outcome of and need for OLT in patients with TT1 in our centre.

Methods: A retrospective analysis was performed of patients treated for TT1 at our institution from 1989 – 2010.

Results: 38 patients were treated with no significant difference in the annual number of patients seen before and after 1992 (P = 0.47). 6/7 (85.7%) seen prior to 1992 and 7/31 (22.6%) initially treated with nitisinone underwent OLT. The primary indication for OLT prior to 1992 was hepatic dysplasia in all with rising α-fetoprotein in 4. Post 1992 indications were suspected/high risk of HCC in 5 patients, proven HCC in 1 and failure to respond to nitisinone in 1. In patients treated with nitisinone who subsequently required OLT, treatment was started at a median age of 428 days compared to 52 days in those who have not required OLT (P = 0.03). Survival following OLT was 4/6 (66.7%) pre- and 0.47). 6/7 (85.7%) seen prior to 1992 and 7/31 (22.6%) initially treated with nitisinone underwent OLT. The primary indication for OLT prior to 1992 was hepatic dysplasia in all with rising α-fetoprotein in 4. Post 1992 indications were suspected/high risk of HCC in 5 patients, proven HCC in 1 and failure to respond to nitisinone in 1. In patients treated with nitisinone who subsequently required OLT, treatment was started at a median age of 428 days compared to 52 days in those who have not required OLT (P = 0.03). Survival following OLT was 4/6 (66.7%) pre- and 7/7 (100%) post-nitisinone. Early complications included acute rejection in 4, hepatic artery thrombosis in 1, biliary reconstruction in 1, redo portal vein anastomoses in 1, burst abdomen in 1 and primary non function in 1 patient. Late complications included chronic rejection in 3, hypertension in 3, post transplant lymphoproliferative disease in 2, denovo hepatitis in 2, pulmonary metastasis in 1 and renal failure in 1 patient. 3 patients required a second transplant. Mean calculated glomerular filtration rate decreased post OLT with no significant difference between the pre- and post-nitisinone groups. Mean tubular reabsorption of phosphate remained within the normal range for both groups up to 5 years post OLT. Mean urinary protein:creatinine ratio normalised post OLT in the nitisinone group and was significantly lower than the non-treated group in which it remained raised up to 5 years post OLT (P = 0.005). Quality of life following transplant is good with unrestricted diet in all.

PO-N-0199/PD-N-0211

Nutrition, Metabolism, and Experimental Approaches
PERIPARTUM ANTIBIOTIC ALTERS PIG ILEAL BARRIER FUNCTION DEVELOPMENT AND MODIFIES ILEAL RESPONSE TO A HIGH-FAT DIET LATER IN LIFE
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Objectives and Study: Intestinal barrier function is a key parameter of gut homeostasis. An increased barrier function has recently been described in diet-induced obese rats. Other data also describe deviance of neonatal microbiota colonisation in over-weight or obese young adults. Early colonisation of the intestine drives the development of many intestinal functions, including epithelial permeability. We hypothesized that deviance in microbiota colonisation such as that induced by peripartum antibiotics would modify the post-natal development of ileal barrier function but also ileal barrier function response to an obesogenic diet.

Methods: Two groups of sows were administered or not amoxycillin per os (40 mg/kg/d) from 10 days before term to 21 days after parturition (ATBQ group, n = 11 and CTRL group, n = 12). Piglets were weaned at 28 days of age. At 150 days of age, two sex- and weight-matched littersmates per litter were fed either a low fat (LF) or a high fat (HF) diet for 30 days (n = 10 per group). Ileal paracellular permeability (FD-4 flux across the mucosa and epithelial conductance, G), transcellular permeability (HRP flux) and ion fluxes across the epithelium (short circuit current, Isc) were studied in pig ileum mounted in Ussing chambers at 14, 21, 28, 42 and 180 days of age.

Results: During the neonatal period, ileal paracellular permeability was higher in ATBQ piglets than CTRL ones at 14 days of age (FD-4 flux: 1026 ± 186 vs 598 ± 70 ng/cm²/h, P < 0.05 and G: 34.2 ± 3.2 vs 25.3 ± 3.4 mS/cm², P = 0.08). Ileal paracellular permeability was similar between the two groups thereafter (d21 and 28 and two weeks after weaning (d42)). Ileal Isc was significantly lower in ATBQ compared to CTRL piglets at d21 (24.0 ± 12.3 vs 50.9 ± 17.9 μA/cm², P < 0.05). Later in life, permeability to FD-4 was increased by the HF diet in CTRL pigs (HF 823 ± 92 vs LF 561 ± 67 ng/cm²/h, P < 0.05) as already described. However, this increase was not observed in ATBQ pigs (HF 700 ± 137 vs LF 655 ± 115 ng/cm²/h, P > 0.05). Permeability

Conclusion: OLT remains an effective treatment for TT1. Since the introduction of nitisinone the need for OLT has been reduced. Early introduction of nitisinone therapy may prevent need for OLT. Treatment with nitisinone prior to OLT results in improved renal tubular function.

Disclosure of Interest: P. Mckiernan Speaker Bureau with: Swedish Orpahn Ltd. D. Bartlett: None declared, D. Mirza: None declared, C. Lloyd: None declared, P. Newsome: None declared.
to large molecules (HRP) was altered neither by the maternal antibiotic treatment nor by the HF diet at any age studied.

**Conclusion:** Modifications of intestinal microbiota colonisation alter epithelial barrier function development with a higher ileal permeability in piglets born and suckling antibiotic-treated mothers. Moreover, intestinal defaults induced by a HF diet are dependent upon early microbiota colonisation.

**References:**
1. de la Serre et al., *AJP* 2010.
2. Luoto et al., *JPGN* 2011.

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**Disclosure of Interest:** None declared.

**PO-N-0200/PD-N-0212**

**Nutrition, Metabolism, and Experimental Approaches**

**SUPPLEMENTATION OF THE MATERNAL DIET WITH C18:3N-3 HAS LONG-TERM EFFECT ON OFFSPRING GUT IMMUNE FUNCTION IN PIGS**

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**Objectives and Study:** Consumption of n-3 polyunsaturated fatty acids (n-3PUFAs) is low and should be increased, especially in pregnant and lactating woman. Indeed, n-3PUFAs have many beneficial effects on newborn development, including systemic and mucosal immune function. Studies on the impact of n-3PUFA upon newborn immune function have mainly focused on long-chain n-3PUFAs. The objective of our study was to investigate the effect of the precursor C18:3n-3 in the maternal diet on gut barrier and immune function in a piglet model of human babies.

**Methods:** Two groups of sows were fed either a low (ALA3) or a high (ALA27) C18:3n-3 diet throughout gestation and lactation. Piglet jejunal barrier function was followed every week during the suckling period, using Ussing chambers (FD-4 flux across the mucosa). Immunoglobulin concentration was measured in sow colostrum and 36hr-old piglet plasma. Intestinal sensitivity to LPS was determined at the end of the suckling period, jejunal sensitivity to LPS was lower in ALA27 piglets despite the higher jejunal permeability at birth. At the end of the suckling period, jejunal sensitivity to LPS was lower in ALA27 piglets despite the higher intestinal permeability. Oral tolerance acquisition to OVA was not modified by the higher intestinal permeability in ALA27 piglets. Later in life, however, MLN cells proliferative response to concavalin A was reduced (proliferative index: 226 ± 28 vs 412 ± 54, $P < 0.05$) while that to LPS was increased (proliferative index: 9.9 ± 2.1 vs 4.1 ± 0.5, $P < 0.05$).

**Conclusion:** Supplementation of the maternal diet with C18:3n-3 modifies the post-natal development of jejunal barrier function with an enhanced permeability at key points of the neonatal period. This had, however, no immediate consequence on gut immune function and sensitivity during the neonatal period. Conversely, gut immune response to mitogen and inflammatory mediators was altered later in life.

**Disclosure of Interest:** None declared.

**PO-N-0191/PD-N-0213**

**Nutrition, Metabolism, and Experimental Approaches**

**GLUCAGON-LIKE PEPTIDE-2 INDUCES ADAPTATION AND IMPROVES FUNCTION OF THE REMNANT INTESTINE FOLLOWING INTESTINAL RESECTION IN PRETERM NEONATES**

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**Objectives and Study:** Short bowel syndrome (SBS) is a frequent complication after intestinal resection in preterm infants suffering from necrotizing enterocolitis (NEC). We hypothesized that exposure to elevated levels of the intestino-trophic hormone glucagon-like peptide-2 (GLP-2) will improve intestinal structure and function in the period immediately following massive intestinal resection in preterm neonates.

**Methods:** Preterm pigs were fed colostrum for 48 hours before undergoing resection of 50% of the small intestine and establishment of a jejunostomy. Following resection, pigs were maintained on total parenteral nutrition (TPN) either without (SBS, $n = 8$) or with GLP-2 (3.5 μg/kg/h, SBS+GLP-2, $n = 9$). TPN was stopped on day 5 and enteral feeding was introduced with bovine colostrum (15 mL/kg/3 hours) to perform a 24 h nutrient balance study before the pigs were euthanized for collection of intestinal tissue. At the same time, intestinal samples were collected from a group of unresected control preterm pigs (control, $n = 5$) subjected to the same age and feeding regimen as the SBS groups.

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Results: Plasma GLP-2 levels were about 50-fold higher (~1400–3000 pM) in the SBS+GLP-2 group compared with other groups (~20–60 pM, P < 0.001). GLP-2 increased the relative intestinal absorption of food wet weight (46 vs 22%), energy (79 vs 64%) and macronutrients (all P < 0.05), and decreased the intestinal loss of wet weight (99 vs 154 mL), energy (157 vs 279kJ) and macronutrients from the jejunostomy (all P < 0.05). This improvement in intestinal function in the SBS+GLP-2 group was supported by increased sucrose and maltase activities (+200%), and increased villus height and crypt depth (742 ± 21 vs 537 ± 16 μm and 93 ± 2 vs 62 ± 1 μm, respectively, P < 0.01). Intermediate results were found in the unrected control group for villus height (653 ± 11 μm) and crypt depth (72 ± 1 μm, both P < 0.05), but decreased maltase activity compared to SBS (P < 0.05).

Conclusion: Following intestinal resection GLP-2 induced a rapid increase in intestinal structure and function leading to enhanced fluid and nutrient absorption. Immediate post-surgical GLP-2 treatment could be an effective therapy to induce adaptation and improve intestinal function in preterm infants with SBS.

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PO-N-0215/PD-N-0214

Nutrition, Metabolism, and Experimental Approaches

COMPARISON OF THE GLYCEMIC AND INSULINEMIC RESPONSES OF AN OPTIMIZED-PROTEIN INFANT FORMULA AND HUMAN MILK IN INFANT RHESUS MONKEY

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Objectives and Study: Little is known about the glycemic, insulineinic and metabolic responses of human milk and infant formula. The objective of this work was to assess the acute postprandial responses of blood glucose and serum insulin to human milk and a commercially available infant formula in an infant rhesus monkey model.

Methods: In a cross-over study with a 1-week washout, ten infant rhesus monkeys, aged 8–10 weeks, were randomized to receive 1 of 2 experimental feeds: a commercially available infant formula (Pfizer Nutrition) or human milk. On the day of the testing procedure, animals were separated from mothers and fasted for four hours. Following this fast, infants were subjected to a blood draw (0 time) and were fed 13.1 g protein/ liter infant formula (Pfizer Nutrition) or human milk. On the day of the testing procedure, animals were separated from mothers and fasted for four hours. Following this fast, infant monkeys were fed infant formula or human milk at time 0 were 93 ± 4 (mean ± SEM) mg/dL and 103 ± 6 mg/dL, respectively. Maximal glucose concentrations were reached at 10 minutes postfeeding and were 123 ± 5 mg/dL and 130 ± 5 mg/dL for infant formula and human milk groups, respectively. For both treatment groups, glucose returned to preprandial values by 60 minutes. Serum insulin levels for the infant formula- or human milk-fed animals at time 0 were 879 ± 118 pg/mL and 950 ± 99 pg/mL, respectively. At 40 minutes postfeeding, insulin concentrations increased to 1867 ± 317 pg/mL in the infant formula group and 1846 ± 258 pg/mL in the human milk-treatment group. Insulin concentrations for both treatment groups returned to preprandial concentrations by 120 minutes.

Conclusion: These data demonstrate that the acute glycemic and insulineinic effects of human milk and an optimized-protein infant formula do not significantly differ in an infant, non-human primate model. These data suggest that an optimized-protein infant formula may promote postprandial glucose and insulin responses that are similar to those of human milk.


PO-N-0210/PD-N-0215

Nutrition, Metabolism, and Experimental Approaches

EFFECT OF CHOLINE AND URIDINE-MONOPHOSPHATE ON CONDITIONED TASTE AVERSION IN INFANT RATS

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Objectives and Study: To study the influence of the oral supplementation with choline (Chol), CDP-chol (citicoline), or UMP (uridine-monophosphate) + Chol through the lactation period and infancy on conditioned taste aversion (CTA) in rats.

Methods: Nine day-old rat pups were paired by both body weight and litter and were distributed into 4 study groups (n = 12). The pups were hand-fed, using special bottles and nipples, with a rat milk substitute from postnatal day 9
(PND9) to weaning (PND21), when an AING-93 powder diet was introduced. The study compounds were prepared in a water solution and given to the animal as a daily supplement until PND57. The study groups were: Group A, CDP-Chol; Group B, Chol; Group C, water; and Group D, Chol+UMP. The CTA test was performed from PND38–57. An i.p. injection of the visceral distress-inducing agent lithium chloride was administered 15 min after a sodium saccharin solution. After 2 recovery days, the strength of saccharin aversion was evaluated in a one-bottle test followed by a two bottle-test (saccharine versus water). All the experimental procedures were approved by the local Ethics Committees, and were in accordance to the Directive 86/609/EEC. The data were analyzed by a factorial design and post-hoc comparisons by LSD test.

Results: The survival rate of the animals during the lactating period was 85.4%, resulting in evaluation of 10, 11, 11 and 9 animals for groups A, B, C and D, respectively. All groups showed a significant reduction of consumption of liquids as a consequence of learned aversions in the one-bottle test. However, when the saccharin-water choice-test was analyzed, only group D drank less saccharin than the rest of the groups revealing learned saccharin aversion (saccharine solution intake in group A: 3.89 ± 0.45, group B: 4.40 ± 0.50, group C: 4.02 ± 0.50 and group D: 2.36 ± 0.42 (mean in ml ± SD), P < 0.05).

Conclusion: CTA is a well-established learning and memory paradigm in rodents that is considered to be a special form of classical conditioning. It involves brain areas located at different levels such as parabrachial nucleus, amygdala, and insular cortex that process taste sensation and gastrointestinal function. It is likely that the rearing procedure and mother deprivation protocol used in this study, suppresses or reverses the CTA ability of rats by affecting taste discrimination, the learning process or both. Only supplementation with Chol+UMP during the lactating period and infancy was able to restore this capability.

Disclosure of Interest: None declared.

PO-N-0211/PD-N-0216

Nutrition, Metabolism and Experimental Approaches
CHOLINE AND ITS COMBINATION WITH URIDINE-MONOPHOSPHATE IMPROVE SPATIAL LEARNING IN INFANT RATS
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Objectives and Study: To study the influence of the oral supplementation with choline (Chol), CDP-chol (citicoline), or UMP (uridine-monophosphate) + Chol through the lactation period on the performance of the Morris Water maze (MWM) in rats.

Methods: Nine day-old rat pups were paired by both body weight and litter and were distributed into 4 study groups (n = 12). The pups were hand-fed, using special bottles and nipples, with a rat milk substitute from postnatal day 9 (PND9) to weaning (PND21), when an AING-93 powder diet was introduced. The study compounds were prepared in a water solution and given to the animal as a daily supplement until PND57. The study groups were: Group A, CDP-Chol; Group B, Chol; Group C, water; and Group D, Chol+UMP. The maze evaluation was performed from PND24–32. The animals received 6 training blocks (hidden platform), applied in daily sessions during 6 consecutive days and 2 probe trials (no platform) 24 and 48 hours later. All the experimental procedures were approved by the local Ethics Committees, and were in accordance to the Directive 86/609/EEC. The data were analyzed by a factorial design and post-hoc comparisons by LSD test.

Results: The survival rate of the animals during the lactating period was 85.4%, resulting in evaluation of 10, 11, 11 and 9 animals for groups A, B, C and D, respectively. All the groups showed spatial learning. Nonetheless, group D had shorter latency and shorter path lengths than the rest of the groups (P < 0.05), indicating better performance in the navigation task. In the probe trial at 24 h, Groups B and D searched longer in the target quadrant than in the opposite quadrant, while the time spent in both quadrants by A and C groups did not differ. Improved memory of platform location in Groups B and D was also supported by reduced latency to reach the target quadrant during the probe trial. Moreover, group D spent longer (P < 0.05) in the proximal zone than the rest of the groups. After 48 hours, only groups B and D remembered the position of the platform, but differences were statistically significant only for group D.

Conclusion: Supplementation with Chol or Chol+UMP during the postnatal period had improved memory as measured by the MWM in rats. However, the combination of both compounds was more effective than Chol alone because both memory recall and spatial learning during training were improved and because the effect lasted longer. CDP-chol, which has been reported as an effective memory enhancer in adults, had no effect in this experimental model of infancy.

Disclosure of Interest: None declared.

PO-N-0259/PD-N-0217

Nutrition, Metabolism, and Experimental Approaches
EFFECT OF ENTERAL SUPPLEMENTATION OF NEUTRAL AND ACIDIC OLIGOSACCHARIDES IN PRETERM INFANTS ON ALLERGIC AND INFECTIOUS DISEASES DURING THE FIRST YEAR OF LIFE
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Objectives and Study: Enteral administration of a prebiotic mixture of neutral and acidic oligosaccharides during the neonatal period in preterm infants may affect the immune response later in life. Aim was to determine the effect of enteral supplementation of neutral and acidic (scGOS/lcFOS/AOS) oligosaccharides during the neonatal period in preterm infants on the incidence of allergic and infectious diseases during the first year of life.

Methods: In a randomised controlled trial, preterm infants (GA < 32 wks and/or BW < 1500 g) were allocated to receive enteral scGOS/lcFOS/AOS supplementation or placebo (maltodextrin) between day 3 and 30 of life. Incidence of physician-diagnosed allergic (atopic dermatitis, bronchial hyperreactivity and milk protein allergy) and infectious diseases (infections of the upper respiratory tract (URI), lower respiratory tract (LRI), gastrointestinal tract, sepsis and meningitis) was assessed by validated questionnaire at 1 year of age. Data were analysed by logistic regression analysis and adjusted for confounding factors.

Results: In total, 113 preterm infants were enrolled in the initial study; 98/113, were eligible for follow-up (12 died, 3 were excluded). To date, 90/98 (92%) infants participated in our follow-up study. Baseline infant, maternal, environmental and nutritional characteristics were not different between scGOS/lcFOS/AOS- (N= 48) and placebo group (N=42).

After adjustments for confounding factors (maternal education, family history of atopy, smoking, exclusive breast-feeding in the neonatal period and presence of pets at home) incidence of bronchial hyperreactivity (odds ratio [OR], 1.00; 95% confidence interval [CI], 0.36–2.77) and atopic dermatitis (OR, 1.26; CI, 0.34–4.71) was not different between both groups. Likewise the incidence of URI (OR, 1.00; CI, 0.39–2.60), LRI (OR, 0.98; CI, 0.35–2.75) and gastrointestinal tract infections after the neonatal period (OR, 1.96; CI, 0.58–6.63) was not different between both groups, also after adjustment for confounding factors (maternal education, smoking at home, siblings and child care attendance). The incidence of milk protein allergy, sepsis and meningitis after neonatal period in the first year of life did not allow statistical analysis.

Conclusion: Short time enteral administration of a prebiotic mixture of acidic and neutral oligosaccharides during the neonatal period in preterm infants does not decrease the risk of allergic and infectious diseases during the first year of life.

Disclosure of Interest: N. Niele: None declared, A. van Zwol: None declared, G. Boehm Employee of: Also Employe of Danone Research. Danone Research provided the preterm formula (Nenatal Start®) and post-discharge formula (Nenatal 1), neutral and acidic oligosaccharides and placebo supplementation. The funding source had no role in the study design, data collection, data analysis and interpretation of the study results., E. A. Westerbeek: None declared, H. Lafeber: None declared, R. Van Elburg: None declared.
PO-N-0219/PD-N-0219

**Nutrition, Metabolism, and Experimental Approaches**

**EFFECTS OF PASTEURIZATION ON THE PROTEIN CONTENT OF HUMAN MILK: HIGH-TEMPERATURE SHORT-TIME VS HOLDER**

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**Objectives and Study:** Holder pasteurization is the recommended method in human milk banks, ensuring the microbiological safety of human milk (HM). However, loss of some biologically active milk components due to heat treatment is a main concern. Studies aiming to find the pasteurization method providing the best compromise between the microbiological safety and the biological quality of the milk are promising. High temperature short-time (HTST) pasteurization is one of the alternative methods and may be effective in maintaining the nutritional and immunological quality of HM. A study was performed to compare the impact of two pasteurization methods-Holder and HTST-on the protein profile of donor HM.

**Methods:** Raw and pasteurized milk samples by Holder and HTST methods were analyzed. HM protein profile and the carboxylation degree, modified proteins, lipase activity, and total available lysine quantity have been evaluated.

**Results:** These analyses showed that protein patterns of HTST-treated milk and raw milk were similar, whereas Holder method substantially modified several proteins; modified proteins were identified as bile salt-stimulated lipase, lactoferrin and components of the immune system (Ig variable region, MHC class I antigen). HTST method preserved the integrity of bile salt-stimulated lipase, lactoferrin and, to some extent, of IgAs; Holder pasteurization not only decreased the amount of bile salt-stimulated lipase, but also completely inactivated the remaining lipase molecules, while HTST method preserved its activity. Pasteurization, particularly Holder, increased the bioavailable lysine quantity.

**Conclusion:** According to these preliminary results, HTST pasteurization seems to be superior to Holder method in retaining the protein profile, and some of the key biologically and immunologically active components of donor HM.

**Disclosure of Interest:** None declared.

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PO-N-0252/PD-N-0220

**Nutrition, Metabolism, and Experimental Approaches**

**SUPPLEMENTATION OF AN ARACHIDONIC ACID–DOCOSAHEXAENOIC ACID MIXTURE DURING EARLY LIFE REDUCES BODY WEIGHT GAIN, PLASMA LIPIDS, AND ADIPOSITY LATER IN LIFE**

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**Objectives and Study:** Arachidonic acid (ARA) and docosahexaenoic acid (DHA) are n-6 and n-3 long-chain polyunsaturated fatty acids, respectively, that have been proven to support brain and visual development in infants. Their dietary supplementation during early life is therefore considered to be health beneficial. Some have speculated that high intake of n-6 polyunsaturated fatty acids in the context of declining n-3 fatty acids ingestion may be associated with an increased risk of obesity. Furthermore, the prevalence of obesity early in life is increasing. This study addresses whether supplementation of a specific ARA/DHA mixture during early life has effects on body weight development and lipid metabolism later in life. This was tested in ApoE3L-transgenic mice, a humanized animal model for hyperlipidemia with mild obesity.

**Methods:** Four-week-old male ApoE3L-mice were fed ad libitum chow with or without supplementation of 0.129wt% ARA and 0.088wt% DHA for 8 weeks. Then for another 8 weeks, both groups of mice were placed on a high-fat (28wt%)/high-carbohydrate (42wt%) (HFHC) diet. Control mice received chow during the entire experimental period. Body weight and food intake were determined every 2 weeks and plasma levels of total cholesterol, triglycerides, fasting glucose, fasting insulin, leptin and adiponectin were measured at 4-week intervals during the course of the experimental feeding period. At the end of the experiment, fat tissue and liver weights were determined.

**Results:** Mice that received ARA/DHA early in life gained ~5% less body weight compared to the group without ARA/DHA supplementation. Food intake was not affected. ARA/DHA supplementation also lowered plasma levels of total cholesterol and triglycerides over time compared to mice without ARA/DHA supplementation. Furthermore, fasting plasma glucose was decreased after ARA/DHA supplementation, but plasma insulin was not different between the dietary treatments. ARA/DHA supplementation substantially reduced plasma leptin to healthy control levels in comparison to mice without supplementation. Plasma adiponectin levels were not different between the dietary groups. Mice that received ARA/DHA during early life had ~25% lower weights of total white adipose tissue, particularly gonadal fat mass, than mice without supplementation. No effects were found on liver weight.

**Conclusion:** This study shows that ARA/DHA dietary supplementation early in life reduces body weight gain, plasma levels of total cholesterol and triglycerides and adiposity during a HFHC diet later in life.

PO-N-0213/PD-N-0221

**Nutrition, Metabolism, and Experimental Approaches**

**GENETIC INACTIVATION OF THE HEPATIC BILE SALT EXPORT PUMP IN MICE LEADS TO PROFOUND MALABSORPTION OF DIETARY LIPIDS**

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**Objectives and Study:** Cholestatic liver diseases are characterized by defective bile secretion, which can lead to fat malabsorption and failure to thrive. In order to improve (pre-transplantation) survival of cholestatic patients, it is essential to optimize nutritional status. To investigate therapeutic and dietary strategies in sufficient detail, an appropriate animal model is crucial. We have characterized a mouse model that has recently become available (PNAS 2001; 98(4):2011–6) in which the bile salt export pump (BSEP) is genetically inactivated (Bsep<sup>−/−</sup> mice). BSEP transports bile salts from hepatocytes to biliary canaliculi. Mutations in human BSEP result in progressive familial intrahepatic cholestasis type 2 (PFIC-2). We compared nutritional status and intestinal absorption of fat and cholesterol in Bsep<sup>−/−</sup> and control mice.

**Methods:** We measured body weight, food intake and fecal output of Bsep<sup>−/−</sup> and their wildtype littermates. We determined fat levels in food and feces and calculated fat absorption (ingestion - excretion)/ ingestion×100%). In addition, we determined plasma appearance of 13C-labeled, long-chain fatty acid palmitic acid after its intragastric administration. In a separate group of Bsep<sup>−/−</sup> mice and controls, we determined cholesterol levels in food, feces and plasma.

**Results:** Body weight was not significantly different in Bsep<sup>−/−</sup> mice compared with controls (31±3 versus 34±6 g). Ingestion of dietary fat was not different in Bsep<sup>−/−</sup> mice compared with control mice (892±139 g vs. 763±145 g mol day<sup>−1</sup>). However, fecal fat excretion was significantly increased in Bsep<sup>−/−</sup> mice compared with control mice (127±18 g vs. 36±9 g mol day<sup>−1</sup>, respectively; P < 0.01), indicating decreased fat absorption in Bsep<sup>−/−</sup> mice compared with control mice (85±3 g vs. 95±2%; P < 0.01). In accordance, plasma uptake of 13C-palmitic acid was decreased in Bsep<sup>−/−</sup> compared with control mice (AUC 0.7±0.4 vs. 1.9±1.0% of administered dose/ L plasma in 6h, P < 0.01). Dietary cholesterol intake was similar in Bsep<sup>−/−</sup> and control mice (3.2±0.4 g vs. 2.9±0.3 g mol day<sup>−1</sup>). Fecal cholesterol excretion was significantly increased in Bsep<sup>−/−</sup> compared with control mice (32±5 g vs. 4±1 g mol day<sup>−1</sup>, P < 0.01). Plasma cholesterol was significantly lower in Bsep<sup>−/−</sup> mice compared with control mice (0.8±0.2 g vs. 2.3±0.5 mmol/L, P < 0.01).

**Conclusion:** Our data show that absence of Bsep in mice is associated with profound lipid malabsorption. We conclude that Bsep<sup>−/−</sup> mice provide a valuable model to investigate therapeutic and dietary strategies to improve the nutritional condition and dietary fat malabsorption under conditions of impaired bile formation.

**Disclosure of Interest:** None declared.

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PO-N-0201/PD-N-0222

**Nutrition, Metabolism, and Experimental Approaches**

**BUTYRATE MODULATES EPITHELIAL DRA EXPRESSION IN CHILDREN WITH CONGENITAL CHLORIDE DIARRHEA**

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**Objectives and Study:** Congenital chloride diarrhea (CLD-OMIM 214700) is an inherited intestinal electrolyte transport disorder determined by mutations in the SLC26A3/DRA gene. Oral butyrate has been proposed for the treatment of CLD, but the exact mechanism is not completely defined. We hypothesize that butyrate could regulate DRA expression in epithelial cells. In this study, we aimed to investigated the relationship between clinical effect of butyrate and DRA expression in children affected by CLD with different genotype.

**Methods:** We enrolled 3 CLD children with 3 different type of mutation on SLC26A3 gene: missense (C1484A>C, C1640C>T) and deletion (c2008–151_2061+1546del). Enrolled subjects were treated with oral butyrate (100 mg/kg/d) for 1 week. Data regarding fecal ion concentrations and stool pattern before and after butyrate treatment were collected and considered for analysis. Primary epithelial cell culture was obtained by nasal brushing before starting butyrate therapy. At confluence cells were treated with 5 mM of sodium butyrate for 24 hours. RNA was extracted and DRA expression was analysed using Real time PCR (SYbr Green and Taqman chemistry) before and after in vitro butyrate exposure.

**Results:** The child carrier of missense mutation showed a good clinical response to butyrate (normalization of stool pattern and of fecal ion loss concentrations) and their epithelial cells showed an increased expression of DRA after butyrate exposure (>5-fold). A partial clinical response was observed in patient with deletion on SLC26A3 gene (reduction of 50% in fecal ion loss, without modification in stool pattern). Paralleling, in cells obtained from this subject, after exposure to butyrate, we observed a partial
increasing of DRA expression (5-fold). In patient with nonsense mutation butyrate was unable to induce any in vivo or in vitro effect.

**Conclusion:** The therapeutic effect elicited by butyrate in CLD patients is largely dependent on a modification in DRA expression at epithelial level.

**Disclosure of Interest:** None declared.

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**PL-G-0223**

**Intestinal Motility**

**RECURRENT ABDOMINAL PAIN IN INFANCY AND RISK OF RECURRENT ABDOMINAL PAIN IN 12-YEAR-OLD SWEDISH CHILDREN**

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**Objectives and Study:** The course over time (prognosis) of recurrent abdominal pain (RAP) in infancy remains poorly described. The objectives of this study were to assess prevalence proportions of recurrent abdominal pain at ages 1, 2 and 12 years and to test the hypothesis that RAP in infancy predisposes to RAP at 12 years of age.

**Methods:** A Swedish unselected birth cohort (BAMSE) of 4,089 newborn infants was followed for 12 years. Parental questionnaires were used to collect information of RAP in infancy and of potential confounders. RAP was asked for at 1 year (for the last 6 months) and at 2 years (for the last 12 months). Our main outcome at 12 years was RAP as self reported by the children. In 2682 children (66%), there were data available regarding RAP at all 3 ages (1, 2, and 12 years). Prevalence proportions of RAP in different age groups were compared. We used logistic regression to calculate odds ratios (OR) and 95% confidence intervals (CI) for RAP at 12 years as a function of RAP at 1 or 2 years. Adjustment was made for potential confounders.

**Results:** Prevalence proportions of parent reported RAP at 1 and 2 years was 4.0% and 3.6%, respectively. RAP at 12 years was reported by 9.3 % of children. Out of the 2682 children with information regarding RAP at all 3 ages, 15 % (n = 390) had RAP at some point. RAP over 2 or more assessments was observed in 2% (n = 44). RAP at 12 years was significantly more common among children with a history of RAP in infancy (between ages 6 and 24 months) (OR 1.92, CI 1.25–2.93, P = 0.003). The association between RAP in infancy and RAP at 12 years was not altered by adjustment for sex, socio-economic status, nationality or parental smoking.

**Conclusion:** RAP is a common complaint both in infancy and among 12-year-old children. RAP in infancy seems to double the risk of RAP at 12 years.

**Disclosure of Interest:** None declared.

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**PL-G-0224**

**Coeliac Disease and Enteropathies**

**GENOTYPE-PHENOTYPE CORRELATION AND IMMUNOHISTOPATHOLOGY**

**CHARACTERIZATION OF 38 CASES OF CONGENITAL TUFTING ENTEROPATHY (CTE)**

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**Objectives and Study:** Congenital Tufting Enteropathy (CTE) is a rare and severe congenital intestinal insufficiency that has recently been first ascribed to mutation in EpCAM gene. Then, a syndromic form of CTE, associating keratitis and choanal atresia, has been shown to be associated with mutation of SPINT2 gene. Now, the Congenital Sodium Diarrhea (CSD) first described by Müller et al. had previously been ascribed to SPINT2 mutations. No phenotype-genotype correlations of CTE have yet been reported, and the genetic findings involve clarifying the classification between CTE versus CSD. We report the largest cohort of CTE ever analyzed and provide molecular and immunohistochemistry data to establish a genotype-phenotype correlation for EpCAM and SPINT2 genes. We also initiate a discussion about a classification of CSD versus CTE.

**Methods:** 38 patients diagnosed with typical CTE were included. The coding regions of EpCAM and SPINT2 genes were sequenced. Immunostaining of EpCAM and SPINT2 were performed on intestinal andconjunctival biopsies.

**Results:** We identified mutations in EpCAM gene in 14 patients, which resulted in absence of immunostaining of EpCAM on intestinal biopsies. These patients had congenital diarrhea, typical CTE histological abnormalities without extradigestive symptoms. Fourteen patients carried mutations in SPINT2 gene. EpCAM protein was detected on intestinal biopsies, but negative or abnormal immunostaining of SPINT2 was observed. These patients had congenital diarrhea with keratitis and choanal atresia and typical CTE pathological abnormalities. Interestingly, 6 EpCAM mutated patients carried a heterozygous mutation in SPINT2 gene as well; in addition to clinical and histological symptoms of CTE, these patients displayed a clinical and/or histological keratitis without choanal atresia. Four patients were not classifiable.

**Conclusion:** This first genotype-phenotype correlation study of CTE helps in understanding the pathophysiology of the disease, as well as in establishing diagnosis. Prognosis may as well come as a result for this challenging disease whose evolution spreads from parenteral nutrition weaning to intestinal transplantation. Moreover, it opens the discussion regarding the overlap between CTE and CSD.
Objectives and Study: Incidence of esophageal atresia (EA) is estimated between one each 2500 until 4600 live births based on literature. We present the results of a national registry created since 2008 based on the inclusion of all the French living birth babies with an EA. Methods: All the 38 multidisciplinary centres all over the French territory and French overseas departments and territories taking care of EA and belonging to the national network on esophageal atresia. Results: One hundred forty-nine new cases of EA were recorded in 2008 making an incidence of 1.8/10000 live births. The sex ratio was at 1.49 (M/F). The mean birth weight was at 2529 g and gestational age was 37 ± 3.2 gestational weeks with 39% born under 36 gestational weeks. Prenatal diagnosis was only suspected in 17% of cases. According to Ladd classification, they were 16 cases with type I and 128 with type III EA. Diagnosis was made within the first 24 hours in 89% of cases and surgery was scheduled before the 48th hour in 96% of cases of type III EA. Full oral alimentation was possible at the time of first discharge in 87% of infants. Global survival was 95%, 8 patients died in whom 6 died in the neonatal period before discharge from hospital and in 1 case before EA surgery. Associated malformations were present in 53% of the cases with 30%, 27% and 10% of cardiovascular, genitourinary and anorectal abnormalities respectively. Length of stay was significantly greater in the group of premature, and the group of birth weight under 2500 g. Conclusion: This is the first national epidemiologic registry for EA. The survival in France is equivalent to survival reported in Western countries. Prenatal diagnosis remains rare in EA with distal fistula. Postnatal detection of EA is followed by a short lapse of time before newborn’s admission in neonatal surgery department.

Disclosure of Interest: None declared.
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Objectives and Study: Chronic constipation is common in childhood. It has been suggested that a subgroup of patients with refractory constipation have underlying primary colonic motor abnormalities. We studied the relationship between those abnormalities and enteric neuromuscular pathology in children with refractory slow transit constipation (STC).

Methods: 11 children (6 males, mean age 8 y) with refractory STC underwent colonic high resolution manometry (HRM) before segmental colonic resection allowing histological examination of full-thickness colon. HRM was performed for at least 90 min before and after 2 intraluminal instillations of bisacodyl (0.2 mg/kg). The following variables were analysed: number of high-amplitude propagating contractions (HAPC) and of low-amplitude propagating contractions (LAPC), presence of “common cavity,” motility index (MI) [\( \log_e (\text{sum of amplitudes} \times \text{number of contractions} + 1) \)]. All variables were analysed for each colonic segments (ascending, transverse, descending, sigmoid). Immunohistochemistry was performed to identify abnormalities in the enteric nervous system, interstitial cells of Cajal (ICC), and smooth muscle layers. For manometric data 5 age- and sex-matched children served as controls.

Results: In ascending and transverse no statistical differences were found between manometric variables in patients and controls. All patients had segmental manometric abnormalities in the left colon. In descending post bisacodyl HAPC were significantly lower than in controls (mean ± SD 2.7 ± 4.1 vs 4.2 ± 0.5); in sigmoid HAPC were absent in all patients (0 vs 3.6 ± 0.9, \( P < 0.001 \)). “Common cavity” was ubiquitous in the sigmoid of patients. Sigmoid MI was significantly lower in STC children (1 ± 1.8 vs 10 ± 0.9, respectively, \( P < 0.001 \)). Novel manometric qualitative abnormalities, as increased and retrograde LAPC activity, abnormal HAPC waveforms and unstable HAPC propagative velocities were seen in the STC group only. Sigmoid full-thickness specimens were ganglionic in all STC. Neuromuscular abnormalities were seen in 9/11 children including ectopic neurons in the circular muscle (CM) coat (CMC), vertical nerve fibres and ectopic ganglion cells in both lamina propria and CMC, rudimentary ICC networks, myenteric plexus not confined to the intermuscular layer, intermittent additional muscle layer within the submucosa. Many of these have not been described previously.

Conclusion: Colonic HRM abnormalities in children with refractory STC are highly predictive but not diagnostic of primary enteric neuromuscular disease. Our findings confirm the place of colonic HRM in predicting histological abnormality in refractory childhood STC.

Disclosure of Interest: None declared.

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PL-G-0228

Inflammatory Bowel Disease

SEVERE EARLY-ONSET COLITIS DUE TO MUTATIONS IN INTERLEUKIN-10

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Objectives and Study: IL-10 is secreted by many cells, limiting the secretion of pro-inflammatory cytokines such as TNF-α. Loss of IL-10 function cannot be compensated and results in an imbalance of the immune system, leading to excessive inflammation and an IBD-like phenotype. These children can present in the neonatal period with intractable, untreatable mucosal inflammation and severe perianal fistulae with haemorrhage. The aim of the study was to identify mutations in the IL-10 pathway in all cases of infant early-onset colitis, and to understand the natural progression of this condition in children treated and untreated with bone marrow transplantation (BMT).

Methods: Gene Search: All children under 1 year of age with IBD were screened for mutations in the IL-10 receptor chains, alpha and beta (IL10RA and IL10RB). If none were found, the coding regions of the IL-10 gene were sequenced, followed by linkage analysis with direct mutation screening of potential candidate genes if still no mutations were found.

Functional analysis: The impact of detected mutations on function was assessed by using Polymorphism Phenotyping and the affect on the protein tertiary structure was assessed by using Swiss PDB viewer.

Results: Out of 20 children screened with early IBD, we identified 6 patients (3 boys, aged 1–22 years, median 6 years) with IL-10 pathway defects. IL-10 mutation: 2 children from Northern Pakistan with as yet unknown homozygous nonsynonymous single nucleotide polymorphism at codon 113, resulting in an amino acid exchange from glycine to arginine. Both have undergone BMT and are well. IL-10 receptor mutation: 2 children, 1 Arab and 1 Indian, with IL-10RB mutation. Both have NOT been transplanted. One, aged 14 years, has had a colectomy and associated deafness. The other, aged 22 years, previously had a large pelvic EBV-driven lymphoma and several intestinal symptoms including fistulae despite colectomy. IL-10 ligand: 2 Romany brothers, with origins of distant consanguinity. Unique mutations were identified in IL-10 ligand, causing a stop codon and leading to early protein truncation. Both had BMT and are well 6 and 9 years post-BMT. The elder brother had developed EBV-driven Non-Hodgkins lymphoma prior to BMT.

Conclusion: IL-10 receptor and ligand mutations are present in 30% of children with early-onset IBD. Untreated children develop EBV-driven non-Hodgkins lymphoma. In those treated, BMT is curative.

Disclosure of Interest: None declared.
SP-N-0229

Clinical Nutrition
FISH OIL CONTAINING LIPID EMULSIONS PROTECT AGAINST PARENTERAL NUTRITION–ASSOCIATED LIVER DISEASE IN PRETERM PIGS
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Objectives and Study: During their first wks of life preterm infants are dependent on parenteral nutrition (PN). However, PN is associated with the development of liver disease (PN Associated Liver Disease (PNALD)). Studies in children showed that fish oil-based lipid emulsions can reverse PNALD; whether they prevent PNALD in preterm neonates is unknown. Mechanisms that can explain protective action of fish oil emulsions include 1)anti-inflammatory effects of n-3 fatty acids, 2)limiting soybean oil, rich in n-6FAs and phytosterols. In addition, a reduction of lipid load can also play a role, since in some parts of the world lipid intake is reduced to 1 g/(kg.d) whenever fish oil emulsions are used as monotherapy in PNALD. The aim of the study was to test whether lipid emulsions with varying amounts of fish oil prevent the development of PNALD in preterm pigs.

Methods: Preterm pigs bearing venous and arterial catheters were randomly assigned to 4 groups (7–14 pigs/group; equal daily macronutrient intake with 5 g/kg lipid): PN+soybean oil (100%) (Intralipid, IL), PN+fish oil (100%) (Omegaven, OV), PN+oil mixture w/soybean (30%)-coconut (30%)-olive (25%)-fish (15%) (SMOF); a reference group was fed milk formula enterally (EN). Serum AST, ALT, ALP, LDL, GGT, and bilirubin were measured at d0, 7, and 14. At d14 pigs were killed and liver histopathology, triglyceride (TG) content, and bile acid metabolism gene expression were analyzed.

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Results: See Table for significant differences at d14 (mean ± SEM).

Table.

<table>
<thead>
<tr>
<th></th>
<th>IL</th>
<th>SL</th>
<th>OV</th>
<th>EN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight gain (g/kg.d)</td>
<td>46 ± 1</td>
<td>54 ± 2</td>
<td>53 ± 2</td>
<td>59 ± 2</td>
</tr>
<tr>
<td>Liver weight (g/kg)</td>
<td>48 ± 2</td>
<td>42 ± 1</td>
<td>48 ± 1</td>
<td>32 ± 1</td>
</tr>
<tr>
<td>Direct bilirubin (mg/dL)</td>
<td>0.39 ± 0.10</td>
<td>0.07 ± 0.01</td>
<td>0.18 ± 0.04</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>171 ± 29</td>
<td>116 ± 27</td>
<td>70 ± 11</td>
<td>85 ± 13</td>
</tr>
</tbody>
</table>

*a,bSign. different from EN,SL,OV, resp.; P < 0.05. Direct bilirubin and GGT increased markedly from d0–14 in IL pigs, compared to other groups. Liver histopathology showed signs of cholangitis, microvesicular steatosis and neutrophil infiltrate mainly in IL pigs. Compared to EN pigs, all PN treatments suppressed hepatic mRNA of FXR and its target genes BSEP (bile transporter) and CYP7A1 (bile acid synthesis).

Conclusion: In preterm pigs, PN with soybean oil induced hepatic cholestasis and steatosis and this was largely prevented with either 100% or 15% fish oil emulsions. Fish oil did not prevent the PN-induced suppression of FXR target gene expression.

Disclosure of Interest: H. Vlaardingerbroek: None declared, B. Stoll: None declared, N. Benight: None declared, J. Van Goudoever: None declared, O. Olutoye: None declared, D. Burrin Grant/Research Support from: Fresenius Kabi, Germany supplied lipid emulsions. No other support received.

SP-H-0230

Transplantation
HUMORAL REJECTION AFTER INTESTINAL TRANSPLANTATION

Objectives and Study: To describe clinical and pathological features of humoral rejection (HR), antibody (Ab) mediated, after intestinal transplantation (Tx), and to discuss diagnosis and treatment.

Methods: From 1994 on, 88 children received 94 Tx. Anti-HLA Ab were screened pre- and post-Tx. Since 2008: 27 children were enlisted, 19 transplanted. Intestinal biopsies were performed according to our protocol. Staining with C4d was not systematically performed. When HR was suspected on high titers of donor-specific anti-HLA Ab (DSA), the child received high dose methylprednisolone (MP), high dose IV immunoglobulins (IVIg) and plasmapheresis (PP).

Results: Among the 27 enlisted children, 3 had pre-Tx anti-HLA Ab, including 2 waiting for reTx. 2/3 has received a transplant (L-SBTx). A significant titer of DSA was found in 8/19 children after Tx (42%), 4 isolated small bowel Tx (SBTx), 2 combined with liver (L-SBTx), and 2 multivisceral (MVTx, 1 with kidneys). In both the 1st ones (L-SBTx and SBTx), without preformed Ab, DSA appear contemporaneous of severe rejection with disappearance of epithelium. One child died, and graft was removed in the other. In the 6 others, biopsies showed an acute rejection in 3/6 (16% of transplanted patients), 2 mild, 1 moderate. All children received IVIg, and 5 doses MP (except the child MVTx-kidneys, normal biopsies). PP was performed in 4, and was not undertaken in 2 (1 normal biopsy, 1 mild rejection). The only severe complication of PP was a transient renal failure due to vancomycin overdose for a catheter-related infection. All control biopsies were normal. 2 children died 3 and 4 m. after Tx, of surgical and infectious complications. With a follow-up of 4 to 18 months, the 4 other children are at home with a normal graft function.

Conclusion: A significant titer of DSA is common early after Tx, and not always responsible for histological damage.
The pathological diagnosis of HR is not easy, as criteria have not been defined as they are in the kidney. C4d has not helped us to differentiate HR from cellular rejection (1). The dramatic course of 2 patients explains the aggressive protocol even without abnormal biopsies. Further clinico-pathological correlations are needed, in order to understand if cellular rejection was “fortuitously” associated with DSA. The last 2 patients received IVIg, MP and PP only when the biopsy was abnormal. An untreated HR may have a severe course: high risk of early graft losses, increased mortality. Due to the organ shortage, we decided not to reject any combined graft because of DSA (specific protocol designed), but to reject isolated small bowel grafts if the titer of preformed DSA was abnormal. An untreated HR may have a severe course:

References:

Disclosure of Interest: None declared.

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**SP-G-0231**

**Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori**

**EOSINOPHILIC ESOPHAGITIS AND GASTROESOPHAGEAL REFLUX DISEASE: A DEEP RELATIONSHIP FROM DIAGNOSIS TO LONG-TERM FOLLOW-UP**

F. Rea1, L. Dall’Oglio1, F. M. Paone2, T. Caldaro1, N. Cotugno2, L. Di Iorio2, F. Foschia1, E. Romeo1, R. Tambucci2, F. Torroni1, P. De Angelis1.

**1Digestive Surgery And Endoscopy Unit, Ospedale Bambino Gesù, 2Pediatric Gastroenterology and Endoscopy Unit, University of “Tor Vergata,” Rome, Italy.**

**Objectives and Study:** Eosinophilic infiltration of esophageal wall is observed in two clinicopathologic settings: gastroesophageal reflux disease (GERD) and eosinophilic esophagitis (EE). It is well known that EE and GERD can coexist and the overlap of EE with GERD is probably greater than believed, even for consequent disorders of esophageal motility. The aim of our study was to establish the relationship between EE and GERD and to define clinical features in patients with EE.

**Methods:** Between September 2000 and July 2010, we enrolled 70 consecutive patients (mean age 9.7 years; range 0.9–19 years) affected by EE, diagnosed according to Furuta criteria, including also patients with mild GERD (“overlap” EE) who did not respond to high dose proton pump inhibitor (PPI). All patients underwent 24/h pH monitoring or multi-channel impedance (pH-MII) at diagnosis and during the follow up, if moderate or severe GERD was suspected. Clinical evaluation and traditional allergy-testing (specific IgE, skin prick test, and patch test) have been used to search food and environmental allergies. Specific treatment of EE consisted of diet and/or fluticasone; children with mild GERD received also treatment cycles with PPI.

**Results:** At baseline assessment, 38 (54%) patients were affected by “primitive EE” and 32 (46%) were affected by EE coexisting with mild GERD (“overlap EE”). During the mean follow-up of 6 years and 3 months (range: 6 months–10 years), 9 children (6/38 with “primitive EE” and 3/32 with “overlap EE”) developed severe GERD secondary to EE, confirmed by pH monitoring or pH-MII. They were treated with high dose of PPI; 5 of them needed surgical correction of GERD, with complete resolution of symptoms and eosinophilic infiltration of esophageal wall. Of 32 patients with “primitive EE” treated with specific therapy for EE, 19 were asymptomatic, 10 were partially responsive and 1 lost to follow up; of 29 patients with “overlap EE” treated with specific therapy for EE and PPI cycles, 23 patients were asymptomatic, 5 patients partially responsive and 1 lost to follow-up. The difference of prevalence of allergy in “primitive EE” group was statistically significant according to χ² test (P < 0.05) (25/32 patients, 78%) and in “overlap EE” group the difference was not significant (18/29 patients, 62%); all patients with GERD secondary to EE had allergy.

**Conclusion:** Our study demonstrated that EE and GERD have a deep relationship that must always be sought can coexist. Allergy is also present in “overlap EE”. Clinical and instrumental reevaluations are mandatory in any stages of EE, to look for GERD that could condition the outcome of patients, predisposing to long-term complications.

**Disclosure of Interest:** None declared.

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**SP-G-0232**

**Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori**

**ESOPHAGEAL PRESSURE TOPOGRAPHY FEATURES IN CHILDREN WITH REPAIRED ESOPHAGEAL ATRESIA AND TRACHEO-ESOPHAGEAL FISTULA**

P. Karanika1,*, K. J. Lindley1, V. Giorgio1, N. Thapar1, J. Curry2, O. Borrelli1. 1Division of Neurogastroenterology & Motility, Department of Paediatric Gastroenterology, 2Department of General Surgery, Great Ormond Street Hospital, London, United Kingdom.

**Objectives and Study:** The pathogenesis of the esophageal motor abnormalities following repair of esophageal atresia (EA) and tracheo-esophageal fistula (TEF) is still matter of debate. The objective of this study was to investigate the characteristics of esophageal motor activity in patients with corrected EA and TEF by using esophageal high resolution manometry (EHRM).

**Methods:** Nine consecutive children (median age 8.2; range 2–13 y) with type C EA and TEF were enrolled into the study. All patients underwent prolonged EHRM by using a solid-state catheter incorporating 36 unidirectional strain gauge pressure sensors, spaced at 1-cm intervals. Single wet swallows, multiple rapid swallows (MRS), and solid swallow were systematically studied. According to the peristaltic propagation pattern assessed by generating an isobaric contour plot at 30 mmHg threshold pressure, the post-swallowing esophageal motility pattern were defined as
increased significantly (9.6\( < \)E124 P < 0.001) and the number of simultaneous contractions increased significantly (9.6 \( < \) 0.1 \( < \) 0.4, P < 0.001). No differences in the UESP (67.3 \( < \) 63 \( < \) 22, NS) and LESP (14.1 \( < \) 7.6 \( < \) 16.1 \( < \) 36, NS) were found between the 2 groups. The proportion of patients with peristaltic pattern was significantly higher in controls vs EA (8/8, 100\% vs 0/9, 0\%, P < 0.001) and accordingly an aperistaltic pattern characterized by simultaneous contraction was found in all age with EA (9/9, 100\% vs 0/8 0\%, P < 0.001). In 2 EA patients (2/9, 22\%) distal esophageal peristaltic activity was occasionally found. Peristaltic contractions were never recorded in the upper third of the esophagus in EA patients.

Conclusion: Profound esophageal motor abnormalities are ubiquitous in children with EA with an absence of normal peristaltic activity throughout the esophageal length despite a good anatomical result from surgery. It is likely that abnormalities of the esophageal enteric nervous system are contributory to this profound esophageal motor dysfunction in EA and TEF making these children candidates for neuronal stem cell therapy in the future.

Disclosure of Interest: None declared.

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peristaltic, peristaltic dysfunction, esophageal spasm and aperistaltic. Other parameters routinely analysed included: the number of peristaltic contractions, the number of simultaneous contractions, lower esophageal sphincter pressure (LESp) and upper esophageal sphincter pressure (UESp). Eight age-matched children (5 males, median age 7.5 yrs, range 2–15 y) with nonerosive reflux disease served as controls.

Results: The mean number of peristaltic contractions was lower in EA as compared to controls (0.4 \( < \) 0.8 vs 8.3 \( < \) 0.5, P < 0.001) and the number of simultaneous contractions increased significantly (9.6 \( < \) 0.1 \( < \) 0.4, P < 0.001). No differences in the UESP (67.3 \( < \) 63 \( < \) 22, NS) and LESP (14.1 \( < \) 7.6 \( < \) 16.1 \( < \) 36, NS) were found between the 2 groups. The proportion of patients with peristaltic pattern was significantly higher in controls vs EA (8/8, 100\% vs 0/9, 0\%, P < 0.001) and accordingly an aperistaltic pattern characterized by simultaneous contraction was found in all age with EA (9/9, 100\% vs 0/8 0\%, P < 0.001). In 2 EA patients (2/9, 22\%) distal esophageal peristaltic activity was occasionally found. Peristaltic contractions were never recorded in the upper third of the esophagus in EA patients.

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Disclosure of Interest: None declared.

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Gut Infection

HAEMATOPOIETIC STEM CELL TRANSPLANTATION AS A TREATMENT OPTION IN CHILDREN WITH MULTIPLE INTESTINAL ATRESIA AND RECURRENT STRICTURING DISEASE

E. Volonaki1,2, A. Hassan1, K. J. Lindley1, N. Shah1, P. Veys1, M. Elawad3.1 Great Ormond Street Hospital, London, United Kingdom.

Objectives and Study: Severe transmural inflammatory gut condition can result in stricture formation. When such process starts antenatally in children with primary immune deficiency or immune dysregulation, it can result in Multiple Intestinal Atresia (MIA). However, children with inflammatory gut conditions associated with immune dysregulation may develop strictureing inflammatory gut disorder without overt features of Crohn’s disease. Children affected by such conditions usually have refractory disease to all conventional immunosuppressive therapy and will continue to have recurrent strictures requiring multiple surgical resections. Haematopoietic stem cell transplantation (HSCT) is potentially curative for severe and refractory inflammatory gut conditions associated with immune dysregulation.

Methods: We report 3 children with MIA and recurrent intestinal strictures who underwent HSCT. Case 1 presented with congenital multiple intestinal atresias, needing surgical intervention in the first week of life. Case 2 presented in the neonatal period with congenital pyloric stenosis and was found to have severe apoptotic inflammatory panenteric disease. Case 3 presented at the age of 4 months with severe inflammatory gut condition that led to recurrent intestinal strictures. Overt primary immune deficiency has been excluded in all three patients. Despite the use of extensive immunosuppressive therapy, all three children continued to have severe panenteric inflammation and recurrent strictures needing multiple surgical resections. All 3 patients received HSCT with reduced intensity conditioning followed by a matched sibling donor in case 1, mismatched unrelated cord in case 2 and a matched family donor in case 3 and were followed up 1.2 to 4.5 years.

Results: All patients are alive with successful engraftment. Following HSCT there was resolution or substantial improvement in their inflammatory gut conditions. Most important, none of the 3 children developed any further strictures following transplant. All patients were discharged home for the first time following transplant. To our knowledge, patient 1 is the only survivor with MIA.

Conclusion: This is the first report to demonstrate the effect of HSCT in downregulating the severe refractory inflammatory process in children with MIA and recurrent intestinal strictureng disease. Therefore we recommend that HSCT should be considered as a treatment option in this selected group of patients to improve their survival and improve quality of life.

Disclosure of Interest: None declared.

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Intestinal Motility

COLONIC MANOMETRY VERSUS COLONIC SCINTIGRAPHY AS A DIAGNOSTIC TOOL FOR CHILDREN WITH SEVERE CONSTIPATION

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Objectives and Study: Children with severe constipation not responsive to conservative treatment merit further investigation. In adults, colonic manometry and colonic scintigraphy are both valuable studies to help differentiate normal from abnormal colonic motility. The aim of this study was to compare the diagnostic yield and tolerability of colonic manometry vs colonic scintigraphy in children with severe constipation.
Methods: Twenty-two children (mean age 11.4 years, 73% male) with severe constipation referred to our Motility Center were included. Colonic manometry was performed per standard protocol. Scintigraphy was performed by the Nuclear Medicine department per the Mayo Clinic protocol. All patients, except for 1, swallowed a methacrylate-coated capsule containing In-111. One patient had the capsule placed during upper endoscopy. Images were taken at 4, 24, and 48 hours, and geometric centers were calculated. We categorized the results of both tests in 3 different groups: normal, abnormal function in the distal part of the colon, and colonic inertia. Cohen’s kappa was used to assess the level of agreement. Patients and parents completed a questionnaire regarding their experience with the tests.

Results: Placement and maintenance of the catheter in the right side of the colon during the entire colonic manometry was achieved in 48% of the cases. Scintigraphy data was obtained in 21 patients; the capsule did not properly release in 1 patient. Colonic manometry was normal in 38%, abnormal in the proximal colon in 48%, and colonic inertia was diagnosed in 14% of the patients. Colonic scintigraphy showed a normal transit time in 24%, delay in the distal colon in 52%, and colonic inertia in 24% of the patients. All 3 patients diagnosed with colonic inertia by manometry had the same result by scintigraphy. The kappa score was 0.32 for the 2 tests. Data from the questionnaire demonstrated that 86% of the patients preferred scintigraphy over manometry. Despite this preference, only 3 patients described manometry as significantly unpleasant. Parental preferences were more divided, with almost 25% preferring colonic manometry over scintigraphy.

Conclusion: Colonic manometry and colonic scintigraphy provide different diagnostic information in children with medically refractory constipation. The 2 studies have a fair agreement regarding the categorization of the type of colonic dysfunction in constipation. Scintigraphy is a well-tolerated procedure and may be a useful tool for evaluation of pediatric patients with severe constipation.

Disclosure of Interest: None declared.

PO-AHP-0002

Allied Health Professionals (Including Nurses & Dieticians)

TUBE FEEDING PRACTICES IN INSTITUTIONS FOR PATIENTS WITH NEURODEVELOPMENTAL DISABILITIES IN FLANDERS, BELGIUM

M. Van Winckel, A. Vanoppen, S. van de Velde.

Objectives and Study: Descriptive transversal study on the practice of tube feeding in institutions for patients with neurodevelopmental disabilities in Flanders, Belgium.

Methods: All residential institutions in Flanders were asked to participate. A structured questionnaire on tube feeding practices was directed to the medical department of each participating institution. After obtaining informed consent, individual tube feeding practices in 94 randomly chosen residents were recorded. Data were gathered by 3 students, as part of their bachelor thesis. The study was approved by the ethical committee of UZ Gent (reg.nr. B67020097398).

Results: 17/24 institutions agreed to participate. Resident number varies between 13 and 514, 12/17 institutions house more than 188 residents. A total of 314 residents are tube fed. The number of tube-fed residents per institution varies between 2 and 103. 14/17 institutions have a written protocol on tube feeding. 280/314 have a gastrostomy, in 288/314 a feeding pump is used. In 15/17 institutions a gastrostomy is inspected daily by a nurse. In 3 the gastrostomy is disinfected.

PO-AHP-0001

Allied Health Professionals (Including Nurses & Dieticians)

ANTHROPOMETRIC AND BIOMETRIC CORRELATIONS FOR INSERTING PEDIATRIC NASOGASTRIC/OROGASTRIC TUBE


Objectives and Study: To determine whether height and knee height variables could be used to accurately predict lower esophageal sphincter (LES) location in Brazilian children; (N=153; age range from 2 to 12 years).

Methods: The study design was analytical, observational and transversal. Patients with abdominal functional pain and otherwise healthy were invited to participate. Biometric data were included in statistical analysis when upper gastrointestinal endoscopy was concluded as normal. The distance from superior dental arch to the lower esophageal sphincter was correlated to height and knee height measurements. Predictive models were adjusted using multiple linear regression

Results: Height and knee height showed correlation values with LES location were, respectively, r = 0.90 and 0.87. The regression equations that described subjects (L = 0.42 [knee height] + 15, and L = 0.2[height] + 10, where L is the location in centimeters of the distance from the superior dental arch to LES; knee height and height are in centimeters) correctly predicted lower esophageal sphincter location, respectively, in 83% and 77% of subjects.

Conclusion: Correlation values between external measurements and distance from superior dental arch to esophageal gastric transition have pointed height as the best correlated measurement but knee height also showed a high correlation. We conclude that lower esophageal sphincter location can be predicted from height or knee height in subjects from 2 to 12 years. The prediction is sufficiently accurate to allow placement of gastric tubes for nutrition. The regression equation may be used in patients who are unable to standing up as children with cerebral palsy.

Disclosure of Interest: None declared.
daily, in 14 water and soap is used in daily care. Individual tube feeding practices are recorded in 94 residents, 45 male and 49 female, median age 18 years. 67/94 are exclusively tube fed, 27/94 receive supplemental tube feeding. 65 have a button, 24 a PEG, 1 a surgical gastrostomy and 4 a jejunostomy. In 87/94 gastrosomy care consists of daily washing with water and soap. In 7/94 daily desinfection is performed. In 16 tube feeding is delivered continuously, 78 have an individual feeding scheme delivering feeds in 2 to 6 portions. In 47 enteral feeding is given in its original container. Administration tubes are changed daily in 41, every other day in 21, every 3 days in 18, and less frequently in 14 residents. Extension sets for buttons are changed less frequently (weekly to monthly). Tubes are rinsed with water after each administration of feeding in 89, and also before in 18/89. Tap water is used for rinsing in 46, mineral water in 41, distilled water in 6. At the moment of the survey 8 gastrosomies showed granulation tissue or infection, 1 had leakage. 48 had a history of infection and/or granulation, 11 of leakage, 1 of migration of the PEG and 1 of buried bumper. 

Conclusion: Tube feeding by a gastrostomy is the rule in institutional patients with neurodevelopmental disabilities in Flanders. Practices regarding gastrostomy care and administration of tube feeding are diverse. Evidence regarding “best practice” is needed.

Acknowledgments: Thanks to K. Mennes, K. Sercu, and Y. Van Rooy for gathering the data.

Disclosure of Interest: None declared.
The Food Adventures Workshop: A Five Week Nutrition/Science Intervention Program Provided to Children 8–11 Years Old

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Objectives and Study: The purpose of this study was 2-fold: to create a 5-week nutrition/food science curriculum along with cooking activities; to implement the curriculum and evaluate its effectiveness in improving the knowledge and attitudes of the participants toward healthy eating behaviors.

Methods: This 5-week program, called “The Food Adventures Workshop,” was implemented in a cooking class at California State University Northridge (CSUN), through the Summer Academic Program for Elementary School Students (SAPESS). Seven boys and 13 girls with the mean age 8.7 participated in this study. Data were collected from participants and their parents. The participants completed a pretest questionnaire on the first day and posttest at the end of the 5-week workshop. Questions sought opinions and information concerning nutritional knowledge, healthy eating behaviors and attitudes, healthy exercise behaviors and attitudes, and frequency of fruits and vegetable consumption of the participants.

Results: Paired samples t tests indicated that there were statistically significant differences in the participants’ attitudes and behaviors toward fruits and vegetables. Specifically, participants significantly increased their fruit consumption after participating in the class. Parents and/or guardians commented that the Food Adventures Workshop was a great opportunity for their children to learn about nutritional concepts and application.

Conclusion: These findings suggest that such intervention programs might assist in the prevention of childhood obesity and promotion of healthy cooking for children, as well as similar age groups.

Disclosure of Interest: R. Dabboussi Grant/Research Support from: Community Service Learning at the Center for Innovation and Engaged Learning Opportunities (CSUN)

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weight-for age z score = 0.23 ± 1.24) were included in the study. The children were randomly assigned in two treatment groups. In Group A the parents were instructed about their children’s diet with written instructions and examples explained by the pediatric gastroenterologist. In Group B the parents had a further appointment with a dietician and a personalized 7-day dietary regimen was designed according to the needs of every patient, supplemented by high fiber recipes. Dietary intake was assessed by a 24 h dietary recall at baseline (Visit 1) and 1 month after intervention (Visit 2). Analysis of dietary intake was performed with Food Processor 7.40. The changes in water and fiber consumption after intervention were used as compliance criteria. Statistical analysis of the data was carried out using paired and independent samples tests.

Results: The changes (Visit 2-Visit 1) in children’s fiber and water intake in Group B were significantly higher compared to Group A (Table).

<table>
<thead>
<tr>
<th></th>
<th>Personalized dietetic treatment n = 44</th>
<th>Medical instructions n = 42</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber %</td>
<td>84.7 ± 69.5</td>
<td>21.2 ± 79.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>15.6 ± 2.9</td>
<td>3.7 ± 3.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Water (mL)</td>
<td>621 ± 510</td>
<td>148 ± 467</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Water (mL/kg)</td>
<td>37.9 ± 45.3</td>
<td>4.8 ± 39.5</td>
<td>0.001</td>
</tr>
</tbody>
</table>

At the follow-up visit, a month later, water consumption (mL) was found increased significantly in group A, (from 361.8 to 1005.3). Fiber intake was also increased but it was not statistically significant. In group B, fiber intake (g) and water intake (mL) increased significantly (from 10.00 to 25.61 ± 14.77, P < 0.001; from 940.9 ± 353.9 to 1562.1 ± 422.3, <0.001, respectively). The increase in energy and macronutrient intake (Visit 2-Visit 1) was significantly higher in group B compared to group A: energy (kcal) 334 ± 364 vs 38 ± 465, P = 0.002; protein (g) 6.9 ± 26.7 vs −7.0 ± 32.1, P = 0.03; carbohydrates (g) 55.3 ± 58.1 vs 13.2 ± 64.5, P = 0.002.

Conclusion: Children under personalized dietetic management for functional constipation achieved better compliance in increasing fibre, water consumption and overall food intake. These findings suggest that they found their diet more appealing.

Disclosure of Interest: None declared.

PO-G-0013

Coeliac Disease and Enteropathies

CELIAC DISEASE HETEROGENEITY VERSUS HOMOGENEITY IN DUODENAL BIOPSIES AND CLINICAL CORRELATION TO MARSH AND DESCRIPTIVE PATHOLOGIC CLASSIFICATIONS

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Objectives and Study: Potential, silent and atypical celiac disease (CD) is frequently diagnosed today, increasing the debate about clinicopathological correlation. We aimed to compare different pathologic classifications to clinical parameters in search for correlation, also when the Marsh type is not homogenous.

PO-G-0011

Coeliac Disease and Enteropathies

COMPLIANCE TO GLUTEN-FREE DIET IN ADULTS AND CHILDREN WITH CELIAC DISEASE: COMPARISON BETWEEN DIFFERENT MEASURES OF EVALUATION

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Objectives and Study: Increasing numbers of individuals are now being diagnosed with celiac disease (CD). Although gluten free diet (GFD) is extremely important for CD patients, it is known that not all patients are able to have a good compliance. Furthermore, no standardized, objective and accurate tools exists to measure the adherence to GFD. The aim of the study was to evaluate the compliance to GFD in celiac patients by comparing interviewing patients (anonymous and not anonymous) and serologic tests (serum titres of IgA tissue-transglutaminase antibodies).

Methods: 175 patients with biopsy-proven CD (91 children, median age 11 y and 84 adults, median age 45 y) in GFD since at least 1 year were examined. Dietary compliance was assessed by anti-transglutaminase antibodies measurement (anti tTG-test) and 2 questionnaires: the first one was anonymous (children completed it without the help of parents and only in the presence of a paediatrician) and the second was not anonymous (children responded in presence of their patents). Characteristics of the enrolled subjects (age at diagnosis, actual age, symptoms at diagnosis, familiarity for CD, length of GFD, nutritionist’s assistance) were correlated to quality of their GFD.

Results: 24% of children (22 out of 91) and 19% of adults (16 out of 84) declared GFD transgressions by anonymous questionnaire; 8% of children (8 out of 91) and 15% of adults (13 out of 84) declared GFD transgressions by not-anonymous questionnaire. 16% of children (15/91) and 14% (12/84) of adults tested positive to anti-tTG test, but without correlation to questionnaire’s answers. Compliance was bad in adolescents, in asymptomatic children at diagnosis and in children with longest duration of GFD. Receiving nutritionist counseling did not ameliorate the compliance to GFD in adults.

Conclusion: Follow-up in CD is as important as early CD diagnosis, considering that 19–24% of patients did not follow appropriately the GFD. Questionnaire resulted more sensible than serology to detect patients that transgress. Children more truthfully filled in the GFD questionnaire when helped by the pediatrician alone in the absence of their parents.

Disclosure of Interest: None declared.
Methods: A retrospective series of 167 clinically/serologically well-characterized CD cases were evaluated comparing updated Marsh and mucosal lesion (descriptive) classifications. Duodenal biopsies were divided into homogenous/single Marsh type and heterogeneous/patchy/2–4 Marsh types.

Results: 117/167 were children and 60/167 adults. By univariate analysis the following correlations were found: diarrhea, vomiting and swollen abdomen were more frequent in children \( P = 0.023, 0.001 \) and 0.001, and anemia in Marsh 3b, 3c \( (P = 0.027) \). Homogenous Marsh type was found in 52/156(33.3%) – mean age of 17.6 (range 2.4 to 65 years), and heterogenous in 104/156(66.7%)–mean age of 16.9 (range 1.3 to 75 years). In heterogenous group 98/103 had Marsh 3a, 3b, 3c when the total number of fragments/biopsy \( \geq 3.8, P = 0.0001 \)A and the age \( \geq 15, P = 0.015 \). In homogenous group, 13/23 of Marsh 3b, 3c had anemia, versus 3/20 in Marsh 0, 1, 3a \( (P = 0.036) \). TTG levels were higher in advanced Marsh types only in homogenous group: mean 94.81, median = 100 in Marsh 3c \( (P < 0.01) \). Descriptive classification correlates with Marsh classification (the gold standard), with a sensitivity of 100%, specificity 83.3%, PPV 99.4% and NPV 100%. By multivariate analysis only anemia and tTG level were found to be independent predicting factors for the presence of any villous atrophy (Marsh type 3a, 3b, 3c) \( (P = 0.021, OR = 1.23, \text{respectively, as well as for significant villous atrophy (Marsh type 3b, 3c) - } P = 0.029, OR = 2.943 \) and \( P = 0.012, OR = 1.13, \text{respectively.} \) Liver enzymes and anemia were found to be the independent predicting factors for total villous atrophy (Marsh type 3c) \( (P > 0.05, OR = 3.86 \) and \( P = 0.005, OR = 3.61, \text{respectively.} \)

Conclusion: Only classic CD correlated with age at diagnosis, being more frequent in children. In heterogenous group more atrophy was detected in age\( \geq 15 \) years or when \( \geq 4 \) tissue fragments/biopsy. A descriptive pathologic classification is as good as Marsh. Only anemia and tTG level were found to be significant independent predicting factors for the presence of any villous atrophy (Marsh type 3a, 3b, 3c). Liver enzymes and anemia were found to be independent predicting factors for total villous atrophy (Marsh type 3c).

Disclosure of Interest: None declared.

PO-G-0014

Coeliac Disease and Enteropathies

COELIAC DISEASE AND THE RISK OF SUBSEQUENT TYPE 1 DIABETES

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Objectives and Study: Earlier studies suggest that children with type 1 diabetes (T1D) are more likely to have a subsequent diagnosis of coeliac disease. However, research is sparse on the risk of subsequent T1D in the population with coeliac disease. Our aim was to determine the risk of subsequent T1D in coeliac children, adolescents and their first-grade relatives.

Methods: We have determined in coeliac patients: children, parents (n: 61, median age range: 21.22/6.1–48.2/y) and in their asymptomatic family members (n: 128, median age range: 26.2/2.3–49.5/y) and in healthy control (n: 100, median age range: 18.6/2.5–38.7/y) the islet cell antibody (ICA/JDFU with indirect immune-fluorescence assay), glutamic acid decarboxylase autoantibody (GADA/U/mL, RIA), C-peptide (ng/mL, RIA) levels and the occurrence of the gene protein tyrosine phosphatase, non-receptor type 22 (PTPN22, TaqMan SNP genotyping assay).

Results: We have found 2 patients with elevated ICA levels (>10 JDFU) in the coeliac group, 1 elevated ICA level (>10 JDFU) in the asymptomatic family members under 10 years of age and 15 elevated values above 10 years, in the control group 1 elevated ICA level below 10 years and 12 cases above 10 years. The intravenous glucose challenge has proven an exact early insulin response. The other assays (GADA, C-peptide) did not prove any alterations in the different groups. The PTPN22 polymorphism was equal to that in the normal occurrence.

Conclusion: Our coeliac patients following a careful gluten free diet (detected transient failures in 4 patients) regimen have not shown a marked risk for subsequent type 1 diabetes mellitus. The results of the examined groups did not show any significant difference. To determine the occurrence, risk and the interaction of different factors concerning the development of type 1 diabetes mellitus in coeliac children and their family members need further longitudinal control and survey as the medical reviews are controversial.

References:


Disclosure of Interest: None declared.

PO-G-0018

Coeliac Disease and Enteropathies

BONE HEALTH IN CHILDREN WITH CELIAC DISEASE ASSESSED BY DUAL X-RAY ABSORPTIOMETRY: EFFECT OF GLUTEN-FREE DIET AND PREDICTIVE VALUE OF SERUM BIOCHEMICAL INDICES

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Objectives and Study: Bone health is affected in patients with celiac disease (CD). Dual x-ray absorptiometry (DXA) has become the method of choice for the evaluation of bone status. Gluten free diet (GFD) significantly improves bone mineral density (BMD), although the minimum duration required for normalization is unknown. In this study we assessed bone health in children with CD, the effect of a gluten-free diet (GFD) and the predictive value of biochemical indices in determining bone derangement.

Methods: Forty-five children at the time of diagnosis of CD, (group A) and 36 children on GFD for more than 2 years (group B), were included. Additionally, 16 children of group A were reexamined 12 months after initiation of GFD. Laboratory investigation included serum measurements of 25-(OH)-D, parathormone (PTH), calcium (Ca), phosphorus (P) and alkaline phosphatase (ALP). Bone mineral density (BMD) was assessed by Dual x-ray absorptiometry (DXA). Statistical procedures included Fisher’s exact test, Student t test, Wilcoxon’s matched-pairs signed-ranks test and Pearson’s r. The ability of various biochemical indices to distinguish patients with “low bone mineral density for chronologic age” as defined by the International Society of Clinical Densitometry, was assessed non-parametric receiver operating characteristic curve (ROC) analysis.

Results: BMD z score was significantly higher in group B (−0.58 ± 0.80) compared to group A (−1.12 ± 1.54, P = 0.044), however both groups had lower values than the expected in the normal population (P < 0.001 in both groups). Parathormone serum levels were significantly higher (P = 0.002) in group A (49.8 ± 29.5 pg/ml) compared to group B (33.2 ± 15.6 pg/mL) with patients on GFD having significantly lower probability of abnormal PTH status (P = 0.05). In group B, BMD z score was positively correlated with 25-(OH)-D levels (P = 0.009). In the 16 patients with the repeated measurements, BMD z scores (−1.45 ± 0.28 vs −0.61 ± 0.25, P = 0.004) and abnormal 25-(OH)-D status (37.5% vs 0%, P = 0.018) differed significantly between pre and post GFD. No biochemical index was capable of adequately predicting an abnormal BMD z score in the overall population (ROC analysis, all AUCs<0.66).

Conclusion: GFD has a beneficial impact on bone health, although 2 years on diet does not ensure BMD normalization. Biochemical markers are not indicative of BMD disturbances. DXA should be a part of standard management in children with CD.

Disclosure of Interest: None declared.

PO-G-0019

Coeliac Disease and Enteropathies

POTENTIAL CELIAC DISEASE: POSSIBLE MARKERS OF EVOLUTION TO OVERT DISEASE

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Objectives and Study: Potential celiac disease (CD) is characterized by normal histologic features associated with positive gluten dependent autoantibodies and DQ2/DQ8 haplotype. The outcome is variable and unpredictable; sometimes histologic picture remains normal for long time, some others it progresses to overt villous atrophy. At present only a few prospective studies are available. The aim of this observational study was to identify new reliable markers of evolution from potential to overt celiac disease.

Methods: We identified 84 cases of potential CD (51 F, 33 M, age M 7.5 ± 4.4 years) during the years 2002–2010, referred to our gastroenterology pediatric service. A screening test was performed dosing IgA/IgG anti-tissue transglutaminase antibodies (tTG-Ab) by radiobinding assay. Endoscopic bowel biopsy was performed in all patients who were repeatedly tTG-Ab positive. Histological evaluation was done according to Marsh’s classification modified by Oberhuber. Follow-up was performed at 6, 12, 18, 24, 24–48 and over 48 months after diagnosis by the determinations of t-TG-Ab, anti endomysial antibodies (EMA), folic acid, hemoglobin and ferritin; a second biopsy was indicated in the case of clinical symptoms onset or in the presence of a twice higher tTG-Ab value than the basal level.

Results: Out of 84 patients with features according to potential CD, 31 (18 F, 13 M, age M 7.65 ± 4.8) started a gluten-free diet (GFD) for the following indications: gastrointestinal symptoms (n = 7), short stature (n = 12), autoimmune associated condition (n = 1), Turner syndrome (n = 1), peripuberal age (n = 2), ex adjuvantibus (n = 5), significant tTG-Ab values (n = 1), other symptoms (n = 2). The remaining 53 patients (33 F, 20 M) on a gluten-containing-diet were followed up. A second biopsy was performed in 24 patients: 16 of them (30.2%, 9 M, 7 F, age M 8.2 ± 5.1) showed a worsening of the histological picture, suggestive for the diagnosis of CD. The mean time of progression was 3.2 years ± 2.3 years. Among patients who underwent the second biopsy during the follow-up, the only of the considered markers that showed a significant difference between patients who developed CD and those who did not, was tTG IgA titre. This difference became statistically significant starting from 6 months from diagnosis of potential CD (p-value = 0.016), whereas all patients showed a worsening of the histological picture at the biopsies done after 12 months from diagnosis of potential CD.

Conclusion: Potential CD is a fairly common condition in subjects investigated for CD. The evolution from potential to overt CD can occur rapidly, in several years, or never occur. Except for tTG IgA, no more markers have shown the ability to reliably predict this evolution and duodenal biopsy still remains the only way to confirm diagnosis.

Disclosure of Interest: None declared.
PO-G-0022

Coeliac Disease and Enteropathies

OSTEOPENIA PREVALENCE RATIO IMPROVES IN CHILDREN WITH CELIAC DISEASE AFTER 4 YEARS OF GLUTEN-FREE DIET

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Objectives and Study: Bone mineral density (BMD) has been reported to be low in children with celiac disease (CD). An improvement in the BMD when gluten-free diet (GFD) is carried out has been widely shown, although a normal BMD is not always achieved. The aim of our study is to find out the duration of GFD needed to improve the prevalence of osteopenia (<1 BMD standard deviation) in CD patients.

Methods: The study included 166 children over 6 years old (10.95 ± 3.71) with CD in whom BMD had been performed. All patients were on strictly GFD (5.39 ± 3.90 years) BMD was obtained in the lumbar spine (L1–4) using dual energy radiograph bone densitometer and corrected for age and gender (z score). Mean BMD z score obtained was −0.23 ± 1.06. The osteopenia prevalence for the group of children at each moment of the GFD had been studied. Osteopenia prevalence in children that had been at least 2, 3, 4 or 5 years on GFD had been compared with the osteopenia prevalence in children that hadn’t reach 2, 3, 4 or 5 years of GFD duration, respectively. The statistical analysis was performed using the chi-square test with the SPSS 17.0 pack.

Results: Global prevalence of osteopenia was 24.7%, 47.4% of children under 1 year of GFD had osteopenia, and 16.7% of patients that had been at least 10 years on the GFD. The table summarizes the osteopenia prevalence before and after each moment studied (2, 3, 4, and 5 years after the GFD was begun) and the comparison between them.

<table>
<thead>
<tr>
<th>GFD duration, y</th>
<th>Osteopenia prevalence before time studied, %</th>
<th>Osteopenia prevalence after time studied, %</th>
<th>Chi-square (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>44.1</td>
<td>19.7</td>
<td>0.003</td>
</tr>
<tr>
<td>3</td>
<td>36.5</td>
<td>19.3</td>
<td>0.017</td>
</tr>
<tr>
<td>4</td>
<td>31.3</td>
<td>20.2</td>
<td>0.102</td>
</tr>
<tr>
<td>5</td>
<td>26.8</td>
<td>22.6</td>
<td>0.529</td>
</tr>
</tbody>
</table>

Conclusion: Osteopenia is frequent in celiac disease at diagnosis and along the first years of the GFD. GFD in celiac children improves the osteopenia, and the longer the GFD is achieved, the lower the osteopenia prevalence is found. Our study shows that there are significant differences between the osteopenia prevalence before and after 4 years of GFD, but not earlier.

Disclosure of Interest: None declared.

PO-G-0026

Coeliac Disease and Enteropathies

SELF-PERCEIVED BURDEN OF COELIAC DISEASE IN CHILDREN AND THEIR FAMILIES: A NATIONWIDE PROSPECTIVE STUDY

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Objectives and Study: The estimated prevalence of coeliac disease in children is up to 1%, but because of the heterogeneous clinical presentation the disorder is markedly underdiagnosed. However, many of the unrecognized patients have no significant clinical symptoms, and it remains unclear whether such screen-detected cases would benefit from an early diagnosis and treatment. The aim of this prospective study was to assess self-perceived well-being and health and the impact of a gluten-free diet in children with a newly detected coeliac disease. Of particular interest was to compare screen-detected children and those diagnosed due to clinical symptoms.

Methods: All new child members of the Finnish Coeliac Society and their parents received a questionnaire comprising questions about the severity of clinical symptoms, the overall health condition the child and reaction of the family after receiving coeliac disease diagnosis. A second follow-up questionnaire was sent to all original responders after 1 year on a gluten-free diet. The follow-up questionnaire included questions about the strictness and difficulty to maintaining the diet and its effect on the daily life.

Results: All together 133 (60%) out of 222 families responded to the questionnaire and fulfilled inclusion criteria. Of those, a further 131 returned the follow-up questionnaire. Forty-three (32%) children were found by screening at-risk groups and 90 (68%) because of clinical symptoms. Of note, 67% of the screen-detected children also suffered from some clinical symptoms before diagnosis. After 1 year on gluten-free diet adherence was strict in 71% of the screen-detected and 83% of the symptom-detected children; 81% were capable of managing the diet and less than 5% considered it difficult. Symptoms alleviated in 78% of the screen-detected and 86% of the symptom-detected patients and the diet was experienced as positive in more than 90% of the children in both groups. About 80% of the children in both groups and all but 2 parents were content with the coeliac disease diagnosis.

Conclusion: Our results showed that most of the screen-detected children benefit from the early diagnosis and treatment of coeliac disease. In addition, positive attitude towards coeliac disease diagnosis and a relatively good adherence to gluten-free diet can be achieved also in this patient group.

Disclosure of Interest: None declared.

PO-G-0029

Coeliac Disease and Enteropathies

DUODENAL MUCOSA DIFFUSION OF THE HISTOLOGICAL LESIONS IN COELIAC PATIENTS CORRELATES WITH TRANSGLUTAMINASE AB TITERS AND TARGET DOMAINS IMMUNOREACTIVITY
Objectives and Study: Coeliac disease (CD) is an autoimmune gluten-dependent disorder, characterized by typical histological lesions of the small intestinal mucosa. CD patients usually show coeliac-specific serum autoantibodies, in particular anti-transglutaminase autoantibodies (tTGAb) that react against multiple epitopes of the protein. To date, no information is available on who tTG epitope immunoreactivity varies with the different histological picture. The aim of our study was to evaluate a possible correlation between the diffusion of duodenal mucosa lesions and tTGAb titers and the epitope specific humoral immunoreactivity against the combinations of 3 human recombinant constructs of the tTG molecule (full-length aa.1–687, a.a.227–687 and a.a.473–687).

Methods: Sera of 326 CD children at diagnosis (209 f, median age: 7.3 years) were analyzed for tTGAb with a fluid-phase radioimmunoprecipitation assays (RIA). 231 of them were also tested for the two distinct fragments. Patients were classified as having diffuse (D), patchy (P) or only bulb (B) histological lesions.

Results: All the CD patients were found full-length tTGAb positive. tTGAb mean ± SD was 0.84 ± 0.39, 0.57 ± 0.39 and 0.45 ± 0.24 Ab index in patients with D, P and B lesions. A significant difference was found between localized (P+B) and D lesions (0.52 ± 0.34 vs 0.84 ± 0.39, P < 0.0001). The main target of immunoreactivity in CD patients was the tTG(227–687) fragment (D: 89%, P: 75%, B: 40%; D vs B P < 0.0001). The immunoreactivity for the tTG(473–687) fragment was: D: 53%, P: 19%, B: 10%; D vs P p < 0.0001). The immunoreactivity for the tTG(473–687) fragment was: D: 53%, P: 19%, B: 10%; D vs P p < 0.017, D vs B: p = 0.02. The table shows the combined immunoreactivity against the 2 tTG fragments.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Ab+/</td>
<td>104 (50.7%)</td>
<td>78 (38%)</td>
<td>6 (3%)</td>
<td>17 (8.3%)</td>
</tr>
<tr>
<td>Ab-/</td>
<td>3 (18.7%)</td>
<td>9 (56.3%)</td>
<td>0</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>(473–687)</td>
<td>1 (10%)**</td>
<td>3 (30%)</td>
<td>0</td>
<td>6 (60%)***</td>
</tr>
</tbody>
</table>

D vs P P = 0.027, **D vs B P = 0.028, ***D vs B P < 0.0001.

Conclusion: tTGAb titer correlates with the diffusion of histological lesions in CD patients at diagnosis. The immunoreactivities against fragments as well as the combined immunoreactivities of tTG(227–687) and tTG(473–687) were higher in patients with diffuse lesions. Whilst the absence of both immunoreactivities was significantly more frequent in patients with only bulb lesions. This is the first evidence of a distinct humoral immunoreactivity in patients with different duodenal mucosa involvement.

References:

Disclosure of Interest: None declared.

PO-G-0030

Coeliac Disease and Enteropathies
THE INCIDENCE OF CHILDHOOD COELIAC DISEASE IN SCOTLAND: FIRST YEAR OF THE SPSU COELIAC PROJECT
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Objectives and Study: To establish the incidence of coeliac disease (CD) (<16 years) in Scotland using the Scottish Paediatric Surveillance Unit (SPSU) e-reporting system and through strategic contacts within the 3 Tertiary GI Regions - West (W), East (E) and North (N) of Scotland.

Methods: The SPSU began e-mailing Scottish Paediatric Society (SPS) members in September 2009- the question was how many new cases of CD were diagnosed by members in the preceding month. Routine demographic and clinical data was collected on each new patient using a validated questionnaire. Regular e-mail contact was maintained throughout the study period. Only cases diagnosed from 1.09.09 to 31.08.10 were included in the study. Minimal incidence rates for each of the 3 regions were calculated using population data from the General Register Office for Scotland.

Results: There has been steady reporting of new cases over the year. Double reporting has happened in 13 cases. These have been cross-checked and duplicate cases excluded using sex, age and postcode data. Four cases were furthermore excluded as a biopsy to confirm diagnosis was not performed (2W, 1N, 1E). A total of 94 new cases of CD were reported (38E: 39W: 17N), males to females ratio was 1:2. Mean (SD) age at presentation was 7.9 (± 4.1) years. 57% of patients had abdominal pain, 31% diarrhoea and 15% were asymptomatic. 24% of cases were actively screened for CD (60% due to type 1 diabetes). 30% had a first degree relative with CD. There were no significant differences in age at presentation (P = 0.79), asymptomatic (P = 0.29) cases and those actively screened (P = 0.77) between tertiary regions. Of the 94 patients, 90 (94 %) were scoped by a paediatric gastroenterologist, one by an adult gastroenterologist (W) and 3 by paediatric surgeons (N). The under 16 populations within each catchment area are 504,973 in the west, 240,664 in the East and 230,323 in the north (General Register Office for Scotland). The calculated incidence of CD is 7.7 W, 7.4 N and 15.8 E per 100,000 population.

Conclusion: The study has successfully captured 94 new cases of CD within the Scottish population. There is some uniformity in terms of screened groups, presenting...
symptoms and asymptomatics between regions. The majority have diagnosis confirmed endoscopically in a pediatric setting. There is a stark difference, however, in the number of cases diagnosed per head of population, with a rate more than twice the west and north diagnosed in the east of Scotland. The reasons for this are unlikely to be due to different population genetics, given the homogeneity of the population. Further analysis is required.

Disclosure of Interest: None declared.

PO-G-0031

Coeliac Disease and Enteropathies
EXPRESSION STUDIES ON CANDIDATE GENES OF NF-KB PATHWAY IN MONOCYTES OF CELIAC AND POTENTIAL CELIAC PATIENTS
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Objectives and Study: Celiac disease (CD) is a polygenic disease and through genome wide association studies (GWAs) more than 40 non-HLA genes were identified to be associated with the disease. The term ‘potential CD’ is assigned to individuals characterized by positive serology and normal small intestinal mucosa. The aim of the study is to evaluate the expression of 3 candidate genes of the NF-KB pathway (REL, TNFSF14, TNFAIP3) in monocytes of CD patients, potential CD, CD on gluten free diet (GFD) and controls, in order to show a possible different expression of selected genes in this cell type. c-RELI is subunit of the NF-KB complex, while TNFAIP3 is a key player in the termination of NF-KB signaling (negative feedback). TNFSF14is a cytokine that binds to TNFRSF3/LTBR, it activates NF-KB and stimulates the proliferation of T-cells.

Methods: Monocytes were extracted by immunoselection with magnetic beads from peripheral blood of at least 7 controls, 8 celiacs, 6 potential, 5 GFD celiac patients. RNA was extracted and reverse transcribed: since the RNA amount was very low for the analysis, cDNA transcription was linearly amplified before expression studies using the TaqMan PreAmp Master Mix kit. Three genes were analyzed using a Taqman specific probe (AssaysOnDemand, Applied Biosystems).

Results: With regard to REL gene, the comparison between controls and celiacs shows a substantial increase of gene expression in CD patients (P = 0.0059), CD-GFD and potential CD have a similar expression compared to celiacs. Instead, regarding to TNFAIP3 and TNFSF14 controls, CD-GFD, potential CD have a similar expression compared to celiacs.

Conclusion: Through expression studies on peripheral blood monocytes, we can assume that NF-KB pathway plays an important role in the pathogenesis of CD. REL genes show an expression level higher in celiac compared to controls, unlike the data obtained in our previous studies on intestinal biopsies where celiacs showed a trend similar to that of controls. That can lead us to hypothesize a mechanism of early lymphocyte activation at the peripheral level, not only localized at intestinal level. Further studies are needed to confirm these preliminary data.

Disclosure of Interest: None declared.

PO-G-0032

Coeliac Disease and Enteropathies
ANALYSIS OF THE LPP GENE IN A CELIAC POPULATION OF SOUTHERN ITALY
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Objectives and Study: LPP lipoma preferred partner) is a gene located on 3q28. Little is known about LPP, it may have a structural role at sites of cell adhesion in maintaining cell shape and motility. Among 40 non-HLA genes, which are likely involved in the pathogenesis of celiac disease (CD), LPP was considered the best candidate gene. In previous association studies in populations of southern Italy, 638 CD patients and 711 controls were analyzed for the rs1464510 SNP founding an association between this SNPs and CD risk. To reinforce results, we analyzed the allele frequency of another SNP in the LPP gene (rs1136644) found in the frequency of the LPP gene.

Methods: Eighty-nine CD patients and 87 controls were recruited from the Department of Pediatrics of the “Federico II” University. DNA was extracted from peripheral blood cells and genotyped with TaqMangenotyping assays (Applied Biosystems). The expression studies were performed on mRNA extracted from duodenal biopsy of 10 CD patients and 10 controls and from peripheral blood monocytes (8 CD patients and 7 controls) extracted by immunoselection with magnetic beads. LPP expression was analyzed using a normalized relative quantification. We used a Taqman expression assay (Applied Biosystems) and all reactions were performed in triplicate for each sample of RNA.

Results: No significant difference between CD patients and controls was found in the frequency of the rs1136644 SNPs (p = 0.58). No difference in expression study was found between cases and controls for the LPP gene.

Conclusion: Despite rs1464510 SNPs appears particularly associated to CD risk in our cohort of families, we have not found any difference for the SNPs rs1136644 in another our group case-control. Furthermore, since there is not a different expression between the 2 cohorts of patients we can assume that these SNPs has not role in modifying the expression. In progress, we are evaluating the protein expression of LPP product in duodenal biopsies of CD patients.

Disclosure of Interest: None declared.
PO-G-0033

Coeliac Disease and Enteropathies

IMPROVING THE ESTIMATION OF RECURRENCERISK IN CELIAC DISEASE

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Objectives and Study: Celiac disease (CD) is a polygenic trait, characterized by the presence of the HLA-DQ2 heterodimer in more than 95% of the patients even if HLA genes explain only about 35% of the genetic variation. Through large GWAs more than 40 non-HLA genes, which are likely involved in the pathogenesis of CD, were identified. The aim of the study was to confirm, in 183 CD families, the association of the 11 most strongly associated SNPs obtained in the recent GWAs and in their replications, and to validate a model of recurrence risk prediction for the siblings in the same cohort.

Methods: 183 families (794 patients) from southern Italy were recruited: they were characterized by a CD-child (proband), both parents and at least 1 sibling of the proband. HLA genotyping was performed in order to group the subjects into 5 HLA classes of decreasing CD risk, as previously reported. Eleven SNPs were genotyped using TaqMan methodology: rs6441961 (CCR1/CCR3), rs17810546 and rs9811792 (IL12A/SCHIP1 and IL12A, respectively), rs1738074 (TAGAP), rs2816316 (RGS1), rs1464510 (LPP), rs2327832 (OLIG3), rs842647 (REL), rs6822844 (IL2/IL21), rs3184504 (SH2B3). Associated SNPs were identified through the Transmission Disequilibrium Test (TDT) and, by means of a Bayesian approach, an arbitrary risk score was evaluated and assigned to each sib of the proband, allowing us to test if CD recurrence was more frequent in sibs with higher values of the arbitrary risk score.

Results: An association between three SNPs (LPP, REL, and RGS1 genes), and the CD risk was found and the best were obtained the LPP SNP (OR=2.38, 95% CI= 1.66–3.33; P < 0.001). In order to classify sibs in 2 groups (high and low risk) a risk score was computed from the genotype of these 3 SNPs and the HLA haplotype. The recurrence risk in low risk sibs is 10% while in high-risk sibs is 22% with a relative risk (RR)= 2.34 (95% CI= 1.05 - 5.23) and a p value= 0.048.

Conclusion: Three out of 11 SNPs were reconfirmed to be associated to CD in astringent family study. In addition, a Bayesian approach showed that affected siblings line up more frequently in high-risk score independently from HLA type. Although more studies on larger and different populations are needed, these results suggest that the prediction of CD recurrence in siblings might be improved by adding 3 non-HLA genes to the known HLA-related risk.

Disclosure of Interest: None declared.

PO-G-0034

Coeliac Disease and Enteropathies

AN ORAL BOLUS OF GLUCOSE IS NOT EFFECTIVELY ABSORBED DURING METHOTREXATE-INDUCED GASTROINTESTINAL MUCOSITIS IN A RAT MODEL

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Objectives and Study: Pediatric patients with chemotherapy-induced gastrointestinal mucositis often suffer from weight loss and malnutrition. We developed a methotrexate (MTX)-induced mucositis rat model to study nutrient digestion and absorption, and to ultimately design a rational feeding strategy for mucositis patients. We previously showed that during mucositis, lactose digestion is severely decreased while glucose absorption is still intact, when supplied in trace amounts, in spite of decreased mRNA and protein expression of glucose transporters SGLT1 and GLUT 2. Here, we studied glucose absorption during mucositis when administered as an oral bolus (meal size), to see whether bolus feeding is an adequate method to administer glucose during mucositis.

Methods: After recovery from a jugular vein catheter implantation, young Wistar rats (6 wk old) were i.v. injected with MTX (60 mg/kg) or NaCl 0.9% (controls). Four days later, during MTX-induced mucositis, we started a continuous infusion with trace amounts of [6,6–2H2]glucose to calculate endogenous glucose metabolism. Two hours later, we orally administered a [1–13C]glucose-enriched glucose bolus (2 g/kg) and quantified appearance of labeled glucose in the blood for another 4 hours. Furthermore, blood glucose and plasma insulin levels were frequently determined. Finally, we collected the small intestine to assess histology and mucosal myeloperoxidase (MPO) levels, and determined plasma citrulline levels.

Results: MTX-treated rats suffered from severe mucositis, as shown by profound villus atrophy and epithelial damage, increased MPO levels (41.5-fold, indicating neutrophil infiltration) and decreased citrulline levels (6.5-fold, indicating decreased functional enterocyte mass), as compared to controls (both P < 0.01). Shortly after bolus administration, blood glucose and plasma insulin levels started to rise only in control rats, and were significantly increased, as compared to MTX-treated rats (P < 0.05). During the experimental period, total glucose absorption was 5.7-fold decreased in MTX-treated rats, as compared to controls (15% versus 85% respectively of the administered glucose bolus, P < 0.01).

Conclusion: We conclude that glucose absorption is severely decreased during mucositis, when administered as an oral bolus (meal size). Therefore, bolus feeding seems not an adequate method to administer glucose to pediatric patients with mucositis. It remains to be elucidated whether glucose absorption during mucositis can be improved when administered via continuous enteral (tube) feeding, as has

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been shown in other cases of intestinal failure and is the focus of our ongoing research.

Disclosure of Interest: None declared.

PO-G-0036

Coeliac Disease and Enteropathies

FOOD QUESTIONNAIRE FOR ASSESSMENT OF GLUTEN INTAKE FOR CHILDREN 1–4 YEARS OF AGE

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Objectives and Study: The time of introduction of the gluten into the diet of young children and the amount of gluten consumed play a role in the development of coeliac disease and it is important to assess the gluten intake by young children. A food questionnaire to assess gluten intake in infants 0–12 months of age has been developed and validated (FQ-gluten), but an instrument to assess gluten intake in children 1–4 years is not available. The aims of the study were the development and validation of a food questionnaire to assess gluten consumption in healthy young children aged 1–4 years (FQ-gluten4).

Methods: The previously developed FQ-gluten for 0–12 months aged children was adapted according to age related food consumption. The results of a 2-day food record (FR) were compared with the results of this FQ-gluten4.

Results: Seventy-one parents filled in the FR and the FQ-gluten4. The mean amount of gluten consumption calculated from the FQ-gluten4 was comparable with that of the FR, but significant differences were found in the amount of gluten intake in the 1- to 2-year-old children and in the percentage of gluten from porridge among the 1- to 3-year-olds. The bland Altman limits of agreement with an SD of 2600 mg were –5118 to 5630 mg.

Conclusion: This new, short, standardized, validated and easy to use FQ-gluten4 may be a useful instrument to assess gluten intake in young children, both at the individual and at the population level. The use of this method by investigators in other countries provides the opportunity for a better comparison of the results of gluten consumption in (cooperative) international studies. Furthermore, such an instrument can be used to quantify the gluten intake in individuals suspected to have celiac disease but in whom the diagnoses cannot be confirmed.

Disclosure of Interest: None declared.

PO-G-0042

Coeliac Disease and Enteropathies

COELIAC DISEASE IN CHILDREN AND ADOLESCENTS IN DENMARK IS INCREASING: COMBINED INFORMATION FROM NATIONAL REGISTRIES

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Objectives and Study: The objective of the study was to determine the incidence and prevalence of diagnosed coeliac disease (CD) in Denmark and identify trends over time. Furthermore we aimed to describe trends for associated diseases and age at diagnosis.

Methods: All patients admitted to Danish hospitals are since 1977 registered in the Danish National Patient Registry (DNPR) including registration of diagnoses according to the International Classification of Diseases. We included all children registered with the diagnosis CD and included data of associated diseases. A unique personal registration number (CPR number) is assigned to all persons living in Denmark and is attached to all registrations. This allowed us to combine information from DNPR with histological evaluations of small-bowel biopsies registered in the National Registry of Pathology (NRP), which was nationwide complete from 1999. The biopsies were classified according to the modified Marsh classification. If one or more biopsies showed mucosal lesions corresponding to Marsh grade 2–3 we considered the diagnosis verified.

Results: From 1 January 1996 to 1 January 2010 1,188 children and adolescents younger than 18 years of age were registered with CD in the DNPR. The prevalence proportion increased from 0.42‰ [0.38; 0.46] in 1996 to 0.84‰ [0.78; 0.89] in 2010 and the incidence from 0.03‰ [0.02; 0.04] in 1996 to 0.10‰ [0.08; 0.12] in 2009. We found no evidence that the increase has reached its maximum. The increase may be even higher due to probable delay in data registration. A total of 923 (78%) children had one or more biopsies registered in NRP, and 660 (72%) of these children had at least one biopsy showing mucosal lesions corresponding to Marsh grade 2–3. The yearly incidence proportion of verified CD increased from 0.008‰ [0.003; 0.014] in 1996 to 0.014‰ [0.007; 0.021] in 1999 and 0.069‰ [0.054; 0.084] in 2009. The average age increased from 5.1 [3.4; 6.7] years of age in 1996 to 8.1 [7.2; 9.0] years of age in 2009. For verified diagnoses the average age increased from 3.7 [1.9; 5.4] years of age to 8.2 [7.3; 9.2] years of age. The incidence was increasing in all age groups. The proportion of children with associated diseases did not change significantly over time.

Conclusion: The incidence and prevalence of diagnosed CD in Danish children and adolescents has increased but is still low compared to other countries. The average age at diagnosis increased. The proportion of children with associated diseases did not change.

Disclosure of Interest: None declared.

PO-G-0043

Coeliac Disease and Enteropathies

ANTIBODIES AGAINST DEAMIDATED GLIADIN PEPTIDES AND TRANSGLUTAMINASE: PERFORMANCE OF 4 COMMERCIAL ASSAYS FOR DIAGNOSIS AND FOLLOW-UP IN CHILDREN WITH CELIAC DISEASE
PO-G-0044

Coeliac Disease and Enteropathies

CELIAC DISEASE SCREENING ASSAYS IN CHILDREN YOUNGER THAN 3 YEARS OF AGE–IS THE IGA+IGG DGP ASSAY HELPFUL?

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Objectives and Study: Early detection and treatment of CD can prevent growth failure and disease complications. Therefore, screening for CD is recommended for a wide variety of symptoms, but also in asymptomatic patients from different risk groups. It is well known that for the diagnosis of CD highly specific serologic tests are needed. Anti-endomysial antibody (EMA) and anti-tissue transglutaminase (TTG) have high sensitivity and specificity. Other tests include the recently introduced antibodies against deamidated gliadin peptides (DGP) that seems to be useful, but less accurate than TTG. The optimal serologic test for celiac disease (CD) in young children is not known. The aim of our study was to compare the performance of three serological tests (IgA+IgG DGP, IgA TTG and IgA+IgG EMA) in children younger than 3 years of age.

Methods: We identified all subjects younger than 3 years of age (n = 6074) that were tested for CD serology and included those with biopsy data. Patients were classified as group 1 (n = 47): patients with confirmed CD or group 2 (n = 12): patients with normal biopsy findings. There was statistically significant difference between group 1 and group 2 in regard to positivity for IgA+IgG DGP (100% vs 77.78%, P = 0.007), IgA TTG (97.87% vs 50%, P < 0.001), and IgA+IgG EMA (95.65% vs 9.09%, P < 0.001). Suggested manufacturer’s cutoff levels had high sensitivity for all tests (IgA+IgG DGP 100%, IgA TTG 97%, IgA+IgG EMA 96%), however specificity was low for IgA+IgG DGP (44%), IgA TTG (50%) but not for IgA+IgG EMA (91%).

Conclusion: Our current study showed that all 3 used tests (IgA TTG, IgA+IgG DGP and IgA+IgG EMA) have high sensitivity in children younger than 3 years of age. However, EMA was the only test that proved to be specific, and the addition of TTG or DGP did not provide a significant added value. In children younger than 3 years of age, only EMA is both highly sensitive and specific in predicting biopsy findings. This suggests that in patients with positive DGP and negative EMA, biopsy might be postponed as long as good clinical and serological follow up is provided.

Disclosure of Interest: None declared.

PO-G-0046

Coeliac Disease and Enteropathies

ALTERATION OF CELLULAR SHAPE IN FIBROBLASTS AND DENDRITIC CELLS FROM CELIAC PATIENTS

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Objectives and Study: Recent data from our laboratory demonstrated that gliadin peptides cause actin alterations
and cell proliferation in CaCo2 cells. Our aim was to investigate cell shape and actin cytoskeleton organization in different cell types from celiac (CD) patients both in the absence and presence of gliadin peptides.

Methods: Skin grafts were taken from 6 CD patients, in the remission phase of the disease, and from 4 healthy controls. Fibroblasts were isolated and kept in culture. Dendritic cells (DC) from 10 controls and 10 CD patients, 5 in the active and 5 in the remission phase of the disease, were generated from peripheral blood monocytes. Immunofluorescence assay with phalloidin was used to visualize actin cytoskeleton in both cell types.

Results: Fibroblasts from CD patients present different cell shape and actin rearrangements respect to controls. Fibroblasts from CD patients have a significantly bigger area than controls fibroblasts (2065 ± 115.5 μm² vs 1511 ± 125.4 μm² P = 0.0014) and also shorter and broken actin filaments compared to the controls. After 3 hours’ seeding on fibroblasts, a lower percentage of adherent DC in controls showed an altered actin phenotype compared to active CD patients (36.8 ± 8.9% vs 72.5 ± 8.7% P = 0.00065) and remission CD patients (36.8 ± 8.9% vs. 62.2 ± 5.8% P = 0.0014).

Treatment with A-gliadin peptide P31–43 induces a derangement of actin cytoskeleton more evident in controls fibroblasts than in patients. A 3-hour treatment with peptic-tryptic digest of gliadin (PTG) induced a marked rearrangement of actin cytoskeleton in DC from controls inducing an elongation of dendrites, but only a milder effect on cells from CD patients whose shape was already elongated compared to controls. Gliadin peptide P31–43 favoured an elongated morphology of DC, but only in controls.

Conclusion: Our results demonstrate that in CD patients 2 different cell types, such as skin fibroblasts and peripheral blood monocyctic-derived DC, have an altered morphology compared to controls. These cells derive from two compartments far from the gut, the main site of inflammation, suggesting that the observed properties could depend on genetic environment more than on inflammation state. Furthermore gliadin peptides can induce actin rearrangements more evident in controls cells, probably because CD cells morphology is constitutively altered. These data indicate also that some intrinsic biological properties could render CD cells more sensitive to gliadin effects.

Disclosure of Interest: None declared.

PO-G-0047

Coeliac Disease and Enteropathies

HEPATITIS B VACCINE RESPONSE IN CHILDREN WITH CELIAC DISEASE IN ISRAEL

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Objectives and Study: Celiac is an autoimmune disorder of the small intestine that occurs in genetically predisposed people of all ages from middle infancy (after gluten introduction) onward. Almost all celiac patients have the human leukocyte antigen (HLA)-DQ2 DQ8 allele variants. Previous studies have shown decreased response to hepatitis B vaccine in patients with celiac disease. We aimed to determine whether children with celiac disease, who often carry these HLA variants, fail to show a response to hepatitis B virus (HBV) vaccine more frequently than children without celiac disease and whether gender, age, age at diagnosis, family history, clinical presentation of celiac and other clinical and laboratory variables affect the response of celiac patients to HBV vaccine.

Methods: A combined retrospective and prospective study compared the response to HBV vaccine between children with celiac disease and control subjects (patients who presented with elevated liver enzymes but without evidence for celiac disease).

Results: The study population included 72 children with celiac disease and 75 controls. All had received the full array of childhood vaccinations including hepatitis B. A significantly higher proportion of subjects in the celiac group (37 of 72, 52.4% vs. 21 of 75, 28%; P < 0.05) failed to respond to HBV vaccine compared with controls. The control group was 2.5 times more likely to test positive for anti hepatitis B surface antigen than celiac patients. We found that the duration between vaccination and antibody testing affected the response to HBV vaccine. A longer duration was noticed in the nonresponding children (91.52 ± 45.36 months) compared to responders (58.79 ± 42.52 months) (P < 0.05). Nevertheless, the trend was also noted after statistical adjustment. No effect was noted for gender, age, age at diagnosis, family history for celiac disease, the clinical presentation, height, weight, hemoglobin and transaminases levels.

Conclusion: 52.4% of children with celiac disease do not show a response to the standard vaccination regimes for HBV. No effect was noted for gender, age, age at diagnosis, family history, clinical presentation of celiac disease and laboratory parameters. Given the large number of children with celiac throughout the world and in Israel, this observation suggests that there is a large HBV-susceptible population despite widespread vaccination. Current immunization strategies may need to be reassessed in patients with celiac disease to protect this population and achieve the goal of universal protection.

Disclosure of Interest: None declared.

PO-G-0048

Coeliac Disease and Enteropathies

INVERTING THE DIAGNOSTIC PYRAMID IN CELIAC DISEASE: A COST-EFFECTIVENESS STUDY OF GENETIC SCREENING FOR DISEASE DETECTION

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Disclosure of Interest: None declared.
Objectives and Study: Coeliac disease (CD) is a public health problem worldwide. Two possible screening strategies can be considered: a) a two-step strategy based on selection of potential CD individuals by HLA-DQ typing, followed by longitudinal serological screening and b) serological screening only. The advantage of HLA-plus-serology screening is that only 30–40% of the general population needs serological screening however is limited by costs; serological screening only is less expensive however this approach makes it necessary to screen the entire population more than once. The aim of our study was to assess if the 2-step strategy based on selection of potential CD children by HLA-DQ typing is a cost-effective strategy in the setting of a tertiary referral centre for pediatric gastroenterology.

Methods: 938 consecutive children referred in the last 2 years at the outpatient clinic for pediatric gastroenterology of the University of Bari and Catania were studied. 281 were younger while 657 older than 2 years of age. We calculated the mean cost of CD serology and HLA according to available pricelists of different manufacturers. The average cost per patient below 2 years calculated according to the determination of serum-IgA, anti-transglutaminase (TTG)-IgA and anti-gliadin (AGA)-IgA was of 13 Euros, while the cost per patient older than two years calculated according to the determination of serum-IgA and anti-transglutaminase (TTG) was of 9 Euros. The cost of HLA determination was estimated 60 Euros.

Results: The mean number of CD serology requested per patient was of 1.7 time irrespective to age. HLA was determined in 25% of cases and those who were positive CD serology was requested a mean time of 0.5 per patient. The cost of HLA determination was estimated 60 Euros.

Conclusion: The use of such a convenient protocol, easily supported by non-medical staff, makes it necessary to screen the entire population more than once. The primary objective of the present study was to evaluate the feasibility of screening for CD of asymptomatic toddlers at the community level, using rapid antibody testing of finger prick blood by non-medical staff. The secondary objective was to collect data about the sample size calculation and stratification for the main study.

Disclosure of Interest: None declared.
Objectives and Study: Background: Infections may play a role in the pathogenesis of celiac disease (CD). Studies comparing the small intestinal microflora in children with and without CD are contradictory.

Objective: To compare the composition of the duodenal mucosa-associated microbiota of children with untreated CD and control children without CD.

Methods: Microflora in small bowel biopsies of 40 children were evaluated by means of IS-pro, a recently validated 16S-23S interspac (IS) region based profiling method (Budding 2010). Biopsies from 20 children with untreated CD, (9 collected during a mass screening for CD in children from 2 to 4 years, and 11 from at random selected children with clinical suspicion of CD) and from 20 age-matched control children without CD were analysed and compared.

Results: Both groups showed a similar mucosa-associated microbiota pattern, consisting of Streptococci, Rothia and Gemella.

Conclusion: The similar microflora in children with untreated CD and control children without CD suggest that bacteria do not play an important role in the aetiology of CD. Possible explanations for the differences in bacterial composition between these 2 groups described in previous studies are the different microbial identification methods and the fact that the majority of these studies were done in faeces, which may not be a reliable reflection of the duodenal microbiota.

References:

Disclosure of Interest: None declared.

PO-G-0054

Cystic Fibrosis

TRANSIENT ELASTOGRAPHY (TE; FIBROSCAN®) AS SYSTEMATIC SCREENING TEST FOR INCipient CYSTIC FIBROSIS LIVER DISEASE (CFLD)

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Objectives and Study: TE is reported to be well suited for the detection of liver fibrosis, the early hallmark of CFLD. We therefore examined its usefulness as systematic screening tool for CFLD.

Methods: Following a standard procedure liver stiffness was annually measured for 4 consecutive years with a transient elastography device (Fibroscan, Echosens, Paris) in 128 cf patients (age 4–47 y; M 17 y). The 310 results where and trace elements deficiencies seem to be less important. But complete etiopathogenesis is not clear yet. Autonomic test results revealed abnormalities in sympathetic and parasympathetic nervous system function. Heart rate variability (HRV) analysis can detect early subclinical stages of autonomic dysfunction. The aim of this study was to investigate alterations of autonomic nervous system in Slovak children with celiac disease using short-term HRV analysis and respiratory manoeuvres.

Methods: 51 paediatric patients with celiac disease – 28 girls, 23 boys, median age 15 years (IQR 11.0–17.0) and 48 healthy controls matched for age and gender were studied. VariaPulse TF4 system was used for evaluation HRV changes during rest state and respiratory manoeuvres. Evaluated were two time-domain HRV parameters (R-R interval variation, MSSD index) and three frequency-domain HRV parameters (total power, power LF, power HF) and two parameters during respiratory manoeuvres (I/E ratio, CVR-R).

Results: Significant decrease of almost all evaluated parameters-R-R interval variation, MSSD index, total power, power LF, power HF and CVR-R ($P = 0.043$, $P = 0.002$, $P = 0.001$, $P < 0.001$, $P < 0.002$, respectively) in children with celiac disease compare to healthy controls was noticed. Girls were at greater risk to development autonomic neuropathy compare to boys. Long-term compliance with gluten free-diet had protective effect on development autonomic dysfunction.

Conclusion: Although infrequent incidence of autonomic nervous system dysregulations in paediatric patients with celiac disease, they should be considered, especially in cases of presyncope and syncope, palpitations, fatigue, lightheadedness and postural nausea.

Disclosure of Interest: None declared.

PO-G-0053

Coeliac Disease and Enteropathies

DYsfunction of AUTONOMIC NERVOUS SYSTEM IN CHILDREN WITH CELIAC DISEASE

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Objectives and Study: Neurologic complications are estimated to occur in 10–12% of patients with celiac disease. Current evidence suggests that autoimmune-mediated process has an important role on neurological manifestations of celiac disease. Gluten-related immune markers (antiigliadin, anti-tissue transglutaminase and antientomyosial antibodies) in combination with antibodies directed against nervous system epitopes may have neurotoxic effects. Vitamins and trace elements deficiencies seem to be less important. But complete etiopathogenesis is not clear yet. Autonomic test results revealed abnormalities in sympathetic and parasympathetic nervous system function. Heart rate variability (HRV) analysis can detect early subclinical stages of autonomic dysfunction. The aim of the study was to investigate alterations of autonomic nervous system in Slovak children with celiac disease using short-term HRV analysis and respiratory manoeuvres.

Methods: 51 paediatric patients with celiac disease – 28 girls, 23 boys, median age 15 years (IQR 11.0–17.0) and 48 healthy controls matched for age and gender were studied. VariaPulse TF4 system was used for evaluation HRV changes during rest state and respiratory manoeuvres. Evaluated were two time-domain HRV parameters (R-R interval variation, MSSD index) and three frequency-domain HRV parameters (total power, power LF, power HF) and two parameters during respiratory manoeuvres (I/E ratio, CVR-R).

Results: Significant decrease of almost all evaluated parameters-R-R interval variation, MSSD index, total power, power LF, power HF and CVR-R ($P = 0.043$, $P = 0.002$, $P = 0.001$, $P < 0.001$, $P < 0.002$, respectively) in children with celiac disease compare to healthy controls was noticed. Girls were at greater risk to development autonomic neuropathy compare to boys. Long-term compliance with gluten free-diet had protective effect on development autonomic dysfunction.

Conclusion: Although infrequent incidence of autonomic nervous system dysregulations in paediatric patients with celiac disease, they should be considered, especially in cases of presyncope and syncope, palpitations, fatigue, lightheadedness and postural nausea.

Disclosure of Interest: None declared.

PO-G-0054

Cystic Fibrosis

TRANSIENT ELASTOGRAPHY (TE; FIBROSCAN®) AS SYSTEMATIC SCREENING TEST FOR INCipient CYSTIC FIBROSIS LIVER DISEASE (CFLD)

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Objectives and Study: TE is reported to be well suited for the detection of liver fibrosis, the early hallmark of CFLD. We therefore examined its usefulness as systematic screening tool for CFLD.

Methods: Following a standard procedure liver stiffness was annually measured for 4 consecutive years with a transient elastography device (Fibroscan, Echosens, Paris) in 128 cf patients (age 4–47 y; M 17 y). The 310 results where
retroactively reviewed with the knowledge of subsequent 1–3 y hepatic evolution. 7 kPa was derived from the literature as upper limit of normal. The low success rate due to the narrow intercostal space precluded the exam under 4 y.

**Results:** At the end of the study period portal hypertension caused by CFLD was present in 17 patients (13.2%). In 13 the diagnosis was already known for several years (6–28 y; M 15 y). Of the 33 available values (range 6.9–75 kPa; M 16.9 kPa) in these patients 1 (3.0%) was normal causing a sensitivity of 97%. Ultrasonography was grossly abnormal in all with Williams score ranging 6–9 (M9). In 4 with persistently elevated TE values (7.9–10.4 kPa; M 8.1) subsequent evolution revealed CFLD. At the first elevated TE ultrasonography-Williams score was 4 in all. No statistical correlation could be found between duration of CFLD and TE value (r=-0.23; P = 0.15). In 16 patients (12.5%) without established CFLD at least one TE value > 7 kPa was found causing specificity to be 85.6%. The youngest patient with TE value > 7 kPa who developed CFLD was 7 y. The oldest CFLD patient who persistently changed from normal to abnormal TE values had 13 y. None of the 12 pancreatic sufficient patients ever had an abnormal TE measurement, consistent with the suggestion that CFLD does not occur in them.

**Conclusion:** TE is a more sensitive screening tool for CFLD than ultrasonography. It should be performed annually in pancreatic insufficient patients between 6 and 14 y. Since no correlation is found between duration of CFLD and TE values, absolute figures deserve less attention than the qualification normal/abnormal. Because of the lower specificity a first abnormal value warrants control and further investigation.

**Disclosure of Interest:** None declared.

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**PO-G-0056**

**Cystic Fibrosis**

**BORDERLINE SWEAT CHLORIDE TEST VALUES AND CFTR MUTATIONS NOT CLEARLY CAUSING CYSTIC FIBROSIS (CF): THE REAL DIAGNOSTIC CHALLENGE FOR DIAGNOSING CF IN CHILDREN AND ADOLESCENTS WITH RECURRENT PANCREATITIS**

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**Objectives and Study:** A small percentage of patients affected by cystic fibrosis (CF) may present a nonclassic form of the disease, often characterized by a single organ involvement, as recurrent pancreatitis. These patients have nondiagnostic sweat chloride test values and/or they are carrier of 2 CFTR gene mutations, of which at least one mutation has not clearly demonstrated to be CF causing. The aim of our study was to identify atypical forms of CF in a pediatric population affected by recurrent pancreatitis.

**Methods:** We studied, retrospectively, all consecutive pediatric patients affected by recurrent episodes of pancreatitis, defined as 2 or more separate documented episodes of acute pancreatitis with serum amylase and/or lipase levels at least three times the upper reference limit. All patients were tested for CF by a sweat chloride test. A complete CFTR gene sequencing was done for most patients.

**Results:** We enrolled 105 consecutive young patients (53 M, mean age at diagnosis 8.9 ± 5.4 y, range 4 months-18 yrs) affected by recurrent pancreatitis of several etiologies. Sweat test showed pathologic chloride values (>60 mmol/L) in two patients, allowing a diagnosis of classic CF at its onset. Borderline sweat chloride values (40–60 mmol/L) were found in 8 (7.6%) patients. We detected CFTR gene mutations in 35.5% (22/62) of patients. In 10 patients we identified a CFTR gene mutation associated to another CFTR mutation or a polymorphism (IVS8–5T) on the other allele. Only 2 patients with borderline sweat chloride values had 2 CFTR gene mutations (1 surely CF causing); 1 of them was diagnosed as having classic CF when he developed bronchiectasis during clinical follow-up. In 1 patient with borderline chloride values we found only 1 CFTR gene mutation; we detected no mutation in the remaining 5 patients.

**Conclusion:** We observed a high percentage of patients (7.6%) with borderline sweat chloride values. Only 2 of these patients had 2 CFTR gene mutations (but only 1 known CF-causing mutation); clinical follow-up was determinant for diagnosing CF in 1 of them. We also identified 8 patients carrying 2 CFTR gene mutations but they had a negative sweat test. These patient groups currently represent a challenge for a correct CF diagnosis. In the absence of sure diagnostic investigations, only clinical features and follow-up may lead clinicians to confirm or, ultimately, to exclude CF.

**Disclosure of Interest:** None declared.

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**PO-G-0057**

**Cystic Fibrosis**

**LONGITUDINAL CHANGES IN BONE MASS IN CHILDREN WITH CYSTIC FIBROSIS: EFFECT OF SIZE ADJUSTMENT USING BONE MINERAL APPARENT DENSITY (BMAD)**

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**Objectives and Study:** Patients with cystic fibrosis (CF) are at risk of poor growth, suboptimal bone mineralisation and osteoporosis. Bone mass accrual during puberty is critical for bone health in adult life and monitoring is recommended from age 10 years. DXA machine-derived bone mineral
density (BMD) standard deviation scores (SDS) do not fully adjust for body size and may give deceptive results for children small for age.  

**Methods:** 48 children (27 girls) with CF had DXA measurements (GE Lunar Prodigy) of the lumbar spine (L2–L4) at baseline (age 7 to 12 yrs), 2 and 4 y, providing bone mineral content (BMC), bone area (BA) and BMDSDS for age and sex. Adjustment for size of bone; bone mineral apparent density (BMAD) was calculated as BMC/BA raised to the power of 1.5 and BMADSDS derived for age and sex using UK reference data. Lung spirometry was performed to derive percentage of predicted forced expiratory volume in 1 sec (FEV%). 

**Results:** Compared to population reference data, CF patients were significantly short (both sexes) and light (females). Mean baseline BMDSDS was <0 and fell progressively at 2 and 4 y follow-up, especially in girls; (girls; mean (sd); baseline, –0.48 (0.71), 2 y, –0.89 (0.78), 4 y, –1.01 (1.16). Apparent bone deficits were reduced when expressed as BMADSDS, although scores remained significantly <0 in girls; (baseline, –0.47 (0.68), 2 y, –0.56 (0.75), 4 y, –0.66 (0.99)). Paired t test showed no significant change in BMAD SDS between baseline and 2 or 4 y follow-up measurements but a significant difference in BMD SDS between baseline and 4 y in girls only. At baseline FEV% was poorer in girls (boys; 100 (20.6), girls; 75.7 (15.7), P < 0.01). 

**Conclusion:** Mean BMADSDS in these patients was low and fell progressively with age especially in girls, which may be a reflection of their poorer lung function. However, following size adjustment there was no significant difference between baseline and 2 and 4 y BMAD SDS, suggesting that the fall in BMADSDS reflects poor growth rather than poor mineralisation per se. Further follow-up is necessary to identify direction and magnitude of change. Use of BMADSDS may be useful in avoiding mis-diagnosis of low bone mass in children who are small for their age. 

**Disclosure of Interest:** None declared. 

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**Cystic Fibrosis**

**RECURRENT PANCREATITIS IN ISRAEL**

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**Objectives and Study:** Etiologies of recurrent pancreatitis include anatomical anomalies, hereditary, metabolic and autoimmune disorders. A significant number of patients remain with a diagnosis of idiopathic pancreatitis. The advent of genetic analysis and electrophysiologic testing may further assist in the diagnostic process. Evidence has shown that specific genetic mutations in the cationic trypsinogen gene PRSS1 and the SPINK1 gene for pancreatic secretory trypsin inhibitor cause pancreatitis; furthermore cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations have been associated with pancreatitis. The aim of this study is to present the work-up of patients with recurrent pancreatitis referred for genetic analysis and electrophysiological testing. 

**Methods:** Patients with recurrent, acute pancreatitis with no known etiology were referred for PRSS1 and SPINK1 gene mutations as well as evaluation of CFTR function by nasal potential difference (NPD) testing. 

**Results:** 42 patients were evaluated; age 21 ± 14.9 years. One-third of the patients were Ashkenazi, 41% Sephardi, 24% Arab, and 2% others. There was a family history in 8 patients. The patients had a mean of 4 episodes (range 1 – 25). 6 (14%) patients showed PRSS1 gene mutation (p.R112H and p.K23R) including 2 sets of siblings of Georgian Jewish ancestry with p.K23R. No SPINK1 mutations were found. 3 patients out of 21 submitted for cfr test showed mutations (5T, F508del/p.L997F and D1152H/5T). 26 (61%) patients underwent sweat testing, with 13 patients with results >40 mmol/L. 35 (83%) patients had NPD testing, 4 (11.5%) with abnormal results: 3 had sweat chloride >60 mmol/L with no CFTR mutations found but 1 patient with D1152H/5T had a sweat test of 30 mmol/L. None of the 6 patients with PRSS1 gene mutation showed any concomitant CFTR dysfunction (by NPD or sweat testing) or gene mutation. 

**Conclusion:** A prospective study with a larger number of patients may further clarify the impact of genetic mutations and CFTR dysfunction on the clinical presentation and outcome of recurrent pancreatitis. 

**Disclosure of Interest:** None declared. 

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**Endoscopy: Diagnosis and Therapeutic Surgical Procedures**

**GASTROINTESTINAL ENDOSCOPY IN THE FIRST YEAR OF LIFE: INDICATIONS AND OUTCOME**

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**Objectives and Study:** Despite widening indications for gastrointestinal endoscopy in infants, knowledge of gastrointestinal mucosal findings is limited, based on small and selected patient series only. Currently there is no data on the usefulness of endoscopy in children under 1 year of age. 

**Methods:** The aim of this study was to identify all children under 1 year of age referred to a single tertiary paediatric gastroenterology unit during the period June 1987-August 2007 who underwent gastrointestinal endoscopy. Clinical
indicators and histological outcomes were reviewed in 1024 cases (median age 210 days, range 6–365 days) in a total of 823 infants less than 12 months old (433 males, 390 females).

**Results:** A total of 933 gastroscopies and 439 colonoscopies were performed in our selected group of patients in the specified time period. In order of frequency, clinical indications were mainly diarrhea (522/1024, 51%), failure to thrive (428/1024, 41.2%), reflux/vomiting (278/1024, 27.1%) and rectal bleeding (87/1024, 8.5%). The procedure failed to produce adequate samples in 25/1024 cases (2.4%), was normal in 346/1024 cases (33.8%), whereas abnormalities were found in 653/1024 cases (63.8%). In the latter group, foregut histology revealed oesophagitis in 121/431 cases (28%), gastritis in 92/431 cases (21.3%) and enteropathy in 437/588 cases (74.3%), mainly in the form of villous atrophy and inflammatory cell infiltration. Colonic histology was abnormal in 234/325 cases (72%). More specific diagnoses included microvillous inclusion disease in 21/1024 cases (2%), autoimmune enteropathy in 12/1024 cases (1.2%), graft-versus-host disease post bone marrow transplantation in 9/1024 cases (0.9%), tufting enteropathy in 5/1024 cases (0.5%) and disaccharidase deficiency in 2/1024 cases (0.2%).

**Conclusion:** Gastrointestinal endoscopy with biopsies under the age of one year is a highly informative test with low failure rates. In 63.8% a positive result was obtained that the age of one year is a highly informative test with low adverse effect, mainly for chronic patients with complex medical histories.

**Disclosure of Interest:** None declared.

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**PO-G-0063**

**Endoscopy: Diagnosis and Therapeutic Surgical Procedures**

**PAEDIATRIC ENDOscopic PROCEDURES: A 5-YEAR EXPERIENCE IN A TERTIARY PAEDIATRIC GASTROENTEROLOGY CENTRE**

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**Objectives and Study:** Even though paediatric endoscopy has become routine in many centers around the world, little data are available on the actual safety of the procedure. We report our 5-year experience in a large tertiary paediatric gastroenterology centre, with the aim of quantifying workload and complication rates, as well as identifying possible risk factors and providing parents with accurate data when obtaining informed consent.

**Methods:** All patients receiving gastroscopy (OGD), colonoscopy, wireless capsule endoscopies (VCE) and percutaneous gastrostomy (PEG) insertion over a 5-year period (April 2004–March 2009) were retrieved from hospital databases. Systemic infection, blood transfusion/gastrointestinal (GI) bleeding or perforation were the major acute outcomes that were investigated in all children with prolonged admissions (>3 days).

**Results:** In 2969 children (median age 8.3 years, age range 16 days-18.6 years, 1531 males) a total of 6422 procedures (3512 OGD, 2778 colonoscopies, 75 VCE and 57 PEG insertions) were performed. Major outcomes studied were found in 32/287 cases not discharged by day 4. Namely, 4/2969 (0.1%) patients required transfusion after endoscopy, although not related to gross GI bleeding (2 patients with inflammatory bowel disease, 1 with microvillous inclusion disease, 1 with pseudo-obstruction and concurrent systemic infection). 21/25 children (21/2969, 0.7%) treated for presumed systemic infection had positive blood cultures. Most patients had central lines in situ (18/21) and significant underlying illness, such as intestinal failure, immunodeficiency or neurologic conditions. 3/2969 (0.1%) children and 3/6422 (0.05%) procedures had bowel perforation after endoscopy, requiring surgical intervention (1 Crohn’s disease, 1 unspecified inflammatory enteropathy and 1 colonic polyp resection). None of the perforations were related to PEG insertion during the studied period.

**Conclusion:** Our data show that endoscopic procedures in experienced paediatric gastroenterology centres are safe. The perforation rate in our institution for all procedures was only 0.05%. No risk of gastrointestinal bleeding was noticed. Systemic infection was the most common acute adverse effect, mainly for chronic patients with complex medical histories.

**Disclosure of Interest:** None declared.

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**PO-G-0065**

**Endoscopy: Diagnosis and Therapeutic Surgical Procedures**

**APPLICATION OF THE ENDOSCOPIC HEMOCLIPS FOR NONVARICEAL UPPER GASTROINTESTINAL BLEEDING IN CHILDREN**

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**Objectives and Study:** Acute nonvariceal upper gastrointestinal bleeding (NUGB) remains a common medical problem associated with significant morbidity and mortality in children. The majority of patients benefit from conservative treatments; however, for those who have active bleeding, or have high risk of recurrence of bleeding, it is still a serious problem for pediatric endoscopists. Effective methods for control of NUGB include local injection, thermal coagulation and mechanical methods (hemoclips, elastic bands). Among these methods hemoclips can achieve immediate haemostasis by obstructing the vessel and have the special advantage of lack of additional tissue damage. The aim of this study was to investigate effectiveness of endoscopic hemoclips in children for acute NUGB.

**Methods:** Fifteen children (8 male, mean age: 11.5 years, range: 3–15 years) who were given endoscopic treatment
with hemoclip application for acute NUGB were included to the study. Etiology of NUGB was gastric ulcer (n = 10), duodenal ulcer (n = 3), Diallofey’s lesion (n = 1) and post-polypectomy bleeding (n = 1). Six patients were received NSAID. One patient had hypovolemic shock at initial examination. Endoscopies were carried out using Fujinon E250WR5 endoscope under general anesthesis, and endoscopic hemoclip therapy was performed with stainless steel hemoclip (HX-610 135 Olympus Medical Systems). The following outcome measures were recorded; initial gastrostomy, re-bleeding, need for emergent surgery, and 30 days’ mortality.

Results: Endoscopic ulcer grade was F1b in 10, and F1a in 3 according to Forrest classification. Mean hemoglobin concentration was 6.2 ± 2.1 mg/dL at initial admission. Homeostasis was obtained by hemoclip application in all cases (100%), but 1 patient required second endoscopic application (re-bleeding 6.5%). Mean number of applied endoscopic hemoclip was 3 (2 to 6). No adverse event was seen during the endoscopy. Patients were totally require 15 U (1 U/patient) red blood packets. Mean duration of hospital stay was 7.5 days, and on long-term follow-up; none of the patient needed surgery or experienced recurrence of bleeding.

Conclusion: Endoscopic hemoclip application was an effective and safe method for acute NUGB in children with satisfactory outcomes and without any adverse effects.

Disclosure of Interest: None declared.

PO-G-0069

Endoscopy: Diagnosis and Therapeutic Surgical Procedures
PERCUTANEOUS GASTROSTOMY IN CHILDREN USING A PUSH INTRODUCER GASTROPEXY TECHNIQUE
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Objectives and Study: Some children require supplementary or exclusive enteral feeding. A nasogastric tube feeding should not be used more than 2 months to avoid certain complications. When a gastrostomy is indicated, the classic pull technique is not always possible (ie, in small infants or when there are oesophageal disorders such as stenosis). In this study we evaluated a push PEG technique in children.

Methods: We tested the last 4 years a new introducer PEG-gastrostomy kit designed by Fresenius Kabi AG, Bad Homburg, Germany (Freka Pexact-15ch introducer PEG kit) to avoid surgical placement when a classic pull technique placement was not possible. After antibiotic prophylactic injection and under general anaesthesia, the gastric wall was non-surgically sutured to the anterior abdominal wall using a dedicated device and a video-gastroscope for visualisation.

Then the gastrostomy tube was placed using an introducer. Enteral feeding was started after an overnight fasting.

Results: This technique was tempted in 18 children, 10M/8F; median age 0.9 y (range 0.5–19), median weight 8.5 kg (range 6–39). One placement was not possible due to lack of trans-illumination. Few complications were observed: gastric haemorrhage treated endoscopically in 1 patient during the first 24 h, balloon deflation in 2 patients during the first 3 weeks, but the tube was easily replaced without spillage of stomach contents in the peritoneal cavity and without infection. Late complications such as wound infection more than 4 weeks after placement or granulation tissue on the wound orifice were frequent but no more than with a gastrostomy placed by the classic pull technique.

Conclusion: This push introducer PEG placement technique is easily feasible even in small babies, the rate of complications was similar to what is observed with pull techniques and avoids secondary anaesthesia to replace the material.

Disclosure of Interest: None declared.

PO-G-0096

Immunology
EFFECT OF DOCOSAHEXANOIC ACID (DHA) SUPPLEMENTATION DURING PREGNANCY AND LACTATION ON INFANTS’ IMMUNE RESPONSE
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Objectives and Study: Dietary omega3 PUFA have been shown to have a major impact on various functions and components of the immune response. Breast milk is believed to confer protection against infections in infancy and RBC membrane phospholipids of breast–fed infants have a two fold higher level of DHA than infants fed a non–LCPUFA supplemented formula. The effect of dietary PUFA supplementation, in infants and older children, on the immune response is inconsistent. It is currently recommended that the diet of pregnant mothers should contain at least 300 mg of DHA per day. The aim of the study was to determine whether DHA supplementation during pregnancy and lactation affects infants’ humoral immune response, peripheral blood lymphocyte subsets and intracellular cytokine production.

Methods: Of 60 pregnant women in their 3rd pregnancy (~50% in 5th-8th pregnancy), 30 randomly assigned to receive DHA 400 mg/day from 12th week of gestation until 4 months postpartum. Infants exclusively breast-fed for 4 months. Blood obtained at age 4 months for antiHBs antibodies, for immunoglobulin levels and lymphocyte studies. Cells; stained for CD4, CD4CD45RA (naive cells) CD4CD45RO (memory/activated cells), CD8, CD8CD45RA, CD8CD45RO, CD16 (NK cells). Activated, permeabilized and incubated with antibodies to IFNγ and IL4. Cells phenotyped by flow cytometry.
Results: Level of Anti HBs antibodies, total IgA, IgM and IgG did not differ between groups. CD4 did not differ between groups (46.9 ± 1.61 vs. 41.0 ± 2.75 mean ± s.error, P = 0.06) whereas CD8 was sig. lower in the w3 supplemented group (17.7 ± 0.98 vs. 21.2 ± 0.99 P = 0.01). Among CD4 cells CD4CD45RA+/CD4 did not differ between groups. Proportion of activated memory CD4 cells CD4CD45RO+/CD4 did not differ between groups. Proportion of CD8 activated cells CD45ROCD8/CD8 sig. higher in w3 group; 35.5 ± 6.2 vs.16.8 ± 5.4 P < 0.05. CD16 did not differ between groups. Proportion of CD4 and CD8 producing cells lower in w3 group but differences not significant.

Conclusion: Maternal DHA supplementation did not affect infants’ levels of antibodies to HBsAg or total IgA, IgM and IgG. In infants of mothers receiving DHA supplementation, a higher percentage of CD4 naïve helper cells higher in infants of w3 supplemented mothers, constituting 87.2 ± 0.89 % of total CD4 vs.79.8 ± 2.95 % in controls P < 0.05. Percentage of activated memory CD4 cells CD4CD45RO+/CD4 did not differ between groups. Proportion of CD8 activated cells CD45ROCD8/CD8 sig. higher in w3 group; 35.5 ± 6.2 vs.16.8 ± 5.4 P < 0.05. CD16 did not differ between groups. Proportion of CD4 and CD8 IL4-producing cells lower in w3 group but differences not significant.

Disclosure of Interest: None declared.

PO-G-0098

Immunology

DIETARY SUPPLEMENTATION OF SPECIFIC ACIDIC OLIGOSACCHARIDES IMPROVE THE IMMUNE RESPONSE IN BALB/C MICE WITH PSEUDOMONAS AERUGINOSA CHRONIC PULMONARY INFECTION

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Objectives and Study: Pseudomonas aeruginosa (PA) pulmonary infections are the leading cause of morbidity and mortality in cystic fibrosis. According to the literature, severe chronic PA infections are usually associated with a Th2 type immune response, while moderate chronic infections are preferentially associated with Th1 response. Acidoeligosaccharides, which are found in human milk, are prebiotics with immunomodulatory properties by promoting the switch from the Th2 towards the Th1 response. The aim of this study was to evaluate the influence of acidic oligosaccharides derived from pectin (pAOS) in a mouse model of PA chronic pulmonary infection in order to investigate whether the function of human milk derived AOS could be mimicked.

Methods: BALB/c mice, which are known to develop a Th2 response to PA infections, were randomized into 2 groups fed a control diet or a diet containing 5% pAOS. After 5 weeks of diet, animals were infected by endotracheal instillation of 5 × 10^7 P. aeruginosa entrapped in agar beads. The survival of animals, the inflammatory parameters (KC, TNF-α levels and number of neutrophils and macrophages) in bronchoalveolar fluid and the immune markers (INF-γ for Th1 and IL-4 for Th2), markers of Thelper polarization (T-bet for Th1 and Gata3 for Th2), markers of macrophages activation (Nos2 for M1 and Arg1 for M2) in the spleen and lungs were measured from the first to the fourth day of infection. The resistance to a new PA infection was also analyzed by reinfected surviving mice 2 weeks after the first infection and by measuring the bacterial load.

Results: pAOS did not improve significantly the survival of BALB/c mice but reduced significantly the bacterial load (100-fold decrease). This was associated with a significant increase of the inflammatory response in the lung (increase of neutrophils and macrophages recruitment, increase of KC level), pAOS modulate the immune response by promoting a significant switch from the Th2 to Th1 response (2-fold increase of IFN-γ level and 2-fold decrease of Gata3 expression) and promote significant macrophages M1 activation (4-fold increase of Nos2 expression). They also improved significantly the bacterial clearance after a second infection.

Conclusion: In conclusion, pAOS modulate the inflammatory response, the immune response by promoting a switch from the Th2 to Th1 response and limit P. aeruginosa pulmonary infection and reinfection in BALB/c mice. Further confirmation of these results in clinical studies would be needed.


PO-G-0101

Immunology

MINIMAL ENTERAL NUTRITION WITH AMNIOTIC FLUID FAILS TO PROTECT AGAINST NEC IN PRETERM PIGS

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Objectives and Study: In the perinatal period, amniotic fluid (AF), colostrum and milk exert a continuum of beneficial effects on the developing intestine via growth and immunomodulatory factors present in these fluids. Consequently, maternal colostrum and milk (relative to formula) protect against necrotizing enterocolitis (NEC) in preterm neonates. We have shown that AF given enterally to preterm pigs throughout the transition from parenteral to full enteral feeding improves NEC resistance. In this study,
we hypothesized that short-term AF treatment as “minimal enteral nutrition” prior to full enteral feeding, is sufficient to improve the resistance against formula-induced NEC.

**Methods:** Preterm pigs received parenteral nutrition plus enteral boluses of control fluid, porcine AF (pAF), or human AF (hAF) for 2 days, followed by full enteral feeding with formula for 2 days. Pigs were euthanized before (n = 27) or after enteral formula feeding (n = 33). The gastrointestinal tract was evaluated for NEC severity (scores 1–6, with ≥3 defined as NEC), and villous morphology, nutrient uptake capacity and brush border enzyme activities were recorded as markers of intestinal function.

**Results:** NEC incidence and severity did not differ among groups before enteral formula feeding (control 2/9, pAF 2/9, hAF 1/9). After formula feeding, NEC incidence and severity were increased in the hAF group relative to controls (10/11 vs. 4/13, *P* < 0.01). Values did not differ between control and pAF pigs. Neither the pAF or hAF treatments affected intestinal nutrient absorption (galactose, glucose, leucine), brush-border enzyme activities (sucrase, maltase, lactase, aminopeptidases), intestinal dimensions or amount of mucosa.

**Conclusion:** AF given as minimal enteral nutrition does not protect against NEC or improve digestive function in preterm neonates during the later transition to full enteral feeding. Continued AF administration, together with the enteral diet, may be required for AF to improve resistance against feeding-induced NEC development. The tendency to increased NEC incidence in the hAF group suggests that the immunomodulatory effects of AF on the immature intestine may be highly species-specific.

**Disclosure of Interest:** None declared.

**PO-G-0103**

**Immunology**

**THE ROLE OF COMPLEMENT IN HEPATITIS C VIRUS INFECTIVITY**

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**Objectives and Study:** Hepatitis C virus (HCV) is an important human pathogen that infects 3% of the world’s population. HCV replicates in hepatocytes and infected subjects develop progressive liver disease. Complement is known to be involved in the pathogenesis of a number of viruses but its role in HCV infection remains unclear. Our aim was to determine the effect(s) of human complement on HCV infectivity.

**Methods:** We used the recently reported infectious strain of HCV (JFH-1) that can replicate and assemble infectious particles in cell culture to investigate the effects of complement on virus infectivity. Hepatocyte expression of complement regulatory proteins CD55 and CD59 was measured by flow cytometry.

**Results:** The C3 component of complement binds specifically to HCV infected hepatocytes via an interaction with the viral encoded glycoproteins. We failed to observe complement mediated lysis of infected cells due to the expression of complement regulatory proteins CD55 and CD59. Indeed, CD55 expression was significantly increased on HCV infected cells. In contrast, human serum significantly reduced viral infectivity by approximately 50%. The inhibitory effect of human serum was negated following C3 depletion and heat inactivation, confirming a complement-dependent pathway. Mannose binding lectin (MBL) is a member of the collectin family that is produced by the liver and can initiate the complement cascade in the absence of antibodies. We confirmed that recombinant MBL bound HCV infected cells and reduced virus infectivity. Additional experiments confirmed that serum inhibition of virus infectivity was abrogated by prior treatment with mannan, confirming a role for the MBL pathway in complement inactivation of HCV.

**Conclusion:** HCV encoded glycoproteins bind MBL that primes C3 deposition on infected cells and reduces the infectivity of virus particles. HCV infection promotes CD55 expression, providing an explanation for the resistance of infected cells to complement mediated lysis. These studies highlight a role for the MBL pathway in the HCV lifecycle and warrant further studies to assess the clinical significance of MBL gene polymorphism on HCV replication and pathogenesis. Identification of mechanisms by which HCV can modulate the complement system will help us understand the role of the immune system in HCV pathogenesis, and may provide possible therapeutic options in the future.

**Disclosure of Interest:** None declared.

**PO-G-0105**

**Inflammatory Bowel Disease**

**CLOSTRIDIUM DIFFICILE INFECTION IN NEWLY DIAGNOSED PEDIATRIC PATIENTS WITH INFLAMMATORY BOWEL DISEASE: PREVALENCE AND RISK FACTORS**

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**Objectives and Study:** Superimposed infections of pathogenic bacteria may have deleterious effect on the clinical course of inflammatory bowel disease (IBD). *Clostridium difficile* infection (CDI) is one of them. To date, the prevalence and risk factors for CDI in both adults and children...
with new diagnosed IBD have not been established. The aim of the study was to investigate the prevalence and risk factors for *Clostridium difficile* infection in newly diagnosed pediatric patients with IBD.

**Methods:** It was a retrospective, observational study evaluating all new diagnosed pediatric IBD (up to 18 years old) patients in 3 pediatric gastroenterology clinic in Poland between the years 2006–2010. All these patients were diagnosed according to Porto criteria, therefore all have been performed screening test for CDI. Potentially risk factors (established for adult IBD patients) for the diagnosis CDI were recorded. This included prior hospitalization and use of antibiotics within 2 months of the CDI detection, colonic involvement, duration of symptoms and others. Diagnosis of CDI was based on a positive stool enzyme immunoassay and/or on the isolation of toxigenic *Clostridium difficile* strain.

**Results:** We evaluated 233 patients (108 with Crohn disease and 125 with ulcerative colitis; 91% of the patients had colonic disease). The average age of the patients was 12.6 years. The incidence of CDI was 30% CI 95% (24.5%-36.2%). There was no significant difference in the prevalence of *Clostridium difficile* infection between Crohn disease and ulcerative colitis (P = 0.53). CDI was associated with increasing patient’s age (P = 0.000057), presence of bloody diarrhea (P = 0.000057) and longer duration of IBD symptoms (P = 0.0265). There was no significant difference in antibiotic exposure, prior hospitalization or disease activity between IBD patients with and without CDI.

**Conclusion:** The prevalence of *Clostridium difficile* infection in newly diagnosed IBD patients was 30%. The risk of CDI was independent of disease type (Crohn disease or ulcerative colitis) and disease activity.

**Disclosure of Interest:** None declared.

**PO-G-0109**

**Inflammatory Bowel Disease**

**ADHERENCE TO MEDICAL TREATMENT IN CHILDREN WITH INFLAMMATORY BOWEL DISEASE: A PAIRED PATIENT–PARENT STUDY**

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**Objectives and Study:** To evaluate the frequency of medication nonadherence in children with inflammatory bowel diseases (IBD), and to identify risk factors for nonadherence among patients and their parents.

**Methods:** Patients with an established diagnosis of IBD for at least 6 months, age 10–20 years, and the accompanying parent, answered each a separate anonymous questionnaire during their clinic visit. The questionnaires included 19 or 24 items for patients and parents, respectively, including the following categories: demographics, disease type, medications type, schedule, adherence and reasons for nonadherence, communication about the disease, hobbies, parental information- medical screening, medication use, education, profession. In order to identify predictors associated with nonadherence, bivariate analysis using the χ² test of association for categorical variables and t tests for continuous variables was conducted. Parent–child agreement was assessed using a McNemar test. Multiple logistic regression analysis was used to adjust the predictors associated to nonadherence. Reported p values are 2-tailed. Analyses were conducted with SAS 6.1.2 (SAS Institute, Cary, NC).

**Results:** 80 consecutive patients/parent questionnaires (46 (57.5%) males) were included: 61 had Crohn disease, 16 UC and 2 indeterminate IBD. The means and SD of age and adherence rates in children with IBD were 15.24 ± 3.11 and 10.40 ± 4.14 years, respectively. Complete adherence to medications was reported by 60% of the patients and 74% of their parents (NS). In 12 patients (15%) there was disagreement between parent and child, with reporting nonadherence (P = 0.004). Risk factors for medication nonadherence were: patient’s age > 16 years (P = 0.01), need to take medications in the evening (P = 0.03), and lack of communication of the child about the disease (P = 0.001). In multiple logistic regression, Odds ratio was 4.1 (95% CI 1.4–11.4, P = 0.008) for nonadherence at age ≥6 vs. age ≤16 years, and 5 (95% CI 1.5–16.7, P = 0.009) if the child never or almost never talks about the disease. The main reason for nonadherence was forgetfulness, followed by medication side effects. Oral steroids and nutritional treatment had the highest adherence rates, possibly reflecting better adherence during active disease.

**Conclusion:** Nonadherence to medications in pediatric IBD patients is 40% in the current study, similar to previous studies. A significant number of parents are not aware to treatment nonadherence of their children. Adolescent patients older than 16 years and patients who do not communicate about their disease are at increased risk for nonadherence. Preventive measures, such as support groups and better doctor–patient interaction are needed to reduce nonadherence rates in children with IBD.

**Disclosure of Interest:** None declared.

**PO-G-0110**

**Inflammatory Bowel Disease**

**INFLAMMATORY BOWEL DISEASE IN CHILDREN UNDER 2 YEARS OF AGE: CLINICAL CHARACTERISTICS AND OUTCOME**

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Objectives and Study: Although rare, inflammatory bowel disease (IBD) may have an early onset in childhood, before the age of 2 years. The data about long-term evolution and outcome of this group is scarce and limited. We aimed to evaluate the clinical characteristics and outcome of children diagnosed with IBD under age 2 years.

Methods: Patients diagnosed with IBD at age ≤2 years, and followed for at least 12 months were included. A retrospective chart review of identified patients from 7 medical centers, including clinical, endoscopic and histological data, was performed.

Results: Sixteen patients (11 males) were identified. The age of diagnosis was 11.6 ± 7.5 months, and the length of follow-up was 42 ± 38 months. Family history of IBD was positive in 7 patients (44%). All patients were born at term, 15 were appropriate and one was small for gestational age. Eleven patients were initially breastfed. The clinical presentation included: bloody diarrhea (16), failure to thrive (8), perianal fissures and/or fistulae (6), extraintestinal manifestations of arthritis and/or rash (4). Laboratory findings were: iron deficiency anemia (hemoglobin 9.5 ± 1.1 gram%), thrombocytosis (566 ± 176), increased CRP (61.3 ± 61.7), and hypoalbuminemia (3.0 ± 0.8 g/L). Disease location was colonic in all patients, 15 of them had pancolitis. Six out of 11 patients undergoing UGI endoscopy had mild chronic inflammation of the duodenum or stomach, and one had H. pylori gastritis. Ten patients had Crohn disease, and 5 UC or indeterminate colitis. Disease behavior was inflammatory in 15 and stricturing in one patient. Treatment included corticosteroids (16), azathioprine/6MP (11), methotrexate (1), infliximab (4), 5-ASA (9), and cyclosporine (1). At the end of follow-up, 7 patients (44%) were in clinical remission (12–120 months, median 36 months) without steroids; 5 were on azathioprine and 2 on 5-ASA maintenance therapy. Four patients with intractable disease (25%) underwent colectomy and/or ileostomy. The patients achieved remission had good linear growth, with height in the 15th-75th percentile, and weight in the 10th-70th percentile.

Conclusion: Children diagnosed with IBD under age 2 years generally have a severe colonic disease, requiring intensive medical therapy. A quarter of patients require extensive surgery in order to control symptoms, but about half of patients may achieve long-term remission and normal growth.

Disclosure of Interest: None declared.

PO-G-0113

Inflammatory Bowel Disease

CORRELATION OF CROHN DISEASE SEVERITY WITH EXPRESSION LEVEL OF LEUKOTRIENE B4 RECEPTOR

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Objectives and Study: Many functions of inflammatory and immune cells are activated by LTβ4, and its concentration levels are high in Crohn’s disease (CD) lesions. Moreover, LTβ4 receptor (BLT1) expression on T lymphocytes is associated with several inflammatory conditions. Severity of CD may be related to the expression level of BLT1 on T lymphocytes or on monocytes. The aim of this study was to evaluate the expression levels of BLT1 in correlation with CD severity.

Methods: This study included patients (n = 17, 10 females and 7 males, mean age: 15 ± 0.5 years) with CD without any other inflammatory disease or associated infection. Severity of CD was evaluated using the Pediatric Crohn's Disease Activity Index (PCDAI). Expression levels of BLT1 were measured in peripheral blood leukocytes using flow cytometry as well as in intestinal tissue biopsies using immunofluorescence microscopy. Patient BLT1 levels were compared to age-matched healthy controls.

Results: Mean PCDAI was 14 ± 2 (range: 0 - 35). Mean expression level of BLT1 on monocytes (BLT1mono: 59 ± 2.2%) and lymphocytes (BLT1lympho: 3.4 ± 0.6%) were significantly associated (r = 0.5, P = 0.009). BLT1mono expression level was significantly higher in CD patients than in controls (P = 0.004) and was inversely correlated with PCDAI (r = -0.7, P = 0.009). BLT1lympho expression level was almost 3 times higher in CD patients than in controls (P < 0.04). At the tissue level, there was an increased in the number of CD3+ and BLT1+ infiltrating cells in patient suffering from CD.

Conclusion: These results suggest that expression levels of leukotriene B4 receptor in peripheral blood leukocytes are higher in CD and are inversely correlated with CD severity. On the other hand, there is a strong correlation between the tissue level of leukocytes and CD severity, suggesting that these leukocytes have migrated to the inflamed intestinal tissue.

Disclosure of Interest: None declared.

PO-G-0114

Inflammatory Bowel Disease

PANCREATIC ANTIBODIES ARE ASSOCIATED WITH ANTI-SACCHAROMYCES CEREVISIAE ANTIBODIES AND MORE COMPLICATED DISEASE PHENOTYPE IN PEDIATRIC CROHN DISEASE

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Objectives and Study: Antibodies against exocrine pancreas (PAB) are highly specific for Crohn’s disease (CD), but their sensitivity is low. Some studies have shown that the
Generation of PAB may be genetically determined. On the other hand, the presence of PABs may be a secondary phenomenon of the inflammation in the gut mucosa. The exact value of the determination of PAB in CD remains unclear. We aimed to examine the relationship between PAB, disease phenotype, ASCA and NOD2/CARD15 genotype in pediatric CD patients.

**Methods:** 52 patients with CD (2–18 years, M/F = 1.6/1) were tested for PAB by a standardized indirect immunofluorescence method and for ASCA by enzyme-linked immunosorbent assay. All patients had genotyping performed using sequence specific PCR directed against the wild type and the R702W, G908R and 3020insC variants of NOD2/CARD15 gene. Disease activity using Pediatric Crohn’s Disease Activity Index (PCDAI), body mass index (BMI) at the time of diagnosis and the presence of complicated disease phenotype (penetrating/strictureing) were determined.

**Results:** Of the 52 children with CD, 36.5% were positive for PAB and 63.4% for ASCA (IgG and/or IgA). 30.8% of CD patients had at least 1 of the 3 principal mutations of NOD2/CARD15 gene. Disease activity using Pediatric Crohn’s Disease Activity Index (PCDAI), body mass index (BMI) at the time of diagnosis and the presence of complicated disease phenotype (penetrating/strictureing) were determined.

**Conclusions:** PO-G-0115

**Disclosure of Interest:** None declared.

**PO-G-0116**

**Inflammatory Bowel Disease**

**MAGNETIC RESONANCE IMAGING FOR DIAGNOSIS OF SMALL BOWEL DISEASE IN PAEDIATRIC CROHN DISEASE: A SYSTEMATIC REVIEW**

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**Objectives and Study:** Early surgery is generally considered as a predictor of bad prognosis in patients with Crohn disease (CD) who present with complications at diagnosis or early after diagnosis. The aim of this study was to compare the outcome of patients with pediatric CD who were operated on within their first year of diagnosis with those who were operated on later in the CD course. In a population-based derived incidence cohort diagnosed from 1988 to 2004, we identified 404 pediatric CD patients (age 0–17 y at diagnosis) with a follow-up time ≥2 y. Intestinal resection was required in 131 patients including 42 within 1 y after diagnosis and 89 later on. We compared the occurrence of a poor CD outcome arbitrarily defined on the need for at least 1 of the following criteria: 2nd intestinal resection; systemic steroid therapy, immunosuppressive therapy; biologics. Cumulative incidence of poor outcome was calculated for each group using the Kaplan-Meier estimator and compared using the log-rank test.

**Results:** In the early surgery group (n = 42; 21 F), median time between CD diagnosis and 1st intestinal resection was 3.2 months [Q1 = 0.6–Q3 = 8.2], median age at CD diagnosis was 15.3 y [12.4–16.4] and median follow-up duration was 11.8 y [7.7–15.6]. In the later surgery group (n = 89; 50 F), median time between CD diagnosis and first intestinal resection was 40.1 months [24.3–64.3], median age at CD diagnosis was 13.8 y [11.8–15.7] and median follow-up duration was 9.8 y [6.7–12.7]. Cumulative incidence of poor CD outcome was significantly lower in the early surgery group than in the later surgery group: 26.3% vs 41.7% at 3 y, 26.3% vs 54.1% at 5 y and 45.5% vs 67% at 10 y (P = 0.02). This difference was independent from the period of diagnosis (1988–1996 or 1997–2004). Cumulative incidence of need for at least a 2nd intestinal resection was not different between both groups: 16.8% vs 14.5% at 5 y and 26% vs 26% at 10 y.

**Conclusion:** In this pediatric population-based cohort, early surgery was not associated with a higher risk of poor outcome. The cumulative incidence of a 2nd intestinal resection did not differ whether surgery was performed early or later in the course of the disease. These data need to be taken into account when facing a patient with pediatric CD presenting with complications potentially requiring surgery at diagnosis or early after diagnosis.

**Disclosure of Interest:** None declared.
inflammatory bowel disease (IBD) (1). However it is subject to poor sensitivity and involves ionising radiation. MRI enterography (MRE) has recently been reported as an alternative methodology. We aimed to critically appraise the published evidence on the use of MRE in diagnosis of paediatric IBD by systematic review.

Methods: Review of all English language data reporting MRE for the investigation of patients <16 years with known or suspected IBD. Searches of MEDLINE (Jan 1950-Nov 2010) and PubMed (Jan 1950-Nov 2010) were performed using keyword and MeSH terms; IBD; MRI; small bowel imaging. Reference lists of potential studies, handsearching and personal collections of authors were also examined. Two authors independently assessed the quality of studies for inclusion using the QUADAS tool (2). A third author was an arbiter in cases of disagreement.

Results: Database searches yielded 606,291 hits, combination word searches limited this to 968 titles. 38 studies were fully reviewed and 10 potential studies identified. 2 studies were excluded due to lack of separate paediatric data or inadequate methodological rigour. 8 studies were included (QUADAS scores 7–13/14) (table). Studies displayed heterogeneity in bowel preparation, scanning technique, reporting methodology and comparisons with BM, ultrasound and CT. Timing of ileocolonoscopy in relation to MRE was also variable. Two papers reported greater sensitivity and specificity for MRE in comparison to BM.

Conclusion: MRE is a sensitive and specific tool for the diagnosis of paediatric IBD. Technical considerations require refinement and standardisation, but MRE does offer a significant reduction in ionising radiation exposure. Current data suggest that MRE should supercede BM as the small bowel imaging technique in centres with appropriate expertise.

References:

Disclosure of Interest: None declared.

PO-G-0117

Inflammatory Bowel Disease

PSYCHOSOCIAL FACTORS ASSOCIATED WITH THE ACTIVE STATE OF PEDIATRIC INFLAMMATORY BOWEL DISEASE

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Objectives and Study: To examine the role of psychosocial factors associated with active state of inflammatory bowel disease (IBD) in childhood.

Methods: The study comprised of 85 children with IBD (28 with ulcerative colitis and 57 with Crohn’s disease). Forty-three children being in active disease (19 on diagnosis and 24 on relapse; mean age 12.9 ± 2.1 years) and 42 children in remission (mean age = 13.6 ± 2.6 years) and their parents completed self-reported questionnaires measuring child anxiety and depressive symptomatology, emotional/behavioural problems, life events during the year prior to relapse, child-parents and child-peers attachment, family functioning as well as parental psychopathology. Differences between groups were examined through parametric and non-parametric tests and the relation between the self-reported symptoms and the probability of being in active state of the disease was assessed by logistic regression analysis.

Results: Univariate analyses showed that children being in active state of the disease reported significantly higher levels of emotional (P = 0.015) and anxiety symptoms (P = 0.017) whereas their parents reported significantly more life events (P = 0.005) and negative life events (P = 0.048) for the child during the year prior to diagnosis/relapse compared to children being in remission of IBD. Additionally, the parents of children in active state of IBD reported significantly higher levels of psychopathology (P < 0.001). After applying multivariate logistic regression analysis, only parental psychopathology (OR = 5.7, 95% CI: 1.6–20.7) and self-reported anxiety symptoms (OR = 1.08, 95% CI: 1.01–1.15) were significantly related to the disease status.

Conclusion: The aforesaid findings underline the role of psychosocial factors in the course of pediatric IBD and highlight the importance of simultaneously addressing the needs of both children and parents in any effective therapeutic intervention.

Disclosure of Interest: None declared.

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PO-G-0118

Inflammatory Bowel Disease

FAECAL CALPROTECTIN CONCENTRATIONS IN APPARENTLY HEALTHY CHILDREN AGED 0–12 YEARS IN URBAN KAMPALA, UGANDA: A COMMUNITY-BASED SURVEY

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Objectives and Study: Calprotectin is a calcium and zinc binding protein and is extremely stable in faeces. Faecal calprotectin is used as a nonspecific marker for gastrointestinal inflammation. It has a good diagnostic precision to distinguish between irritable bowel syndrome and inflammatory bowel disease. Studies have established normal concentrations in healthy children; all these studies have been performed in high-income countries. The objective of this study was to determine the concentration of faecal calprotectin in apparently healthy children living in urban Kampala, Uganda.

Methods: We tested 302 apparently healthy children aged, age 0–12 years (162 female, 140 male) in urban Kampala, Uganda. The children were recruited consecutively by door-to-door visits. Faecal calprotectin was analyzed using a quantitative enzyme-linked immunosorbent assay. Faeces were also tested for Helicobacter pylori antigen, for growth of enteropathogens and microscopy was performed to assess protozoa and helminths. A short standardized interview with socio-demographic information and medical history was obtained to assess health status of the children.

Results: In the different age groups the median faecal calprotectin concentration was 249 mg/kg in 0–1 year (n = 54), 75 mg/kg in 1–4 years (n = 89) and 28 mg/kg in 4–12 years (n = 159). There was a significantly difference in the faecal calprotectin concentration across all three age groups. There was no significantly difference in faecal calprotectin concentrations and sex, education of female caretaker, wealth index, habits of using mosquito nets, being colonized with H. pylori, having other pathogens in the stool, have used antibiotics or had malaria within last 3 months.

Conclusion: This is the first survey of faecal calprotectin concentration in an apparently healthy population in sub-Saharan Africa. Concentrations of faecal calprotectin among healthy children, living in urban Ugandan, a low-income country, are comparable to those in healthy children living in high-income countries. In children older than 4 years, the faecal calprotectin concentration is low. In healthy infants faecal calprotectin is high. The suggested cutoff concentration in an apparently healthy population in sub-Saharan Africa can be used in apparently healthy Ugandan children. This finding also shows that healthy children living under poor circumstances do not have a constant inflammation in the gut. We see an opportunity to use this relatively inexpensive test for further understanding and investigations of gut inflammation in children living in low-income countries.

Disclosure of Interest: None declared.

PO-G-0119

Inflammatory Bowel Disease

THE COURSE OF LIFE AND HEALTH-RELATED QUALITY OF LIFE OF ADOLESCENTS WITH INFLAMMATORY BOWEL DISEASE

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Objectives and Study: Inflammatory bowel disease (IBD) is a chronic debilitating disorder occurring in young patients, in the most productive period of their lives. Little is known about the effect on the developmental trajectory of adolescents growing up with IBD. The purpose of this study was to assess the course of life, the health-related quality of life (HRQOL) and socio-demographic outcomes in adolescents with IBD compared with peers from the general population.

Methods: Adolescents, aged 16–20 years, with IBD were invited to fill in the Course of Life Questionnaire (which measures developmental milestones and socio-demographic outcomes) and the SF-36 (HRQOL questionnaire). Norm data of healthy peers, representing the Dutch general population, were available for both questionnaires.

Results: A total of 62 adolescents (response rate 74%, male 51.6%, mean age 18.6 years) completed the questionnaires. Patients with IBD achieved fewer milestones on the domains of autonomy, social and psychosexual development compared with their healthy peers (P < 0.02). They went less frequently on holidays without adults (P = 0.002), had fewer jobs during secondary school (P = 0.002), were less frequently going out to a bar/disco during secondary school (P = 0.009) and were older when falling in love for the first time (P = 0.019) and when having their first boy-/girlfriend (P = 0.003). After secondary school, IBD patients were more often unemployment (P = 0.004). The HRQOL of adolescents with IBD was impaired on domains of social functioning (P = 0.006), vitality (P < 0.0001) general health perception (P < 0.0001) and role limitations due to physical health (P < 0.0001).

Conclusion: Negative consequences in terms of development and HRQOL are prevalent in adolescents with IBD. Health care physicians should be attentive to these consequences and provide additional support (emotional and educational guidance) if necessary. During transition to adults’ clinics these topics are of major importance and
should be an integral component of the comprehensive care of chronically ill adolescents and young adults.

Disclosure of Interest: None declared.

PO-G-0120

Inflammatory Bowel Disease

DOES COW’S-MILK PROTEIN ELIMINATION DIET HAVE A ROLE IN INDUCTION AND MAINTENANCE OF REMISSION IN CHILDREN WITH ULCERATIVE COLITIS?
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Objectives and Study: Children with inflammatory bowel disease (IBD) avoid dairy products more than they would need to based on the prevalence of lactose malabsorption and/or allergy to milk proteins because of arbitrary advice from physicians. Aims of the present study were to evaluate the efficacy of a cow’s milk protein elimination diet on induction and maintenance of remission and to define association with atopy in children with ulcerative colitis (UC).

Methods: Twenty consecutive patients (mean age: 10 y and 5 months; range: 4 y and 7 months to 17 y; F/M: 11/9) with newly diagnosed UC were randomised either to receive a cow’s milk protein elimination diet (n = 10) or to continue a free diet (n = 10) associated to concomitant steroid induction and mesalamine maintenance treatment. Children were prospectively evaluated at four time points: within 1 month, 2 months, 6 months and 1 year after diagnosis or at the time of relapse. Pediatric Ulcerative Colitis Activity Index (PUCAI) and a physician’s global assessment were used to measure disease activity. At baseline and at 12 months or at the time of relapse all patients were assessed endoscopically and histologically. A questionnaire was completed including personal and family history of atopy. At diagnosis, patients were tested for atopy by specific serum IgE and/or skin-prick tests.

Results: All 20 patients responded to the IBD induction therapy. At 6 months remission was achieved in 5 patients (50%) treated with elimination diet and IBD therapy and in 4 patients (40%) treated with free diet and IBD therapy (P = 0.31). Preliminary data showed that 5 of 10 (50%) patients treated with elimination diet and IBD therapy and 4 of 10 (40%) patients treated with free diet and IBD therapy relapsed within 6 months of follow-up (P = 0.5; OR = 1.5; CI = 0.25–8.81). At time of enrollment and at time of relapse, endoscopic and histological scores were not statistically different comparing the exclusion diet and free diet groups (P = 0.45; P = 0.68). All 20 patients completed the questionnaire. The prevalence of atopy in our study population was 32%. Four children in the elimination diet group had a diagnosis of atopy compared with 3 patients in the free diet group (P = 0.32).

Conclusion: Even though allergy to milk protein has been reported to play a possible role in the etiopathogenesis of UC, preliminary data of this first pediatric, randomised, controlled trial suggest that cow’s milk protein elimination diet does not modify the time of induction and the risk of relapse.

Disclosure of Interest: None declared.

PO-G-0122

Inflammatory Bowel Disease

TACROLUMUS FOR TREATMENT OF STEROID-DEPENDENT CROHN DISEASE
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Objectives and Study: Tacrolimus has proven to be an effective option in the therapeutic approach for paediatric inflammatory bowel disease. We aimed to evaluate the usefulness of tacrolimus therapy for paediatric patients with steroid-dependent Crohn disease

Methods: 6 children (1 male) aged 7 to 16 years of age diagnosed with non-fistulaing non-stricturing steroid dependent ileocolonic or colonic Crohn disease were treated with oral tacrolimus from September 2006 to December 2010. All of them had failed to respond to exclusive polymeric diet and had relapsed (PCDAI > 30) whilst on treatment with azathioprine during or soon after prednisolone-dose reduction. Median duration of Crohn disease from the diagnosis was 8 months (rank: 6 to 45 months). Oral tacrolimus therapy was started and aimed for serum trough levels of 12–15 ng/mL. After achieving clinical improvement (PCDAI < 10), tacrolimus dose was gradually reduced to trough levels of 2 ng/mL and discontinued after 6 months. Steroids were weaned off from the beginning of the study and azathioprine was maintained for the length of treatment with tacrolimus and after its discontinuation. All children underwent upper and lower endoscopies for diagnosis and in the event of relapse and the histology was consistent with active Crohn disease.

Results: All children achieved remission (PCDAI < 10); 4 children in the first 2 weeks, 1 child in the 3rd week and 1 child in the 4th week of treatment. 2 children relapsed after 1 year from tacrolimus discontinuation, both responded to tacrolimus re-introduction but both eventually relapsed 6 and 9 months later. One was successfully treated with infliximab and the other one was successfully treated with an ileal resection. Four children have not relapsed after 7 to 45 months after tacrolimus discontinuation. Five of 6 children did not suffer from any adverse effect. One girl suffered from recurrent headache, hand tremor, insomnia, raised urea and hypomagnesaemia that resolved when tacrolimus trough levels were reduced to < 7 ng/mL.

Conclusion: Tacrolimus therapy is well tolerated and effective in achieving remission in children with steroid-dependent Crohn disease but is less effective in maintaining remission and preventing relapses.

Disclosure of Interest: None declared.
PO-G-0123

**Inflammatory Bowel Disease**

**Efficacy of Metothrexate in the Treatment of Pediatric Crohn’s Disease – Single Center Report**

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**Objectives and Study:** Limited controlled clinical trial data in adults suggest that methotrexate (MTX) is effective for induction and maintenance of remission in Crohn’s disease (CD). The appropriate place of MTX therapy in pediatric population is still not well established. MTX is usually used as a second line therapy in patients who do not respond to azathioprine/6-mercaptopurine. The aim of our study was to estimate the efficacy and safety of MTX in the maintenance of remission of CD in our patients.

**Methods:** Data of all children with CD diagnosed and treated from January 2004 to December 2010 (n = 72) were retrospectively analyzed. In this report, only children with the disease which was steroid dependent and azathioprine resistant and who were therefore treated with MTX were included. MTX was administrated intramuscularly at a weekly dose of 15 mg/m². We reviewed case records for medications used, number of relapses prior to and after MTX introduction, duration of remission prior to and after MTX introduction, and for complications of treatment. Remission was defined as a state with no symptoms of the disease and defined as PCDAI score <10.

**Results:** During the study period a total number of 24 patients (75% male and 25% female; median age at diagnosis: 13.3 years, range 12.6–16.17 years) were treated with MTX. Before MTX, all patients received azathioprine and 6 patients underwent surgery. Median duration of the disease before introduction of MTX was 15 months (mean: 21.9 months, range: 4.6 months to 6 years). Nineteen patients were followed up more than 6 months after introduction of MTX (median: 2.1 years, range: 2.2 years, range: 0.5 to 3.9 years). Before MTX treatment all patients had moderate to severe disease (PCDAI median 40, mean 43.9, range: 30–60). After 6 months of therapy 15 (78.9%) patients were in remission. Eight patients (42%) had relapse under MTX during follow up, median time of remission duration was 5 months (mean 7.5, range 3–24 months). In one patient remission was not achieved. Ten patients (53%) were in stable remission under the MTX treatment (median follow up time 18 months, mean 10 months, range 6–36 months). Altogether patients were in significantly longer remission under MTX treatment than before (P = 0.014). Median time for remission achievement was 2.5 months. Concerning adverse effects, two patients had elevated liver enzymes and one patient had nausea which improved with decreasing MTX dose.

**Conclusion:** Although data in our study are retrospectively collected and are limited with small number of patients, we can conclude that MTX is well tolerated and effective treatment in remission maintenance in children with CD whose disease is not responsive to usually used first-line maintenance therapy, azathioprine.

**Disclosure of Interest:** None declared.

PO-G-0126

**Inflammatory Bowel Disease**

**Inestinal Alkaline Phosphatase in Mucosa of Pediatric Patients with Inflammatory Bowel Disease**

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**Objectives and Study:** Intestinal alkaline phosphatase (iAP) might be an important factor in the maintenance of the intestinal barrier integrity. iAP binds lipopolysaccharide (LPS), the ligand of Toll-like receptor4 (TLR4) and detoxifies its activity. However, there is no previous study on determination of iAP protein level in the intestinal mucosa of patients with inflammatory bowel disease (IBD).

**Methods:** 15 children with newly diagnosed IBD (10 with Crohn’s disease (CD), 7 boys, 3 girls; median age: 10.5 years, range: 1.5–15 years), 5 children with ulcerative colitis (UC) (3 boys, 2 girls; median age: 11 years, range: 6–17 years) and 10 healthy controls (5 boys, 5 girls; median age: 9.5 years, range: 1.5–16 years) were enrolled in the study. We determined the mRNA expression of iAP by RT-PCR, the protein level of iAP by Western blot analysis and tissue localization of iAP and TLR4 by immunofluorescent staining in colonic biopsy samples from inflamed and non-inflamed mucosa of 15 children with newly diagnosed IBD.

**Results:** The protein level of iAP in the inflamed mucosa of children with Crohn’s disease (CD) and ulcerative colitis (UC) was decreased by 22% and 20% compared with controls. Significant decrease in iAP protein level in the involved mucosa in CD and UC was observed in comparison to uninvolved mucosa in CD. The mRNA expression of iAP in inflamed colonic mucosa of patients with CD and UC was significantly elevated compared to non-inflamed colonic mucosa with CD or to controls. Immunofluorescent staining revealed iAP and TLR4 colocalization and restriction to the epithelial surface of terminal ileum and colon in patients with IBD and controls.

**Conclusion:** The decreased iAP protein level in the inflamed colonic mucosa of children may indicate its role in the pathogenesis of IBD.

**Disclosure of Interest:** None declared.
PO-G-0128

Inflammatory Bowel Disease

CLINICAL VALIDATION OF SIMPLE ENDOSCOPIC SCORE FOR CROHN’S DISEASE (SES-CD) IN CHILDREN

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Objectives and Study: Simple endoscopic Score for Crohn’s disease (SES-CD), validated in 121 CD patients, is based on 4 endoscopic variables (ulcer size, ulcerated and affected surfaces, stenosis) in 5 ileocolonic segments and the endoscopic parameters are scored from 0–3. PCDAI is basic clinical index used to assess the severity of Crohn disease (CD) in children. The aim of this study was to evaluate the correlation between SES-CD and PCDAI in various clinical situations in children with CD.

Methods: PCDAI and SES-CD were analyzed three times in group of children with Crohn disease receiving therapy with infliximab: 66 patients (aged 14.8; 12.9; 16.3 [median; Q1; Q3]) prior to the therapy week 0; 66 patients after induction therapy (22 clinical remission, 26 clinical response, 18 no response) week 10; 32 patients (23 clinical remission) who finished maintenance therapy week 50. Spearman’s rank correlation was used as a statistical method.

Results: The results of PCDAI were as follows [median; Q1; Q3]: week 0 - 18; 12; 22 vs. week 10 - 5.0; 0.0; 12.5. The results of SES-CD were as follows [median; Q1; Q3]: week 0 - 52.5; 45.0; 57.5; week 10 - 15.0; 10.0; 30.0; week 50 - 5.0; 0.0; 12.5. The results of PCDAI variance in subgroup of patients with moderate to severe CD.

Conclusion: SES-CD reflects clinical status in children with CD as well as its improvement measured by PCDAI, but underestimates PCDAI variance in subgroup of patients with moderate to severe CD.


Disclosure of Interest: None declared.

PO-G-0129

Inflammatory Bowel Disease

ADHESION-INVASIVE ESCHERICHIA COLI STRAINS HAVE A POTENTIAL ROLE IN PEDIATRIC IBD

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Objectives and Study: Crohn’s disease (CD) and ulcerative colitis (UC) are chronic, relapsing, immunologically mediated disorders of the gastrointestinal tract, with a complex pathogenesis. They are characterized by various genetic abnormalities that lead to overly aggressive T-cell responses to a subset of commensal enteric bacteria. Recent data reported high numbers of Escherichia coli colonizing the epithelial intestinal layer. In particular, adherent-invasive E. coli (AIEC) is increasing in relevance, due to its higher presence in adult CD patients than in controls. The aim of the study was to identify and characterize adherent-invasive E. coli strains in pediatric patients with inflammatory bowel disease (IBD).

Methods: 34 consecutive patients (24 with CD and 10 with UC) and 18 healthy controls were enrolled for this study. Intestinal mucosal biopsies were taken during ileo-colonoscopy and analyzed for the presence of adherent-invasive Escherichia coli by the adhesive-invasive test. The presence/absence of virulence genes associated with other AIEC was determined by RT-PCR. Phylogenetic analysis of the strains was completed using RAPD–PCR. TNF-alpha, IL-8 and IL-1beta mRNA expression was analyzed in Caco2 cell line after incubation with identified adherent-invasive strains by real time PCR.

Results: 2 AIEC strains, EC10 and EC15, were isolated from 2 IBD pediatric patients (1 with CD and 1 with UC) and resulted positive for the adhesive fimbrial factor H gene, while negative for the principal virulence genes. These strains were able to adhere and invade the epithelial cell line, Caco2, at levels comparable to that of the widely known AIEC strain, LF82, and to upregulate the specific AIEC receptor, carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6); moreover, they induced in vitro an increased mRNA expression of pro-inflammatory cytokines such as TNF-alpha, IL-8 and IL-1beta.

Conclusion: Our data indicate that these E. coli strains, isolated from our patients, belong to the AIEC group spectrum, suggesting their potential role in the mechanisms of pediatric IBD.

Disclosure of Interest: None declared.

PO-G-0130

Inflammatory Bowel Disease

ANTIBODIES AGAINST SACCHAROMYCES CERERVISIAE ARE ASSOCIATED WITH PENETRATING DISEASE BEHAVIOR AND PERIANAL DISEASE, BUT PANCREATIC AUTOANTIBODIES ARE NOT RELATED TO CLINICAL PRESENTATION, MEDICAL THERAPY, AND NEED FOR SURGERY IN PEDIATRIC-ONSET IBD


Disclosure of Interest: None declared.
Objectives and Study: Significance of autoantibodies against exocrine pancreas (PAB), recombinant pancreas antigens (rPAG) and goblet cells (GAB) are not well known in pediatric patients with inflammatory bowel disease (IBD). Our aim was to determine the accuracy of PAB, rPAG, GAB, antibodies against Saccharomyces cerevisiae (ASCA) and perinuclear components of neutrophils (pANCA) in pediatric IBD patients. Moreover, association with NOD2/CARD15 and disease phenotype was determined.

Methods: This prospective multi-center study included 154 pediatric patients with IBD (mean age 13.9 years) 102 patients with Crohn’s disease (CD) and 52 patients with ulcerative colitis (UC). Controls consisted of patients with celiac disease and patients with no disease, respectively. Sera were determined for serum autoantibodies by indirect immunofluorescent assay. NOD2/CARD15 variants were tested by polymerase chain reaction/restriction fragment length polymorphism. Detailed clinical phenotypes were defined.

Results: In 33 pediatric CD patients (32.3%) developed penetrating and/or stricturing disease after a median follow-up 18 months and 7.1% underwent surgery. ASCA positivity (72.5%) was associated with penetrating disease behavior (P = 0.0013) and perianal complications (P = 0.0098). In CD pANCA positivity (32.3%) correlated with positive family history (P = 0.0302). NOD2 variants were associated with steroid refractory disease (P = 0.0485) and infliximab use (P = 0.0352). The presence of PAB was significantly higher in CD (34.3%) compared with UC (21.1%), celiac (26.3%) and control (6.25%) patients. The frequency of antibodies against rPAG was 36.5% in CD, 21.1% in UC, 26.3% in celiac group and 6.25% in controls. GAB detection was low in all groups (UC: 11.5%, CD: 2%, celiac: 5.2%, controls:18.7%). PAB, rPAG and GAB antibodies were not related to clinical presentation, medical therapy or need for surgery in CD. No association was found in UC.

Conclusion: Our study suggests that ASCA would be beneficial in identifying subjects who are more likely to develop complicated disease course in pediatric patients with CD. PAB and rPAG antibodies were specific for CD, but the sensitivity was poor. Antibody response to PAG, rPAG and GAB was not associated with disease phenotype in this pediatric-onset IBD cohort. Antibodies in patients with celiac disease are not uncommon finding.

Disclosure of Interest: None declared.

PO-G-0131

Inflammatory Bowel Disease

Predictive Factors of Disabling Course in Pediatric Crohn’s Disease

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Objectives and Study: Predicting clinical course of Crohn’s disease (CD) is a key step for a right therapeutic approach. This will reserve an early use of immunosuppressive and possibly biological therapy to the most aggressive disease, avoiding risks of overtreatment in favourable outcomes. Adopt a correct therapeutic approach is of importance in children to allow normal development and growth. Aim of this study was to identify at diagnosis predictive factors of disabling disease course in pediatric patients with CD.

Methods: In a retrospective and multicenter study pediatric patients affected by CD with a follow-up that was longer than 2 years were included. A total number of 128 children were recruited from 3 pediatric referral centers that adopt a uniform therapeutic approach. We identified 5 disease localizations: stomach-duodenum, small bowel, ileo-colic, colonic and sigma-rectum. We considered as possible predictors of disease course: age at disease onset and diagnosis, clinical presentation, disease activity assessed by the Pediatric Crohn’s Disease Activity Index (PCDAI) score, disease localization, presence of perianal disease and disease extension. Crohn’s disease course was conventionally considered as disabling when at least 1 of the following criteria was present: at least 1 flare-up in the first year of follow-up, a mean number of relapses/year ≥ 1 in the two years after diagnosis, infliximab therapy or surgery within 2 years after diagnosis. Data were analyzed by multivariate analysis.

Results: The rate of disabling disease was 60 out of 128 (46.9%). Thirty-two children out of 60 showed at least one flare-up in the first year of follow-up, 51 had a mean number of relapses/year ≥ 1 in the first 2 years after diagnosis, 18 received infliximab therapy and 3 underwent surgery. Among all the variables analyzed, disease extension (P < 0.0001) and disease localization in the small intestine (P < 0.002) and sigma-rectum (P < 0.004), were the only independent significant risk factors for disabling course in our children with CD.

Conclusion: This study shows that children with CD with disease extension in many segments of gastrointestinal tract and those who have disease localization in small intestine and sigma-rectum are at high risk of disabling disease course. In these children an aggressive therapeutic approach at diagnosis is warranted.

Disclosure of Interest: None declared.
PO-G-0135

Inflammatory Bowel Disease
INFLAMMATORY BOWEL DISEASE NEUTROPHILS HAVE INCREASED MIGRATORY ACTIVITY ASSOCIATED WITH ENHANCED CXCR1 AND CXCR2 EXPRESSION
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Objectives and Study: Neutrophils transmigrate from the blood into inflamed tissue via the interaction of interleukin 8 (CXCL8), produced in this tissue, with chemokine receptors, CXCR1 and CXCR2 that are expressed on the membranes of neutrophils. We investigated neutrophil migration and components of this pathway in patients with inflammatory bowel disease (IBD) and healthy controls.

Methods: CXCL-8 induced chemotaxis of peripheral blood isolated neutrophils was studied in a transwell system. CXCL8, CXCR1 and CXCR2 expression and CXCL8 release were examined by qRT-PCR, ELISA and immunofluorescence in neutrophils and in intestinal tissue.

Results: IBD neutrophils (n = 24) show a similar chemotactic index in response to CXCL8 when compared with healthy control neutrophils (n = 6), although the basal migratory capacity is 5-fold higher. The expression of both CXCR1 and CXCR2 is increased in IBD neutrophils, while the expression and release of CXCL8 is decreased. CXCL8 protein levels were lower in non-inflamed IBD tissue homogenates (n = 38) and significantly increased in inflamed IBD tissue (n = 50) when compared with healthy tissue homogenates of colorectal cancer patients (n = 20). CXCL8 was mainly found in epithelial cells in inflamed IBD tissue while sporadically neutrophils expressing CXCL8 were found in IBD tissue.

Conclusion: IBD neutrophils have different expression levels of components involved in CXCL8-CXCR1/2 chemotaxis. In addition to the enhanced CXCL8 levels in inflamed IBD tissue, this might explain the enhanced infiltration of neutrophils in IBD.

Disclosure of Interest: None declared.

PO-G-0138

Inflammatory Bowel Disease
OUTCOME IN THE 2 YEARS FOLLOWING A COURSE OF EXCLUSIVE ENTERAL NUTRITION IN A COHORT OF >100 PAEDIATRIC CROHN’S DISEASE PATIENTS
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Objectives and Study: Exclusive enteral nutrition (EEN) is an effective first-line treatment for active paediatric Crohn’s disease (CD). We explored the short- and long-term effects of EEN including anthropometric parameters and evaluated factors that predicted subsequent disease outcomes.

Methods: A retrospective case note review in newly diagnosed CD (<16 y) who completed 8 weeks of EEN. Demographics, anthropometry, disease characteristics and inflammatory markers were taken at EEN initiation and then at 1, 2, 6, 12 and 24 months post diagnosis. Clinical response to EEN was characterised according to the global physician assessment.

Results: 110 patients were included (males 68; median age: 11.2 years). At diagnosis 34% were thin (BMI ≤ 2 SD), 1% obese (BMI ≥ 2 SD), 10% had short stature (height ≤ 2 SD) and 25% were underweight (weight ≤ 2 SD). By 4 weeks of EEN weight and BMI z-score increased significantly (−1.1 cf. −0.6; −1.3 cf. −0.4, respectively, P < 0.05), with a smaller increase between 4 and 8 weeks (−0.6 cf. −0.4 and −0.4 cf. −0.05, respectively, P < 0.05). Children with active disease (n = 12) gained less weight than those in remission (2.2 vs. 5.0 kg, respectively, P < 0.05). There was a strong negative correlation between weight/BMI z-score and magnitude of change at the end of EEN (r = −0.76, P < 0.0001). Compared to baseline albumin, CRP and platelets significantly improved and weeks 1 and 2 (albumin (g/L): 31 ± 6.4 vs 36 ± 4.7; CRP (mg/L): 44.1 ± 48.8 vs 16.1 ± 13.9; platelets (×10^9): 525 ± 147 vs 471 ± 165; all P < 0.01) but not between weeks 4 and 8. ESR improved significantly between 0–4 ((ESR (mm/h): 43.5 ± 23.2 vs 24.3 ± 20.7, P < 0.00001) and continuing to fall to 18.8 at 4–8 weeks (P < 0.01 cf. 4 weeks). 44 patients completed a second course of EEN; median weight gain improved but was less than the initial course (3.3 vs. 5.1 kg, P < 0.05). Of these children, 19 achieved remission after both courses and 2 children who had active disease after the first course went on to achieve remission after the second course. BMI z score at diagnosis was the strongest predictor of BMI z score at any time point of the follow-up. The size of weight or BMI z score change at the end of the primary EEN did not predict time to subsequent clinical relapse or anthropometry at follow up. Median height z score did not change compared to diagnosis.

Conclusion: Anthropometry improves with EEN but the change is smaller in the second half of the initial course or in secondary courses. Weight gain is not a predictor of time to relapse or anthropometry at follow up to 2 years. Systemic inflammatory markers improve during the first 4 weeks of treatment with little improvement at the second half.

Disclosure of Interest: None declared.

PO-G-0139

Inflammatory Bowel Disease
CALPROTECTIN LEVELS AT DIAGNOSIS IN UNTREATED PEDIATRIC CROHN DISEASE
Objectives and Study: Calprotectin is a validated marker of intestinal inflammation in Crohn disease (CD). It may be elevated when other inflammatory markers are normal, and is thus a very useful screening test. Concern has been raised about the possibility that it may be less predictive in isolated small intestinal disease. The objective of the study was to prospectively evaluate fecal calprotectin in newly diagnosed untreated CD patients, and to evaluate if low calprotectin levels are associated with isolated small intestinal disease or low levels of systemic inflammation.

Methods: Consecutive children under age 18 with new onset untreated CD diagnosed by the Porto criteria, participating in the ongoing ESPGHAN GROWTH CD study, were evaluated at diagnosis for PCDAI, extent of disease, CRP and fecal calprotectin.

Results: 60 children met the inclusion criteria (mean age 12.6 ± 4.6 years, 38 (63%) males). Disease activity was evenly distributed, 25 (42%) with mild disease, 17 (28%) with moderate disease and 18 (30%) with severe disease, using the physician global assessment. Disease location was as follows: 15 (25%) had small bowel only (L1/C6), 6 (10%) had only colonic disease, and the others were combined (L3, or L3+C6). Median calprotectin levels were 1862 (range 30–2400 μg/g). Four children (6.6%) had normal calprotectin levels (range 0–50, an additional 2 children had low calprotectin levels <100, all of whom with moderate to severe disease as judged by the PCDAI and PGA. CRP values for these children were normal or near normal (ie, <1 mg/dL). The correlation between PCDAI and PGA values was 0.76 (P < 0.001). However, there was no correlation between calprotectin levels and either PCDAI or PGA or disease activity categories) as classified by both PCDAI and PGA (P>0.1). The correlation between calprotectin and CRP was 0.39 (P = 0.004). In a multivariate regression analysis, none of the following variables were associated with calprotectin values (including age, small bowel disease only, CRP value and gender).

Conclusion: Calprotectin levels at diagnosis do not correlate with disease activity indices, CRP or site of disease. A small proportion of patients with active disease may have normal calprotectin levels, irrespective of site or severity of disease. These patients are likely to have low CRP as well.

Disclosure of Interest: None declared.

PO-G-0140

Inflammatory Bowel Disease

RECTAL INFUSION OF LACTOBACILLUS REUTERI ATCC 55730 IMPROVES INFLAMMATION AND DOWNREGULATES MUCOSAL PRO-INFLAMMATORY CYTOKINES IN DISTAL ULCERATIVE COLITIS

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Objectives and Study: Ulcerative colitis (UC), is an inflammatory bowel disease resulting from an abnormal immune response to specific commensal microbiota antigens. Modulation of the latter by administering probiotics has been proposed as a therapeutic option to manage UC. We aimed at investigating, in patients with ulcerative proctitis, if the rectosigmoid delivery by enema of a human-derived hetero-fermentative Lactobacillus reuteri strain ATCC 55730 could affect mucosal inflammation and expression of pro-inflammatory cytokines.

Methods: Patients with distal UC (range age 5–18 years) were recruited, during a 2-year period, for an 8-week prospective randomized, double blind controlled trial. Disease activity was assessed with Mayo Disease Activity Index (MDAI) (variables: stool frequency, bleeding, physician’s assessment of disease activity, mucosal appearance; maximum score: 12 points; mild-to-moderate activity: < 10). Thirty-six patients with mild-to-moderate activity were included; they were randomized to receive bedtime an enema solution containing 10^9CFU of L. reuteri ATCC 55730 (18 cases) or placebo (18 cases). All were also treated with oral mesalamine that was maintained during the trial. Colonoscopy was performed prior to the trial and the day after the last enema. Mucosal biopsies were taken for histology as well as for mRNA (real time PCR) and protein expression (Western blot assays) of different cytokines. A clinical response was defined as a reduction in the MDAI of ≥50% as compared to basal value.

Results: There was a significant decrease in the MDAI score only in the L. reuteri group (3.2 ± 2.6 vs baseline 10.8 ± 1.5, P < 0.01; placebo: 7.1 ± 2.2 vs baseline: 10.7 ± 1.4, NS). A clinical response was recorded in 14 L. reuteri group (77.7%), but only in 6 placebo group (33.3%; P < 0.01). Evaluation of mRNA and protein expression in mucosal specimens showed that IL-1α, TNF-β, and IL-6 were expressed at markedly lower level in L. reuteri group (P < 0.01).

Conclusion: Rectal administration of L. reuteri is of value in reducing inflammation in distal ulcerative colitis and downregulating pro-inflammatory signals in rectal mucosa.

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Disclosure of Interest: None declared.
PO-G-0142

Inflammatory Bowel Disease

THREE-DIMENSIONAL ENDO-ANAL ULTRASOUND IN PERIANAL CROHN’S FISTULAS: WHAT IS ITS ROLE IN MANAGEMENT?

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Objectives and Study: Management of perianal Crohn’s disease is complicated, due to several limits in diagnosis and therapy. Three-dimensional endo-anal ultrasonography (3D EAUS) has been already validated in investigating anal sphincters and pelvic floor, whereas the use of hydrogen peroxide fistulography optimizes the ultrasound evaluation. The aim of our study was to demonstrate the effectiveness of hydrogen peroxide enhancement of 3D EAUS in the assessment of fistulas and abscesses in perianal Crohn’s disease and in planning the most adequate local treatment.

Methods: Seven pediatric patients (5 female; mean age: 16 years and 7 months; range: 9–22 years) affected by perianal fistulising Crohn’s disease resistant to conventional medical treatment, underwent 3D EAUS (recto-anal rotating probe with a 360° radius and a frequency between 10 and 16 MHz with a built-in 3D mover) with infusion of hydrogen peroxide throughout the visible external opening of the fistula. Parks classification was used to identify the fistulous tract anatomy. The fistulas were treated by placement of vessel-loops under general anaesthesia or without sedation depending on patient compliance.

Results: In all cases, the hydrogen peroxide-enhanced 3D EAUS detected the tracts of the perianal fistulas and the locations of the internal opening. Four patients presented a trans-sphincteric fistula (in 1 case associated to abscesses); 3 patients had an inter-sphincteric fistula with abscess. The drainage of fistulas with vessel-loop placement was performed without complications; in 4 children general anaesthesia was necessary. After a mean follow-up of 4 months, 6 patients presented a clinical improvement with local disappearance of inflammation. In one patient who had undergone vessel-loop application 1 year before, surgical fistulectomy was mandatory because of the persistence of abscess.

Conclusion: Even in pediatric age, hydrogen peroxide-enhanced 3D EAUS represents a useful tool to perform a correct diagnosis and to guide towards a suitable therapy in the management of perianal Crohn’s disease, as it favours an accurate detection and characterization of fistulas.

Disclosure of Interest: None declared.

PO-G-0143

Inflammatory Bowel Disease

WIRELESS CAPSULE ENDOSCOPY AS PART OF ROUTINE WORK-UP OF PEDIATRIC PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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Objectives and Study: Wireless capsule endoscopy (WCE) is a non-invasive method for visualizing mucosal changes of small bowel (SB) in patients with inflammatory bowel disease (IBD). It is mostly used to evaluate the extent of disease within SB; it may also help in reclassification of IBD from indeterminate colitis (IC) to either Crohn’s disease (CD) or ulcerative colitis (UC). In addition, previously diagnosed patients with CD may be found to have a more significant burden of SB disease.

Methods: From October 2006 to January 2011 WCE was performed in 53 pediatric patients (33 male, 20 female) with confirmed or suspected IBD at our unit. All patients had upper and lower GI endoscopy and most also had SB follow-through prior to WCE study to minimize the possibility of capsule retention due to stenosis. From June 2009 Agile patency capsule became available and was used in 9 patients prior to WCE, confirming SB patency in all, without unnecessarily exposing patients to radiation.

Results: Patient mean age was 13.2 y (min 2, max 18). In 7 patients (6 patients <6y/o, 1 pt 10y/o) endocapsule was inserted endoscopically into the duodenum. WCE was performed in 33 patients with established CD (62.3%), 12 patients with previously diagnosed IC (22.6%), and 8 patients with suspected IBD (15.1%). In 34 patients WCE confirmed SB mucosal lesions; total diagnostic yield was 64.1% (72.7%, 58.3% and 37.5% for CD, IC and suspected IBD, respectively). In 7 patients with IC the disease was reclassified to CD (6 patients) upon newly diagnosed SB mucosal lesions and to UC (1 pt) upon negative result of the WCE. In 24 of 33 patients with previously diagnosed CD more extensive SB disease was confirmed. In 3 patients suspected of having IBD the disease was confirmed as CD based on the new SB mucosal changes seen on WCE. The procedure was well tolerated and safely performed in all our patients; we had no report of capsule retention or any other adverse reactions.

Conclusion: At our unit we use WCE as part of routine diagnostic work-up in all suspected IBD patients as well as in all patients with previously diagnosed CD/IC to evaluate the extent and severity of the SB disease. It is performed safely and has good diagnostic yield, comparable to that in adult WCE series and studies using different diagnostic modalities, as reported in literature.

Disclosure of Interest: None declared.
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Objectives and Study: Gastro-ileocolonoscopy is considered the gold standard for diagnosing pediatric inflammatory bowel disease (IBD). Magnetic resonance enterography (MRE) is an attractive method for further evaluation of the small bowel as a noninvasive method without radiation exposure. The aims were to study the diagnostic accuracy of MRE in diagnosing intestinal inflammation and to evaluate the clinical significance of the MRE results on the management of pediatric IBD patients.

Methods: Forty pediatric patients (median age 13.8 years, range 10.0–17.7) with suspected (n = 35) or confirmed IBD (n = 5) were included and underwent gastro-ileocolonoscopy with biopsies followed by MRE (median interval 20 days, range 6–49). The MRE results were compared with macroscopic and microscopic assessment of the ileum. The clinical impact of the MRE results was registered.

Results: Crohn’s disease (CD) was diagnosed in 25 cases, ulcerative colitis (UC) in 12, and IBD unclassified (IBDU) in three. Macroscopic ileitis was detected in 15/25 (60%) of CD cases and in 2/12 (17%) of UC (backwash ileitis). Microscopic inflammation was found in another 4 CD cases and 1 IBDU patient. In total, discrepancy between macroscopic and microscopic inflammation was found in 9 CD, 2 UC and one IBDU patient. MRE demonstrated terminal ileitis in 13/25 (52%) CD cases and in 1 UC patient. The most common MRE findings in ileum were increased contrast uptake (n = 12) and/or bowel wall thickening (n = 9). Other MRE findings among CD patients were lymphadenopathy (n = 14), stenosis (n = 4), jejunal inflammation (n = 2) and luminal narrowing in the jejunum (n = 1). When compared with macroscopic assessment of terminal ileum, MRE sensitivity was 71%, specificity 92%, positive predictive value 86%, negative predictive value 81% and observed agreement 83%. When compared with the findings of macroscopic and/or microscopic inflammation, MRE sensitivity was 64%, specificity 100%, positive predictive value 100%, negative predictive value 69% and observed agreement 80%. MRE was decisive for diagnosis in 4/40 (10%) and led to treatment changes in 11/40 (28%) in the following 6 months.

Conclusion: MRE is a reliable method for imaging of intestinal inflammation in pediatric IBD. It also provides important information about stenoses and extraintestinal manifestations, and can be supportive for clinical treatment decisions.

Disclosure of Interest: None declared.

PO-G-0145

Inflammatory Bowel Disease

MOLECULAR PHYLOGENY OF ESCHERICHIA COLI STRAINS ISOLATED FROM PEDIATRIC CD PATIENTS

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Objectives and Study: In both adult and pediatric Crohn’s disease (CD) it has been recognized an increased predominance of potentially harmful Escherichia coli species, leading to the hypothesis of a particular adherent/invasive E. coli (AIEC) prototype role in CD pathology. The specific aim of this study was to characterize overall 770 E. coli strains isolated from 5 IBD pediatric patients and 5 non-IBD ones, both in a phylogenetical and phenotypical way, in order to assess intra-patient genotypical relationships and search AIEC prototypes.

Methods: 77 E. coli strains were isolated from each patient, and underwent such analysis: random amplified polymorphic DNA (RAPD) genomic profiling, phylogenetic group belonging (A, B1, B2, D), adhesive/invasive characterization. Results were analyzed through multivariate statistics.

Results: First results obtained show: a CD intrapatient median 20% presence (15/77) of E. coli strains with invasive properties analogous to AIEC prototype, a major genomic homogeneity in RAPD profiles from CD (P < 0.0001), the existence of particular genomic (RAPD) subtypes associable to CD.

Conclusion: Our study points to have a deep knowledge of the E. coli intraspecies genomic variability, both in normal and altered (inflamed) habitats. Strains with higher genomic mutability could be preferred in an already established pathological habitat, thanks to their enhanced fitness properties, so leading to their overpopulation in the gut community. As establishment is completed, E. coli strains tend to homogenize their genomes due to the continuously inflamed habitat. Such studies will give further insights on the gut bacterial microevolution in relation to gut environment.

Disclosure of Interest: None declared.

PO-G-0071

Food Allergy

PREDICTIVE FACTORS OF FAILURE OF THE FIRST COW’S-MILK PROTEIN CHALLENGE IN CHILDREN WITH COW’S-MILK PROTEIN ALLERGY

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Objectives and Study: The prevalence of cow’s-milk allergy (CMA) is elevated in infancy. The majority of
children become tolerant before 4 years, but 15% remain allergic after the age of 10. Previous studies have shown that approximately half of the children tolerated cow’s-milk protein (CMP) after the first challenge. Moreover, the timing of this challenge represents an important issue for clinicians. The aim of this study was to identify clinical or biological factors present at diagnosis of CMA or during follow-up, which could be related to the risk of failure of the first attempt of CMP reintroduction.

**Methods:** 130 children followed for CMA in the Besancon Teaching Hospital were eligible for this retrospective analysis. Those with success of the first attempt were compared to those with failure. Clinical and biological factors possibly associated with failure of the test were analysed with nonparametric tests.

**Results:** 63 children were included in the final analysis (34 males), the median age at diagnosis of CMA was 3 months. Before the diagnosis of CMA, 10 infants were exclusively breast-fed, 35 had artificial milk and 34 had a mixed feeding. The symptoms of CMA at diagnosis were mainly digestive (70%) or cutaneous (55%). Initial diet consisted of exclusive hydrolysed formula in 70% of cases and exclusive breast-feeding in 30%. One third of infants required an amino acid based formula during follow-up. The challenge test was performed after a median time of 9 months (6–13). Tolerance to CMP was successful in 65% of infants. Signs of failure were mainly delayed (in 60% of cases): vomiting, diarrhea and failure to thrive. There was no relation between gender, age of CMA diagnosis, parental smoking, familial atopy and the risk of failure of milk reintroduction.

In multivariate analysis, the only factor associated with an increased risk of failure of the test was an anti-alpha-lactal-bumine antibody (AAA) rate greater than 2 kUI/L at reintroduction after 9–12 months of diet exclusion should be based on clinical presentation and AAA reduction. A rate of AAA less than 2 kUI/L at the time of reintroduction predicts a great chance of success.

**Disclosure of Interest:** None declared.

**PO-G-0073**

**Food Allergy**

**ILEOCecal INTUSSUSCPTIONS AND ABDOMINAL BLOATING SECONDARY TO MULTIPLE FOOD ALLERGIES**


**Objectives and Study:** IgE and non-IgE mediated mechanisms are involved in several gastrointestinal (GI) disorders as proctocolitis, enterocolitis, diarrhea, constipation, or gastroesophageal reflux. Prevalence of non-IgE mediated multiple food allergies has increased in recent years, presenting often as atypical forms like intussusception.

**Methods:** We describe the evolution of 4 children with recurrent ileocecal intussusception episodes, abdominal bloating and other GI symptoms that disappeared after dietary restriction.

**Results:** In 3 of 4 children symptoms started after cow’s-milk–based formula was introduced between 1 and 3 months of age and in one child at 23 months of age after an acute gastroenteritis. All children had suffered 3 to 7 episodes of ultrasound scan-diagnosed intussusceptions that had resolved after air/hydrostatic enemas or spontaneously and from recurrent episodes of abdominal distension and vomiting: 3 had recurrent diarrhoea and 1 oral ulcers. Temporal association between the ingestion of new foods and intussusception episodes was discovered between 1 to 7 months after the onset of symptoms. Ultrasound scan performed during intussusception episodes showed multiple mesenteric adenopathies. Total IgE was normal and specific IgE (CAP-RAST) against multiple food allergens were negative in all of the children. Skin-prick test against multiple food allergens was negative except in 1 child (soy and sole positive). Three children had positive skin patch testing against multiple foods. Complete resolution of symptoms was achieved when children were started on an amino acid–based formula. Intussusceptions and GI symptoms relapsed during multiple food challenges and resolved after the withdrawal of specific new food. Histopathology of endoscopic biopsies performed right after intussusception episodes showed pronounced nonspecific nodular lymphoid hyperplasia in the duodenum and colon in 3 children and was normal in the fourth child; histopathology of biopsies performed after 2 months on amino acid–based formula was normal in all patients. After 1 to 4.5 years from diagnosis all children are asymptomatic, growing normally (weight: p25–90; height: p65–95) and on a restricted diet with amino acid-based formula and none to a maximum of 10 different foods.

**Conclusion:** Recurrent intussusceptions and abdominal bloating mimicking chronic intestinal pseudoobstruction can be secondary to food allergy. Mesenteric adenopathies, intestinal lymphoid hyperplasia, positive skin patch testing, temporal association between food challenges and intussusceptions, and complete resolution of all GI symptoms and lymphadenopathies on dietary exclusion support the idea of an allergic, non-IgE-mediated mechanism.

**Disclosure of Interest:** None declared.

**PO-G-0079**

**Food Allergy**

**INTESTINAL PERMEABILITY AND FECAL EOSINOPHILS-DERIVED NEUROTOXIN ARE GOOD DIAGNOSIS TOOLS FOR DELAYED-ONSET DIGESTIVE COW’S-MILK ALLERGY IN TODDLERS**

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Objectives and Study: Food allergy is a common problem in 
France involving 4–6% of toddlers. IgE-mediated cow’s-
milk allergy (CMA) is easily diagnosed with skin prick tests 
(SPT) and Rast IgE measurement guided by clinical history, 
whereas delayed-onset CMA, mostly, non IgE-dependant, 
still remains difficult to diagnose in toddlers. Our study 
assessed the diagnostic performances of intestinal permea-
Bility (IP) and fecal markers compared with a standard 
allergic work-up in children referred for CMA diagnosis.

Methods: A prospective study was performed in 25 con-
secutive children, mean age (SD) 6.3 mo (4.8) with digestive 
and/or extra-digestive manifestations suggesting CMA, 
based on a standardized allergic and digestive work up 
(specific cow’s-protein IgE and IgG, cow’s-milk SPT, 
cow’s-milk protein atopy patch test Finn chambers (APT), 
open cow’s-milk challenge (OCMC), IP determination (urin-
ary lactitol/mannitol ratio), faecal markers, i.e. al antitryp-
sin, TNF-α, calprotectin, bdefensin2, SlgA and eosinophils-
derived neurotoxin (EDN). ROC curves were calculated 
for all markers in order to define cutoff levels.

Results: The OCMC was positive in 11 children (CMA), and 
negative in 14 (controls). The global test performances i.e. 
the number of true positive + negative cases/ the total 
number of cases, were 76% and 72% for IP and fecal 
EDN, respectively contrasting with 68%, 60%, 55% and 
52% for APT, IgE, SPT and IgG.

Table.

<table>
<thead>
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<th></th>
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<th>Negative Predictive Value, %</th>
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<td>100/93</td>
<td>100/80</td>
<td>58/65</td>
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<td>93/100</td>
<td>75/100</td>
<td>62/58</td>
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<td>Fecal EDN</td>
<td>55</td>
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Conclusion: In this routine diagnosis allergy work-up for 
CMA in toddlers, the best efficacy was seen for IP compared 
IgE, IgG, SPT and APT. Moreover, fecal EDN in a single spot 
displayed a similar performance.

Disclosure of Interest: None declared.

PO-G-0082

Food Allergy

COMPARISON OF 2 EXTENSIVELY HYDROLYZED FORMULAS FOR THE TREATMENT OF CHILDREN 
WITH COW’S-MILK INTOLERANCE

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Switzerland.

Objectives and Study: Cow’s-milk protein allergy (CMPA)- 
associated atopic manifestations are frequent in clinical 
practice and require a specific feeding regimen. For infants 
who are not breast-fed, AAP and ESPGHAN recommend an 
extensively hydrolyzed formula. The present study aimed to 
compare the efficacy of a new extensively hydrolyzed 
hypoallergenic formula using an innovative peptide technol-
ogy with an existing extensively hydrolyzed formula. The 
study was designed with an assumption of noninferiority of 
the test formula as compared to the control. Efficacy was 
assessed by the CMPI (cow’s-milk protein intolerance) score 
after a 4-week intervention period. The CMPI is a composite 
score ranging from 0 to 33, evaluating the following symp-
toms: crying, regurgitation/vomiting, stools, atopic eczema, 
urticaria and respiratory symptoms.

Methods: This was a prospective, multicenter, randomized, 
reference-controlled study. 86 infants between 0 to 6 
months, with suspicion of a mild/moderate CMPI (CMPI 
score 12), were randomized to receive either the test 
formula or a control exclusively during the first 4 weeks 
and then for up to 8 months. The noninferiority boundary 
was defined in advance of the study with 3 CMPI points. A 
positive treatment difference points in the harmful direction, 
while a negative difference points in the beneficial direction.

Results: A total of 75 patients were included in the intent-to-
treat dataset (N=40 in experimental group and N=35 in 
control). The overall CMPI score with both groups pooled, 
showed a significant change from baseline to 4 weeks −8.04 
(95% CI=−8.87, −7.21, P<0.001), thereby showing 
construct validity of the CMPI score. The change in CMPI 
score observed in the experimental group was −8.45 (SD 
3.85) and −7.57 (SD 3.33) in the control group. The treat-
mment difference was −0.879, the 95% CI (−2.79, 1.03) 
showing a statistically significant noninferiority of the 
experimental group with respect to the control 
(P<0.0001). Treatment differences between the 2 groups 
were evaluated for change in scores from baseline for each of 
the individual symptoms of the CMPI, i.e. crying, regur-
gitation, stool consistency, atopic eczema, urticaria and respi-
atory symptoms. There were no significant differences 
between the 2 groups, all symptoms improved as compared 
to baseline. Growth (weight, length, and head circumfer-
ence) was evaluated at baseline, 1, 2, 4, 6, 8 and 10 months. 
Normal growth was observed in both groups with no sig-
ificant differences in any of the growth measurements. 
Both formulas were safe and well tolerated. There were no 
adverse events related to the study formula in either of 
the groups.

Conclusion: This new extensively hydrolyzed formula is 
safe and effective for infants with CMPI.

Disclosure of Interest: Y. Vandenplas Consultant for: Uni-
ted Pharmaceuticals and Biocodex, Conflict with: Member

PO-G-0083

**Food Allergy**

THE CORRELATION BETWEEN INTERLEUKIN-10 GENE POLYMORPHISMS AND PATHOGENESIS OF FOOD ALLERGY IN INFANTS

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**Objectives and Study:** To explore the relations between the single nucleotide polymorphisms (SNP) of interleukin-10 (IL-10)-1082A/G and IL-10–819C/T and pathogenesis of food allergy in infants.

**Methods:** 71 food allergy infants (food allergy group) and 86 healthy infants (control group) were involved in this study. The IL-10–819C/T and IL-10–1082A/G polymorphisms of all infants were determined by amplification refractory mutation system polymerase chain reaction (ARMS-PCR). 15 infants in each group were selected randomly for sequencing to confirm further the results of the ARMS-PCR.

**Results:** The genotype frequency of IL-10–1082A/G was AA38.0%, AG62.0%, and GG 0.0% in the control group, and AA 47.7%, AG 50.0%, and GG 2.3% in the food allergy group, and AA 47.7%, AG 50.0%, and GG 2.3% in control group, there were significant differences between the 2 groups (χ²=10.627, P = 0.005). The frequency of each allele was C 50.0%, and T 50.0% in the food allergy group, and C 37.2%, T 62.8% in the control group, there were significant differences between the 2 groups (χ²=5.192, P = 0.023). The genotype frequency of IL-10–1082A/G was AA 38.0%, AG 62.0%, and GG 0.0% in food allergy group, and AA 47.7%, AG 50.0%, and GG 2.3% in control group, it showed no significant differences between the 2 groups (χ²=3.493, P = 0.174). The frequency of each allele was A 69.0%, G 31.0% in the food allergy group, and A 72.7%, G 27.3% in the control group, the differences were not significant between the 2 groups (χ²=0.506, P = 0.477).

**Conclusion:** IL-10–819C/T gene polymorphisms have been involved in food allergy. The decreased T allele frequency is associated with the increase in susceptibility of food allergy.

**Disclosure of Interest:** None declared.

PO-G-0084

**Gut Infection**

EARLY INTESTINAL BACTERIAL COLONIZATION AND NECROTIZING ENTEROCOLITIS IN PREMATURE INFANTS

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**Objectives and Study:** Necrotizing enterocolitis (NEC) is an acute inflammatory disease that affects the intestinal tract of neonates. The pathophysiology of NEC is multifactorial and gastrointestinal bacteria are thought to play an important role. The aim of this project was to determine the importance of bacterial flora in the development of NEC in premature infants. Furthermore, the microflora from infants who developed NEC was compared to the flora of preterm infants who did not.

**Methods:** 163 neonates <30 weeks of gestation were enrolled and faecal samples taken during the first month of life were analysed. The bacterial flora was analysed by faecal culturing and DNA analysis by 16 s rRNA gradient gel electrophoresis (16 s rRNA DGGE). A total of 482 faecal samples were examined.

**Results:** Of the 163 neonates, 21 developed NEC. The gestation days played an important role. NEC decreased by 8% (3.4; 13.2) per day of gestation. In the faecal samples few bacterial species were detected by cultur. Assessing the bacterial diversity of the faecal flora it was seen that neonates whom from the faeces less than 3 species were cultured tended to have increased risk for the development of NEC (OR on 4.6 (95% CI 0.98–21.8)). When comparing the faecal microflora in prematurity, it was observed that neonates who did not develop NEC overall were colonised with a diverse flora comprising of Gram-negative and Gram-positive bacterial species. Premature infants who developed NEC were colonised with pronominally a flora consisting of Gram positive bacteria. The 16S rRNA DGGE profiles for each infant appeared individually unique. Bacterial diversity was assessed by the band richness of each sample. There was no difference in band richness between the profiles obtained for faecal samples form infants who developed NEC and controls. Principal component analysis was performed by considering each DGGE profile a unique fingerprint for each child at each point in time. By inspection of score plots of the primary principal components no discrimination was found associated to any particular band position, indicating that no specific bacterium was present in the one patient group and absent for the other.

**Conclusion:** Our study is the largest to date, analysing the faecal flora of premature neonates during the first month of life. By culture we showed that premature with NEC are colonised predominately by Gram-positive bacteria compared to control neonates who did not develop NEC. The control premature neonates were found to be colonised with a significant diverse microflora. Surprisingly, by molecular methods we found no association between microflora profiles and development of NEC.

**Disclosure of Interest:** None declared.
PO-G-0085

Gut Infection
COST-EFFECTIVENESS OF ADD-ON PROBIOTICS (SACCHAROMYCES BOULARDII) IN CHILDREN WITH ACUTE ROTAVIRUS DIARRHEA IN TURKEY (SB-COSTR)

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Objectives and Study: Rotavirus (RVGE) is a major cause of acute gastroenteritis in children with significant direct health care cost including visits and hospitalizations as well as indirect cost including loss of working days of parents. A meta-analysis on the efficacy of Saccharomyces boulardii (Sb) in children showed that the probiotic reduces the duration of diarrhea and length of hospital stay with about 24 hours. Up to date, there are no relevant data about the potential pharmacoeconomic effect of probiotics in acute diarrhea.

Methods: The pharmacoeconomic model consisted in a theoretical scheme offering the possibility to conduct simulations of health processes associated with cost of first line and emergency care visits, hospitalizations, use of medications through estimates obtained from the efficacy data available from clinical trials performed in Turkey. We evaluated the potential cost effect of administration of Sb on treatment cost of RVGE. After the exclusion of the deaths we hypothetically subdivided all RVGE cases that were seen by general practitioners, at emergency care units, or needed hospitalizations in 2 equal groups. Cost was calculated in the first group with add-on Sb to conventional treatment (ORT, iv fluids) and in the second group without Sb.

Results: Standard mean cost of first line visit due to RVGE is 25 US$, increasing to 31.5 US$ with Sb. However when we calculated the cost of RVGE including indirect costs (transportation to hospital, loss of working days for parents) standard cost of RVGE per case increases to 175 and 159.6 US$ per case with add on Sb (considering the shortening of duration of diarrhea duration with 1 day). In a country based 1-year perspective, standard treatment cost is 17,495,275 and is reduced to 15,955,690 US$ when Sb is added. In mild-moderate RVGE, mean cost of hospitalization of 1 case is 565 US$ with standard treatment, and was reduced to 449.5 US$ when Sb was added. In one year perspective, total cost of hospitalization due to mild to moderate RVGE is 12,317,000 and is reduced to 9,799,100 US$ with Sb. Total RVGE related cost was 43,934,895 US$ in the standard treatment group and was reduced to 39,284,582 US$ when Sb was added.

Conclusion: If Sb would be administered in all RVGE cases during one year in all children under 5 years, the additional cost of Sb is 1,860,014 US$. However, the total cost of RVGE would be reduced to 78,568,164 US$ from 87,869,690 US$ (10.5% reduction) and 32.5 US$ per patient with RVGE would gain. With 1 day reduced diarrhea duration and length of hospitalization in large birth cohort and higher disease burden, potential effect of Sb is significantly higher than expected.


Gut Infection
INHIBITION OF ENTERIC BACTERIA ADHESION BY SIALIC ACID AND GANGLIOSIDES IN CACO-2 CELLS

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Objectives and Study: Evaluate the inhibition of newborn disease bacteria, in Caco-2 cells by sialic acid and gangliosides present in bioaccessible fraction from commercial infant formulas.

Methods: Caco-2 cells were seeded grown in DMEM and experiments performed 8–10 days post seeding. The method described by Laparra & Sanz (2009) was followed for the adhesion assays. Briefly, bacteria from 20-hour-old-culture were collected, washed with PBS and resuspended in PBS for a optical density of 0.5 (A_600), incubated with 75 mmol/L carboxyfluorescein diacetate at 37°C for 30 min, washed and resuspended in PBS. Four hundred microlitre of bacterial labelled suspensions were incubated at 37°C for 1 hour with 0.4 mL of each ganglioside standard solution (for control, medium without gangliosides nor sialic acid was added). Work suspensions of gangliosides and sialic acid were prepared in cell medium without antibiotics and foetal bovine serum, according to the concentration found in the bioaccessible fraction of infant formulas obtained by simulated gastrointestinal digestion. Afterwards, the resultant solution was loaded into 24-well plates and incubated at 37°C for 1 h. Cells were washed with PBS and added 1 ml per well of 1% (w/v) sodium dodecyl sulphate in 0.1 mol/L sodium hydroxide and incubated again at 37°C for 1 h. Mixtures were homogenised, 0.3 mL transferred to black 96-well plate and fluorescent measured (λ_exc = 485 nm, λ_em = 538 nm). For each bacteria and ganglioside/sialic acid, assay was performed in 12 replicates divided in 2 independent experiments. A 1-way ANOVA (P < 0.05) was done to evaluate differences in the adhesion inhibition between control and treatments.

Results: The inhibition of the adhesion are (expressed as the percentage of inhibition respect to control).
Conclusion: Inhibition rate depends on the pathogen and ganglioside, being the effect always higher with sialic acid than with gangliosides. GM3, GD3, GM1, and Neu5Ac at the concentration found in the bioaccessible fraction of commercial infant formula are able to diminish the adhesion of the pathogens evaluated.

References:

Disclosure of Interest: None declared.

PO-G-0088

Gut Infection

BENEFICIAL EFFECT OF SPECIFIC PECTIN-DERIVED ACIDIC OLIGOSACCHARIDES ON SALMONELLA ENTERITIDIS INFECTION IN MICE
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Objectives and Study: One of the main functions of acidic oligosaccharides in human milk is to act as anti-infective agent by inhibiting adhesion of pathogens throughout the gastrointestinal tract. In this study it was examined whether dietary intervention with different concentrations of specific pectin-derived acidic oligosaccharides (pAOS) can prevent or reduce the infectious symptoms in an in vivo Salmonella enteritidis infection model.

Methods: Two concentrations of pAOS (1% and 5% w/w) in an AIN-93G-based diet were tested in a Salmonella enteritidis infection model in young male BALB/c mice. Mice (26 per group) were supplemented via the diet with these acidic oligosaccharides during the entire experiment (34 days). After 28 days the mice (20 per group) were challenged with 10⁵ cfu of a virulent S. enteritidis strain by oral gavage and subsequently sacrificed 6 days after infection. An infection control group was also included in this study as positive control, receiving an AIN-93G diet.

Results: Salmonella enteritidis infected mice fed with specific acidic oligosaccharides showed improved health-related behavior in a dose-dependent manner. The illness score, food intake post-infection and infection-related weight loss were all significantly ameliorated in the pAOS supplemented mice. An increase of cecum content (dose-dependent) may indicate that the acidic oligosaccharides are mainly fermented in the cecum. No relevant differences of pAOS were seen in shedding of Salmonella in their feces. Translocation of Salmonella to liver (P = 0.07) and spleen (P = 0.03) was reduced in the 5% pAOS group. Especially the incidence of organs affected by Salmonella translocation was strongly reduced (to all organs tested: P < 0.001). For the low dose pAOS only a tendency was seen towards reduced severity and incidence of translocation. After infection not only the elevation of the pro-inflammatory plasma cytokines, IL-6 and TNF-α were reduced by pAOS feeding, but also the Th1 cytokine IL-12 and the regulatory cytokine IL-10 levels were less elevated.

Conclusion: Specific pectin-derived acidic oligosaccharide supplementation reduces the severity and incidence of severe Salmonella enteritidis infection in mice in a dose-dependent manner. pAOS may therefore at least partly simulate the action of acidic oligosaccharides in human milk on this specific relevant pathogen.


PO-G-0089

Gut Infection

A PROMISING METHOD FOR RAPID DIAGNOSIS OF SALMONELLA ENTEROCOLITIS VIA REAL-TIME PCR ASSAY
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Objectives and Study: Salmonella enterocolitis has been a leading problem of food-borne disease worldwide. Conventional methods for Salmonella strains’ isolation required at least 3 to 5 days depending on the quantity of viable organisms within the fecal specimen. Clinically, developing a more sensitive detecting tool is in demanding. In addition, we also tried to determine the relationship between the quantity of Salmonella’s DNA copies isolated and the severity of the disease.
Methods: Patients admitted into the pediatrics ward with the diagnosis of acute enterocolitis characterized by blood-tinged or mucoid stool were included in this study from September 2007 to December 2008. Rectal swab was performed for real-time polymerase chain reaction (PCR) and culture. Another two specimens of traditional stool cultures were performed as control. Blood culture was done to exclude bacteremia. We also compared the efficacy of molecular detecting method using direct real-time PCR to samples of trypticase soy broth (TSB) pre-enriched culture for 3 hours at 35°C. The sequences for the Salmonella-specific oligonucleotide primers (ttr-6 and ttr-4) and the Salmonella target probe (ttr-5) were designed based on a multiple alignment of the ttrBCA sequences. All the real-time PCR cases were reconfirmed by the conventional PCR assay. McNemar test and Binomial test analysis were used to evaluate the data. A p value of < 0.05 was considered to be significant.

Results: A total of 118 patients’ specimens were collected. The positive rate for bacteria culture, direct real-time PCR assay and pre-enrichment real-time PCR assay were 23.9% (22/92), 26.9% (7/26), and 37.0% (34/92), respectively. Among these cases, 24 of the 92 samples showed conflicting results, in which 6 specimens determined positive bacteria culture but negative real-time PCR assay. Conversely, 18 specimens that tested positive for real-time PCR assay yield negative stool culture. The sensitivity, specificity, positive predictive value, and negative predictive value of the real-time PCR assay versus conventional bacteria culture were 84.6% vs 56.4%, 98.1% vs 100%, 97.1% vs 100% and 89.7% vs 75.7%, respectively. The overall analysis time for realtime PCR assay was less than 6 hours.

Conclusion: This study indicated that molecular detecting of Salmonella enterocolitis by using minimal pre-enrichment real-time PCR assay is highly sensitive, accurate, time-saving and cost-effective. Mass application of this method may allow physician to give appropriate therapy for the patients in the shortest possible time.

Disclosure of Interest: None declared.

PO-G-0091

Gut Infection

PROBIOTIC LACTOBACILLUS PLANTARUM 299V AND LACTOBACILLUS RHAMNOSUS G INDUCE THE SECRETION OF BETA-DEFENSIN-2 DURING HUMAN INTESTINAL ORGAN CULTURE

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Objectives and Study: The probiotic Lactobacillus strains Lactobacillus plantarum 299v (Lp299v) and Lactobacillus rhamnosus GG (LGG) adhere to intestinal epithelial cell lines and increase mucin gene expression and secretion. This has been proposed as a protective anti-infective mechanism for these probiotics. We have previously presented data demonstrating that during in vitro organ culture (IVOC) of human intestinal biopsies, Lp299v and LGG do not significantly adhere to epithelial cells of small intestinal and colonic biopsies and do not induce mucin gene expression, although they do associate with intestinal mucus. Therefore we sought to extend our investigation of potential protective mechanisms by examining the gene expression and secretion of the anti-microbial alpha defensins and beta defensins.

Methods: IVOC co-cultures of duodenal biopsies with Lp299v and LGG were carried out for 6, 8 and 12 hours. Real-time PCR was used to measure expression of the defensin genes hBD-1, hBD-2 and hBD-3. The secretion of hBD-2 into IVOC culture supernatants was measured after 24 hours co-culture using a commercial ELISA kit. Biopsies were taken with fully informed parental consent and Local Ethical Committee approval.

Results: At each time point basal mRNA expression for all defensin genes was detected in duodenal biopsies. The median expression of hBD-2 was increased 5.7-fold (P < 0.05, n = 6) at 8 hours and 4.2-fold at 12 hours during coculture with LGG and 3.1-fold at 8 hours and 2.4-fold at 12 hours with Lp299v. No significant difference in expression was seen for any other gene at each time point. After 24 hours hBD-2 peptide secretion was increased in IVOC supernatants from LGG co-cultures relative to matched uninoculated controls (mean 235 pg/mL vs 45 pg/mL, n = 2).

Conclusion: Although Lp299v and LGG do not directly adhere to the surface of intestinal epithelial cells during IVOC and do not induce mucin gene expression in duodenal biopsies (previous data), we have demonstrated induction of hBD-2 mRNA expression and secretion. This represents a potential anti-infective protective mechanism for these probiotic strains. Future work will examine the secretion of hBD-2 during Lp299v and LGG IVOC with terminal ileal and colonic biopsies and investigate which bacterial ligands induce hBD-2 secretion.

Disclosure of Interest: None declared.

PO-G-0093

Gut Infection

SACCHAROMYCYES BOULARDII INHIBITS THE ENTEROTOXIC AND CYTOTOXIC EFFECTS AND REDUCES THE OXIDATIVE STRESS INDUCED BY ROTAVIRUS IN HUMAN ENTEROCYTES

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Objectives and Study: Saccharomyces boulardii (Sb) is a probiotic yeast used in the treatment of childhood gastroenteritis (AGE). Rotavirus (RV) is the most severe agent of gastroenteritis and induces a sequence of enterotoxic and...
cytotoxic effects in enterocyte human model. The aim of this study was to investigate the effects of Sb culture supernatant (SbS) on ion secretion and cell damage induced by RV in enterocytes and the mechanisms involved.

**Methods:** We used a RV infection model, which consisted in a virus infection of Caco-2 cell monolayers with an early enterotoxic effect and a later cytotoxic damage, electrically measured in Ussing chambers (*J Infect Dis* 2009;200:813). SbS was added to Caco-2 cells before or after RV infection. Epithelial damage was evaluated by the transepithelial resistance (TER) and ion transport by the intensity of short circuit current (Isc). Reactive oxygen species (ROS) and glutathione (GSH) and oxidated (GSSH) forms were assessed using dichlorofluorescein (DCF) and a colorimetric assay respectively. NFkB was evaluated by western blot analysis.

**Results:** The addition of SbS to Caco-2 cells before or after RV infection reduced tissue damage by 82% ± 0.5 and 42% ± 0.3 respectively (P < 0.05) peaking at 96 h post-infection, when RV damage is maximal. SbS added before, but not after, RV infection inhibited ion secretion by 58% ± 0.3 (P < 0.05). RV induced a significant increase in ROS intra-cellular level (223.76 vs 25 ± 19 DCF fluorescence units, P < 0.05) and a reduction of GSH/GSSH ratio compared to controls (0.08 vs 4.48, P < 0.05) indicating that the virus alters the oxidative status and impairs antioxidant defences. SbS counteracted RV-induced oxidative stress, reducing ROS increase by 43% ± 0.2 and restore GSH/GSSH ratio to the control level. Finally, western blot indicated that SbS also reduced RV-activated NFkB inflammatory pathway.

**Conclusion:** Sb counteracts ion secretion induced by RV and restores epithelial integrity. These protective effects are directly exerted on enterocytes and involve intracellular oxidative and inflammatory pathways. These data provide an entirely new intraepithelial pathway for both RV pathogenesis and high efficacy of Sb against childhood diarrhea observed in clinical trials.

**Funding:** The work was partially supported by a grant from Biocodex, Paris, France.


**PO-G-0149**

**Intestinal Motility**

**THE EFFECT OF PROBIOTICS ON SEROTONIN SIGNALLING IN PLASMA AND INTESTINAL GG TISSUE IN PEDIATRIC IRREDIBILE BOWEL SYNDROME**

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**Objectives and Study:** To evaluate serotonergic signalling in plasma and intestinal tissue in children’s IBS, and further to assess therapeutic efficacy of probiotics and effect of probiotics on serotonergic signalling in plasma and intestinal tissue in children’s irritable bowel syndrome (IBS).

**Methods:** In 2 years, 40 children with IBS were randomized to receive probiotics and antidiarrheal/fecal softener as study group, and a single anti-diarrheal/fecal softener as control group over a period of 8 weeks. Twenty age-matched healthy children were enrolled as normal group. Patients are categorized as IBS-Constipation (IBS-C), IBS-Diarrhea (IBS-D), and IBS-Mixed (IBS-M). Blood test and colonoscopy were performed in each patient to measure serotonin signalling in plasma (ELISA method) and colon tissues [immunohistochemical (IHC) staining] before and after the 8-week treatment. Measures of content, release, and reuptake of serotonin were analyzed with these samples; serotonin transporter (SERT) was used to evaluate the release and uptake of serotonin in intestinal tissue.

**Results:** After 8-week treatment, the improvement of abdominal pain was significantly in both groups, while the improvement of abdominal pain was achieved more significantly in study group patients (P < 0.05, paired t test). Significant differences of IHC staining of serotonin and SERT in colon tissues, and serotonin levels in plasma were found between IBS patients and healthy children (P < 0.05, paired t test). The staining of serotonin and SERT in intestinal tissues positively correlated with the severity of abdominal pain. Marked reduction of serotonin staining in intestinal tissues was found in IBS-C patients after treatment, while no significant differences were found in those IBS-D and IBS-M patients. The serotonin level in plasma was significantly higher than in IBS-D (373.95 ± 201.70 ng/mL) than IBS-C (124.10 ± 210.46 ng/mL) and IBS-M patients (270.43 ± 199.73 ng/mL). After 8-week treatment, significant increase of levels of plasma serotonin was found in study group (117.10 ± 120.56 to 346.49 ± 276.12, P < 0.001, Student t test), while no significant differences were found in the control group (220.66 ± 263.91 to 261.60 ± 150.85). Marked increase in the levels of plasma serotonin was found in IBS-C patients after treatment, while no significant differences were found in IBS-D and IBS-M patients.

**Conclusion:** Probiotics reduce abdominal symptoms and influences serotonin signalling in plasma and intestinal tissues in pediatric IBS patients. Change of distribution on serotonin signalling in plasma and intestinal tissues is found in IBS-C patients after probiotics treatment.

**Disclosure of Interest:** None declared.

**PO-G-0150**

**Intestinal Motility**

**CLINICAL MANIFESTATION OF INTUSUSCEPTION BEFORE AND AFTER INTRODUCTION OF AN ORAL ROTAVIRUS VACCINE IN AUSTRIA**

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**Objectives and Study:** New orally administered, live attenuated rotavirus vaccines have been licensed recently in
Europe, and introduced in Austria. During prelicensure studies these vaccines-in contrast to a historic vaccine-showed no increased risk for intussusception. Primary goal of the study was to document the clinical symptoms of children suffering intussusception and to determine the incidence of intussusception in children in Austria prior, during, and after introduction of oral rotavirus vaccine.

**Methods:** During 3 time periods (January 2005 to December 2008) almost all cases of intussusception in Austria were documented and evaluated. Patient and disease characteristics were assessed with a standardized questionnaire based on the case definition for intussusception developed by the Brighton Collaboration.

**Results:** The mean age of the 264 patients with intussusception was 2.8 ± 1.3 years, ratio males: females was 1.65:1. The incidence of intussusception during the pre-vaccination period was 42/100,000 and 28/100,000 during the period of introduction of rotavirus vaccine. Incidence was highest in infants, declining during the 2nd and 3rd year of life. Almost 50% of all cases had a history of coinciding gastroenteritis. 15% and 20% respectively required surgical treatment.

**Conclusion:** This nationwide surveillance of intussusception in Austria showed a wide variation in incidence rates during the observation periods. The association with rotavirus gastroenteritis was rare, no case was found in temporal relationship to oral rotavirus vaccination.

**Disclosure of Interest:** None declared.

PO-G-0151

**Intestinal Motility**

**EFFECT OF AN INFANT FORMULA CONTAINING MILK FAT, ALPHA-LACTALBUMIN, NUCLEOTIDES AND LCPUFA ON STOOL PATTERNS IN INFANTS**

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**Objectives and Study:** Healthy bowel habit is related with decreased risk of colon cancer, as well as reducing constipation and associated symptoms. We compared indicators of bowel habit in infants fed a formula containing milk fat, α-lactalbumin, nucleotides and LCPUFA, and infants fed a control formula without additional ingredients.

**Methods:** A prospective, double-blind, controlled trial randomly assigned healthy, full-term infants (n = 36) to receive exclusively either supplemented (n = 18) or control formula (n = 14) from 12 to 91 days of age. A group of exclusively breast-fed infants (BF) served as reference (n = 34). For 7 days prior to monitoring at 15, 28, 50, and 90 days of age, parents recorded frequency, consistency and color of infants’ stool, frequency of regurgitation and vomiting, as well as flatulence. Infants’ growth parameters were measured and their health assessed.

**Results:** Compared to the control (CF), supplemented fed group (SF) showed higher stool frequency (P < 0.05): 2.3 ± 1.5 vs. 2.1 ± 1.2; 1.4 ± 0.6 vs. 1.3 ± 1.0; 1.1 ± 0.6 vs. 1.5 ± 1.0; 1.4 ± 1.3 vs. 0.9 ± 0.6 at 15, 28, 50 and 90 days of age, respectively. In the first 2 months, the media number of stool per day was significantly higher (P < 0.05) for BF infants but after 3 months, the results of the SF group did not differ significantly (P < 0.05) from BF infants (1.4 ± 1.3 vs. 1.3 ± 1.4, respectively). Stool consistency changed according to age. Among 1 month old infants, 83% of BF infants had watery stools and 49% had soft stools whereas the frequency of watery stools was 8% and 27% for CF and SF respectively and the frequency of soft stools was 31% and 47% for CF and SF groups. At 3 months of age, 41%, 46% and 2% of BF infants had watery, soft and formed stools, respectively whereas 0% of CF group and 36% of SF group had watery stools, 32% of CF group and 52% of SF group had soft stools and 47% of CF group and 40% of SF group had formed stools. Incidence of yellow and green stool was higher (P < 0.05) in BF group compared to CF and SF groups and SF group had lower incidence of brown stool and similar to HM group compared to CF group. Frequency of regurgitation, vomiting and flatulence and growth parameter values were similar for all groups.

**Conclusion:** The data suggest that an infant formula containing milk fat, α-lactalbumin, nucleotides and LCPUFA provides superior bowel habit than a control formula.


PO-G-0152

**Intestinal Motility**

**ATTENTION-DEFICIT/HYPERACTIVITY DISORDER IS FREQUENTLY DIAGNOSED IN CHILDREN WITH FUNCTIONAL CONSTIPATION AND/OR FECAL INCONTINENCE REFERRED FOR TREATMENT BY A MULTIDISCIPLINARY TREATMENT TEAM**

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**Objectives and Study:** Psychosocial co-morbidity affects treatment of children with functional constipation (1),
constipation was made according to the criteria recommended by the European Society of Paediatric Gastroenterology, Hepatology, and Nutrition. Fifteen healthy non-obese prepubertal children without any infection or chronic illness were included as a control group. Constipated patients were divided into 2 groups. Group I consisted of 43 children with functional constipation and group II consisted of 10 children with organic reasons (1 case of colonic atresia, 4 cases with congenital megacolon, 2 cases of intestinal pseudo-obstruction, 3 cases with anorectal malformation). After an overnight fasting, blood samples were drawn from patients and controls. Serum ghrelin levels were analyzed initially in all groups. Ghrelin levels were reanalyzed at the second month of treatment in functional constipated patients. Treatment protocol was the same in all patients, including dietary modification, administration of pediatric enema and lactulose. Serum fasting ghrelin levels were measured using radioimmunoassay method (Ghrelin RIA Kit).

**Results:** The initial median value of serum ghrelin levels in group I, group II and controls were found as 778.25 pg/mL, 1844.76 pg/mL, and 3543.87 pg/mL, respectively. The initial serum ghrelin levels were significantly different between group I, group II and controls (P<0.0001). Serum ghrelin levels were found to be increased at the second month of therapy in group I (P<0.001). Good response to therapy was observed in all children in this group.

**Conclusion:** Serum ghrelin levels might be helpful for discrimination of functional and organic constipation. Normalization of ghrelin levels may also be a good indicator for therapeutic response.

**Disclosure of Interest:** None declared.

**PO-G-0154**

**Intestinal Motility**

**DO STOOL CONSISTENCY AND FREQUENCY CORRELATE WITH TOTAL GASTROINTESTINAL TRANSIT TIME? RESULTS FROM AN ITALIAN STUDY IN CONSTIPATED AND HEALTHY CHILDREN**


**Objectives and Study:** Functional constipation is a very common disorder in childhood and is mainly due to a delayed colonic transit time. Although most patients with functional constipation generally do not require diagnostic tests, radiopaque marker (ROM) study is a validate method still used for measuring total gastrointestinal transit time when testing is required. However, in research and in clinical practice stool consistency and stool frequency are often used as surrogate markers of bowel transit. The aims of our study were to evaluate: the correlation between stool characteristics (consistency and frequency) and gut transit in children;

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**PO-G-0153**

**Intestinal Motility**

**GHRELIN IN CONSTIPATED CHILDREN**

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**Objectives and Study:** Ghrelin is a hormone released from the gastrointestinal tract that is known to have a motilin-like effect. The purpose of this study is to investigate the possible role of ghrelin in the pathophysiology of constipation in childhood.

**Methods:** Newly diagnosed 53 constipated children aged between 1 and 6 years old were included in the study [24 boys (45.28%) and 29 girls (54.71%)]. The diagnosis of functional constipation was made according to the criteria recommended by the European Society of Paediatric Gastroenterology, Hepatology, and Nutrition. Fifteen healthy non-obese prepubertal children without any infection or chronic illness were included as a control group. Constipated patients were divided into 2 groups. Group I consisted of 43 children with functional constipation and group II consisted of 10 children with organic reasons (1 case of colonic atresia, 4 cases with congenital megacolon, 2 cases of intestinal pseudo-obstruction, 3 cases with anorectal malformation). After an overnight fasting, blood samples were drawn from patients and controls. Serum ghrelin levels were analyzed initially in all groups. Ghrelin levels were reanalyzed at the second month of treatment in functional constipated patients. Treatment protocol was the same in all patients, including dietary modification, administration of pediatric enema and lactulose. Serum fasting ghrelin levels were measured using radioimmunoassay method (Ghrelin RIA Kit).
whether Bristol Stool Form Scale (BSFS) may be considered as a reliable method of assessing intestinal transit rate also in children.

**Methods:** We recruited 50 children (M/F: 22/28; mean age: 8.2 years; range: 4–12 years) referred to our general pediatric outpatient Department from March 2010 to November 2010. Thirty-two (64%) subjects were affected by functional constipation according to the Rome III criteria. All participants were required to maintain a 1-week stool diary, recording the time and the date of every bowel movement and the stool form, through the use of BSFS. Whole gut transit time was then assessed in every child, by ROM test. During the study period, the use of fecal softeners was not allowed. Spearman’s rank correlation was used to establish a correlation between stool consistency, bowel frequency and transit time.

**Results:** Our data report a significant correlation between stool consistency and whole-gut transit time measured by ROM in our study population (r = 0.4, P < 0.001). We did not find any correlation between bowel frequency and gut transit time, nor between stool consistency and bowel frequency. Children with constipation demonstrated an average BSFS score of 2.3 compared with a score of 3.7 for healthy children.

**Conclusion:** Stool consistency is able to differentiate a normal from a pathologic intestinal transit time in children. In addition, we found that stool frequency is a poor surrogate for transit, even in patients with reduced stool frequency. Our results demonstrate the role of BSFS as a simple and reliable method to assess intestinal transit rate in children.

**Disclosure of Interest:** None declared.

**PO-G-0155**

**Intestinal Motility**

**EARLY-LIFE RISK FACTORS FOR FUNCTIONAL CONSTIPATION: PRELIMINARY RESULTS OF AN ITALIAN MULTICENTRE PROSPECTIVE STUDY**

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**Objectives and Study:** This multicentre prospective study sought to assess the incidence of FC and to establish possible risk factors associated to this condition during the first year of life.

**Methods:** The study was conducted from 1 June to 30 September 2009. Parents of 600 consecutive healthy newborns, with a weight appropriated to gestational age, were invited to participate in the study. Four hundred sixty-five newborns completed the study. At the child’s age of 3, 6 and 12 months, parents were contacted by telephone to complete a questionnaire on FC defined according to the Rome III criteria. Breast-feeding, drugs (acetaminophen, anti-inflammatory drugs, corticosteroids, antibiotics and antiemetics), intake of vitamins and food supplements, family history of functional gastrointestinal disorders (FGIDs) and atopy, weaning and nursery-school age, episodes of fever within 2 weeks before the onset of FC and socio-demographic factors were screened.

**Results:** Our preliminary data show that out of 465 infants, 54 (11.6%) and 64 (14.1%) presented FC respectively at 3 and 6 months after birth. Family history of atopy was a significant risk factor for the development of FC both at 3 and 6 months of life (P = 0.04 and P = 0.02 respectively). Breast-feeding was significantly related to a normal evacuation pattern at 3 months (P = 0.05), while it resulted to have no influence at 6 months of age (P = 0.12). Acetaminophen didn’t result to be a risk factor for the onset of FC at 3 months of age (P = 0.13), but at 6 months of life we found a trend toward the significance for the use of this drug in FC infants compared with no FC infants (P = 0.06). After adjustment for all analyzed variables, FC in infants was significantly associated with family history of atopy at 3 and 6 months (OR: 2.11; 95% confidence interval (CI): 1.15–3.89). None of the other analyzed variables resulted to be associated with the onset of FC.

**Conclusion:** These preliminary results show that a family history of atopy is associated with the development of FC. Our results confirmed that breast-feeding is a protective factor for FC in the first 3 months of life. In addition, we found that the use of acetaminophen could be partially involved in FC onset in infants; however further investigation is necessary to explain this association trend and the possible correlation with drug formulation.

**Disclosure of Interest:** None declared.

**PO-G-0158**

**Miscellaneous Pathologies**

**PROTEIN-LOSEING ENTEROPATHY IN A PAEDIATRIC POPULATION: ITS SPECTRUM OF PRESENTATION, DIAGNOSTIC WORK-UP, AND YIELD**

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**Objectives and Study:** Protein-losing enteropathy (PLE) presents in many disorders of the various specialities, mainly gastrointestinal, immunology, cardiology and dermatology. The diagnosis is made by an elevated faecal alpha 1-antitrypsin (A1AT) > 0.48 mg/g stool. However when the underlying condition is not obvious, determining the etiology can be challenging. The aim of this study is to review the spectrum of PLE, its presentation and investigations done,
and hence propose a staged approach to the diagnostic workup of PLE.

**Methods:** We retrospectively reviewed the cases of elevated faecal A1AT over a 10-year period. There were 101 patients, mean age of 45 months (range 14 days-218 months). Their disease presentation, laboratory results, histopathological and radiological findings were identified.

**Results:** The majority had a primary gastrointestinal disorder (62/101), followed by immunological disorder (16/101) and cardiac condition (10/101). Among the gastrointestinal diagnoses, the common causes were eosinophilic/food allergic enteropathy (n = 15), intestinal lymphangiectasia (n = 12), congenital enteropathy (n = 11), gut inflammation (n = 8), post-infectious (n = 5) and autoimmune enteropathy (n = 4). The most common presenting complaint was diarrhea (69/101), followed by failure to thrive (50/101) and vomiting (40/101). All patients had a stool culture to exclude infectious causes, although only 1 was positive for a bacterial infection (diagnostic yield of 1.2%). 77 patients had immunoglobulin E testing for possible food allergy, which was elevated in 31 patients (40%). Immune deficiency workup was done in 41 patients, with a primary immunological disorder found in 16 patients (39%). Barium studies were performed in 16 patients, with abnormal findings in only 3 (19%). 65 patients underwent endoscopy, with a good yield of 92% showing abnormalities in the biopsies. 6 patients had video capsule endoscopy (VCE), all (100%) with abnormalities seen in the small intestine such as intestinal lymphangiectasia and gastrointestinal inflammation.

**Conclusion:** To our knowledge, this is the largest series of PLE reviewed so far. Based on our spectrum of PLE and diagnostic yields of the individual investigations, we propose a staged approach in the diagnostic workup of PLE, with basic tests such as stool investigations, immunoglobulin levels and immunodeficiency workup in the first phase, followed by more advanced investigations in the second phase. We also find that barium studies give a lower yield of diagnostic imaging and we recommend the combined use of endoscopy and VCE instead with the additional benefit of guidance to treatment options such as surgery for localized intestinal lymphangiectasia.

**Disclosure of Interest:** None declared.

**PO-G-0159**

**Miscellaneous Pathologies**

**GASTROENTEROLOGICAL PROBLEMS IN CONGENITAL MYOTONIC DYSTROPHY (CDM)**

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**Objectives and Study:** Congenital myotonic dystrophy (CDM) is an autosomal dominant condition with variable phenotype. Literature on its gastrointestinal manifestations at birth and infancy is extremely limited. We report the gastrointestinal manifestations on 4 patients.

**Methods:** We identified 4 patients with CDM who presented with gastrointestinal manifestations during the neonatal period. The management of gastroenterological problems was documented till the age of 1 year.

**Results:** Gestational age ranged from 33 weeks to 40 weeks at birth and all 4 patients were females. There was polyhydramnios in 3 pregnancies. Delivery was by emergency caesarean section in 3 patients due to reduced fetal movements. Enteral feeding was started between day 2-4 of life and all of them had abdominal distension, bilious aspirates or bilious vomiting. Two of the patients had a trial of prokinetic medications and hydrolysed feeds without any improvement in enteral food tolerance. Three patients had a barium follow-through study and enema and 2 of them showed slow small bowel motility. 1 of them showed thickened pylorus, ileal atresia and microcolon and 1 showed subtotal small bowel obstruction and microcolon. One patient had an ileostomy and a feeding jejunostomy and one had an ileostomy and gastrostomy after which there was improved tolerance to enteral feeds. The third patient gradually had improved food tolerance to nasogastric feeding. The fourth patient died from overwhelming sepsis at 15 weeks of age. Three patients were on parenteral nutrition (PN) from week 1 of life and full enteral feeding was established between 22 and 39 weeks of age. Meconium was passed between day 2-3 of age in all 4 patients and all patients required glycerine suppositories to open bowels every 4-5 days. Rectal biopsy in all 4 patients was normal. PN-related cholestasis was noted in all 4 patients by 3-6 weeks of age. In 2 patients the PN-related cholestasis completely resolved after PN was stopped however one patient continued to have significant liver disease at 1 year of age. Oral feeding has not been possible due to unsafe swallow in the surviving patients. All 4 patients had multiple septic episodes.

**Conclusion:** The symptoms were attributed to the well reported abnormal motility of gastrointestinal tract. However investigations showed additional causes like a thickened pylorus, microcolon and ileal atresia. All patients required nutritional support with PN but developed PN related cholestasis early in life. It is unclear whether this is peculiar to CDM or secondary to recurrent infections. With optimal management and early establishment of enteral feeding this would resolve in time. Clinicians should look for upper and lower gastrointestinal symptoms in all patients with CDM.

**Disclosure of Interest:** None declared.

**PO-G-0160**

**Miscellaneous Pathologies**

**DETECTION OF SMALL BOWEL ENZYMES IN COLON SPECIMENS OF PATIENTS WITH SHORT BOWEL SYNDROME**

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Objectives and Study: A successful management of SBS patients essentially depends on the extent of intestinal adaptation, the possibility of enteral feeding and finally weaning of parenteral nutrition. In animal models, disaccharidases were detectable in the colon of SBS indicating the intestinalization of the colon and its potential to take over digestive functions. In the present study, we investigated enzymes of the small bowel within the colon of SBS patients and compared their duodenal and colonic levels with healthy and sick controls. Duodenal and colon biopsies from nine SBS patients and nine control patients were investigated.

Methods: Immuno-electron microscopy (IEM) was used to localize and quantify the enzymes aminopeptidase N (AP), lactase (L) and sucrase-isomaltase (SI) in the brush border membrane. Quantification was performed by counting labeling density of gold particles per μm membrane length of the brush border (gp/μm) and numbers of positively marked enterocytes. In addition, enzymatic activities of lactase, SI and maltase-glucosaminase were assessed.

Results: Respective values for the colon varied considerably between the SBS patients and the different enzymes. Most of the SBS patients showed a significant enzyme expression and activity in colon specimens. In the colon of SBS patients 71.3% of enterocytes were positive for AP, 29.6% for SI, and 3.0% for L in contrast to 16.9% of control enterocytes for AP, 0.0% for SI, and 0.4% for L (values are median).Labeling density of the brush border was 18.4 for AP, 3.4 for SI, and 0.2 for L in the colon specimens of SBS patients in contrast to 1.9 for AP, 0.0 for SI, and 0.0 for L of control enterocytes (values in gp/μm). Compared to the control patients the amount of enzymes in the duodenum of SBS patients was increased by a factor of up to 3. AP in SBS colon specimens (median 18.4 gp/μm) was stronger as that in duodenum of control patients (median 10.6 gp/μm).

Conclusion: Our results demonstrate the presence of small bowel enzymes in the colon of SBS patients and point to a digestive function of the large bowel as part of the intestinal adaptation process in SBS, which could be used more systematically in the therapy of SBS.

Disclosure of Interest: None declared.

PO-G-0161

Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori

ESOPHAGAL ATRESIA AND TRACHEOESOPHAGEAL FISTULA: OUTCOMES, SHORT- AND LONG-TERM COMPLICATIONS

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Objectives and Study: Esophageal atresia and tracheoesophageal fistula (EA/TEF) are the most common congenital anomalies of the esophagus, affecting 1 in 2400–4500 neonates. Children with EA/TEF suffer from complications related to the anomaly; the various repair operations and associated anomalies. We aimed to describe an up to date portrayal of the outcomes, natural history and rate of the various complications that follow EA/TEF repair operations in children.

Methods: A retrospective analysis of children who were operated for EA/TEF at Tel Aviv Medical Center between the years 1999–2008. Data was recorded from their medical records and by parental phone call questionnaire.

Results: There were 87 children in the cohort; male to female ratio was (1.4:1). Mean follow up time was 5.6 ± 3.1 years (range 1–12 years). The most common type of EA/TEF was type C (83% of the children, Gross classification), primary anastomosis was the most common repair operation (81.2%), followed by delayed repair (17.5%) and gastric pull up (1.2%). Ten percent of the children were in need for a secondary repair. Sixty percent had additional anomalies. The incidence of immediate complications was 31%, the most common of was anastomotic leak (18.9%). The total incidence of long-term morbidity was 94.6%. The most common were recurrent pneumonias (79%), gastroesophageal reflux (69%) and esophageal strictures (54.5%). More than half of our patients were diagnosed with developmental delay. The weight and height percentiles at the ages of 1 and 3 years old were significantly lower in the study group than those of the normal population (according to the WHO growth standards).

Table 1. Immediate Complications Following AE/TEF Repair Operations (n = 87)

<table>
<thead>
<tr>
<th>Complication</th>
<th>Incidence, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anastomotic leak</td>
<td>18.9</td>
</tr>
<tr>
<td>Sepsis and mediastinitis</td>
<td>9.4</td>
</tr>
<tr>
<td>Vocal cord paralysis</td>
<td>3.0</td>
</tr>
<tr>
<td>Chylothorax</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Conclusion: Despite the great improvement in the immediate outcomes of the EA/TEF repair operations, these children suffer from a significant morbidity in the first years of life which requires close follow-up by a multidisciplinary team.

Disclosure of Interest: None declared.

PO-G-0163

Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori

SEQUENTIAL VERSUS CONCOMITANT THERAPY FOR HELICOBACTER PYLORI INFECTION IN CHILDREN: A PILOT STUDY

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**Objectives and Study:** Helicobacter pylori eradication rates with standard triple therapy, which originally achieved 90% eradication, are now being observed to be consistently lower than 70–80%, requiring therefore a search for novel therapeutic approaches. In few pediatric controlled trials, sequential therapy (ST) with a proton pump inhibitor (PPI) and amoxicillin for the first 5 days followed by a PPI, clarithromycin, and an imidazole agent for a further 5 days have been reported to have a better rate of eradicating H. pylori infection than PPI, amoxicillin, and clarithromycin triple therapy. Though the concomitant administration of these 4 drugs for the entire 5-day duration of therapy has also been proposed in the hopes of reducing the complexity associated with ST and enhancing adherence to therapy, no efficacy data are yet available on the concomitant therapy in children.

**Methods:** Thirty consecutive children with H. pylori infection were randomized to receive either ST (omeprazole plus amoxicillin for 5 days, followed by omeprazole plus clarithromycin plus tinidazole for another 5 days) (n = 15; 7 boys [46.6%]; median age, 7.6 years [range, 4.8–14.1 years]) or concomitant therapy (omeprazole, amoxicillin, clarithromycin, and tinidazole) for 5 days (n = 15; 6 boys [40%]; median age, 11 years [range, 5.8–16.7 years]). H. pylori infection was based on 2 out of 3 positive tests results: 13C-urea breath test, rapid urease test, and histologic analysis. Eradication was assessed by 13C-urea breath test 8 weeks after therapy.

**Results:** All patients completed the study. H. pylori eradication was achieved in 13 children receiving sequential treatment (86.6%; 95% confidence interval, 62.1 to 96.3) and 14 children receiving concomitant therapy (93.3%; 95% confidence interval, 70.2 to 98.8) (P = NS). Compliance with therapy was good (>95%) in all.

**Conclusion:** Our pilot study shows, for the first time in children, that concomitant therapy for 5 days achieves a high eradication rate, similar to ST. Sequential and also concomitant therapy are promising treatment approach that deserves consideration as a treatment strategy for H. pylori infection. However, further assessment across a much broader range of children is required before sequential and also concomitant therapy could supplant existing treatment regimens and be generally recommended in clinical practice.

**References:**

**Disclosure of Interest:** None declared.

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**PO-G-0164**

Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori

**BASELINE ESOPHAGEAL IMPEDANCE DIFFERS ACCORDING TO AGE**

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**Objectives and Study:** Several studies evaluate the baseline impedance (BImp) in the distal esophagus. If the BImp of different studies are combined, infants have a smaller BImp than adults (1750 Ω (range: 1500–2050 Ω) vs 4342 Ω (range: 3838–4889 Ω)). Therefore, we measured the BImp in the proximal and distal esophagus in children according to age.

**Methods:** We evaluated 81 multichannel intraluminal impedance/pH metries (MII/pH) recordings performed in children (mean age ± SD: 48.2 ± 53.9 months; range 1–203 months) to assess the variation of the BImp during the 24 h recording and according to age (3 groups: group 1: 1–12 months; group 2: 13–84 months; group 3: >85 months). We calculated the mean esophageal BImp every 4 hours during the 24 h tracing during 1 min without reflux (acid, non-acid) and gas episodes, in channels 1, 2, 5 and 6. The results were evaluated with one-way ANOVA test and P < 0.05 was considered statistically significant.

**Results:** The results show an increase of BImp in all the channels in relation to age: the older the children, the higher the BImp (Table 1).

**Table 1.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Channel 1</th>
<th>Channel 2</th>
<th>Channel 5</th>
<th>Channel 6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (Range)</td>
<td>Mean (Range)</td>
<td>Mean (Range)</td>
<td>Mean (Range)</td>
</tr>
<tr>
<td>1</td>
<td>2044</td>
<td>2008</td>
<td>2195</td>
<td>2226</td>
</tr>
<tr>
<td>2</td>
<td>2440</td>
<td>2233</td>
<td>2171</td>
<td>2338</td>
</tr>
<tr>
<td>3</td>
<td>2941</td>
<td>2464</td>
<td>2412</td>
<td>2614</td>
</tr>
</tbody>
</table>

We calculated the 24-h mean BImp of proximal and distal esophagus. All data were evaluated with one-way ANOVA test (P < 0.0001 according to the age). No circadian rhythm was found in mean BImp in proximal and distal esophagus.

**Conclusion:** The evaluation of the BImp shows an increase according to age. This result could be explained by the fact that the esophagus diameter increases with increasing age, allowing more air around the probe in the older children, considering that the MII/pH probes have the same diameter in infants, in children and in adults.

**Disclosure of Interest:** None declared.

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**PO-G-0166**

Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori

**THE CHARACTERISTIC OF GASTROESOPHAGEAL REFLUX IN CHILDREN WITH OTITIS MEDIA USING MULTICHANNEL IMPEDANCE (MII) COMBINED WITH DUAL PH-METRY**

Objectives and Study: The aim of the study was to characterize the laryngopharyngeal (LPR) and gastro-esophageal reflux (GER) in children with otitis media with effusion (OME).

Methods: A prospective study of 21 children with otitis media with effusion. The diagnosis of OME was based on otomicroscopic examination, tympanometry and audiometry. All patients underwent multichannel channel impedance combined with dual pH-metry. Proximal pH probe was positioned 1 cm above upper esophageal sphincter; distal pH was measured 3–5 cm above lower esophageal sphincter. Pathologic GER was defined as at least one abnormal result in either distal pH metry or MII and LPR was defined as at least one episode of reflux in proximal channel of MII combined with pH drop < 5.0 in the proximal pH probe.

Results: The mean age of the patients was 7 years (range 6–10) and there were 15 males and 6 females. 7 patients had unilateral OME, 14 had bilateral OME. Mean cochlear reserve was 16.1 in right ear and 16.2 in left ear. There were 2 patients with tympanograms type C and 19 with type B. GER was diagnosed in 14 patients, although total fraction time (fT) in distal pH probe was >4.2% only in 5 patients. LPR was found in 16 patients. Mean number of LPR episodes amounted to 3.6 (range 1–13). Median duration of the longest LPR episode was 24.6 s (range 1.2–1080). Among all LPR episodes 6 were gas, 32 liquid and 20 mixed.

Conclusion: Most children with OME have pathologic LPR as well as pathologic GER. LPR can be an important risk factor of OME.

Disclosure of Interest: None declared.

PO-G-0170

Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori

COW’S-MILK CHALLENGE INCREASES WEAKLY ACIDIC REFLUX IN CHILDREN WITH COW’S-MILK ALLERGY AND GERD

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Objectives and Study: Cow’s-milk allergy (CMA) and gastroesophageal reflux disease (GERD) commonly occur in childhood. The prevalence of CMA in infants with GERD has been reported to be as high as 15%–42% although such an association has not been proven in a scientifically robust manner. The association between CMA and GERD has been examined methodically using esophageal pH-monitoring that failed to detect reflux especially when little or no acid is present in the refluxate. Multichannel intraluminal impedance and pH (MII-pH) monitoring detects both acid and non-acid reflux into the esophagus. We prospectively assessed the reflux pattern in a selected population of infants with CMA.

Methods: Fourteen children (median age 14 months; range 3 months–2 y) with a proven diagnosis of CMA and suspected GERD were enrolled into the study. All patients underwent upper endoscopy followed by 48 h MII-pH monitoring. During the first 24 h the infants were kept on neocate (N) and during the following 24 h were challenged with CM. The following variables were analyzed: 1. total number of reflux episodes; 2. number of acid reflux episodes (AR)(pH<4.0);
3. number of weakly acidic reflux episodes (WAc)(pH 4–7);  
4. number of weakly alkaline reflux episodes (Walk)(p>7.0);  
4. height of reflux episodes, defined as proximal, intermediate, and distal;  
5. acid reflux index (ARI);  
6. number of reflux episodes lasting >5 min per 24 hrs (R>5 min).  

**Results:** The mean number of total reflux episodes was significantly higher during CM than during Neo (78 ± 37 vs 47 ± 30.5, P < 0.001) feeding. Similarly, the mean number of WAc was significantly higher during CM administration than during N (49.1 ± 17.5 vs 20.5 ± 13.6, P < 0.001), whereas no significant difference was observed for AR (28.2 ± 23.2 vs 24.5 ± 23.7, NS) and Walk (0.6 ± 1.1 vs 2 ± 4.8 NS). The mean number of WAc reaching the proximal oesophagus was significantly higher during CM challenge (43.5 ± 16.4 vs 17.9 ± 13.3 P < 0.001), whereas between the 2 periods no difference was found in the height reached by the AR (21.5 ± 16.4 vs 20 ± 20.5, NS) and Walk (0.2 ± 0.4 vs 0.4 ± 1. NS). Finally, no significant difference between the 2 periods was found in both aRI (CM 5.8 ± 6.6, N 3 ± 2.8, NS) and R>5 min (CM 3.5 ± 5.3, N 2.5 ± 3.6, NS).

**Conclusion:** In children with CMA and GERD, CM increases the number of weakly acidic reflux episodes reaching the proximal esophagus. CM challenge during 48 h MII-pH monitoring increases the yield in identifying this subgroup of patients. Based on our results it should become part of the routine diagnostic work-up for children with CMA in whom GERD is suspected.

**Disclosure of Interest:** None declared.

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**PO-G-0172**

*Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori: Antimicrobial Resistance of* *Helicobacter pylori* To Clarithromycin and Metronidazole in a Tertiary Hospital in the Netherlands*

**P. Mourad-Baars**1, **K. E. Veldkamp**2, **H. Wunderink**3, **L. Mearin**4. 1Dept Of Pediatrics, 2Microbiology, Leiden University Medical Centre, Leiden, Netherlands.

**Objectives and Study:** Antimicrobial resistance of *Helicobacter pylori* (Hp) is increasing worldwide and is one of the main reasons for eradication failure. Data are known on the resistance to clarithromycin and metronidazole between 1993 and 2003 in adults in The Netherlands. Since 2003 no further data on resistance have been published. So far, data on resistance in Dutch children were not available. As failure of first eradication treatment diminishes significantly eradication success in the future, we investigated the prevalence of resistance of Hp in both children and adults to the most commonly used antibiotic components of triple therapy regimen: clarithromycin and metronidazole.

**Methods:** Single-centre retrospective database study from January 2000 to January 2010. All patients undergoing an upper endoscopy with Hp-positive culture from the antral and/or corpal biopsies confirmed with gram stain and positive oxidase, catalase and urease tests were included. Antimicrobial susceptibility of the Hp-positive strains was determined by E-test with cutoff values for clarithromycin at ≤0.25 mg/L = sensitive and > 0.25 mg/L = resistant and for metronidazole at ≤8 = sensitive, > 8 and ≤16/16 mg/L = intermediate and > 16 mg/L = resistant. Results were compared to data from literature on Dutch adults and to data from a European multicentre study of children in Europe, in which no Dutch children were included (Koletzko et al 2006).

**Results:** 1144 cultures from 1092 adults and 78 cultures from 77 children were included. Resistance prevalence of Hp to clarithromycin in adults was 10.2% and in children 7.2%. Resistance to metronidazole in adults was 22.2%, in children 11.8%. In earlier studies in the Netherlands the prevalence of
resistance in adults to clarithromycin was less than 5% and to metronidazole 7–33%. Reported primary resistance prevalence of Hp in children living in western Europe is 22% for clarithromycin and 32% for metronidazole.

**Conclusion:** Resistance prevalences of Hp to clarithromycin and metronidazole in Dutch children are relatively low compared to the rest of western Europe, possibly due to the relatively low prescription rates of antibiotics in the Netherlands. The prevalence of resistance of Hp to clarithromycin in adults is rising, while the prevalence of resistance to metronidazole seems to be stable. In our study clarithromycin resistance in adults is higher than in children. Based on these results we conclude that regular surveillance of local Hp resistance is recommended in order to determine the success of the empirical eradication treatments.

**References:**

**Disclosure of Interest:** None declared.

**PO-G-0174**

**Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori**

**HELICOBACTER PYLORI AS A POSSIBLE CAUSE OF FUNCTIONAL DYSPEPSIA**

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**Objectives and Study:** Functional dyspepsia (FD) is a common, nonspecific clinical manifestation in children and adolescents that may be consequent to numerous disorders of the proximal digestive tract. Helicobacter pylori (H. pylori) infection is mainly acquired in early childhood, being frequently associated with several clinical manifestations and early diagnosis and treatment is recommended to reduce morbidity and the potential for malignancy. However, the role of H. pylori infection recognized as a cause of FD is one of the most debated issues. In fact, the pediatric guidelines do not suggest screening children with dyspeptic symptoms for H. pylori.

Our prospective case-control study evaluated the causal relationship between H. pylori infection and FD in children.

**Methods:** From August 31, 2006 to September 1, 2008, 241 children with FD (diagnosed according to Rome III criteria) (median age 9.8 years, range 4–18; M:F 120:121) and 130 controls, who were non dyspeptic children undergoing diagnostic endoscopy for celiac disease (median age 9.9; range 4–18; M:F 52:78), matched for age and sex, were enrolled. In all patients, gastric biopsies were collected for detection H. pylori infection (histology and culture). Before and 1 year later, a questionnaire (Glasgow Dyspepsia Severity Score) was given in order to assess the severity of dyspepsia and to evaluate the possible relationship between FD and H. pylori.

**Results:** H. pylori infection was detected in 41 (17%) children with FD (median age 10.7; range 5–18; M:F 22:19) and 9 (6.9%) controls (median age 12.6; range 4–18; M:F 4:5) ($P = 0.02$). The mean dyspepsia score was 11.14 (range 6–16) before eradication and 3.73 (range 0–12) after eradication ($P < 0.01$).

**Conclusion:** Our study confirmed that the overall prevalence of H. pylori infection in children of developed countries, such as Italy, is low. However, the infection rate was significantly higher in children with FD than in controls. Furthermore, we found that the H. pylori eradication relieved symptoms of FD and gave a significant benefit. In conclusion, concomitantly with other possible causes, an association between FD and H. pylori infection in childhood should be examined and investigated by a larger multicenter study.

**Disclosure of Interest:** None declared.

**PO-G-0175**

**Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori**

**I-GERQ-R SCORE, IMPEDANCE AND PH-MONITORING RESULTS: ANY CORRELATION?**

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**Objectives and Study:** Infection by Helicobacter pylori is a worldwide disease, sometimes of difficult eradication attributed to different strains; different antibiotics, proton pump inhibitors, bismuth subcitrate, probiotics must be tried with irregular success. We have studied if dogs, in close cohabitation with the dog. After a 12 h fast a C13 urea breath test was done. The dog breath was caught in plastic bags and immediately put in other bag to be connected to an Iris 1Gastroenterology and Nutrition Unit, Via Augusta, Barcelona, Spain.

**Methods:** Fifteen dogs were studied whose owners were children or adults affected by H pylori infection on a close cohabitation with the dog. After a 12 h fast a C13 urea breath test was done. The dog breath was caught in plastic bags and immediately put in other bag to be connected to an Iris Bremen Analyser. The results were pathologic if the $P$ after eradication (11.14 (range 6–16) before eradication and 3.73 (range 0–12) after eradication ($P < 0.01$).

**Conclusion:** Our study confirmed that the overall prevalence of H. pylori infection in children of developed countries, such as Italy, is low. However, the infection rate was significantly higher in children with FD than in controls. Furthermore, we found that the H. pylori eradication relieved symptoms of FD and gave a significant benefit. In conclusion, concomitantly with other possible causes, an association between FD and H. pylori infection in childhood should be examined and investigated by a larger multicenter study.

**Disclosure of Interest:** None declared.
OBJECTIVES AND STUDY: Diagnosis of gastroesophageal reflux disease (GERD) is challenging in infants. No symptom is specific and the only validated questionnaire is a revised version of I-GERQ (I-GERQ-R). The aim of the study was to assess the correlation among the pathological I-GERQ-R score, acid reflux index (RI) and (total) bolus exposure index (BEI).

METHODS: Consecutive infants (range 0–18 months) referred for suspected GERD with I-GERQ-R assessment the same day of pH-monitoring or esophageal impedance (MII-pH) were enrolled. As previously reported a score of ≥16 was considered as positive I-GERQ-R (I-GERQ-R pos) and <16 as negative (I-GERQ-R neg). Patients with gastrointestinal malformations, neurological diseases or with tracing artefacts were excluded from the study. Different RI and BEI cut-off values and 4 age-groups (0–1, 1–6, 6–12 and 12–18 months) were considered in the analysis. Chi-square was used for statistical analysis.

RESULTS: 88 infants (median age 3 months) were included in the study. All underwent I-GERQ-R assessment, 37 pH-monitoring and 51 MII-pH. 22 patients were on acid inhibitors (11 in I-GERQ-R pos and 11 in I-GERQ-R neg group) during the investigations. 48 infants (31 with MII-pH) had a I-GERQ-R score ≥16. In this I-GERQ-R pos group the RI was 3% in 26 (54%), >5% in 21 (44%), >7% in 18 (38%) and >10% in 12 (25%). The BEI was 1% in 27 (87%), >1.5% in 22 (71%) and >2% in 13 (42%). In 40 infants (20 with a MII-pH) the I-GERQ-R score was <16. In this I-GERQ-R neg group the RI was >3% in 16 (40%), >5% in 11 (28%), >7% in 6 (15%) and >10% in 4 (10%). The BEI was 1% in 13 (65%), >1.5% in 12 (60%) and >2% in 11 (55%). No significant difference was found between RI and BEI percentages between the 2 I-GERQ-R groups, between patients on and off treatment and among different age groups.

CONCLUSION: In our population the I-GERQ-R cutoff score did not discriminate infants with normal and pathological acid exposure index. No correlation was found between pathological I-GERQ-R score and BEI.

REFERENCES:

DISCLOSURE OF INTEREST: None declared.

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PO-G-0176

Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori

INFLUENCE OF HELICOBACTER PYLORI INFECTION ON QUALITY OF LIFE AND SYMPTOM SCORES IN CHILDREN WITH ABDOMINAL PAIN AND DYSEPSIS SYMPTOMS

S. Sari1,*, A. Poyraz2, B. Dalgic1,1 Pediatric Gastroenterology, 2 Pathology, Gazi University Faculty of Medicine, Ankara, Turkey.

OBJECTIVES AND STUDY: Dyspepsia is common in children with chronic or recurrent abdominal pain. The relation between Helicobacter pylori infection and abdominal pain and/or dyspeptic symptoms is not clear. We prospectively evaluated the role of H. pylori infection in children with abdominal pain and/or dyspeptic symptoms.

METHODS: Patients who were referred for gastroscopy were evaluated between August 2007 and February 2009. Dyspeptic symptoms and severity of epigastric pain were evaluated by using “The Gastrointestinal Symptoms Rating Scale” and “Wong-Baker Face Scale,” respectively. The Turkish versions of the Kinder Lebensqualität Fragebogen (KINDL) questionnaires were used as a quality of life (QoL) measure. Gastric biopsies were examined for H. pylori by staining of hematoxylin eosin and Wartin-Starry. Modified Sydney classification was used for the evaluation of gastritis. Patients who were positive for H. pylori received triple antibiotic therapy. H. pylori eradication was evaluated by using C13 urea breath test. Patients who had H. pylori infection were re-evaluated for symptoms and QoL at the end of therapy (4 weeks) and 8 weeks after initiation of therapy.

RESULTS: Gastroscopy was offered to 410 children. Forty-one of them denied endoscopy. Two hundred seventy-one patients with duodenal or gastric ulcer, gastroesophageal reflux, giardiasis, duodenogastric reflux, celiac disease, any other chronic disease or history of acid inhibiting drug or antibiotic use were excluded. Ninety-eight children were eligible for the study. Fifty-four of them had H. pylori infection (group 1) and 44 patients had not (group 2). Group 1 had severe histopathological findings than group 2. The symptom and QoL scores were similar between group 1 and 2 at admission. H. pylori was eradicated in 30 (group 1a) and persisted in 24 of 54 children (group 2). Symptom and QoL scores were found to be similar between group 1a and 1b at 8 weeks. Symptom scores were significantly decreased between the first and the last visit in group 1a and 1b (P < 0.05). While both physical and emotional well-being subscale scores significantly increased in group 1a, only physical well-being subscale increased in group 1b at 8 weeks (P < 0.05).

CONCLUSION: Although the patients with H. pylori infection had severe gastritis than noninfected patients, symptom and quality of life scores were found to be similar in both infected and noninfected patients at admission and after H. pylori eradication.

DISCLOSURE OF INTEREST: None declared.

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PO-G-0178

Oesophagus, GDR, Ulcer Disease, and Helicobacter pylori

ERADICATION OF H. PYLORI IMPROVES BODY WEIGHT, HEIGHT GROWTH, AND SERUM ACYLATED GHRELIN LEVELS IN CHILDREN

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2011 ESPGHAN Abstracts
Objectives and Study: Successful eradication of 

H. pylori with noninfected controls after 1-year follow-up. 

There were significant differences among NAFLD and healthy children in serum/plasma total cholesterol-TC (179.32 ± 22.37 vs 163.16 ± 29.31 mg/dL), TG (111.19 ± 27.23 vs 87.72 ± 40.10 mg/dL), LDL-C (116.52 ± 18.10 vs 95.31 ± 30.28 mg/dL), HDL-C (40.94 ± 6.90 vs 47.94 ± 12.08 mg/dL), VLDL-C (21.87 ± 5.13 vs 19.41 ± 21.69 mg/dL), Apo B (0.9116 ± 0.18 vs 0.79 ± 0.18 g/L), Apo AI (1.25 ± 0.28 vs 1.2953 ± 0.40 g/L), GSH (746.823 ± 44.51 vs 776.88 ± 26.40 μmol/mL), GPx (31.048 ± 1.41 vs 32.747 ± 3.60 U/gHb) compared to controls. No difference was found between NAFLD and healthy children serum/plasma for LCAT, Apo AI and Lp(a).

Conclusion: Fatty liver disease is associated with significant disturbances in lipid metabolism when compared to BMI/age/gender matched overweight/obese healthy controls. Glutathione and glutathione peroxidase activity seems to decrease in association with fatty liver disease.

Disclosure of Interest: None declared.

PO-H-0273

Hepatology

FEASIBILITY OF ROUX-EN-Y LOOP ENTEROSCOPY IN CHILDREN WITH LIVER DISEASE USING SINGLE AND DOUBLE BALLOON ENTEROSCOPY

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Objectives and Study: The Roux-en-Y loop (RNY) is used in children to correct biliary obstruction due to biliary atresia (BA) and choledochal malformation. It may be a source of problems postoperatively such as cholangitis due to stenosis or anastomotic bleeding, which can be difficult to diagnose by conventional endoscopic techniques. We now report our experience of using the single (SBE) and double balloon enteroscopes (DBE) in children with liver disease.

Methods: Case 1: A 12-year-old boy (weight 32 kg) represented with jaundice, having undergone portoenterostomy at 25 days of age for BA, clearing his jaundice completely. Laboratory findings showed conjugated jaundice [106 μmol], and an elevated liver enzyme profile (ALP 2366 IU/L, GGT 1106 IU/L, AST 233 IU/L). Liver ultrasound showed features of chronic liver disease but there were no dilated biliary radicals. HIDA scan showed normal...
PO-H-0274

Hepatology

PREVALENCE AND PREDICTIVE FACTORS OF NONALCOHOLIC FATTY LIVER DISEASE IN SEVERELY OBESE ADOLESCENTS: ASSESSMENT USING MAGNETIC RESONANCE SPECTROSCOPY

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Objectives and Study: Limited data are available regarding the exact prevalence of nonalcoholic fatty liver disease (NAFLD) in unselected cohorts of children due to lack of accurate noninvasive diagnostic tools. Proton magnetic resonance spectroscopy (1H-MRS) is a noninvasive tool to detect hepatic fat content and has shown to correlate well with liver biopsy results. Our aim was to prospectively determine the prevalence of NAFLD using 1H-MRS in a cohort of severely obese adolescents and identify clinical parameters related to the prevalence of hepatic steatosis.

Methods: Children with severe obesity (age corrected BMI equivalent >35 kg/m2) admitted to a lifestyle intervention program in a tertiary obesity centre between February 2008 and April 2010 were included. Exclusion criteria were presence of other liver diseases, alcohol abuse and use of steatogenic medication. Clinical evaluation, blood tests and 1H-MRS of the liver were performed before starting the lifestyle intervention. 1H-MRS measurements were performed on a 3.0T Philips Intera scanner. A validation study comparing 1H-MRS measurements in our institution and histopathological assessment of hepatic steatosis has shown an excellent correlation (r = 0.86) and a good sensitivity/specificity for detecting hepatic steatosis (2). Logistic regression analysis was performed to identify clinical parameters related to the presence of hepatic steatosis. All parameters with P < 0.10 in univariate analyses were included in multivariate regression analyses.

Results: A total of 117 children (59% female) were included with a mean age of 14.2 (±1.2) years, BMI ± score 3.34 (±0.35) kg/m² and HOMA-Insulin Resistance index (HOMA) 3.86 (±2.5). None was diabetic. The prevalence of NAFLD measured using 1H-MRS in this cohort was 48%.

In multivariate regression analysis serum ALT (OR 4.8, 95% CI 1.9–12.4, P = 0.001) and HOMA (OR 1.5, 95% CI 1.2–1.8, P = 0.001) were significantly related to the presence of hepatic steatosis. Positive and negative predictive value of ALT>35 U/L were 75% and 65%, respectively, and the positive and negative predictive value of HOMA> 4.0 were 70% and 70%, respectively. BMI-SD score, abdominal circumference, blood pressure, serum lipids serum AST and GGT did not correlate to the degree of hepatic steatosis in multivariate analyses.

Conclusion: NAFLD is common among severely obese adolescents. Serum ALT and HOMA index are the clinical parameters most strongly related to presence of NAFLD, although their predictive value is limited.

Disclosure of Interest: None declared.

PO-H-0275

Hepatology

TAKING PAEDIATRIC LIVER DISEASE INTO ADULTHOOD: INTERVENTIONS FOR YOUNG PEOPLE AND FAMILIES

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Objectives and Study: The adolescent period is characterised by specific biological, psychological and social development tasks resulting in young people feeling invulnerable and engaging in risk taking behavior, eg, nonadherence. Evidence suggests that a combination of educational, behavioural and social support interventions will be most successful in enhancing adherence.1 Our study aims to develop
PO-H-0276

*Hepatology*

**RANGE OF NICU PRACTICE IN ENGLAND AND WALES REGARDING THRESHOLDS FOR NEONATAL CONJUGATED HYPERBILIRUBINÆMIA AND RELEVANT INVESTIGATION**

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**Objectives and Study:** Conjugated jaundice is not an uncommon problem in a neonatal intensive care setting and is generally seen as a reversible complication of prolonged parenteral nutrition. However, several investigations are performed to exclude underlying liver disease. Opinion differs on diagnostic value of such investigations. The aim of the study was to evaluate practice related to investigation of conjugated jaundice in neonatal units across England and Wales.

**Methods:** Questionnaire survey of lead neonatal consultants from all neonatal units in England and Wales. Questions included definition of conjugated jaundice, bilirubin cutoff that prompted investigations and tests performed. Clinicians were also requested to give their opinion on the yield from these investigations.

**Results:** 102/194 neonatal units (52%), responded to the survey of which 33 were level 3 units, 50 level 2 and 19 level 1 units. 96 units (94%) performed conjugated jaundice screen and 6 units (6%) did not. 77 units (75%) had a written policy. 49% of responders defined conjugated jaundice as conjugated bilirubin >20% of total bilirubin and 46% as >15% of total bilirubin and 5% of units did not have a clear definition. Conjugated bilirubin levels that prompted investigations varied between units with 28 (30%) using conjugated bilirubin >20% of total, 33 (36%) a conjugated bilirubin >15% of total and 20 (21%) with no definite threshold. Majority (>76%) of units performed liver and thyroid function tests, Galtosamaemia screen, a-1 antitrypsin and liver ultrasound. In addition to above investigations, 65% of units performed urine culture and hepatitis serology, 32% performed urine organic acids, NH3 and lactate. 19 units performed CF genetics and 23 HIDA scan. 71% of responders (which included 2 out of 3 neonatal units with in-house paediatric hepatology services) thought “diagnostic yield” from these tests were “poor” and 44% based this on their personal view, 29% on local data and 27% on anecdotal evidence.

**Conclusion:** Our study identified a wide variation in definition and investigation of conjugated jaundice in neonates. Most neonatal pediatricians believe yield from these investigations is poor. Further studies are needed to support or refute this view. National consensus guidelines are required to standardize practice.

**Disclosure of Interest:** None declared.

E178
**Objectives and Study:** Various lines of evidence have hitherto suggested that malfunctioning gut-liver axis may contribute to hepatic damage of rodents and humans with nonalcoholic fatty liver disease. Here we re-evaluate the effects of a short-term treatment with *Lactobacillus rhamnosus* strain GG in a group of children affected by obesity-related liver disease who were un-compliant to slimming diets and lifestyle changes.

**Methods:** Twenty obese children (age $10.7 \pm 2.1$ years) with persisting hypertransaminasemia and bright liver were studied. At baseline all patients underwent clinical and laboratory anthropometric evaluation, liver ultrasonography (US) with regions of interest (ROI) ratio computation, standard liver function tests, glucose H2 breath test and serum antibodies to anti-peptidoglycan-polysaccharide (PG-PS) polymers as surrogate markers of small intestinal bacterial overgrowth, and TNFα. After OGTT, and exclusion of possible causes of liver disease, patients received an 8-week probiotic treatment (12 billion CFU/day) in a double-blind, placebo-controlled pilot study.

**Results:** As shown in the Table, ALT and bright liver were confirmed to be the only liver abnormalities. Anti-PG-PS IgA antibodies were elevated in spite of normal H2BTs. A multivariate analysis of studied parameters showed that the significant decrease of ALT (average variation vs. placebo $p = 0.03$) and of anti-PG-PS IgA antibodies (average variation vs. placebo $p = 0.03$) values after probiotic treatment was independent from changes of BMI z score and visceral fat. TNFα, and US bright liver parameters remained fairly stable. The baseline t test (T0 placebo vs T0 probiotic) was not significant for all variables evaluated (ALT, BMI z score, US visceral fat, TNFα, US ROI ratio, anti-PG-PS IgA antibodies).

**Conclusion:** Results of the present pilot study confirm that *L. rhamnosus* strain GG deserves consideration as a therapeutic tool for improving hypertransaminasemia but not bright liver in hepatopathic obese children who are unable to follow slimming diets and/or to change lifestyle. Further studies on a larger scale are therefore warranted.

**Disclosure of Interest:** None declared.

**PO-H-0280**

**Hepatology**

**SERUM FIBROSIS MARKERS IN CHILDREN WITH AUTOIMMUNE HEPATITIS**

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**Objectives and Study:** Liver biopsy is a gold standard for the assessment of staging of liver diseases thus liver biopsy is periodically performed in children with autoimmune hepatitis (AIH). The procedure of liver biopsy is invasive, painful and there is a risk of complication thus alternative methods of liver fibrosis assessment are under investigation. The aim of the study was to measure potential fibrosis markers: hialuronic acid (HA), laminin, MMP-9 and TIMP-1 in the serum of children and adolescents with AIH and to correlate the results distribution with staging of liver disease assessed by liver biopsy.

**Methods:** Blood samples and standard liver biopsies were taken from 29 children (F:21, M:8) aged 5.5–18 (14.5 ± 3.8) years with AIH without HBVand/or HCV co-infection. Serum fibrosis markers: HA, laminin, MMP-9, TIMP-1 were assessed with ELISA technique (HA: Corgenix, laminin: Takara, MMP-9 and TIMP-1: R&D Systems). Batts and Ludwig scoring system was used to determine staging of liver disease. Patients were divided into two groups: group 1-mild fibrosis-staging 0–2; group 2-advanced fibrosis-staging 3–4. The distributions of serum fibrosis markers between 2 groups were compared and Späerman correlation test of fibrosis markers with staging was done. Receiver operating characteristics (ROC) analysis was used to calculate the power of the assays to detect advanced liver fibrosis (AccuROC, Canada).

**Results:** Mild liver fibrosis was present in 23 and advanced fibrosis was found in 6 patients. There were no differences in female to male ratio (17:6 vs 4:2) or age distribution (14.1 ± 4.0 vs 16.3 ± 1.9 years) between group 1 and group 2. Children with advanced fibrosis had significantly higher HA (138 vs 53 ng/mL, $P < 0.02$) and TIMP-1 (352 vs 254 ng/mL, $P < 0.03$) than children with mild fibrosis. No differences in laminin or MMP-9 between both groups were observed. Significant positive correlation was found between staging and HA ($r = 0.53$, $P = 0.0028$) and staging and TIMP-1 ($r = 0.42$, $P = 0.024$), while no correlation between laminin or MMP-9 and staging was observed. Significant ability to differentiate children with advanced fibrosis from those with mild fibrosis was found for HA (AUC=0.7681, $P = 0.02$) and TIMP-1 (AUC=0.7935, $P = 0.015$). HA >85.1 ng/mL had a sensitivity of 83% and a specificity of 87% and TIMP-1 >228 ng/mL had a sensitivity of 83% and specificity of 74%. Laminin and MMP-9 did not allow a useful prediction.

**Conclusion:** HA and TIMP-1 can differentiate children with advanced fibrosis from those with mild fibrosis and thus these noninvasive parameters can be useful to track the progression of the fibrosis in AIH children.

**Disclosure of Interest:** None declared.

**PO-H-0282**

**Hepatology**

**LONG-TERM OUTCOME OF ALPHA1-ANTITRYPSIN DEFICIENCY–RELATED LIVER DISEASE IN CHILDREN: A SINGLE-CENTRE EXPERIENCE**
Objectives and Study: Only about 10% of homozygous alpha-1 antitrypsin deficiency (A1ATD) carriers develop significant liver disease, only a fraction of those will develop end stage liver disease in infancy. Aims of our study was to analyse our large patient cohort and identify risk factors of progressive liver disease.

Methods: All homozygous PiZZ carriers admitted to our centre since 1978 were identified. A retrospective systematic review of all patient’s case notes included family history including smoking, gestational age, maternal age at delivery, date of birth, sex, neonatal history, breast-feeding, symptoms and age at presentation, clinical and laboratory data and date of orthotopic liver transplantation (OLT) and/or death. The statistical analysis was performed using programming language R and SPSS.

Results: Fifty-three patients (age at first visit 5 days-10 years) were identified of whom 36 (70%) presented within the neonatal period with neonatal cholestasis. Sixteen (30%) presented with elevates liver enzymes or chronic liver disease without history of neonatal jaundice. Twenty-two (41.5%, 14 boys, 8 girls) children required OLT due to portal hypertension and/or liver insufficiency. There was no mortality on transplant waiting list, 1 is currently awaiting transplant. Median age at OLT was 5.8 years (range 5 month-15 years). Between 1986 and 2004 eight patients died 0.4 years (9 days-4 years) after OLT because of transplant failure (n=3), PTLD (n=2), infection (n=2) or aortic rupture (n=1). Fourteen patients after OLT and all with native liver are currently alive 1.5–20 years after first presentation. At an early stage of the disease predictors of outcome (OLT vs. non-OLT) were thrombocytes (P<0.001), bilirubin (P<0.001), cholinesterase (P<0.001) and INR (P<0.001). None of other analysed parameters incl. age and symptoms at presentation can divided into patients with good or bad prognosis.

Conclusion: As known only some of PiZZ A1ATD patients develop liver cirrhosis and portal hypertension. Additional factors other than phenotype predisposed a group of PiZZ patients to A1ATD-related liver disease. Some laboratory results can help to predict outcome.

Disclosure of Interest: None declared.

PO-H-0283

Hepatology

PHENOTYPIC VARIATION AND LONG-TERM OUTCOME OF HEPATOBILIARY AND RENAL MANIFESTATIONS IN CHILDREN WITH CONGENITAL HEPATIC FIBROSIS

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Objectives and Study: Congenital hepatic fibrosis (CHF) is a developmental disorder due to defective remodelling of the ductal plate (ductal plate malformation) and is characterised with development of portal hypertension with or without nonobstructive dilatation of intrahepatic bile ducts (Carolii’s syndrome). CHF is usually associated with renal diseases as part of a hepatorenal fibrocystic disease. The lack of studies about the natural history of CHF and the variability in progression makes prognostication difficult. We aim to describe the clinical characteristics and long term outcome of patients with congenital hepatic fibrosis seen at a single centre.

Methods: We conducted a retrospective analysis of children who were diagnosed with CHF at our institution between Jan 1990 and Nov 2009 based on clinical, ultrasonographic, endoscopic and histopathological features. Hepatobiliary complications (varices, GI bleeding, hypersplenism, cholangitis) and renal complications (portal hypertension, chronic renal insufficiency, end-stage renal disease) were recorded at baseline and follow-up. Based on clinical outcome, patients were divided as transplant recommended (Group 1) or not necessary (Group 2).

Results: There were 40 children with 21 males, median age at diagnosis of CHF was 5 y (range 7 months-16 yrs). This included 20 children with CHF and 20 had Caroli’s syndrome. Portal hypertension was present in all children based on endoscopic findings or ultrasonographic evidence. Group 1 included 20 children of whom 80% presented in neonatal period or early infancy with renal insufficiency. The main indication for transplant was end stage renal disease and combined liver/kidney transplant was performed at a median age of 7 y. Moderate to severe portal hypertension was noted in 8/20 with variceal bleeding in 5 children while cholangitis occurred in 8/20 in this group. Group 2 included 20 children who presented with hepatosplenomegaly at a median age of 5 y. Moderate to severe portal hypertension was noted in 8/20 with variceal bleeding in 5 and cholangitis occurred in 3/20. Portal hypertension progressed in 12/20 (60%) and renal insufficiency developed in 6/20 (30%) in group 2.

Conclusion: The children who were transplanted had early presentation with end stage renal disease being the primary indication for transplant. There was no difference in the severity of portal hypertension between the transplanted and nontransplanted children while cholangitis was more common in the transplanted children prior to their transplant. Portal hypertension and renal insufficiency progressed independently in nontransplanted children. Children with Caroli’s syndrome were more likely to develop renal insufficiency.

Disclosure of Interest: None declared.

PO-H-0284

Hepatology

CATCH-UP GROWTH AFTER LIVER TRANSPANTATION IN ALAGILLE SYNDROME

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Objectives and Study: Alagille syndrome (AGS) is a multi-organ disease inherited in an autosomal dominant manner, associated with 5 major features: chronic cholestasis, characteristic facial features, cardiovascular abnormalities, ophthalmologic anomalies and skeleton defects. Growth failure is a common manifestation of AGS, which has been attributed mainly to malabsorption in the course of cholestasis, but also to other factors, such as genetic predisposition, pancreatic insufficiency, cardiovascular anomalies. Children with chronic cholestasis of other etiology usually improve their growth status after liver transplantation (LTx). The aim of our study was to analyze the pre- and post-transplant growth pattern of children with AGS observed in our unit.

Methods: Out of 55 children with AGS, 10 patients underwent LTx. Two patients died in the early postoperative period and were excluded from the study. The mean age at LTx of the remaining 8 patients was 7.31 ± 4.13 (SD) years. At the moment of LTx all children were cholestatic. The mean follow-up time after Ltx is 7.06 ± 2.63 (SD) years. Presently, all 8 patients are alive with good liver function. Two patients obtain small dosages of steroids. The standardized height (z score) before LTx and at present has been compared using the Wilcoxon matched pair test.

Results: found significant improvement of height after Ltx (P < 0.05): median (quartile) standardized height z score was −3.34 (−5.56; −1.12) before Ltx and is −1.76 (−3.08; −0.81) at present.

Conclusion: LTx has a positive impact on growth in children with AGS. However, a complete normalization of growth after LTx should not be expected. Additional factors, except for liver damage, seem to play a significant role in the etiology of growth failure in this group of patients.

Disclosure of Interest: None declared.

**PO-H-0285**

**Hepatology**

**HEPATIC LUMICAN EXPRESSION AND PAEDIATRIC NONALCOHOLIC FATTY LIVER DISEASE**

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Objectives and Study: Lumican is a glycoprotein involved in collagen cross-linking and modulation of the innate immune system. Overexpression of lumican was recently described in a group of adults with histologically progressive NASH but has not yet been evaluated in paediatric NAFLD. The aim of this study was to determine the degree of lumican expression in the liver of children with varying stages of NAFLD.

Methods: 24 children (17 boys), median age 13.1 years, with liver biopsy-proven NAFLD and 6 children with chronic liver disease other than NAFLD (4 with autoimmune hepatitis and 2 with Wilson disease) were included in the study. Paraffin-embedded biopsy sections were scored according to the NAFLD Activity Score (NAS). Sections were immunostained for lumican using HRP-DAB. Quantitative analysis was performed using imageJ (NIH, USA); staining was expressed as percentage of the total area. Relative quantification real-time PCR for lumican was undertaken on frozen biopsies.

Results: Median BMI z score of those with NAFLD was 2.2 and median HOMA-IR; 4.4. 58% had splenomegaly. Thirteen children scored ≥5 (NASH), 6 scored 3–4 (borderline) and 5 scored ≤2 (simple steatosis). Fibrosis was minimal in 10 (F<2) and significant in 14 (F≥2). The pattern of lumican staining followed the sinusoidal contour, and marked the portal vascular endothelium and the luminal border of bile ducts. There was no clear staining of hepatocytes. Lumican was overexpressed in those with significant fibrosis (F≥2) versus those with minimal fibrosis (F<2); (168%, P < 0.01). Lumican was also overexpressed in NASH versus simple steatosis (215%, P < 0.01). At gene level, lumican was upregulated (compared to normal control liver) in those with F≥2 (15.8-fold) and in those with F<2 (10.9-fold). Lumican expression was not related to age, BMI z score, HOMA-IR, splenomegaly or transaminase levels. There was variable expression of lumican in the biopsies of those with chronic liver disease other than NAFLD. Percentage area stained did not correlate with degree of fibrosis in these patients.

Conclusion: Lumican is expressed with increasing severity of paediatric NAFLD. Upregulation at gene level in those with both minimal and histologically more severe disease is also evident. The role of lumican in progression of disease has not yet been elucidated and should be the focus of further investigation.

Disclosure of Interest: None declared.

**PO-H-0288**

**Hepatology**

**ASSESMENT OF PROBIOTIC THERAPY IN CHILDREN OPERATED ON FOR BILIARY ATRESIA IN A RANDOMIZED PLACEBO CONTROLLED TRIAL: PRELIMINARY STUDY**

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Objectives and Study: Hepatoperoenterostomy improves prognosis of children with biliary atresia, still in most of them liver damage progresses to liver failure. Ascending cholangitis is one of major complications worsening prognosis. The aim of our study was to assess efficacy and safety of probiotic therapy in children with biliary atresia after hepatoperoenterostomy.

Methods: We investigated 27 children aged 92 ± 18 days (mean ± SD). Infants 1–2 weeks after hepatoperoenterostomy were randomized to 2 groups-treatment group of 13 children who received Lactobacillus GG added to milk.
formula and placebo group of 14 children who received glucose as a placebo. Both LGG and placebo were distributed in capsules and the content of capsules was administered for 6 months. We analyzed ascending cholangitis and liver tests. 20 infants completed the whole study, 7 of them dropped out because of liver transplantation.

**Results:** At the end of the study (after 6 months or at drop out) patients in the LGG group and in the placebo group presented with similar numbers of ascending cholangitis episodes 0.54 ± 1.2 vs 0.71 ± 1.1 which occurred in 3 vs. 6 patients, total bilirubin level (mg/dL) 7.2 ± 7.2 mg/dL vs. 8.2 ± 14.0, direct bilirubin level (mg/dL) 5.5 ± 5.7 vs. 6.4 ± 11.3, ALT activity (U/L) 143 ± 111 vs. 133 ± 117, AST activity (U/L) 170 ± 134 vs. 130 ± 103, GGTP (U/L) 211 ± 133 vs. 188 ± 1143. Per protocol analysis (for those who completed the study) performed in 20 patients showed similar results. Both LGG and placebo were well tolerated.

**Conclusion:** In this pilot study LGG is not superior to placebo in prevention of ascending cholangitis and ameliorating cholestasis.

**Disclosure of Interest:** E. Orłowska Industry of: LGG was provided by Vitis Pharma, P. Socha Industry of: LGG was provided by Vitis Pharma.

**PO-H-0289**

**Hepatology**

**RELATIONSHIP BETWEEN SERUM INSULIN-LIKE GROWTH FACTOR-1, INSULIN-LIKE GROWTH FACTOR-BINDING PROTEIN-3, AND ANTHROPOMETRIC MEASUREMENTS IN CHILDREN WITH CHRONIC LIVER DISEASE BEFORE AND AFTER LIVER TRANSPLANTATION**

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**Objectives and Study:** In chronic liver disease (CLD), the decrease in synthesis capacity of insulin-like growth factor-I (IGF-I), secondary to hepatocellular dysfunction and malnutrition cause rearrangement of growth axis. A successful liver transplantation (LT) improves growth retardation due to CLD and repair growth axis. Our aim was to evaluate nutritional status of children with CLD before and after LT, determine serum IGF-I/insulin-like growth factor-binding protein-3 (IGFBP-3) levels, and analyze the relationship between them.

**Methods:** 33 LT patients (median 34,24 m (5 m - 11 y) and 54 healthy children were included. IGF-I/IGFBP-3 levels and anthropometry (weight (w), height (h), weight for height (w/h), TST, MAC, MAMA (mid-arm muscle area) were obtained before and 1, 3, 6 and 12 months after LT. The control group’s same measurements were taken once at the beginning of study and 1 year later. IGF-I/IGFBP-3 levels were studied once.

**Results:** Before LT acute malnutrition rate was 21%, 21% and 36% according to w/age, TST and MAC z scores. H/age z score was under –2 SD in 30%. w/h z score underestimated acute malnutrition rate (3%). All measurements except w/h z score were lower in CLD patients (P < 0.0001). A significant negative relationship was detected between Child-Pugh score and TST (r = -0.387, P = 0.026), and MAC (r = -0.448, P = 0.009) z scores. 3 months after LT a significant increase in MAMA, TST, and MAC measurements were detected. After 6 months an increase in w/age (P < 0.0001), h/age (P < 0.05), and w/h (P < 0.05) z scores were seen. 1 year later, no difference was detected for w/age, w/h, and MAC z scores between study and control groups. TST and MAMA measurements were better in the LT group. However, h/age z scores (−0.7 ± 1.46) of LT patients were lower than that of healthy children (0.08 ± 0.9) P < 0.05. As short as the patient before LT, the rate of growing was more rapid after LT (r = -0.381, P = 0.02). In our study, IGF-I and IGFBP-3 levels were lower in CLD patients than the controls (35.24 ± 14.68 versus 69.88 ± 67.45 ng/mL, P < 0.001). There was no relationship between IGF-I, IGFBP-3 levels and any of the anthropometric measurements. No relationship was detected between these proteins and Child-Pugh score (IGF-1 r = -0.194, P = 0.280), IGFBP-3 (r = -0.27, P = 0.882). IGF-I levels were found to increase 1 month after LT, with peak levels of on 3rd months. 1 year after LT, it was still higher than the controls.

**Conclusion:** In CLD, TST, MAC, MAMA were reliable. Before LT, no relationship was detected between IGF-I and IGFBP-3 levels and anthropometry. These proteins did not reflect the presence and severity of malnutrition in CLD group. Likewise, no relationship was detected after LT between IGF-I/IGFBP-3 levels and anthropometry. Improvement of nutrition parameters after LT could not be explained only by growth factors.

**Disclosure of Interest:** None declared.

**PO-H-0290**

**Hepatology**

**APRI AND PASD AS PREDICTORS OF HEPATIC FIBROSIS IN INTESTINAL FAILURE-ASSOCIATED LIVER DISEASE**

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**Objectives and Study:** In children with intestinal failure-associated liver disease (IFALD) invasive tests (endoscopy, liver biopsy) are used to stage the liver disease. To assess effectiveness of APRI and PASD ratio (noninvasive markers) in assessment of hepatic fibrosis in children with IFALD, where normally invasive tests are used.

**Methods:** Inclusion criteria: children undergoing intestinal transplantation assessment, with the following variables: staging on histopathology. AST (U/L); platelets (10^9/L);
(APRI = AST (U/L)/upper normal X 100/platelets); at 2 points: transplant assessment and day of transplant. Spleen diameter on USS (PASD = age adjusted) recorded at transplant assessment.

Results:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Transplant assessment N=28</th>
<th>At the time of transplant N=28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>227 (5–510)</td>
<td>300 (10–777)</td>
</tr>
<tr>
<td>Staging of fibrosis</td>
<td>3 (1–6)</td>
<td>4 (1–6)</td>
</tr>
<tr>
<td>APRI</td>
<td>5.32 (0.1–95.2)</td>
<td>9.44 (0.31–57.7)</td>
</tr>
</tbody>
</table>

APRI paralleled the worsening of liver disease (blood parameters and histological progression) Thirty-nine cases had PASD documented. The median PASD value was 68.8 (range 5.1 – 364.1). PASD value of 120 has an 81% sensitivity, 85% specificity; p-value 0.001.

Conclusion: APRI and PASD ratio index may be useful noninvasive markers which are easily available, cost-effective and providing safe alternatives to invasive tests, avoiding morbidity and mortality, for documenting degree of fibrosis and its progression in children with IFALD.

Disclosure of Interest: None declared.

PO-H-0291

**Hepatology**

LONG-TERM ZINC THERAPY IN WILSON DISEASE CHILDREN WITH MILD LIVER DISEASE

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Objectives and Study: Wilson disease (WD) is a disorder of copper metabolism. In the pediatric age most cases have a hepatic presentation: the percentage of WD children presenting with isolated elevated aminotransferases ranges from 14% to 88%. It is widely accepted that penicillamine is the first-choice therapy for children with liver disease while zinc is indicated in sympotmatic patients and as maintenance therapy. The optimal medical therapy in patients presenting with isolated elevated serum aminotransferases remains unestablished. This reflects the absence of an agreement on classification of WD patients with isolated hypertransaminasemia as symptomatic cases, requiring zinc, or cases with hepatic onset, requiring chelating agents. The aim of our study was to evaluate the efficacy of exclusive zinc monotherapy in WD children with isolated hypertransaminasemia.

Methods: All WD patients referred to our Department of Pediatrics for diagnostic investigation of elevated serum aminotransferases were analyzed. The diagnosis of WD was established in presence of at least 2 of the following features: a low ceruloplasmin level (<20 mg/DL), an increased basal urinary copper level (>100 mcg/24 hours), an increased urinary copper level after the penicillamine challenge test (PCT, >1575 mcg/24 hours), an increased liver copper level (>250 μg/g of dry weight). Among 43 enrolled WD patients, 29 were treated with zinc for a median period of 12 years (range 3–25). Zinc was the initial therapy of choice in 12 cases. Normalization of serum ALT was the main parameter of treatment efficacy in this study. Compliance to therapy was evaluated on the basis of clinical history and serum and urine copper and zinc levels.

Results: Among 17 (58%) children, treated with penicillamine as first choice, 4 (24%) normalized ALT within a median of 14 months (range, 4 to 48), and started maintenance therapy with zinc. The remaining 13 (76%) patients with persistent hyper-ALT during penicillamine switched to zinc; nine of these (70%) normalized ALT on zinc within a median period of 9.5 months (range, 5 to 151). Eleven (92%) of the 12 patients, given zinc alone as first choice, normalized ALT within a median period of 14 months (range, 2 to 46). The patient with persistent hyper-ALT on zinc showed a poor compliance to treatment. According to 24-hour urinary copper excretion (56 ± 4 versus 37 ± 2 μg) at the end of follow-up, the efficacy in terms of decopperization was comparable in 2 groups.

Conclusion: Although penicillamine therapy is generally used for the initial treatment of WD, the present study has showed that zinc monotherapy may be used, as first line therapy, in WD children with isolated hypertransaminasemia at presentation.

Disclosure of Interest: None declared.

PO-H-0293

**Hepatology**

NONINVASIVE ASSESSMENT OF LIVER FIBROSIS IN CHILDREN PRE- AND POST-LIVER TRANSPLANTATION COMPARING TRANSIENT ELASTOGRAPHY AND FIBROTEST

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Objectives and Study: Liver histology is considered the gold-standard for staging of hepatic fibrosis, but is invasive and prone to sampling error. Limited data exists concerning non-invasive transient elastography (Fibroscan) in children. Our study aims at assessing the diagnostic value of Fibroscan examination and of serological assessment of fibrosis (Fibrotest) in children with liver disease before and after liver transplantation.

Methods: Transient elastography was performed in n = 69 children (39 M, 30 F; 7.3 (0.2–18.5) years, 32 transplanted patients) from our pediatric hepatology clinic. 31 underwent diagnostic liver biopsy (LBx). Fibroscan results were accepted if the ratio of interquartile range and median of 10 successive readings was <30%. Blood samples for determination of Fibrotest were obtained within 2 two days of the Fibroscan examination. Calculation of the Fibrotest algorithm was provided by Biopredictive (France). Liver biopsies were scored for fibrosis according to Ishak score.
Quantitative data are given as median (range). Correlation between quantitative variables is calculated using Pearson’s correlation coefficient.

**Results:** In patients undergoing LBx, liver stiffness was 9.1 (4.5–75) kPa (n = 30). Fibrotest was 0.42 (0.02–0.99, n = 17). Stratified according to ISHAK score, liver stiffness was F0 7.3 kPa (5.6–11.4, n = 1), F1 5.9 kPa (4.5–45.7, n = 5), F2 14.2 kPa (9.6–19.8, n = 2), F3 9.15 kPa (7.2–27.6, n = 4), F4 6.6 kPa (n = 1), F5 36.4 kPa (10.9–65.2, n = 4) and F6 71 kPa (16–75, n = 3). Elastography results and Fibrotest correlated with ISHAK score with r = 0.65 and r = 0.41, respectively. Correlation increased to r = 0.68 (Fibroscan/ISHAK) and r = 0.52 (Fibrotest/ISHAK) when patients with acute inflammatory histological changes (allograft rejection, autoimmune hepatitis) were removed from the analysis. Fibrotest/Fibroscan correlation was r = 0.51. Compared to ISHAK, Fibrotest correctly classified 35% of patients, underestimated fibrosis in 29% and overestimated fibrosis in 35%. Overestimation of fibrosis was associated with cholestatic liver disease (chi-square test, P < 0.05).

**Conclusion:** Liver stiffness measurements with Fibroscan show good correlation with histological grading of liver fibrosis and appear useful for the diagnosis of cirrhosis (F5/F6). Cutoff levels for lower degrees of fibrosis are more difficult to determine, possibly due to confounding factors such as inflammation, patient age and varying aetiology of liver disease. Studies with larger numbers are needed to address these issues. Accuracy of staging fibrosis with Fibrotest seems to be influenced by cholestasis.

**Disclosure of Interest:** None declared.

**PO-H-0298**

**Hepatology**

**THE BILE SALT EXPORT PUMP (BSEP) POLYMORPHISM V444A POTENTIALLY CAUSES JAUNDICE AS THE PRESENTING SYMPTOM OF ACUTE LYMPHOBLASTIC LEUKEMIA**

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**Objectives and Study:** Jaundice as a presenting symptom of acute lymphoblastic leukemia (ALL) is a well-described phenomenon. However, the pathogenetic basis and genetic markers remain unknown. Recently, a polymorphism (c.V444A) in exon 13 of the hepatic bile salt export pump (BSEP, ABCB11) was observed to be significantly more frequently expressed in patients with intrahepatic cholestasis...
of pregnancy as well as in patients suffering from drug-induced liver disease. Proinflammatory cytokines cause sepsis-associated cholestasis by impairing hepatocellular bile formation.

Methods: We performed genetic analyses of several bile acid transporters (MRD3; ABCB4, MRP2; ABC2, BSEP) in two pediatric patients, who were both diagnosed with ALL after an episode of fever with scleral jaundice and elevated bilirubin and aminotransferase levels. In detail, the initial blood tests of the first patient, a 15-year-old boy, revealed not only impressive hepatocellular damage (AST/ALT 1195/15.85 UI/L) but also severe leucopenia (0.9 x 10^9/L) and hyperbilirubinemia (direct/indirect bilirubin 15.85/1.78 mg/dL), but also severe leucopenia (0.9 x 10^9/L), thrombocytopenia (58 x 10^9/L) and reticulocytopenia (2.1%). The second patient, a 4-year-old girl, presented with less increased aminotransferase and bilirubin levels (AST/ALT 185/305 UI/L, direct/indirect bilirubin 4.2/0.4 mg/dL) and had thrombocytopenia (57 x 10^9/L) and anemia (erythrocytes 2.86 x 10^12/L). Viral serological studies (hepatitis viruses A, B and C, HIV, HHV6, EBV, adenovirus, parvovirus B19, CMV) and screening for relevant autoantibodies (smooth muscle, anti-nuclear, anti-LKM and anti-mitochondrial antibodies) were negative in both patients. Bone marrow aspirations showed 85% (boy) and 96% (girl) blast cells, resp. (both FAB L1). The diagnoses of ALL were confirmed by immunological studies and flow cytometry.

Results: Sequencing of the hepatocellular efflux transporter genes revealed in both cases a nucleotide polymorphism (c.V444A) of BSEP. Mutations of other relevant hepatobiliary transporters could not be detected.

Conclusion: To the best of our knowledge this is the first proof of the V444A polymorphism in 2 patients with jaundice as the presenting symptom of ALL. We suspect a “two-hit-concept”: proinflammatory cytokines in the febrile neutropenic pre-leucemic phase could be the “second hit” in patients with genetically determined impairment of BSEP function due to the V444A polymorphism (“first hit”). Proinflammatory cytokines might lead to a total BSEP collapse with subsequent loss of hepatocytes and transient elevation of liver enzymes. We hypothesise that the BSEP V444A polymorphism is a risk factor for jaundice as a presenting symptom of ALL.

Disclosure of Interest: None declared.

PO-H-0299

Hepatology

METHACETIN C13 BREATH TEST IN CHILDREN WITH AUTOIMMUNE HEPATITIS

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Objectives and Study: Methacetin is metabolized exclusively in the liver with the first pass effect. It is degraded to acetaminophen and CO2, thus C13 labeled methacetin may be used to evaluate the liver function. The aim of the study was to compare the results of C13-methacetin breath tests with liver histology in children with autoimmune hepatitis (AIH).

Methods: C13-methacetin breath test and standard liver biopsy were done in 29 AIH children (F-22, M-7) aged 13.9 ± 3.5 years. Exhaled air was collected prior and for 120 minutes after oral administration of 75 mg C13-methacetin. To peak C13 exhalation (TTP) and cumulative dose of C13 exhaled after 120 minutes of the test (CD120) were used as the primary outcome parameters. Patients were divided into the groups according to Batts and Ludwig grading (group G0-1: grading 0–1; group G2–4: grading 2–4) and staging (group S0–1: staging 0–1; group S2–4: staging 2–4). The distributions of CD120 and TTP between groups were compared. Receiver operating characteristics (ROC) analysis was used to calculate the optimal cutoff point for TTP and CD120 as well as their specificity and sensitivity for differentiation of children with minimal or present injury in liver biopsy (AccuROC, Canada).

Results: 9 children (31%) were in group G0–1 and 20 (69%) in group G2–4. 7 children (24%) belonged to group S0–1 and 22 (76%) to group S2–4. The groups did not differ according to sex or age distribution. TTP in group S2–4 was greater than in group S0–1 (30.1 ± 18.2 vs 7.1 ± 7.6, P = 0.036). No differences were observed in the distribution of: TTP for grading (24.4 ± 15.1 vs 29.0 ± 18), CD120 for grading (20 ± 5.0 vs 25, 1 ± 6.5) nor CD120 for staging (24.8 ± 5.7 vs 23.2 ± 6.8). The optimal cutoff point TTP=40 had a weak power for differentiation of children from groups G0–1 and G2–4 (AUC-0.56) and optimal cutoff point for CD120=22.2 had mild power to differentiate these groups (AUC-0.77). TTP and CD120 specificity for detecting the presence of active disease was, respectively, 0.89 (0.52 to 0.99) and 0.89 (0.51 to 0.99). The sensitivity of TTP was 0.25 (0.09 to 0.49) and sensitivity of CD120 was 0.75 (0.51 to 0.91). The optimal cutoff point TTP=20 had a mild power for differentiation of children from groups S0–1 and S2–4 (AUC-0.75) and optimal cutoff point for CD120=21.1 had weak power to differentiate these groups (AUC-0.60). TTP and CD120 specificity for detecting the presence of fibrosis was, respectively, 0.43 (0.09 to 0.81) and 0.86 (0.42–0.99) and sensitivity was 0.91 (0.71–0.99) and 0.41 (0.21–0.64).

Conclusion: Patients with fibrosis have greater TTP than those with minimal fibrosis. TTP is slightly better parameter for fibrosis detection while CD120 is better for detection of inflammatory changes however sensitivity and specificity of these parameters in AIH children are low.

Disclosure of Interest: None declared.

PO-H-0301

Hepatology

THE DISCRIMINANT ANALYSIS OF RISK FACTORS IN CHILDREN WITH ACUTE LIVER FAILURE DUE TO AMANITA PHALLOIDES POISONING
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Objective and Study: Amanita phalloides poisoning is a common cause of acute liver or multiorgan failure in paediatric patients in summer and autumn every year in Poland. Mortality is still high (12% to even 50%) and liver transplantation (LTx) is necessary in many cases. As the death occurs usually no earlier than 5 days after poisoning and some scores used to qualify patients to LTx is based on results obtained even up to 10 days after poisoning, it is crucial to establish which early clinical and laboratory factors are able to discriminate between patients with good and fatal outcome. The aim of the study was to construct the best model of clinical and laboratory factors up to 4 days after poisoning to predict clinical outcome in children after Amanita phalloides poisoning.

Methods: We retrospectively estimated data obtained from 78 children with acute liver failure (INR > 2.0 or INR > 1.5 and encephalopathy) due to Amanita phalloides poisoning hospitalized in our center from 1983 to 1990 (before LTx and extracorporeal liver support therapy in children were available in Poland). 35 (aged 8.2 ± 3.5) died, 43 (aged 8.9 ± 3.6) remained alive. The following factors were taken into considerations: age, time between poisoning and diarrhea, maximal grade of encephalopathy up to 4 days and ALT, INR, total serum bilirubin, creatinine, urea taken from 3 and 4 days after poisoning. The forward stepwise discriminant analysis was used to establish the best discrimination model.

Results: The following factors were assessed as the best to the model: INR Day 4, bilirubin Day 4, creatinine Day 4, urea Day 4, grade of encephalopathy, time between poisoning and diarrhea. Using the grouping classification functions the sensitivity and the specificity of the model was 0.65 (95% CI 0.47 to 0.80) and 0.95 (95% CI 0.84 to 0.99), respectively. When a priori probability of bad outcome was increased to 0.99 the sensitivity and the specificity of the model was 0.65 (95% CI 0.47 to 0.80) and 0.95 (95% CI 0.84 to 0.99), respectively.

Conclusion: Our results indicate that mitochondrial biogenesis is impaired within hours after BDL. Early glucocorticoid treatment can reverse the untoward effect and modulate the intrinsic but not extrinsic pathway of apoptosis following BDL.

Disclosure of Interest: None declared.

PO-H-0304

Hepatology

GENETIC ABNORMALITIES OF OXYGEN-SENSING PATHWAY,ERYTHROPOYETIN, AND JAK2 ARE NOT INVOLVED IN THE DEVELOPMENT OF POST-TRANSPLANT ERYTHROCYTOSIS OF PEDIATRIC LIVER RECIPIENTS

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Objectives and Study: We previously described that erythrocytosis may occur in the follow-up of liver transplantation (OLT). Since etiology of post-OLT erythrocytosis remains still unclear and better knowledge of genetic abnormalities possibly playing a role in erythrocytosis is now available, we aimed to extensively investigate this genetic background in 5 affected patients who presented this hematological abnormality after OLT.

Methods: The 5 patients (4 males, 1 female) developed normocytic erythrocytosis at a median age of 14.8 ± 4.3 y, ie, 9.1 ± 5.1 years after OLT. OLT indication was biliary atresia...
in 4 and Crigler-Najjar in 1. Post-OLT erythrocytosis was defined as an elevated hematocrit (Ht) ≥ 51% ± hemoglobin (Hb) level ≥ 16 g/dL or > 2 SD on 2 or more consecutive clinic visits, in the absence of other causes. JAK2 V617F mutation in exon 12 of JAK2, oxygen sensing pathway (Von Hippel Lindau protein-VHL, prolyl hydroxylase domain protein 2-PHD2, hypoxia inducible factor 2α-HIF2α) and high oxygen-affinity haemoglobins, and erythropoietin (EPO) receptor mutations were evaluated.

**Results:** Mean values of Hb and Ht were 17.38 ± 0.6 g/dL and 50.3 ± 2.46%, respectively. MCV mean value was 85.9 ± 4.7 fl. EPO blood levels were within normal range (3–20 μU/mL) in 4 patients, and pathologic (38 μU/mL) in 1. The latter had renal cysts, and was clinically symptomatic thus requiring treatment. None of our patients had genetic mutations of the examined genes underlying several possible pathways involved in congenital or acquired erythrocytosis. A heterozygous C>T transition at nucleotide 552 in VHL gene causing a synonymous variation was found in 1 subject.

**Conclusion:** The genetic factors here investigated are not involved in development of post-OLT erythrocytosis. This condition might therefore be the result of the combined effect of multiple and interrelated factors such as erythropoietin, renin-angiotensin system, male gender, renal cysts and other conditions not yet well recognized.

**Disclosure of Interest:** None declared.

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**PO-H-0307**

**Hepatology**

**LIVER INVOLVEMENT IN CHILDREN WITH FAMILIAL MEDITERRANEAN FEVER**

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**Objectives and Study:** Familial Mediterranean fever (FMF) is autosomal recessive disease characterized by recurrent, self-limiting, febrile, inflammatory attacks of the serosal membranes. Exaggerated and prolonged inflammatory response is triggered secondary to cytokines stimulation including IL (interleukin)-6, IL-8, IL-1 and TNF (tumor necrosis factor)-alpha in FMF due to reduced activity of pyrin protein. Inflammatory cytokines especially TNF-alpha and IL-6 play the major role in the pathogenesis of acute septic liver injury. It showed that IL-6 level may be used as marker of global liver injury. Therefore, acute liver injury may be seen during the attacks of FMF, and acute injury may progress to chronic hepatitis/cirrhosis if undiagnosed, because of chronic, recurrent proinflammatory cytokine production. We aimed to analyze liver involvement (LI) in children with FMF.

**Methods:** The study includes 58 patients with FMF. The diagnosis of FMF was made according to Tel-Hashomer criteria. Patients with amyloidosis or concomitant vasculitic diseases like Henoch-Schonlein purpura, Behçet’s disease or polyarteritis nodosa were excluded. Mutation analyses for the predominant mutations were studied in all patients. Patients with LI were examined in detail including demographic and clinical findings.

**Results:** LI was seen in 12 of 58 patients (20.6%). Three patients (5.1%) had abnormal liver enzymes during the diagnostic evaluation of FMF, while 9 patients (15.5%) was admitted with the clinical and laboratory features of liver diseases and have final diagnosis of FMF on the follow-up. Two of 9 patients had Budd-Chiari syndrome, 5 had chronic hepatitis/cirrhosis and 2 had acute hepatitis. None of
the demographic factors or laboratory findings was different between the patients with and without LI. M694 V allele was more common in patients with LI but did not reach the significant difference (54.1% vs. 32.5%, \(P = 0.08\)) (Table). All the patients had clinical and laboratory improvement after colchicine.

**Table.** Comprehension of the patient with and without liver involvement

<table>
<thead>
<tr>
<th>Alleles (frequency/alleles)</th>
<th>Liver involvement (+ (n = 12))</th>
<th>Liver involvement (− (n = 46))</th>
</tr>
</thead>
<tbody>
<tr>
<td>M694V</td>
<td>13 (54.1)</td>
<td>30 (32.6)</td>
</tr>
<tr>
<td>R202Q</td>
<td>3 (12.5)</td>
<td>—</td>
</tr>
<tr>
<td>V726A</td>
<td>2 (8.3)</td>
<td>5 (5.6)</td>
</tr>
<tr>
<td>M680I</td>
<td>1 (4.1)</td>
<td>3 (3.2)</td>
</tr>
<tr>
<td>P369S</td>
<td>1 (4.1)</td>
<td>—</td>
</tr>
<tr>
<td>E148Q</td>
<td>—</td>
<td>8 (16.8)</td>
</tr>
<tr>
<td>A744S</td>
<td>—</td>
<td>1 (1)</td>
</tr>
<tr>
<td>K695R</td>
<td>—</td>
<td>7 (16.8)</td>
</tr>
<tr>
<td>M680L</td>
<td>—</td>
<td>5 (5.4)</td>
</tr>
<tr>
<td>Undefined</td>
<td>4 (16.6)</td>
<td>33 (35.8)</td>
</tr>
<tr>
<td>Total</td>
<td>24 (100)</td>
<td>92 (100)</td>
</tr>
</tbody>
</table>

**Conclusion:** Our study shows that FMF may involve the liver more frequently than previously thought. Pediatric hepatologist must keep FMF in mind in the patients with cryptogenic hepatitis/cirrhosis especially in regions where hereditary inflammatory diseases are common.

**Disclosure of Interest:** None declared.

**PO-H-0309**

**Hepatology**

**CALPROTECTIN IN PORTAL HYPERTENSION**

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**Objectives and Study:** Measurement of fecal calprotectin (FCP) has proven a useful tool in diagnosing inflammatory bowel disease. There is some evidence to suggest that patients with portal hypertension may have some degree of exsudative enteropathy (EE). The aim of the present study was to measure FCP in children diagnosed with PHT and symptoms of EE, both before and after PHT treatment.

**Methods:** From November 2009 to November 2010, 8 children with PHT, aged between 6 and 72 months (mean: 25 m), were prospectively included in the present study. FCP was measured before and after the etiologic treatment of PHT. Stool samples were obtained at time of routine follow-up clinics. FCP was measured by means of ELISA test and results were expressed as FCP mg/g of dried stools (normal values < 50 μg/g).

**Results:** PHT was related to cirrhosis secondary to biliary atresia (n = 4), to hepatoblastoma (n = 2), and to portal vein stenosis after liver transplantation (n = 2). All 8 children presented with failure to thrive (FFT): weight \(z\) scores were −2.5 in 4 children and −1.5 in the other 4. All had symptoms of exsudative enteropathy (watery diarrhea without blood). None had elevated blood markers of inflammation. In the 8 patients, FCP was increased before treatment (range: 150–1477 μg/g, mean: 417 μg/g), which returned to normal (<50 μg/g) between 1 and 6 weeks after PHT treatment. The FFT resolved with a normal body weight (\(z\) scores = 0).

**Conclusion:** These preliminary observations suggest that measurements of FCP, routinely used for inflammatory bowel disease screening, could be a valuable non invasive marker of exsudative enteropathy due to portal hypertension.

**Disclosure of Interest:** None declared.

**PO-H-0309**

**Hepatology**

**CYCLOSPORIN-INDUCED BIOCHEMICAL REMISSION IN CHILDHOOD AUTOIMMUNE HEPATITIS**

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**Objectives and Study:** The conventional treatment of autoimmune hepatitis (AIH) in children, which includes prednisone either alone or in combination with azathioprine, induces remission in most cases but is often associated with poorly tolerated side effects. To avoid the adverse effects of steroids, Alvarez et al introduced back in 1999 an alternative treatment regimen, using cyclosporin A (cys) as primary immunosuppression with similar remission rate. We carried out a retrospective study to evaluate the efficacy and tolerance of cyclosporin treatment in 9 children and adolescents with AIH treated in our center.

**Methods:** During 2000–2010 period, 9 children (6 female) were diagnosed with AIH according to established international criteria. Following the suggested protocol, cys was administered orally in 2 divided doses (3–5 mg/kg/d), adjusted to maintain therapeutic serum cys levels within 200–300 ng/mL. After 3 months, when the transaminase activity tended to normalise, oral dose of cys was adjusted to achieve serum concentrations of 100–200 ng/mL. Conversion to low dose of prednisone and azathioprine was started after 6 months, with gradual tapering of cys dose and drug discontinuation over a period of 2 weeks.

**Results:** All nine patients, aged 4.0–17.7 years (median 11.2 y), had elevated transaminases and gammaglobulin levels (Table 1), with proven histological changes typical for AIH in 8 patients that underwent liver biopsy (in one patient biopsy was contraindicated due to the prolonged prothrombin time). Serum ANA/SMA autoantibodies were positive in all but 1 patient, who had positive anti-LKM1. Complete or
near complete and persistent normalisation of transaminase activity was observed in 8/9 patients within first 3–6 months of therapy. In one male adolescent where complete biochemical response was absent, an overlap syndrome was established. After ursodeoxycholic acid was added, complete biochemical and clinical remission was observed. All patients had excellent clinical course and histological improvement on follow-up liver biopsy. During the long-term follow-up (median 4.6 years), biochemical relapse occurred in one patient after discontinuation of maintenance corticosteroid dose. Despite registered improvement, none of the patients fulfilled the criteria for therapy discontinuation, so all of them are still receiving maintenance doses of prednisone or azathioprin.

Table 1. Basic laboratory values at presentation and after 6 mo of cys therapy

<table>
<thead>
<tr>
<th>Test</th>
<th>At presentation, average</th>
<th>After 6 mo, average</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR</td>
<td>56</td>
<td>30</td>
</tr>
<tr>
<td>ALT, U/L</td>
<td>1219</td>
<td>40</td>
</tr>
<tr>
<td>Gamma globulin, g/L</td>
<td>44.6</td>
<td>16.8</td>
</tr>
</tbody>
</table>

**Conclusion:** The applied protocol allowed for the control of the liver inflammatory disease in all of our patients and protected them from the side effects related to steroid treatment. Side effects of cys were minimal and were well tolerated.

**Disclosure of Interest:** None declared.

**PO-H-0310**

**Hepatology**

**OUTCOME OF CHILDREN WITH HEREDITARY TYROSINAEMIA TYPE 1 DIAGNOSED BY SELECTIVE NEONATAL SCREENING IN THE NITISINONE ERA**

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**Objectives and Study:** Nitisinone has transformed the management of hereditary tyrosinaemia type 1 (HT1). However the eventual risk of developing hepatocellular carcinoma is related to the age treatment is started. If treatment is started prior to 6 months this risk is very low in childhood. Universal neonatal screening for HT1 is not routine and there is still little data on the outcome of children treated preemptively. The aim of the study was to describe the current outcome of children with HT1 from a single centre treated with nitisinone following selective neonatal screening.

**Methods:** 10 children with HT1 were detected by neonatal screening following second line investigation following detection of raised tyrosine levels on routine neonatal screening for phenylketonuria at 5–8 days of age. HT1 was subsequently confirmed in all cases by mutation detection. Nitisinone and dietary treatment were commenced at median 4 (1–52) days old. 6 children had a coagulopathy at diagnosis but this resolved after median 4 (1–7) days treatment. Currently at median age 6 (1.5–9) years all are clinically normal, with normal biochemical liver and renal function tests, normal alphafetoprotein and normal liver and renal imaging. Mean weight and height SDs are −0.465 and −0.67. One child developed epilepsy requiring treatment aged 3 years. The 6 children of school age are in normal classes but three have reported learning difficulties.

**Conclusion:** Children with HT1 treated with nitisinone following neonatal screening have an excellent outcome. Consideration should be given to universal neonatal screening for HT1.

**Disclosure of Interest:** P. McKiernan Speaker Bureau with: Swedish Orphan Ltd, M. Preece: None declared, A. Daly: None declared, A. McDonald: None declared, P. Gissen: None declared.

**PO-H-0311**

**Hepatology**

A MULTICENTRE RANDOMISED CONTROLLED PILOT TRIAL OF VARICEAL BAND LIGATION FOR PRIMARY PROPHYLAXIS OF OESOPHAGEAL VARICEAL BLEEDING IN CHILDHOOD PORTAL HYPERTENSION

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**Objectives and Study:** Primary prophylaxis of variceal hemorrhage in adults is the established evidence based standard of care. In childhood portal hypertension there are no evidence based guidelines and there have been no randomised controlled trials of primary prophylaxis with variceal band ligation (VBL). This pilot study in 3 UK centres was established to determine the feasibility and safety of such a study.

**Methods:** Children with portal hypertension but without previous gastrointestinal bleeding or beta-blocker treatment were recruited prior to routine endoscopy. Those found to have large oesophageal varices were randomised to receive either prophylactic VBL or no active treatment. Follow up was for 2 years.

**Results:** 65 children were recruited, of whom only 22 had large oesophageal varices. 12 were randomised to receive VBL. After the first year the treatment protocol was relaxed to allow fewer VBL sessions. All have completed at least 6 months follow-up and 10 have completed the study. One
child randomised to VBL died of unrelated causes, 2 children did not complete the study. 1 developed idiopathic thrombocytopenic purpura and underwent prophylactic VBL and 1 randomised to VBL underwent liver transplantation. 3/10 children randomised to no VBL have developed variceal bleeding and were treated with VBL. 1/12 children randomised to VBL had a variceal bleed 1 week after elective VBL which responded to repeat VBL. VBL was well tolerated. A definitive study would require large subject numbers and multinational collaboration.

Disclosure of Interest: None declared.

PO-H-0312

Hepatology

PREDICTING THE RISK OF JAUNDICE IN HEALTHY TERM BREAST-FED INFANTS

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Objectives and Study: Breast-fed infants have higher serum bilirubin levels than formula-fed infants. In recent years, the policy of exclusive breast-feeding and a short hospitalization increases the risk for hyperbilirubinemia and kernicterus in term neonates. The aim of our study is to establish a model for identifying the healthy term breast-fed infants at risk of developing significant hyperbilirubinemia in Taiwan.

Methods: A prospective study was designed to investigate the effects of these factors [breast-feeding, birth body weight, mode of delivery, cephalohematoma, glucose-6-phosphate dehydrogenase (G6PD) deficiency, predischarge total serum bilirubin, variant UDP-glucuronosyltransferase 1A1 (UGT1A1) gene and hepatic solute carrier organic anion transporter 1B1 (SLCO1B1) gene] on significant hyperbilirubinemia in Taiwanese breast-fed neonates. Umbilical cord blood samples have been collected for study of UGT1A1 and SLCO1B1 gene. All term breast-fed babies routinely received blood sampling to do newborn screening for inborn errors of metabolism by tandem mass spectrometry and the analysis of total serum bilirubin (TSB) level were obtained at 3 days old (between 64 to 72 hours in postnatal life) before they were released from the hospital. The PCR-restriction fragment length polymorphism (RFLP) method was applied to detect the known variant sites of UGT1A1 and SLCO1B1 genes in Taiwanese. Those breast-fed neonates with known risk factors for neonatal hyperbilirubinemia, such as blood type ABO incompatibility, hemolytic anemia, hyoxia/aphxia, dehydration/vomiting, sepsis, liver dysfunction, hypothyroidism, and small for gestational age babies, were excluded. Significant hyperbilirubinemia was diagnosed if a full term infant needed phototherapy and had a bilirubin level ≥256.5 µmol/L (15.0 mg/dL) in serum within 1 week after birth. We analyzed the risk factors for significant hyperbilirubinemia using univariate logistic regression models.

Results: A total of 252 full term breast-fed infants were enrolled in this study. Of these, 59 (23.4%, 34 males and 25 females) infants received phototherapy with significant hyperbilirubinemia. The results of univariate logistic regression revealed odds ratios (ORs) of 3.79 [95% confidence interval (CI): 2.57–5.57; P < 0.001] and 2.97 [95% CI: 1.21–7.26; p= 0.017], for the predischarge total serum bilirubin and the variant UGT1A1 gene at nucleotide 211, respectively. The ORs, adjusted for covariates, for the other risk factors were not statistically significant.

Conclusion: Combining the predischarge total serum bilirubin and the variant UGT1A1 gene at nucleotide 211 can predict hyperbilirubinemia in healthy term breast-fed infants in Taiwan.

Disclosure of Interest: None declared.

PO-H-0316

Hepatology

BIOCHEMICAL AND HISTOPATHOLOGICAL EFFECTS OF GHRELIN ON CCL4-INDUCED EXPERIMENTAL ACUTE LIVER INJURY IN RATS

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Objectives and Study: The aim of this study was to evaluate the mechanisms of the protective effects of ghrelin in rats with CCl4 (carbon tetrachloride) induced acute liver injury.

Methods: In experimental studies, 24 Spraque-Dawley albino rats from genus were divided into three groups equally as follows: control, CCl4 and CCl4+Ghrelin. 4 mL/kg olive oil was administered intraperitoneally (i.p.) to the control group, 4 mL/kg CCl4 (1.1) dissolved in olive oil) was administered i.p. to the animals in other 2 group. After 3 and 6h, 80 µg/kg ghrelin was administered i.p. to the CCl4+Ghrelin group. Twenty-four hours after administrating CCl4, all of the rats were sacrificed. Biochemical assessments were performed using serum AST, ALT, MDA (malondiadehyde), tissue MDA, MPO (myeloperoxidase) and NO (nitric oxide) levels. Histopathological assessments were performed using haematoxylin and eosin staining in light microscope.

Results: Serum AST, ALT, MDA and tissue MDA, MPO levels all increased in CCl4 group. But they were decreased in group treated with ghrelin. Tissue NO levels decreased in CCl4 group, but they were a more limited decrease in group treated with ghrelin. Histopathological comparison of the groups showed that a decrease in vacuolar degeneration and necrosis of hepatocytes, hemorrhage, sinusoidal congestion, PMNL(polymorphonuclear leukocytes) and MNL (mononuclear leukocytes) infiltration in group treated with ghrelin.

Conclusion: Our study supports that ghrelin prevents experimental acute hepatic injury by preventing oxidative stress.

Disclosure of Interest: None declared.
PO-H-0317

Hepatology

BIOCHEMICAL AND HISTOPATHOLOGICAL EFFECTS OF N-ACETYLCYSTEINE ON CARBON TETRACHLORIDE-INDUCED EXPERIMENTAL ACUTE LIVER INJURY IN RATS

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Objectives and Study: Glutathione is an endogenous anti-oxidant and has a ubiquitous role in many of the body’s defences. Treatment with N-acetylcysteine (NAC) has been shown to increase levels of glutathione. NAC has been proposed as a treatment for several illnesses. In recent years, N-acetyl-L-cysteine has been widely investigated as a potentially useful protective and antioxidative agent to be applied in many pathological states. The aim of the present work was further evaluation of the mechanisms of the NAC protective effect under carbon tetrachloride-induced acute liver injuries in rats.

Methods: In experimental study 24 albino rats from Spraque-Dawley genus were divided into three equal groups as Control, CCl4 and CCl4+NAC. 4 mL/kg olive oil was administered intraperitoneally (i.p.) to the control group, 4 mL/kg CCl4 (1.1 dissolved in olive oil) was administered i.p. to the CCl4 and CCl4+NAC groups. Three and 6 hours after, 150 mg/kg NAC was administrered i.p. to the NAC group. Twenty-four hours after administering CCl4, all of the groups were sacrificed. Biochemical assessments were performed using serum AST, ALT, and serum MDA, MPO, NO levels. Histopathological assessments were performed using haematoxylin and eosin staining in light microscope.

Results: Serum AST, ALT, tissue and serum MDA, MPO, NO levels were all increased in CCl4 group, but they were decreased in group treated with NAC. Tissue MDA, MPO were increased in CCl4 group, but they were decreased in group treated with NAC in also limited NO reduction was found. Histopathological comparison of the groups showed a decrease in congestion, polymorphonuclear leukocytes. Mononuclear leukocytes, vascular degeneration of hepatocyte and hepatocellular necrosis decreased in group treated with NAC.

Conclusion: As NAC is currently used in humans intoxicated with paracetamol, it can be tested in acute hepatic failure for other reasons. Our results suggest that NAC prevents experimental acute hepatic failure by preventing oxidative stress.

Disclosure of Interest: None declared.

PO-H-0318

Hepatology

FUNCTIONAL COMPARISON OF SPLENORENAL AND MESO-REX-SHUNT IN EXTRAHEPATIC PORTAL VEIN OBSTRUCTION (EHPVO)

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Objectives and Study: The Meso-Rex-shunt (MRS) has been established as an alternative to surgical splenorenal shunts (SRS) in patients with EHPVO. Metabolic, hemato-logic and hepatic implications of both operation methods were retrospectively analysed.

Methods: A chart review of all patients with EHPVO presenting in our clinic for operation or follow-up was conducted. End points were patency of shunt, complications, spleen length, parameters of hypersplenism (blood count), liver function tests (LFT) and serum ammonia levels. Both operation methods were analysed in whole groups and in subgroups with patent and closed shunt. A correlation of intrahepatic portal vein flow and serum ammonia levels was investigated across all shunts. Data are given as median and range, statistics are calculated using the Wilcoxon signed rank test and the Pearson correlation with a significance level of \( P < 0.05 \).

Results: Nine patients received a MRS and eight a SRS in the time period between 1997 and 2010. Age at MRS was 5.9 years (2.0–16.0) with a follow-up of 7.7 months (2–108). Age at SRS was 6.9 years (2.8–12.5; \( P = 0.962 \)) with a follow-up of 11.6 months (0–168; \( P = 0.665 \)). Shunt closure was present in 4/9 MRS at the end of follow-up (closures detected at 2, 36, 49 and 73 months post-OP), and in 2/8 SRS (closures detected at 34 and 150 months post-OP; \( P = 0.451 \)). Shunt closure was observed in patients after MRS with recanalized umbilical vein as vascular graft (n = 3) and due to multiple adhesions after previous operations (n = 1). After SRS, closure occurred in patients with low portal vein pressure (n = 2). No significant difference between MRS and SRS was found with regard to spleen length reduction and normalization of hypersplenemic syndrome, with significantly better outcome in both subgroups with patent shunt. LFTs showed no difference between all groups and subgroups. Serum ammonia levels were significantly lower in MRS than in SRS, both in the whole group (69 vs. 117.5 \( \mu \)g/dL, \( P = 0.043 \)) and in the patent-shunt subgroup (44 vs. 140.5 \( \mu \)g/dL, \( P = 0.009 \)), with no difference in the closed-shunt subgroup (116 vs. 89 \( \mu \)g/dL, \( P = 0.533 \)). Among all patients, an inverse correlation could be demonstrated between intrahepatic portal-vein flow either by shunt or by collaterals and serum ammonia \( r = -0.603; P = 0.01 \).

Conclusion: This study confirms the equivalency of MRS and SRS with regard to safety as well as to control of portal hypertension and hypersplenism. However, the risk of late encephalopathy by high ammonia levels in SRS has to be considered. The finding of an inverse correlation between intrahepatic portal vein perfusion and serum ammonia levels is interesting, possibly indicating that the extent of collateral perfusion into the liver should be taken into account in the decision making process for surgical interventions.

Disclosure of Interest: None declared.

PO-H-0319

Hepatology

LIVER CHANGES IN EPILEPTIC CHILDREN ARE MAINLY ASSOCIATED WITH FIRST-GENERATION ANTICONVULSANTS

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Objectives and Study: Antiepileptic drugs (AEDs) show a wide range of side effects. Because of its anatomical and physiological characteristics, the liver is a well-known major target for their toxicity. However, liver involvement due to second and third generation AEDs has not yet been extensively studied. Therefore, we aimed to assess in greater detail the overall hepatic side effects of available AEDs in children.

Methods: Liver tests of 56 children (30 M/26 F) being followed for epilepsy at the Department of Pediatrics of Federico II University and receiving treatment with various AEDs were retrospectively reviewed. Causes of muscular, viral, metabolic, nutritional, and autoimmune hypertransaminasemia were excluded on the basis of clinical and laboratory appropriate tests.

Results: Patients had a mean age of 9 yrs (range: 0.4 – 22 yrs), and had been treated for a mean period of 5.8 years. 34 pts were in monotherapy (76% with 1st-generation drugs: VPA > PB > CBZ, 24% with 2nd- and 3rd-generation drugs: TPA > LEV > LTG > OXC). VPA + LEV, PB + LTG + LEV were the most common drug associations used for dual (n = 17) and triple therapies (n = 5), respectively. Liver changes were found in 16 out of 56 patients: isolated hyper-GGT (n = 9) was seen mainly during PB treatment [alone (n = 2) or in association with other 1st (2 VPA, 1 CBZ) and/or 2nd- and 3rd-generation (1 TPM, 1 LTG, 1 VPA+TPM AEDs] as a consequence of enzyme induction as shown by normal serum bile acids. The remaining 7 cases showed [isolated (n = 3; 2 VPA, 1 TPM) or hyperGGT associated (n = 4: 3 PB alone, 1 PB+LEV+LTG)] modestly elevated hypertransaminasemia. Except for 2 VPA treated pts with noticeable increase of transaminases requiring therapy change, hepatotoxicity was not clinically relevant. Twenty patients reported weight gain which was observed, mainly (17/20) in the course of a VPA-containing therapy. One of them developed NAFLD and insulin resistance, both disappearing after drug discontinuation.

Conclusion: Liver changes in epileptic children are mainly associated with first-generation AEDs, notably PB and VPA. Excluding VPA cases, these changes are usually both clinically irrelevant and self limited. Valproic acid related over-weight/obesity is a stimulating new field of study. Although drugs of second and third generation seem to have a low propensity to induce liver changes, further studies on more patients are necessary.

Abbreviations: CBZ, carbamazepine; GGT, gamma glutamyltranspeptidase; LEV, levetiracetam; LTG, lamotrigine; OXC, oxcarbamazepine; PB, Phenobarbital; TPM, topiramate; VPA, valproic acid.

Disclosure of Interest: None declared.

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PO-H-0320

Hepatology

IMPACT OF SPECIALISED SYSTEMATIC ULTRASOUND EXAMINATION ON THE INVESTIGATION OF INFANTS WITH NEONATAL CHOLESTASIS

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Objectives and Study: No single investigation can confirm the diagnosis of biliary atresia (BA). However between 2002 and 2005 our department investigated the use of detailed systematic abdominal ultrasound scans (USU) with a high-frequency transducer to differentiate infants with BA from those with other causes of neonatal cholestasis. The overall accuracy was 98% and this method has become routine practice in our unit. The aim of our study was to assess how the incorporation of this technique has affected the need for invasive investigations.

Methods: Case notes were reviewed retrospectively from 2 groups of 50 consecutive infants referred for investigation of neonatal cholestasis before (Gp 1) and after (Gp 2) the USS study. Patient characteristics, stool colour, laboratory tests, radiological and histological reports and final diagnoses were recorded. The range of investigations needed to exclude a diagnosis of BA was noted.

Results: Notes were available for 48 infants in Gp 1 (24 male, median age 5 weeks (range 1 – 19 weeks)) presenting between April 2000 and February 2002, and 49 infants in Gp 2 (29 male, median age 6 weeks (range 1 – 20 weeks)) presenting between November 2005 and July 2007. 10 infants in Gp 1 and 7 infants in Gp 2 had a final diagnosis of BA. All infants had the standard first-line laboratory investigations for neonatal cholestasis and an USS; standard USS in Gp 1 specialised USS in Gp 2. 4 infants in Gp1 and 5 infants in Gp 2 needed no further investigations for diagnosis. The number of other investigations required to achieve or refute a diagnosis of BA in the remainder is seen in Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Gp 1 (n = 48)</th>
<th>Gp 2 (n = 49)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioisotope scan</td>
<td>37 (77%)</td>
<td>41 (84%)</td>
<td>NS</td>
</tr>
<tr>
<td>Liver biopsy</td>
<td>22 (46%)</td>
<td>4 (8%)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Operative cholangiogram</td>
<td>4 (8%)</td>
<td>1 (2%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Conclusion: The introduction of specialist USS into our routine investigation of infants referred with neonatal cholestasis has been associated with a significant decrease in the number of liver biopsies performed to diagnose BA.

References:

Disclosure of Interest: None declared.
PO-H-0322

Hepatology
MECHANISM OF HEPATOCYTE DEATH IN ACUTE-ON CHRONIC LIVER FAILURE IN CHILDREN WITH BILIARY ATRESIA

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Objectives and Study: Acute-on chronic liver failure (ACLF) is being increasingly recognised. Cellular death mechanism of this injury is not well described but it is believed to be secondary to inflammation leading to apoptosis and necrosis. CK18 M65 and M30 are serum biomarkers of acute cellular necrosis and apoptosis, respectively. We aim to evaluate CK18 M30 and M65 as markers of mechanism of hepatocyte death in children with ACLF and biliary atresia (BA).

Methods: Demographic, clinical and laboratory data were collected retrospectively in a cohort of children diagnosed with ACLF on the background of BA (group1) from 1999–2003 in our centre. Equal number of patients from the same time period diagnosed with BA and listed for liver transplantation, matched for age and sex, were allocated in group 2, as controls. The CK18 M30 Apoplosense enzyme-linked immunosorbent assay kit was used to quantify apoptosis-associated neoepitope of CK18 in stored (−80°C) serum samples collected at time of listing or development of ACLF. The M65 ELISA was used to measure soluble CK18 released from dying cells and thus a measure of overall cell death at the same time point.

Results: Twenty (9 male) children were identified in each group. Median age was 0.5 [0.34–5.8] years and 0.7 [0.2–12.5] for group 1 and 2, respectively. Median values were for serum bilirubin 306 μmol/L [44–680] and 222 μmol/L [27–236], INR 1.8 [1.0–3.5] and 1.19 [0.9–1.8], platelet count 153×10^9/L [51–577] and 195×10^9/L [29–475], albumin 30 g/L [20–38] and 31 g/L [17–38] and sodium 136 mmol/L [127–143] and 136 mmol/L [130–142] for group 1 and 2, respectively. Median levels of CK18 M30 were 749.5 IU/L [392–1320] and 745.5 IU/L [195.5–1360.8], respectively. Median levels of CK18 M65 were 1163 IU/L [354–2350] and 754 IU/L [112–2450] for group 1 and 2, respectively.

Conclusion: Acute cell necrosis appears to be responsible for the development of ACLF. CK18 M65 is 1.5 times higher in ACLF although it does not reach statistical significance. This study may help towards future biotherapies.

Disclosure of Interest: None declared.

PO-H-0324

Hepatology
QUALITY OF LIFE IN YOUNG ADULT SURVIVORS OF BILIARY ATRESIA WITH OR WITHOUT LIVER TRANSPLANTATION: RESULTS FROM A NATIONAL COHORT

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Objectives and Study: Biliary atresia (BA) is a cholestatic disease of infancy characterized by obliteration of the extrahepatic bile ducts. Kasai portoenterostomy and liver transplantation are the two sequential treatment options. With the advent of these treatment options, an increasing number of patients survive into adulthood. Little is known about the long-term quality of life (QOL) of survivors of BA. We aimed to determine the QOL in a cohort of young adult survivors of BA.

Methods: The RAND-36 and Liver Disease Symptom Index (LDSI) score 2.0 questionnaires were sent to all eligible 54 surviving adult BA patients, who were born between 1977 and 1991. The RAND-36 encompasses eight QOL domains from which a physical and mental summary score can be computed. The LDSI questionnaire consists of 24 items which scores symptoms on a 1–5 scale. Clinical patient characteristics were obtained from the NeSBAR database. Results of the RAND-36 scores were compared to those of an age-matched Dutch reference group (ANOVA), and relationships between the RAND-36 scores and liver biochemistry parameters and LDSI scores were studied by Pearson’s correlational analysis.

Results: Eighty-three percent (25/30) of the nontransplanted and 63% (15/24) of the transplanted patients responded. The mean RAND-36 and summary scores of either transplanted or transplant-free BA patients did not significantly differ from the reference group’s scores (Table 1). The single exception was general health perception, which was decreased in transplant-free females (63±21) compared to the reference group (75±17; P=0.004). RAND-36 summary scores of all BA patients were not correlated to serum bilirubin, ASAT or albumin levels, but significant relationships were found with LDSI total scores (physical summary score, r=0.62, P<0.001; mental summary score, r=0.50, P=0.001).

Table 1: RAND-36 summary scores

<table>
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<tr>
<th></th>
<th>BA transplant-free</th>
<th>BA OLT</th>
<th>Comparison group</th>
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<tbody>
<tr>
<td>(n=25)</td>
<td>(n=15)</td>
<td>(n=500)</td>
<td></td>
</tr>
<tr>
<td>Physical score</td>
<td>51 ± 10</td>
<td>53 ± 6</td>
<td>50 ± 10</td>
</tr>
<tr>
<td>Mental score</td>
<td>50 ± 9</td>
<td>54 ± 6</td>
<td>50 ± 10</td>
</tr>
</tbody>
</table>

Conclusion: Young adult BA patients had a QOL similar to an age-matched reference group. In transplant-free females, however, perception of general health was lowered. QOL is
correlated to liver disease symptoms and not to serum liver biochemistry parameters. In order to maintain or improve the QOL of the individual BA patient, focus on the improvement of the various liver disease-associated symptoms seems warranted.

Disclosure of Interest: None declared.

PO-H-0326

Hepatology
ABDOMINAL PAIN AND GASTRITIS AS THE SIDE EFFECTS OF ZINC THERAPY IN CHILDREN WITH WILSON DISEASE
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Objectives and Study: Zinc compounds are commonly used for treatment of Wilson disease and are regarded safe and well tolerated. However, occasionally patients report abdominal pain and nausea and require change of pharmacotherapy. Except for reporting abdominal pain these side effects were not investigated thoroughly. The aim of our study was to determine the prevalence and characterize side effects of zinc therapy in children with Wilson disease.

Methods: We retrospectively analyzed a group of 37 patients (20 females, 17 males, aged mean 10.7yrs) with confirmed diagnosis of Wilson disease (according to the Ferenci score and mutation analysis). They were treated with zinc sulphate for 83.3 (8–344) weeks [median (range)]. All patients’ complaints were considered as the potential drug adverse reactions.

Results: Side effects were observed in 12 children (9 females, 3 males, aged mean 10.7yrs) and all were of gastrointestinal origin: abdominal pain, nausea or vomiting. They occurred after 67.1 (8–344) wks on zinc sulphate therapy. Esophagogastroduodenoscopy (EGD) was performed in 4 patients with persistent and severe abdominal pain and it revealed gastritis with mucosal ulceration and negative \(H. pylori\) test in all subjects investigated. In 2 children symptoms resolved on proton pump inhibitors, in other 2 cases additional conversion to penicillamine was necessary. 2 patients with abdominal pain did not give consent to EGD. In the remaining patients clinical improvement was observed after a change to zinc acetate (2 patients), D-penicillamine (4 patients) or introduction of the alternative zinc sulphate dosage scheme (2 patients).

Conclusion: It seems that adverse reactions as abdominal pain, nausea and even gastritis are relatively common in patients treated with zinc sulphate. They may occur at the different stage of therapy. In selected patients EGD should be done to detect and treat inflammation of upper gastrointestinal tract. Discontinuation of zinc sulphate is often inevitable and conversion to penicillamine or zinc acetate may be safer option for these patients.

Disclosure of Interest: None declared.

PO-H-0330

Hepatology
VARIANTS IN PPARGC1A AND TNF-LPHA GENES ARE ASSOCIATED WITH THE RISK OF Pediatric NONALCOHOLIC FATTY LIVER DISEASE
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Objectives and Study: The heritability of pediatric non-alcoholic fatty liver disease (NAFLD), as estimated by family aggregation study, is almost 100%. So far, PNPLA3 rs738409 variant is the most influential single nucleotide polymorphism (SNP) for NAFLD. The aim of this study was to examine the associations between 24 SNPs in 11 NAFLD-related candidate genes and the risk of pediatric NAFLD in obese children with conditioning on the effect of PNPLA3 rs738409 polymorphism.

Methods: 24 SNPs were selected by a pathway-driven approach, including autophagy (ATG16L1, PIK3C3, IRGM), toll-like receptor (CD14, TOLLIP), inflammatory (TNF-a), fatty acid metabolism (PPARG, PPARGC1A), and adiponectin signaling (ADIPOQ, ADIPOR1, ADIPOR2) pathways. SNPs were chosen based on minor allele frequency higher than 5% among Han Chinese. NAFLD was determined by liver ultrasonography. Associations between SNPs and pediatric NAFLD were examined using multiple logistic regression models.

Results: A total of 95 cases and 91 controls were studied. The two groups matched each other in terms of age, gender and body mass index. With conditioning on the effects of waist circumference, triglyceride, adiponectin and PNPLA3 rs738409 polymorphism, one PPARGC1A SNP (rs8192678) was significantly associated with an increased risk for pediatric NAFLD [odds ratios (OR), 2.21; 95% confidence interval (95% CI), 1.04–4.69] and one TNF-a SNP (rs1799964) was significantly associated with a decreased risk for pediatric NAFLD (OR, 0.49; 95% CI, 0.24–0.99).

Conclusion: The variant PPARGC1A rs8192678 and TNF-a rs1799964 genotypes significantly modified the risk of NAFLD independent of the effect of PNPLA3 rs738409 polymorphism in our population of obese Taiwanese children.

Disclosure of Interest: None declared.

PO-H-0334

Transplantation
FEASIBILITY AND TECHNICAL ASPECTS OF TRANSIENT LIVER ELASTOGRAPHY IN PAEDIATRIC LIVER TRANSPLANT RECIPIENTS
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Objectives and Study: Little experience exists concerning the use of transient elastography (Fibroscan) after pediatric liver transplantation. This study aims at exploring the technical feasibility of transient elastography in pediatric liver transplant recipients.

Methods: 35 pediatric liver transplant recipients (20 M, 15 F; age 0.4–18 (median 6) years) were consecutively recruited from our outpatient clinic. Fibroscan examinations were performed at up to 4 different sites in each patient. The localisation of the liver was determined by percussion and transient elastography first attempted in the highest possible intercostal space (ICS) in the anterior axillary line (AAL1). If liver size permitted, a second reading was taken one ICS below the first (AAL2). A reading was then attempted in the mid-clavicular line (MCL1), and, size permitting, a second reading was done 1 ICS below (MCL2). Measurements were judged acceptable if the ratio of interquartile range and size permitting, a second

Results: 24 (68.5%) patients had left lateral split grafts, 3 (8.5%) had right lobe grafts, and 8 (22.9 %) had full size liver grafts. Acceptable Fibroscan examinations were obtained in AAL1/AAL2/MCL1/MCL2 in 86%/71%/71%/43% of cases in full-size livers and in 75%/50%/25%/50% of patients with right lobe grafts. In contrast, in patients with left lateral split grafts, acceptable readings in AAL1/AAL2 were only obtained in 29%/12.5%, whereas acceptable readings were obtained in 58%/20.8% in MCL1/MCL2. At least one acceptable reading could be obtained in 100% of full-size liver and right lobe recipients, and 75% of left segment recipients. Reasons for no acceptable reading at all were excessive movement in small children in 2 cases, refusal to cooperate in 3 cases and midline position of the graft in 1 case. Choice of probe: Full-size recipients could be measured with their recommended probe size in 100%. Right lobe recipients needed downscaling from M to S2 probe in 33% and S2 to S1 in 66%. All left segment recipients only yielded adequate results with the S1 setting. Fibroscan results: Liver stiffness measured 9.1 (3.2–75kPa). No correlation was found between time after transplantation (0.1–13 years, median 19 months) and liver stiffness (r=0.19).

Conclusion: Transient elastography using the Fibroscan is feasible in pediatric liver transplant recipients. Choice of examination site and probe setting need to be adapted to the type of graft. Left segment recipients are more likely to yield acceptable results for examinations performed in MCL with the S1 probe.

Disclosure of Interest: None declared.

PO-H-0335

Transplantation

THE COMPARISON OF LIPID PROFILE AND OXIDATIVE STRESS MARKERS BETWEEN SIROLIMUS AND TACROLIMUS IN PAEDIATRIC RECIPIENTS AFTER LIVER TRANSPLANTATION

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Objectives and Study: Sirolimus (SRL) is an immunosuppressive drug increasingly used in children after liver transplantation. Lipid disturbances are the most frequent side effect of the drug. We compared the effect of oral administration of SRL and tacrolimus (TAC) on the lipid profile and oxidative stress markers in liver-transplanted children.

Methods: In 17 children with stable liver function who received SRL on average for 4.1 years (SD ± 2.9) and in 16 children who received TAC for 6.3 years (SD ± 2.9) the concentrations of lipids and oxidative stress markers were estimated: cholesterol (Ch), triglycerides (TG), lecithin-cholesterol acyltransferase (LCAT), apolipoprotein A-I (Apo AI), apolipoprotein B (ApoB), apolipoprotein E (ApoE), lipoprotein (α) [Lp(a)], low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), high-density lipoprotein (HDL), total cholesterol to HDL ratio (TC/HDL), reduced glutathione (GSH), glutathione peroxidase activity (GPX), oxidized low-density lipoprotein (oxyLDL) and asymmetric-dimethylarginine (ADMA).

Results: There were statistical differences between patients from SRL and TAC groups in cholesterol levels (175.7 mg/dL vs. 144.5 mg/dL, P < 0.05), TG (92.2 mg/dL vs. 144.5 mg/dL, P < 0.02), ApoAI (1.3 g/L vs. 1.5 g/L, P < 0.01), VLDL (16.9 mg/dL vs. 11.7 mg/dL, P < 0.01) and TC/HDL ratio (3.8 vs. 3.0, P < 0.01). LCAT, ApoB, ApoE, Lp(a), LDL, HDL, GSH, GPX, oxyLDL and ADMA values were similar between the groups and did not differ statistically.

Conclusion: There was a stronger influence of sirolimus on lipid profile in comparison with tacrolimus. Oxidative stress parameters were similar in both groups.

Disclosure of Interest: None declared.

PO-H-0338

Transplantation

MEDICATION EVENT MONITORING SYSTEMS (MEMS) MONITORING OF ONCE-DAILY IMMUNOSUPPRESSION FOLLOWING PEDIATRIC LIVER TRANSPLANTATION: STABLE ADHERENCE, BUT IMPROVING QUALITY OF LIFE?

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Objectives and Study: Nonadherence to immunosuppressive therapy following solid organ transplantation may lead to rejection and graft loss. The aim of this study was to compare safety (pharmacokinetics), adherence and quality of

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life in a once-daily versus a twice-daily immunosuppression protocol following pediatric liver transplantation.

Methods: All our patients post orthotopic liver transplantation (OLT) were screened for the following inclusion criteria: twice-daily tacrolimus based immunosuppression, minimum age of 10 years, no graft rejection in the previous 12 months, and at least 12 months post OLT with normal transaminases (AST below 36 U/L and ALT below 46 U/L) and normal renal function (cystatin-c based glomerular filtration rate above 90 mL/min).

Patients who met these criteria were offered conversion from twice-daily tacrolimus (Prograf) (respectively, 3 patients from cyclosporin/azathioprine) 1:1 to tacrolimus prolonged-release (Advagraf) once-daily. We identified 10 patients with twice-daily immunosuppression as controls and provided the memory device Medication Event Monitoring Systems (MEMS), measuring date and time of drug intake to each patient. Patients also answered the KIDSCREEN questionnaire for assessment of quality of life. The investigation was backed by pharmacokinetic profiles before (d0) and after conversion (day14). We also monitored liver and renal function (cystatin-c clearance), histological evidence of graft rejection in case of abnormal liver function tests and CMV status (pp65) before and after introduction of Advagraf.

Results: Of 16 potential candidates 9 patients (56%) declined the offer to participate in the study because they did not want to jeopardize their stable medical situation. Preliminary data with an observational period ranging between 3 and 12 months shows that adherence does not differ significantly between the group with once-daily immunosuppression as compared to the control group. The overall quality of life was rated similar in both groups, however patients with a once-daily tacrolimus application noted a strong preference to the new regimen. In this latter group, the area under the curve of tacrolimus concentration (0 to 12 hours) before (d0) and 14 days after the switching (d14) showed equivalent drug exposure. After conversion to a once-daily immunosuppressive regimen, liver and renal function remained stable, and no CMV infection/reactivation measured by CMV pp65 was observed.

Conclusion: Conversion of a tacrolimus based immunosuppression in stable pediatric liver transplant recipients from twice-daily to once-daily application appears safe and is not associated with graft rejection, change of liver function tests or renal function. Preliminary data suggests an improvement of quality of life.

Disclosure of Interest: None declared.

PO-H-0339

Transplantation

VITAMIN D STATUS IN CHILDREN FOLLOWING LIVER TRANSPLANTATION

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Objectives and Study: Vitamin D insufficiency is common in children with chronic liver disease, but there is scarce information on vitamin D status in children who have undergone liver transplantation.

Aim: To evaluate vitamin D status in children post liver transplantation.

Methods: Between June and December 2010 serum 25-(OH)-vitamin D levels were measured in 53 children (25 girls) aged 16 months to 19 years (median 8 years), who had undergone liver transplantation at our unit. Time post-transplantation, immunosuppression regime, use of steroids, PTH, calcium and phosphate levels, calculated glomerular filtration rate (GFR) using Schwartz formula, history of bone disease, diagnosis, ethnicity, season and vitamin D supplementation were studied as possible risk factors. Vitamin D insufficiency was defined as vitamin D level < 20 ng/mL. Results: Median 25-(OH)-vitamin D level was 16.6 ng/mL (range: 3.9 – 47.3 ng/mL), 30 children (56%) showed vitamin D insufficiency. 25-(OH)-vitamin D levels were obtained 1 month to 15 years following liver transplantation. Mean post-transplantation time for those with vitamin D insufficiency was 5 years. Current immunosuppression was prednisolone (28), tacrolimus (47), mycophenolate (15), cyclosporin (4), and sirolimus (4). Calculated GFR was determined in 41 patients. Median GFR was 139 mL/min/1.73 m² (range 55–206 mL/min/1.73 m²). Two patients had history of bone disease, 1 of them had vitamin D insufficiency. Indication for transplantation was extrahepatic biliary atresia in 60% of the patients. Ethnicity was recorded on all the patients, of whom 33 were Euro-Caucasoid. 11 children (20%) were receiving Vitamin D supplements and despite this, 3 of them had vitamin D insufficiency.

The results of the statistical analysis showed a significant relation only between vitamin D levels and PTH (p 0.009). There was no relation with any of the other studied variables.

Conclusion: Vitamin D insufficiency is common in children post liver transplantation. There was no relationship between vitamin D levels and the studied variables except for PTH levels. Nevertheless, given the clinical relevance of detecting and treating vitamin D deficiency, vitamin D levels should be measured in this population even after the first year post-transplantation.

Disclosure of Interest: None declared.

PO-H-0341

Transplantation

HISTOLOGICAL OUTCOMES FOLLOWING LIVING-RELATED LIVER TRANSPLANTATION IN CHILDREN

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Objectives and Study: We have previously reported that the percentage of children with normal liver function tests increases with time following living-related liver transplantation (LRLT). Histological analysis following paediatric cadaveric liver transplantations however has shown a progressive increase in the percentage of abnormal liver biopsies with time post-transplant, in particular those showing hepatitis and fibrosis (1). The aim of this study was to report on the histological changes seen on liver biopsies following LRLT at our centre.

Methods: One hundred and forty-nine nonprotocol liver biopsies taken from forty children following LRLT were analyzed. Biopsies were classified into normal or non-specific changes, chronic hepatitis (mild with no progressive fibrosis, with bridging fibrosis, cirrhotic), acute rejection, chronic (ductopenic) rejection, changes of biliary or vascular pathology and other pathology.

Results: The mean post-transplant biopsy time was 636 days (3–4708 days) and the mean number of biopsies per patient was 3.7 (1–18 biopsies); 13.4% of biopsies were normal or showed non-specific changes, 32.9% showed a chronic hepatitis (57.1% with no progressive fibrosis, 40.9% with bridging fibrosis and 2.0% with cirrhosis), 29.5% showed changes consistent with acute rejection, 10.1% showed biliary changes, 6% showed vascular changes and 6% showed other pathology. Chronic hepatitis was demonstrated in 28%, 43% and 35.3% of biopsies taken within 1, 5 and 10 years of LRLT respectively and bridging fibrosis in 19.2%, 62.5% and 83.3% of chronic hepatitis biopsies over the same time intervals. There were 3 cases of graft loss during the study period.

Conclusion: These findings suggest that protocol biopsies may provide further insights into the pathophysiology of graft loss. Long-term follow-up is desirable to see whether the increasing incidence of non-specific hepatitis and bridging fibrosis is associated with future graft loss.


Disclosure of Interest: None declared.

PO-H-0343

Transplantation

NUTRITIONAL RISK AND BENEFITS OF ANTHROPOMETRIC EVALUATION AFTER PEDIATRIC LIVER TRANSPLANTATION

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Objectives and Study: The aim of this study was to analyze the nutritional status of a group of pediatric patients immediately after orthotopic liver transplantation (OLT) and its relationship with their short-term clinical outcome (mortality and length of stay in the pediatric intensive care unit (PICU).

Methods: We performed the anthropometric nutritional evaluations of 60 children and adolescents after deceased donor or living-related donor OLT, during the first 24 hours in PICU of tertiary hospital, between January 2006 and December 2009. Anthropometric assessment included weight (W), height (H) or length (L), arm circumference (AC) and triceps skin fold thickness (TST). Nutritional status was determined from the z score for the following indices: W/age (A), H/A or L/A, W/H or W/L, BMI/A, AC/A and TST/A. We used reference values from the World Health Organization (WHO). The severity of liver disease was evaluated by pediatric end-stage liver disease (PELD).

Results: We found 50.0% undernutrition by H/A; 27.3% by W/A; 11.1% by W/H or W/L; 10.0% by BMI/A; 67.4% by AC/A and 51.0% by TST/A. There was no correlation between nutritional status and PELD and mortality. We found a correlation between nutritional status assessed by AC/A and length of stay of hospitalization at the PICU.

Conclusion: Children with chronic liver diseases have significant degree of undernutrition which makes nutritional support an important aspect of therapy for patients. Despite the difficulties in assessing and technical limitations, anthropometric evaluation of the upper limbs is useful for assessment of nutritional status of children before or after liver transplantation.

Disclosure of Interest: None declared.

PO-H-0344

Transplantation

PLASMA SUCCINYLACETONE IS RAISED AFTER LIVER TRANSPLANTATION FOR Tyrosinaemia TYPE 1 AND ASSOCIATED WITH REDUCED PORPHOBILINOGEN SYNTHASE ACTIVITY SUGGESTING IT IS FUNCTIONAL

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Objectives and Study: Tyrosinaemia type 1 (TT1) is a rare disorder of tyrosine metabolism leading to accumulation of toxic metabolites such as succinylacetone (SA) and a high risk of hepatocellular carcinoma. Children with TT1 traditionally required liver transplantation (OLT) and while the need for this has been reduced by the introduction of nitisinone some still go on to require OLT. Circulating SA inhibits the enzyme porphobilinogen (PBG) synthase and its activity can be used as a marker of functional circulating SA. Elevated urinary SA post OLT thought to be due to local
Production has been reported. This study describes a novel finding of elevated plasma SA following OLT for TT1.

**Methods:** A retrospective analysis was performed of patients treated for TT1 at our institution from 1989-2010.

**Results:** 13 patients underwent OLT for TT1. In patients who received nitisinone prior to OLT, mean urinary and plasma SA were elevated prior to treatment but both normalised by the time of OLT ($P < 0.05$). Mean PBG synthase activity increased from abnormally low to levels well within the normal range at the time of OLT ($P < 0.01$). Mean urinary SA in patients not treated with nitisinone was elevated prior to OLT; plasma levels and PBG synthase activity were not available prior to OLT for this group. Following OLT, mean urinary and plasma SA were elevated in all for the duration of follow up and in those treated with nitisinone PBG synthase activity fell from pre-OLT levels as plasma SA recovered.

**Conclusion:** Urinary and plasma SA levels are elevated following OLT for TT1. Low-normal PBG synthase activity suggests the circulating SA may be functional. The clinical significance of this is unclear.

**Disclosure of Interest:** None declared.

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**PO-H-0347**

**Transplantation**

**POST TRANSPLANTATION GROWTH CHANGES IN PRIMARY HYPEROXALURIA TYPE1 PATIENTS**

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**Objectives and Study:** Primary hyperoxaluria type I (PH1) is a rare autosomal recessive disorder with increased oxalate production, nephrocalcinosis, nephrolithiasis, and progressive renal failure caused by a deficiency in the liver peroxisomal enzyme, alanine-glyoxylate aminotransferase (AGT). Due to progressive renal failure and systemic oxalosis growth retardation is a major problem in these patients. Either preemptive liver transplantation (PLTX) or combined liver and kidney transplantation (LKTX) are the treatment options for this metabolic defect and its unworthy consequences. The aim of the study was a retrospective analysis of growth changes in children who underwent transplantation until the age of 18 years, as part of the treatment of PH1 between 1995 and 2009.

**Methods:** Age, sex, clinical parameters, glomerular filtration rate (GFR) and immunosuppressive treatment were retrieved. Measurements of standard deviation scores for weight (WtSDS) and height (HtSDS) were taken before transplantation and on follow up.

**Results:** Twenty one children underwent either preemptive liver (7) or liver/kidney (14) transplantation. Median age at time of surgery was 6 years (range: 1.9–12.8 years). Mean follow-up duration was 8.46 ± 3.45 years. At presentation (prior transplantation) average WtSDS was $-0.47 ± 0.81$ and average HtSDS was $-1.16 ± 0.86$. Both parameters improved at the last visit to $-0.15 ± 0.6$ and 0.86 ± 0.69, respectively, but did not reach statistical significance, most probably due to sample size. Mean GFR at the end of follow up was $83.9 ± 22.4 \text{mL/min/1.73m}^2$.

**Conclusion:** Although this is the largest cohort of transplanted patients with PH-1, we were unable to demonstrate significant improvement of growth.

**Disclosure of Interest:** None declared.

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**PO-H-0349**

**Transplantation**

**THE INCIDENCE OF TUBERCULOSIS IN PATIENTS REFERRED FOR LIVER TRANSPLANT ASSESSMENT AT RED CROSS WAR MEMORIAL CHILDREN’S HOSPITAL**

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**Objectives and Study:** To determine the incidence of tuberculosis (TB) in patients referred for liver transplant assessment over a 5-year period.

**Methods:** A retrospective review of referral forms and folders of patients referred to the Liver Transplant Clinic at Red Cross War Memorial Children’s Hospital (RXH) from June 2004 to June 2009.

**Results:** During this period 156 patients were referred for liver transplant assessment, of which 95 were seen at RXH. There were 60 female and 35 male patients. All the patients are screened for tuberculosis and 20 patients were diagnosed with TB, 16 female and 4 male. Twelve patients were started on first-line TB treatment—Rimcure and 8 patients were started on second line TB treatment. Outcome-6 patients died from chronic liver disease, not secondary to TB. Five had liver transplants, 2 patients were not accepted onto the list and 3 were deferred. The rest are on the inactive transplant list.

**Conclusion:** Although a small group of patients, the incidence of tuberculosis was 21% and the majority of patients tolerated first line TB treatment. None of the patients died secondary to TB but the fact that they were started on TB treatment does take them off the active transplant list for 6 months in patients who need a transplant urgently. These patients need frequent screening for TB as they may not present with the common symptoms of TB.

**Disclosure of Interest:** None declared.
PO-H-0350

Transplantation

TYROSINEMIA TYPE I IN THE NITISINONE ERA: WHICH FACTORS CAN PREDICT HEPATOCELULAR CANCER?
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Objectives and Study: Hereditary tyrosinemia type I (HT1) is a metabolic disorder of autosomal recessive inheritance associated with a high risk of hepatocellular carcinoma (HCC). Nitisinone treatment has transformed the outcome of HT1 but has not abolished the risk of HCC, especially if treatment is delayed. Monitoring for HCC includes serial imaging and alpha-fetoprotein (AFP) measurements. Liver transplantation is indicated for those with proven or suspected HCC. In our centre 6 children treated with Nitisinone have undergone transplant for suspected HCC which was subsequently confirmed in only one case. The aim of this study was to review the clinical, laboratory and radiological findings in children with HT1 treated with Nitisinone who underwent transplantation for suspected HCC in a single centre.

Methods: We retrospectively reviewed the records of all 6 patients. Factors analysed included age at Nitisinone treatment; duration of treatment and plasma levels; biochemical control and AFP trend; histopathology and radiological findings.

Results: All presented clinically with established liver disease. The average age at diagnosis and starting Nitisinone was 1.3 years with 2 patients starting before 6 months old. The patient with HCC started treatment at 21 months. No difference was found in the average Nitisinone levels or measures of biochemical control between the patient with HCC and the other 5 patients. In the patient with HCC, AFP normalised and showed a secondary increase. In 4 patients the level decreased but failed to normalise and 1 patient had normal levels. Radiological findings showed multinodular liver with one dominant nodule in 3 patients including the one with HCC, and a multinodular liver without a single dominant lesion in 3 patients. Histopathology of the explanted liver showed macronodular cirrhosis in 4 with hepatocyte dysplasia in 3, in 1 cirrhosis with a hepatocellular adenoma and in the last one a moderate to poorly differentiated HCC with extensive vascular invasion. The mean follow up is 7.2 years. All patients are alive. The subject with HCC had a retransplant after 9 months because of chronic rejection and then developed a lung metastasis 3 years later requiring resection.

Conclusion: Liver transplantation is very effective in HT1. In our experience the incidence of proven HCC is low in Nitisinone treated patients; however, once HCC is proven there is a risk of metastatic disease post OLT. Rising AFP is the only specific marker for established HCC. There is a need for more specific markers of HCC in patients with HT1 treated with Nitisinone.

Disclosure of Interest: None declared.

PO-H-0351

Transplantation

THE ROLE OF LIVER TRANSPLANTATION FOR UREA CYCLE DISORDER
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Objectives and Study: Urea cycle disorder (UCD) result in hyperammonemia and life-threatening illness. Although liver transplantation (LT) is a useful treatment, the indication and timing of LT is still controversial.

Methods: Ten patients with UCD underwent live donor liver transplantation at our institute. They consisted of 5 patients with ornithine transcarbamylase deficiency (OTCD, 4 males and 1 female) and 5 patients with carbamoylphosphate synthetase 1 deficiency (CPS1D, 1 male and 4 females). Their preoperative clinical course, operation, postoperative clinical course, and outcome were reviewed. The median follow-up period was 1.0 year (range: 3 months – 2 years).

Results: Nine patients (90.0%) were categorized into neonatal-onset UCDs. Seven patients (70.0%) required hemodialysis before LT, and 6 patients (60.0%) developed neurological impairments. The age at LT ranged from 3 months to 2 years and 9 months (median: 6 months). They received 7 left lateral segments and 3 reduced left lateral segments as a graft from their parents. Three patients (30.0%) suffered from surgical complications, consisting of portal stenosis, biliary stricture, and biliary leakage. All patients survived without any episode of hyperammonemia after LT.

Conclusion: Prompt recognition and management for UCD are needed to minimize neurological impairments before LT. Liver transplantation should be considered as a curative therapy for UCD at an early age.

Disclosure of Interest: None declared.

PO-N-0180

Clinical Nutrition

EVALUATION OF HEART FUNCTION IN CHILDREN WITH INTESTINAL FAILURE ON LONG-TERM PARENTERAL NUTRITION
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Objectives and Study: Children with intestinal failure receiving long-term parenteral nutrition have several risk factors potentially deteriorating heart function, such as volume overload, the presence of permanent central catheter with the tip in right atrium, recurrent anemia, carnitine

Disclosure of Interest: None declared.
deficiency, and often, catheter-related sepsis in medical history.

Methods: The aim of this cross-sectional study was to evaluate heart function in children on long-term parenteral nutrition with the use of echocardiographic cardiac function and anatomy parameters such as: IVSd (thickness of interventricular septum at end diastole); LVEDD (left ventricular end diastolic dimension); LVESD (left ventricular end systolic dimension); LVPWd (left ventricular posterior wall thickness at end diastole); SF-shortening fraction, E/A wave ratio and cardiac-thoracic index assessed on a chest x-ray. Results were compared to normal values determined by epidemiological studies with respect to body surface area (1). Sixteen children with intestinal failure in the analyzed group aged 0.25–7.25 year (mean: 2.5 year) received parenteral nutrition for average 2.3 year. Children with known cardiac disease or other diseases with potential influence on heart function were excluded.

Results: Mean centiles of measured parameters were respectively: LVEDD – 0.52, LVESD-0.44, IVSd-0.66, LVPWd–0.54. Mean shortening fraction was 38% (normal values: 28–44%), E/A wave ratio – 1.44 (normal values: 1–2) and cardiac-thoracic index 0.53 (normal value <0.5).

Conclusion: Systolic and diastolic heart function assessed by shortening fraction and E/A wave ratio were in the normal values. Thickness of intraventricular septum at end diastole and cardiac-thoracic index were augmented. Long-term parenteral nutrition might influence cardiac function and anatomy, represented mainly by intraventricular septum hypertrophy. Further prospective studies based on larger groups are needed.

Reference:

Disclosure of Interest: None declared.

PO-N-0181

Clinical Nutrition

THE USEFULNESS OF WAIST CIRCUMFERENCE TO HEIGHT RATIO IN SCREENING OF OBESITY IN KOREAN CHILDREN AND ADOLESCENTS

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Objectives and Study: The obesity of childhood and adolescents has become increasingly common in recent decades. Appropriate early diagnosis and intervention are important to reduce the risk of obesity related disorders. Body mass index (BMI) has become a tool in assessing obesity. But, for parents and other non-professionals, complicated variables such as age, sex, and ethnic specific standards have made the use of BMI less feasible. The present study aims to evaluate the feasibility and usefulness of waist circumference to height ratio (WHTR) and propose the optimal cutoff values of WHTR in the screening of obesity in Korean children and adolescents and to contribute to the information comparing the feasibility of WHTR to BMI.

Methods: The data including BMI, waist circumference (WC) and height were obtained from the national growth surveys for children and adolescents in 2005. Overweight and obesity were determined by BMI for age and sex, and WHTR was calculated by WC divided by height in 57,819 boys, 53,700 girls aged 2–18 years old. The influence of age on the WHTR was analyzed by SAS. The receiver operating characteristic (ROC) analyses were performed to find out the optimal cutoff values of WHTR that could match up with BMI determined overweight and obesity using a STATA program. The area under curve (AUC), a measure of diagnostic power, of WHTR was compared to that of WC.

Results: Subjects were divided into 25,223 boys and 22,778 girls, with an age between 2 and 5 years old, and 32,596 boys and 30,922 girls, with an age between 6 and 18 years old. In both sexes, WHTR sharply decreased with age up to 5 years, and then changed little further. Especially, WHTR showed less correlation with age in the group 6–18 years of age than the group 2–5 years of age. Furthermore, WHTR also showed less correlation with age than WC in the group of 6–18 years of age. The AUC of WHTR in identifying overweight and obesity were significantly higher than WC in those 6–18 years old. The optimal cutoff values were 0.51 in boys, 0.49 in girls for obesity, 0.48 in boys, 0.47 in girls for overweight, with all having the AUC>0.9. The optimal cutoff values of WHTR had a higher sensitivity for diagnosing obesity than WC ≥90 percentiles.

Conclusion: WHTR is a simple, accurate, and less age-dependent index with high applicability in screening for being overweight and obesity in children and adolescents. Further research is warranted to determine the optimal cutoff values to predict metabolic syndrome or cardiovascular disease risks.

Disclosure of Interest: None declared.

PO-N-0182

Clinical Nutrition

LONG-CHAIN POLYUNSATURATED FATTY ACIDS IN PHENYLKETONURIA: A SYSTEMATIC REVIEW

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Objectives and Study: The treatment of children with phenylketonuria (PKU) is mainly based on restricted dietary intake of phenylalanine (Phe)-containing foods. However, dietary protein restriction may not only reduce Phe intake, but may be associated with low intake of long-chain polyunsaturated fatty acids (LCPUFAs) as well. This systematic review focuses on the consequences of dietary restriction in PKU on the bioavailability of LCPUFAs.

Methods: We searched Ovid MEDLINE, EMBASE, SCOPUS, CINAHL, LILACS and Cochrane Library CENTRAL databases from inception to December 2010.
We used formal inclusion/exclusion criteria and applied standard operation procedures for data extraction, quality assessment and meta-analysis.

**Results:** We identified 8 case-control studies (divided into 12 arms) investigating LCPUFA status in patients with PKU, and 5 randomised controlled trials (RCTs) reporting effect of LCPUFA supplementation to the diet of patients with PKU. We did not find any difference in the n-6 essential fatty acid, linoleic acid status, and in the n-3 essential fatty acid, α-linolenic acid status between patients and healthy controls. In contrast, values of the principal n-6 LCPUFA, arachidonic acid (AA) were significantly lower in total plasma lipids (10 studies with 533 participants, pooled effect: −1.07 [−1.57, −0.56], %wt/wt, mean [95% CI]), whereas in the other four biomarkers (plasma phospholipids, total erythrocyte lipids, erythrocyte phospholipids and plasma cholesteryl esters) AA levels did not differ in patients and controls. Even more marked reduction was observed in the values of the principal n-3 LCPUFA, docosahexaenoic acid (DHA) in patients with PKU: DHA status was significantly lower in patients compared to healthy controls in all the lipid classes investigated (Table). At the end of the intervention, patients receiving LCPUFA supplementation showed significantly higher DHA values than patients receiving placebo in all 5 RCTs. The greatest effect was seen in the total plasma DHA level (2.94 ± 0.88 vs. 0.73 ± 0.88 % wt/wt, mean ± SD, treatment group n = 10 vs. placebo group n = 11). None of the studies reported any adverse reactions to LCPUFA supplementation.

**Table.** Case-control studies on docosahexaenoic acid (DHA) status in phenylketonuria

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>No. studies</th>
<th>Pooled effect size</th>
<th>%wt/wt, mean, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total plasma DHA</td>
<td>10 studies; n = 533</td>
<td>−0.91 [−1.32, −0.49]</td>
<td></td>
</tr>
<tr>
<td>Plasma phospholipid DHA</td>
<td>6 studies; n = 268</td>
<td>−1.34 [−1.72, −0.96]</td>
<td></td>
</tr>
<tr>
<td>Total erythrocyte DHA</td>
<td>6 studies; n = 285</td>
<td>−0.66 [−1.28, −0.04]</td>
<td></td>
</tr>
<tr>
<td>Erythrocyte phospholipid DHA</td>
<td>2 studies; n = 107</td>
<td>−1.39 [−1.70, −1.08]</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusion:** In observational studies, patients suffering from PKU showed lower contribution of DHA to the fatty acid composition of various plasma and erythrocyte membrane lipids than healthy controls. In RCTs, LCPUFA supplementation effectively improved DHA status without detectable adverse reactions.

**Disclosure of Interest:** None declared.

**PO-N-0190**

**Nutrition, Metabolism, and Experimental Approaches**

**EVALUATION OF LIVING DONOR LIVER TRANSPLANTATION FOR PATIENTS WITH METHYL MALONIC ACIDEMIA**

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**Objectives and Study:** Methylmalonic acidemia (MMAemia) is inborn error (autosomal recessive) caused by deficiency of Methylmalonyl-CoA mutase. These errors result in metabolic acidosis, developmental delay, renal insufficiency, failure to thrive, and often carry a poor prognosis. The survival is less than 30% during first five years of life. Recently, liver transplantation is indicated for MMAemia to decrease the incidence of metabolic acidosis. The aim of this study is to evaluate efficiency of living donor liver transplantation (LDLT), we reviewed our 10 cases of MMAemia patients with LDLT in National Children’s Hospital.

**Methods:** Between November 2005 and December 2010, we performed LDLT for 147 pediatric patients. Of these 147, 10 patients underwent LDLT for vitamin B12-unresponsive MMAemia (all patients were confirmed to have mut8 by gene analysis). We reviewed the clinical records of these 10 patients to collect the following data: age of onset, age at LDLT, preoperative 24-h creatinine clearance (mL/min), chronological changes in serum levels of MMA (nmol/mL) and urinary levels of MMA (mmol/mol Cr) in the perioperative period.

**Results:** The patients’ age varies from 7 months to 7 years, there are 4 girls and 6 boys, and younger 3 patients received preemptive LDLT without recurrent acidosis. Growth retardation was seen in all patients. Renal dysfunction was seen in 1 patient. All patients received strict protein restriction before and after LDLT. Median follow-up period was 3.5 years. Six patients had viral infection and 2 patients with severe acidosis after liver transplantation. One patient died from severe metabolic acidosis following rejection and sepsis. Nine patients are doing well with protein restriction and carnitin supplementation. Both serum and urine MMA level was reduced to 10% after LDLT in all the patients, but however, they were not normalized even 3 years after LDLT. Pre- and post-operative concentration of MMA in cerebrospinal fluid which showed no significant change. Changes in IQ level pre- and post-operation also showed no significant improvement in all the patients. But in our cases, older patient not always showed severe neurological disability. All survival patients start oral intake 2 months after transplantation and they are freed from feeding tube. Hospital admission was dramatically decreased after LDLT in all patients.

**Conclusion:** LDLT for MMAemia is not a curative operation, however, LDLT can avoid lethal acidosis and achieve improved oral food intake and quality of life.

**Disclosure of Interest:** None declared.

**PO-N-0194**

**Nutrition, Metabolism and Experimental Approaches**

**FATTY ACIDS IN BREAST MILK AND IN BABIES PLASMA IN MATERNAL OBESITY**

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Conclusion: Obese mothers had lower w3 fatty acids in breast milk, and conventional intervention improved the w6/w3 balance towards that seen in normal-weight mothers. BMI limiting weight increase during pregnancy to 6 kg or less. Mothers participated in an intervention program aimed at preventing gestational weight gain with low-calorie diet and physical activity with emphasis on fat composition.

Methods: Fatty acids in infant plasma phospholipids at 3 days after birth and in breast milk were analyzed. Breast milk samples at 3 days, 10 days, 1 and 2 months after delivery were analyzed. Fatty acids were extracted by capillary gas chromatography.

Results: No differences were found in length of gestation, breast-feeding or birth weight of infants between the different groups. BMI at 1 year did not differ. In babies plasma phospholipids, there were significantly lower concentrations of eicosapentaenoic acid (20:5w3 EPA) in the obesity control group than in BMI <25 control group (P < 0.01) and intervention group (P < 0.05). In breast milk, no difference was found regarding saturated and monounsaturated fatty acids. The DHA concentration decreased during the first months of postnatal life. Objectives and Study: Low w3 fatty acids have been associated with obesity. Uncontrolled experimental research long-term effects are obtained in the offspring related to the balance between w6/w3 during pregnancy and early postnatal life. Objective is to study fatty acid composition in breast milk in non-diabetic obese women and in plasma phospholipids of their children. 70 obese (BMI >30) and 41 normal-weight (BMI <25) were included. 29 of the 70 obese mothers participated in an intervention program aimed at limiting weight increase during pregnancy to 6 kg or less. Intervention was low-calorie diet and physical activity with emphasis on fat composition.

Disclosure of Interest: None declared.
Results: Fasting induced a significant ($P < 0.05$) increase of the Overall Index of the CPT II (a global index of inattention) and the TOMAL Visual Selective Reminding (a test of verbal memory), whereas no changes were found with breakfast consumption. Fasting was associated with a reduction in insulin and an increase of glucagon without changes of glucose. The increase of inattention was associated with a reduction of carbohydrate oxidation ($r = -0.66$, $P < 0.05$), an indirect index of brain metabolic activity. The AUC of PYY and GLP-1 were not different after breakfast than after fasting whereas that of Ghrelin was significantly lower. No association between postprandial hormones variation and cognitive performance was found.

Conclusion: Maintenance of carbohydrate oxidation by adequate carbohydrate intake at breakfast may play a role in maintaining attention and visual memory performance in the morning.

Disclosure of Interest: None declared.

Table: Fatty acid composition of plasma phospholipids in expecting women.

<table>
<thead>
<tr>
<th>w/w%</th>
<th>First trimester</th>
<th>Second trimester</th>
<th>Third trimester</th>
<th>Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[n = 7; m = 7271]</td>
<td>[n = 13; m = 1333]</td>
<td>[n = 25; m = 2184]</td>
<td>[n = 23; m = 1736]</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>9.28 (9.18–9.38)</td>
<td>8.80 (8.53–9.06)</td>
<td>8.48 (8.06–8.91)</td>
<td>8.54 (7.57–9.52)</td>
</tr>
<tr>
<td></td>
<td>[n = 8; m = 7280]</td>
<td>[n = 13; m = 1236]</td>
<td>[n = 26; m = 2135]</td>
<td>[n = 24; m = 1915]</td>
</tr>
<tr>
<td>Alpha-linolenic acid</td>
<td>0.18 (0.18–0.19)</td>
<td>0.26 (0.24–0.28)</td>
<td>0.29 (0.24–0.34)</td>
<td>0.25 (0.18–0.32)</td>
</tr>
<tr>
<td></td>
<td>[n = 5; m = 3997]</td>
<td>[n = 9; m = 1169]</td>
<td>[n = 18; m = 1831]</td>
<td>[n = 16; m = 1609]</td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>4.75 (4.69–4.81)</td>
<td>4.52 (4.2–4.71)</td>
<td>4.88 (4.55–5.21)</td>
<td>3.85 (3.36–4.34)</td>
</tr>
<tr>
<td></td>
<td>[n = 7; m = 7271]</td>
<td>[n = 14; m = 1750]</td>
<td>[n = 27; m = 2411]</td>
<td>[n = 27; m = 2202]</td>
</tr>
</tbody>
</table>

References:

Disclosure of Interest: None declared.

PO-N-0198

Nutrition, Metabolism, and Experimental Approaches
PROTEIN DIGESTION CHARACTERISTICS OF HUMAN BREAST MILK IN A DYNAMIC MODEL OF THE GASTROINTESTINAL TRACT

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Objectives and Study: Human breast milk (HBM) is the optimal nutrition for neonates. Therefore, it is considered the reference in the development of infant formula (IF) with the aim to mimic its composition and functionality as close as possible. The postprandial amino acid (AA) kinetics in plasma have been reported to differ between HBM and IF [1]. This is hypothesized to be based on differences in the dynamics of luminal gastrointestinal (GI) digestion and absorption. The objective of this study was thus the design of an in vitro approach in order to investigate protein digestion and absorption kinetics of HBM in young infants.

Table: Fatty acid composition of plasma phospholipids in expecting women.
**Methods:** Pooled HBM from healthy volunteers was digested in vitro in the dynamic TNO intestinal model (TIM-1). TIM-1 is an advanced, computer-controlled, multicompartmental system for the simulation of digestive processes in the GI tract. It enables close emulation of the gastric and small intestinal environment in vitro under different physiological conditions. Based on in vivo data, the model’s settings (gastric pH kinetics, digestive enzyme loads) were adjusted to mimic the digestive conditions of babies below 6 months of age. As a novelty, the hollow fibre membranes (molecular cutoff 10 kD) which allow permeation of digestion products by means of dialysis in the system’s jejunal and ileal compartments were replaced by membranes with a cutoff of 1 kD mimicking the in vivo conditions after pancreatic digestion. The functionality of the new membranes was extensively characterized and validated. Over a course of 6 hours of HBM digestion, hourly samples of membrane dialysates and ileal delivery were dated. Over a course of 6 hours of HBM digestion, hourly samples of membrane dialysates and ileal delivery were analysed for free and peptide bound AAs by means of acid hydrolysis and HPLC quantification.

**Results:** Absorption kinetics of digestion products found in the dialysate followed a Gaussian distribution. The absorption maxima after HBM ingestion were detected after two hours in the system’s jejunal compartment and three hours in the ileal compartment. During six hours of digestion, 42.0±0.1% of total dietary AAs were retrieved from the jejunal compartment and 21.6±0.7% from the ileal compartment. The total individual amino acid absorption varied significantly by more than a twofold. The highest absorption was found for tyrosine and the lowest for serine. Of the measured essential amino acids, the total absorption was the lowest for threonine.

**Conclusion:** Novel insights into the kinetics of the generation of protein digestion products of human breast milk in time and space were gained. The applied modifications render the TIM-1 system a versatile tool for the investigation of the particular GI digestion and absorption conditions in infants.

**Disclosure of Interest:** None declared.

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**PO-N-0202**

**Nutrition, Metabolism, and Experimental Approaches**

**FATTY ACID COMPOSITION OF PARENTERAL LIPIDS DIRECTLY AFFECTS THE FATTY ACID COMPOSITION OF RED BLOOD CELLS AND NEURONAL TISSUES IN PRETERM PIGS**

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**Objectives and Study:** Studies in enterally-fed infants have shown a positive effect of n-3 long-chain polyunsaturated fatty acid (LCPUFA) supplementation on neurodevelopment. The effect of n-3 LCPUFA in fish oil-based parenteral (PN) lipid emulsions on neuronal tissues of PN-fed preterm infants is unknown.

**Objective:** Test whether different PN lipid emulsions directly modify the tissue composition and metabolism of lipids in preterm pigs.

**Methods:** Preterm pigs bearing venous and arterial catheters received 1 of 4 treatments (7–14 pigs/group): PN+soybean oil (Intralipid, IL), PN+fish oil (Omegaven, OV), PN+oil mixture w/soybean (30%)-coconut (30%)-olive (25%)-fish (15%) (SMOF, SL), or milk formula fed enterally (EN). On d11 pigs were subjected to 13C-palmitate oxidation and whole-body respiratory calorimetry measures; on d14 blood and tissues were collected for analysis of lipid composition.

**Results:** Heat and 13CO2 production from 13C palmitate, respiratory quotient (RQ), serum cholesterol, triglycerides, and VLDL were not different between PN groups. FA proportions at d14 are shown in the Table.

**Table.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(20:4)n-6</th>
<th>(20:5)n-3</th>
<th>(22:6)n-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake (mg/kg)</td>
<td>IL 50</td>
<td>1050</td>
<td>1250</td>
</tr>
<tr>
<td></td>
<td>OV 60</td>
<td>1050</td>
<td>1250</td>
</tr>
<tr>
<td></td>
<td>SL 50</td>
<td>235</td>
<td>220</td>
</tr>
<tr>
<td></td>
<td>EN NP</td>
<td>NP</td>
<td>NP</td>
</tr>
<tr>
<td>RBC* (mol%)</td>
<td>IL 5.96</td>
<td>0.06ab</td>
<td>2.49bc</td>
</tr>
<tr>
<td></td>
<td>OV 4.46</td>
<td>3.74bc</td>
<td>6.11bc</td>
</tr>
<tr>
<td></td>
<td>SL 5.10</td>
<td>1.20bc</td>
<td>4.45bc</td>
</tr>
<tr>
<td></td>
<td>EN 4.58</td>
<td>0.05bc</td>
<td>0.92bc</td>
</tr>
<tr>
<td>Brain* (mol%)</td>
<td>IL 9.83a</td>
<td>0.02ab</td>
<td>8.61bc</td>
</tr>
<tr>
<td></td>
<td>OV 8.17c</td>
<td>0.54bc</td>
<td>12.12bc</td>
</tr>
<tr>
<td></td>
<td>SL 8.71c</td>
<td>0.19bc</td>
<td>10.08bc</td>
</tr>
<tr>
<td>Retina* (mol%)</td>
<td>IL 5.75</td>
<td>0.10bc</td>
<td>9.95bc</td>
</tr>
<tr>
<td></td>
<td>OV 5.61</td>
<td>0.81bc</td>
<td>14.93bc</td>
</tr>
<tr>
<td></td>
<td>SL 6.19</td>
<td>0.12bc</td>
<td>12.17bc</td>
</tr>
<tr>
<td></td>
<td>EN 6.95</td>
<td>0.00</td>
<td>10.87bc</td>
</tr>
</tbody>
</table>

NP: not provided; *means for LCPUFA within tissue; a,b,c significantly different from OV, SL, EN resp., P < 0.05.

**Conclusion:** Parenteral infusion of fish oil-containing lipid emulsions for 14 days results in higher proportions of n-3 LCPUFA in blood, brain and retina of preterm pigs. Lipid metabolism was not affected by the different lipid emulsions. The enrichment of tissue with n-3 LCPUFA may have beneficial metabolic and functional actions in preterm infants.

**Disclosure of Interest:** H. Vlaardingerbroek: None declared, B. Stoll: None declared, W. Heird: None declared, J. Van Goudoever: None declared, O. Olutoye: None declared, D. Burrin Grant / Resarch Support from: Fresenius Kabi, Germany provided lipid emulsions. No other support received.
PO-N-0203

Nutrition, Metabolism, and Experimental Approaches CAN EARLY LIPID ADMINISTRATION INCREASE PROTEIN SYNTHESIS IN PREMATURE INFANTS?
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Objectives and Study: Nowadays, parental glucose and amino acids (AA) are routinely initiated from birth onwards in premature neonates. However, lipids are frequently started later or in low amounts, resulting in low energy intakes. Optimizing energy intake might enhance anabolism further, but risk of hyperglycemia prevents administration of higher amounts of glucose. We hypothesized that protein synthesis rates increase upon administration of additional energy via parenteral lipids from birth onwards in premature infants.

Objective: To quantify protein metabolism using stable isotope techniques in premature infants receiving different caloric intakes but similar amounts of AAs.

Methods: From birth onwards premature infants received glucose and 2.4 g AA/(kg/d), supplemented with lipids in a dose of either 0.5–1 g/(kg/d) on day 1 and 2 (control group, C), or 2–3 g/(kg/d) on day 1 and 2 (lipid group, L). On the second day of life, primed, continuous infusions of [1– 13C]phenylalanine and [ring-D 4]tyrosine were administered. Phenylalanine (phe) and tyrosine (tyr) enrichments in plasma were measured at steady state using mass spectrometry techniques. Protein breakdown, synthesis, and hydroxylation rates were calculated from phe and tyr fluxes. Data of this ongoing trial are presented as median (min-max).

Results: The nutritional intake on day 2 of twelve infants (gestational age 26 (24–32) wks and birth weight 810 (593–1240) g, not significantly different between groups) is presented in the Table.

Table.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Lipid group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (g/(kg/d))</td>
<td>8.9 (7.9–9.8)</td>
<td>9.1 (5.7–12)</td>
</tr>
<tr>
<td>Amino acids (g/(kg/d))</td>
<td>2.4 (1.6–2.4)</td>
<td>2.5 (2–3.3)</td>
</tr>
<tr>
<td>Lipid (g/(kg/d))</td>
<td>0.7 (0.6–1.6)</td>
<td>2.8 (1.9–5)</td>
</tr>
<tr>
<td>Energy intake (kcal/(kg/d))</td>
<td>51 (46–60)</td>
<td>75 (56–95)</td>
</tr>
</tbody>
</table>

Conclusion: Increasing energy intake with parenteral lipids directly following birth does not seem to affect protein synthesis.

Disclosure of Interest: None declared.

PO-N-0205

Nutrition, Metabolism, and Experimental Approaches INFANT DIETARY PATTERNS AND FUNCTIONAL CONSTIPATION IN CHILDHOOD: THE GENERATION R STUDY
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Objectives and Study: The influence of infant nutrition on the development of constipation beyond the period of weaning and breast-feeding is relatively understudied. Since the last few years a new approach within nutritional research has been developed by using dietary patterns analysis which takes into account that food products can be highly correlated. The aim of this study was to determine whether common dietary patterns during infancy are associated with functional constipation in childhood.

Methods: This study was embedded in the Generation R study, a population-based prospective cohort study from foetal life onwards. Information on dietary intake was obtained by food frequency questionnaire between 12 and 24 months (N=2420). Accordingly, the adherence score on a Prudent and Western diet was extracted from principal component analysis. The Prudent pattern was characterized by high intakes of fruits, vegetables, legumes and fish; the Western pattern was characterized by high intakes of snacks, confectionary and sugar containing beverages. Functional constipation was defined by questionnaire according to the Rome II criteria at the age of 24 months.

Results: At the age of 24 months, 8% of the children had functional constipation. High adherence to a Prudent diet was significantly associated with a lower prevalence of functional constipation in childhood after adjustment for potential confounders (Table). High adherence to the Western diet did not appear to be significantly associated with functional constipation.

Table: Adherence to a Prudent and Western dietary pattern and functional constipation in Dutch infants

<table>
<thead>
<tr>
<th></th>
<th>Univariate model</th>
<th>Multivariate model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95%CI)</td>
<td>OR (95%CI)**</td>
</tr>
<tr>
<td>Prudent diet score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>High</td>
<td>0.67 (0.47–0.97)*</td>
<td>0.66 (0.44–0.98)*</td>
</tr>
<tr>
<td>Western diet score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>High</td>
<td>1.23 (0.84–1.79)</td>
<td>1.29 (0.85–1.96)</td>
</tr>
</tbody>
</table>

OR: odds ratio; 95% CI: 95% confidence interval; *P < 0.05; **adjusted for total energy intake, timing of introduction of solids, duration of breast-feeding, maternal history of intestinal disorders, maternal smoking, maternal alcohol consumption, maternal BMI, maternal educational background, birthweight, gestational age and infant history of food allergy.

Conclusion: These results suggest that high adherence to a Prudent diet during infancy is independently associated with
a lower prevalence of functional constipation in childhood. No support was found for an association between a Western dietary pattern and functional constipation.

Disclosure of Interest: None declared.

PO-N-0206

Nutrition, Metabolism, and Experimental Approaches

EFFECT OF ORAL SPECIFIC PREBIOTICS IN CHILDREN ON LONG-TERM PARENTERAL NUTRITION: RESULTS OF A RANDOMIZED DOUBLE-BLIND PLACEBO CONTROLLED CROSS-OVER TRIAL


Objectives and Study: A beneficial effect of specific prebiotics on the gut microbiota and on mucosal immunity has been demonstrated in healthy infants. The study was designed to identify such an effect by clinical markers in children under long-term parenteral nutrition.

Methods: Nineteen patients under long-term parenteral nutrition between 1 and 13 years of age participated in a double blind, placebo controlled, and randomized clinical trial. In a crossover design the children were given a prebiotic mixture and maltodextrine as placebo orally for 6 months each. The prebiotic mixture contained short-chain galacto-oligosaccharides, long-chain fructo-oligosaccharides and acidic oligosaccharides produced from food grade pectin (scGOS/lfGOS/pGOS). Blood and stool samples were taken every 3 months and a questionnaire was to be completed by the parents. Thirteen patients completed the study.

Results: In stool samples more Bifidobacteria (32% versus 17% under placebo) and higher amount of D-lactic acid (11.6 mmol/kg faeces versus 5.7 mmol/kg) were found under specific prebiotics. The inflammation marker CRP in blood was lower under prebiotics (1.3 mg/dL versus 6.5 mg/dL). Parents reported more often diarrhoea-free periods (34% versus 15% under placebo) and considered their children’s health status more often as “better.” Prebiotic supplementation was also associated with a significantly reduced frequency of antibiotic medications; 39% of the patients under prebiotic intake had to take antibiotics versus 78% under giving the placebo. No clinically manifest D-lactic acidosis was reported under specific prebiotics.

Conclusion: For children under long-term parenteral nutrition oral supplementation with prebiotics could be a reasonable step to prevent infections, reduce diarrhoea frequency and promote wellbeing. Attention should be paid to the risk of D-lactic acidosis although it was not observed in this study. Given the high mortality of sepsis in patients under parenteral nutrition the use of prebiotics is a simple, cheap and low-risk intervention.

Disclosure of Interest: J. Heller Industry of: Danone Research, M. Krawinkel: None declared.

PO-N-0207

Nutrition, Metabolism, and Experimental Approaches

ADIPOPHILIN LEVELS IN HUMAN COLOSTRUM AND FULL BREAST MILK

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Objectives and Study: Adipophilin is a major constituent of the breast milk (BM) lipid globule surface and was previously described in cow’s and goat’s milk. The aim of our study was to detect and analyze adipophilin concentrations in human BM during 12 months of lactation.

Methods: Adipophilin levels were determined using high sensitive ELISA method (Biovendor) in colostrum (D0) and BM of 72 healthy mothers after uncomplicated delivery. BM samples were collected in 1, 3, 6, and 12 months of lactation (M1, 3, 6, 12).

Results: Mean adipophilin levels in D0 were 1.98 ± 0.12, in M1 2.83 ± 0.21, in M3 2.39 ± 0.17, in M6 2.57 ± 0.16, and in M12 3.25 ± 0.21 µg/mL. Significantly higher levels of adipophilin were found in M1 and M12 when compared to D0 and in M12 when compared to M3 (overall P = 0.0001). Trend for adipophilin levels was intradividually highly conserved from M1 onwards throughout the whole lactation. Significant positive correlation between adipophilin levels was observed at M1 and M3 (r = 0.3091; P = 0.0103). Moreover, adipophilin levels at M3 correlated with levels at M6 (r = 0.2739; P = 0.0227) and M12 (r = 0.4476; P = 0.0043) and levels at M6 also correlated with levels at M12 (r = 0.3699; P = 0.0173). Adipophilin levels at M6 correlated negatively with birth weight of infants (r = −0.3066; P = 0.0083) and birth length of infants (r = −0.36; P = 0.0018). There was no other correlation throughout the lactation between BM levels of adipophilin and body weight of infants, their birth length, body weight gain during the first year of life or BMI of mothers before pregnancy.

Conclusion: Adipophilin was detectable in human BM during the whole 12-month lactation period. Higher levels in M12 might be caused by longer intervals between breastfeeding due to introduction of complementary food.

Disclosure of Interest: None declared.

PO-N-0209

Nutrition, Metabolism, and Experimental Approaches

RELATION BETWEEN KIND OF FEEDING AND ADIPOnectin CONCENTRATIONS IN THE FIRST MONTHS OF LIFE

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Objectives and Study: Adiponectin, an adipocyte-secreted hormone, regulates lipid and glucose metabolism and exerts anti-inflammatory and antiatherogenic effects. Adiponectin, such as others adipokines is present in cord blood and it has been also found in breast milk (BM); these findings suggest that the hormone is involved in the regulation of infant nutritional status and metabolic development. However its physiological role in early infancy has not been completely elucidated. The aim of the study was to evaluate adiponectin concentrationin the first six months of lifeand to compare hormone concentration in breast-fed (BF) and formula-fed (FF) infants. We also determined adiponectin level in serum of lactating mothers and in breast milk (BM) and we investigated the relationship between adiponectin in infants’ and mothers’ serum and in BM.

Methods: We enrolled 85 AGA healthy infants less than 6 months of age, of which 60 exclusively BF and their lactating mothers and 25 FF. Adiponectin has been determined by RIA test in serum and by ELISA test in BM. Statistical analysis: Mann-Whitney test and Spearman correlation; statistical significance was set at P < 0.05.

Results: Median (interquartile range) serum adiponectin concentration in infants was 38.48 ± 22.48 μg/mL; in BF infants (n = 60) was 60.49 (29.07) μg/mL and in FF infants (n = 25) 58.96 (28.43) μg/mL. The median (IR) of serum adiponectin in mothers (n = 38) was 21.14 (19.01) μg/mL and in BM (n = 46) was 9.99 (7.05) ng/mL. We did not observe statistically significant differences between adiponectin concentration in BF and FF infants. Positive correlations were observed between BF infants’ serum adiponectin concentration and hormone values in BM (P = 0.015; r = 0.374) and between serum adiponectin in mothers and in BM (P = 0.001; r = 0.6).

Conclusion: This study presents data of adiponectin levels in BM samples and its correlation with adiponectin concentration in serum of infants and lactating mothers. In our research no significant difference in adiponectin concentration has been shown between BF and FF infants. We confirmed the presence of adiponectin in BM at a lower concentration than that found in infants serum, in accordance with literature (1,2). We observed a positive correlation between serum adiponectin levels in BM and BF infants’ serum. Based on these data, adiponectin levels in infants’ serum may be influenced by those in BM and then we hypothesize that adiponectin in the BM could be absorbed from the gastrointestinal tract of infants and exert its metabolic function in infant growth and development.


Disclosure of Interest: None declared.

PO-N-0212

Nutrition, Metabolism, and Experimental Approaches

INCREASED INTESTINAL CHOLESTEROL EXCRETION IN MICE BY DIETARY MANIPULATION

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Objectives and Study: Augmentation of fecal cholesterol excretion by dietary means could be of therapeutic value, for example in patients with hypercholesterolemia. We previously showed in mice that diet-induced essential fatty acid (EFA) deficiency altered the small intestinal function, as demonstrated by fat malabsorption. We determined cholesterol excretion in diet-induced EFA deficiency in mice, to test the principle that fecal excretion of cholesterol can be manipulated by dietary means.

Methods: EFA deficiency was induced in mice by feeding an EFA-deficient diet during 8 weeks. We determined body weight, dietary intake and dietary, biliary, intestinal and fecal cholesterol levels.

Results: Dietary cholesterol intake and biliary cholesterol secretion were similar in EFA-deficient and control mice (0.2 and ~3 μmol.100g BW−1.day−1, respectively). EFA-deficiency significantly increased fecal cholesterol excretion, compared with control conditions (8.1 ± 1.6 versus 4.5 ± 1.0 μmol.100g BW−1.day−1, P < 0.01). In control mice, the amount of fecal cholesterol excretion was similar to the sum of biliary and dietary cholesterol input, indicating a net intestinal cholesterol balance. In EFA-deficient mice, however, the amount of fecal cholesterol excretion was profoundly higher than the sum of biliary and dietary cholesterol input (2.5 μmol.100g BW−1.day−1, P < 0.05), indicating non-hepatobiliary cholesterol secretion into the intestinal lumen. In EFA-deficient mice, cholesterol amounts recovered from the intestinal lumen were increased by 100% in the middle part of the small intestine, compared with controls (P = 0.01).

Conclusion: Our data show that fecal excretion of cholesterol can be manipulated by dietary means. Induction of intestinal cholesterol secretion by dietary means could represent an attractive target in prevention and treatment of hypercholesterolemia and thereby cardiovascular disease later in life.

Disclosure of Interest: None declared.

PO-N-0214

Nutrition, Metabolism, and Experimental Approaches

CHEEK CELL GLYCEROPHOSPHOLIPIDS: A NONINVASIVE MARKER FOR THE OMEGA-3 STATUS IN HUMANS

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Objectives and Study: Fatty acid status is commonly determined in red blood cells and plasma phospholipids,
which requires invasive blood sampling. Cheek cell phospholipids have been suggested as non-invasive marker for the dietary fatty acid uptake. Published cheek cell fatty acid analysis methods differ widely and methodological reliability or precision data are not available. Aim of this study was to develop and validate a method for the fatty acid analysis of cheek cell glycerophospholipids (GPs).

**Methods:** A high-throughput method for plasma GP fatty acid analysis was adapted for cheek cell GP profiling. Polar lipids were extracted with methanol followed by selective base catalysed synthesis of fatty acid methyl esters from GPs. Inter batch precision was determined, minimally required sample size was identified and the effect of contaminations was evaluated. The method was validated with plasma and cheek cell samples obtained from participants of a life quality study by correlation analysis. Cheek cell samples of newborns were collected and analysed to evaluate the applicability in infants.

**Results:** Inter coefficients of variation in samples with 8.5x10^5 cells ranged from 1.1% (C18:1n-9) to 7.2% (C20:3n-6), with CVs for docosahexaenoic acid (DHA) of 6.9% and arachidonic acid (AA) of 6.4%, respectively. Samples with less than 10^5 cells were found not to reflect the actual fatty acids profile, as contaminations of solvents with palmitic and stearic acid significantly falsify the proportion of all fatty acids. The range of linearity was determined for 10^5-10^6 cells with r > 0.990 for all fatty acids. The average cell yield of the study participants was 7.3 x 10^5 ± 3.7 x 10^5 cells (mean ± SD, n = 29). The comparison of plasma and cheek cell fatty acids showed a significant correlation of DHA and eicosapentaenoic acid (r = 0.82 and 0.84; P < 0.001, respectively), but not for AA. The percentage for DHA (r = 0.98 ± 0.52% and for AA 3.6 ± 0.76% in cheek cells was 0.98 ± 0.52% and for AA 3.61 ± 0.76% Cheek cell sampling in newborns (n = 3) obtained lower cell numbers compared to adults (1.9 x 10^5 – 3.6 x 10^5), but these numbers were sufficient for analysis. Cheek cell DHA and AA contents seemed increased in newborns (1.41 ± 0.26% and 4.21 ± 0.39%, respectively) compared to adults.

**Conclusion:** The developed method for cheek cell fatty acids analysis is highly sensitive and reliable, even if cell numbers are lowered. The procedure is applicable for newborn fatty acid profiling and results obtained agree with published data. The nonchromatographical extraction of GPs allows a high sample throughput. The methodical improvements increase the precision of the cheek cell fatty acids analysis, which is comparable to conventional fatty acids analysis of red blood cells or plasma.

**Disclosure of Interest:** None declared.

PO-N-0217

**Nutrition, Metabolism, and Experimental Approaches**

**PROTON MAGNETIC RESONANCE SPECTROSCOPY IN PROTEIN ENERGY MALNUTRITION PATIENTS**

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**Objectives and Study:** The present study was carried out to assess the diagnostic and prognostic role of proton MRS of the brain in protein energy malnutrition (PEM) patients.

**Methods:** The study included 16 PEM patients (8 edematous and 8 nonedematous) with age range of 6–24 months (mean age was 13 ± 5.5 months) recruited from the inpatient department at the Children’s Hospital, Faculty of Medicine, Ain Shams University. Nine clinically healthy age- and sex-matched infants served as the control group. All studied cases were subjected to full history taking laying stress on dietetic history, thorough anthropometric and clinical examination. Laboratory investigations had been done including complete blood count and liver and kidney functions besides the application of Bayley scale of infant development. Additionally, the radiological study with proton MRS of the brain was performed for all cases using multivoxel study on three brain regions (frontal lobe, basal ganglia and thalamus) bilaterally and assessment of three brain metabolites namely N-acetylaspartate (NAA), choline (Cho) and creatine (Cr) was done and was interpreted in the form of metabolic ratios NAA/Cr, Cho/Cr, NAA/Cho and NAA/Cho+Cr. Nutritional rehabilitation was carried out for 2–4 months then the PEM patients were re-evaluated using the same methods mentioned before.

**Results:** The results showed statistically significant decrease of all ratios in both types of malnourished infants as compared to the controls. The basal ganglia and the thalamus showed the least values in both groups of malnutrition while the frontal areas were less affected. Additionally, the edematous group showed lower values of all studied MRS ratios compared to the nonedematous one yet this finding was not of statistical significance. Regarding, neurodevelopmental assessment using BSID-II; the results showed that their mean values were significantly decreased in both groups of malnourished infants as compared to the controls. All the previous changes showed improvement after nutritional rehabilitation and most of them reached statistical significance yet not reaching the control values.

**Conclusion:** PEM patients whether edematous or non-edematous show developmental and cognitive delay coupled by changes in their MRS finding of the brain denoting metabolic brain affection and some of these persist in spite of the apparent success of the nutritional rehabilitation program. This observation urges us to continue following up these patients for longer durations to make sure no permanent damage occurs due to the PEM insult to the growing brain.

**Disclosure of Interest:** None declared.

PO-N-0220

**Nutrition, Metabolism, and Experimental Approaches**

**HOW DOES GOAT MILK INFANT FORMULA COMPARE TO COW MILK FORMULA? A RANDOMISED CONTROLLED TRIAL**
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Objectives and Study: While the use of goat milk infant formula is increasing worldwide, there has been only one randomised controlled trial (RCT) assessing growth and no RCTs assessing blood biochemistry of infants fed goat milk formula. The objective of the study was to compare the growth rates and nutritional status of infants fed formulas based on either goat milk or cow milk.

Methods: Healthy full-term exclusively formula-fed infants were randomly allocated to receive either goat milk or cow milk based infant formula within 14 days of birth. The study formulas were provided to infants as the sole source of nutrition from enrolment until at least 4 months of age. A reference group of exclusively breastfed infants was included for comparison. Body weight, length and head circumference was assessed at 2 weeks, 1, 2, 3, 4, 6 and 12 months of age. Blood biochemical markers of nutritional status were assessed at 4 months of age.

Results: Two hundred formula fed infants and 101 breast-fed infants took part in the study. The baseline characteristics of the participants and infant growth over the 12-month study period were comparable between the two formula groups. Haemoglobin, serum albumin, ferritin, folate and urea levels of formula or breast fed infants at 4 months were within reference range for this age group. There were some minor differences in the plasma amino acid profile between the 2 formula groups, but there was no indication that any amino acids were limiting in either formula. Compared with breastfed infants, infants fed formula were heavier at 3, 4 and 6 months of age. There were no differences in the percentage of children who had a serious adverse event, defined as death or hospitalisation over 24 hours, between the two treatment groups or in comparison with breastfed reference group.

Conclusion: The growth rate and nutritional status of infants fed goat milk formula are comparable to infants fed cow milk formula.

Disclosure of Interest: None declared.

PO-N-0225

Nutrition, Metabolism, and Experimental Approaches

LEPTIN IS NOT AN OBLIGATORY SIGNAL FOR ONSET OF PUBERTY

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Objectives and Study: To assess the effect of pubertal induction on leptin levels and growth indices in boys with constitutionally delayed growth and puberty.

Methods: Eighty-two boys 13.6–15.5 years of age, who were referred to the growth clinic because of short stature and delayed puberty were randomly allocated to one of the following treatment groups: oxandrolone therapy, 5 mg/d for six months (n = 15), testosterone depot, 100 mg monthly for 3 months, (n = 15) or for 6 months, (n = 20), nutritional program, (n = 17), oxandrolone and nutritional program (n = 15) or passive observation (n = 20). Boys in the nutritional programs received 12-mg/day iron and 6000IU/week of vitamin A. The outcome measurements were height,
weight, pubertal signs, dietary intake, serum vitamin A, iron, Insulin – like growth factor –1 and leptin levels in blood.

**Results:** A 72 hours dietary recall revealed suboptimal intake in all participants. Six months of vitamin A supplementation induced growth acceleration similar to that seen in the oxandrolone and testosterone treated children, and significantly higher than the observation group (9.3 ± 2.9 vs. 4.0 ± 0.9 P < 0.001). While in the vitamin A supplemented group, puberty (increase in testicular volume ≥6 ml) was induced within 6 months, no pubertal signs were noted in the other groups during this time. BMI and leptin blood levels remained constant in all groups throughout the study with no significant differences among groups (4.4 ± 1.7 in observation group versus 4.2 ± 1.8 in other groups).

**Conclusion:** Onset of puberty was not associated with the expected increase in leptin levels. These data suggest that leptin is not an obligatory signal for induction of pubertal onset.

**Disclosure of Interest:** None declared.

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**PO-N-0226**

**Nutrition, Metabolism, and Experimental Approaches**

**Intestinal Microbiota Establishment in Breast-Fed Neonates Delivered Vaginally at Term**

PO-N-0226

**Methods:** Fecal samples were obtained from 7 neonates at 3 different time points postpartum (days 4–6, 9–13 and 25–29 postpartum). Culture-based methods were performed to enumerate the major gut-associated anaerobic and facultative anaerobic populations, and validated using a culture-independent method, quantitative real-time PCR (qPCR).

**Results:** Cultivation revealed that a highly dense anaerobic microbiota had established in all 7 neonates within the first week of life. Presumptive total anaerobes reached adult levels of 10.5 ± 0.4, 10.7 ± 0.3 and 10.5 ± 0.6 log cfu·g⁻¹ feces, for each of the 3 successive samplings, respectively; while total facultative anaerobes ranged 5 to 15 times lower. Already at the first sampling point high levels of *Bifidobacterium* (10.2 ± 1.4 log cfu·g⁻¹ feces), *Bacteroides* (9.0 ± 3.3 log cfu·g⁻¹ feces) and clostridia (9.0 ± 2.6 log cfu·g⁻¹ feces) were enumerated. This rapid colonization was confirmed by qPCR, with total and average levels of 11.4 ± 0.4 log 16S rDNA copies·g⁻¹ feces, *Bifidobacterium* (10.9 ± 0.8 log xfp copies·g⁻¹ feces), as well as *Bacteroides* (10.6 ± 2.0 log 16S rDNA copies·g⁻¹ feces). Although the Firmicutes population was high (10.1 ± 0.4 log 16S rDNA copies·g⁻¹ feces), members of the clostridial cluster XIV and IV (butyrate-
producers), which are major constituents of the Firmicutes in adults, could not be detected within the neonatal period.

**Conclusion:** The microbiota of breastfed neonates becomes rapidly colonized by anaerobic bacteria, especially by species of the genera *Bifidobacterium*, *Bacteroides* and some members of the clostridia, suggesting that anaerobic populations act as pioneer bacteria. In contrast to the clostridial composition typically encountered in adults, colonization with major butyrate-producers, which play an important role in healthy physiology of colonocytes, is apparently delayed in breastfed neonates.

**Disclosure of Interest:** None declared.

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**PO-N-0185**

**Clinical Nutrition**

**MACRONUTRIENT COMPOSITION OF BREAST MILK**

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**Objectives and Study:** In most NICUs calculation of caloric intake for preterm infants is based on a standard, average, breast milk composition. Considering the known variation in macronutrient composition of breast milk there will often be under- or overestimation of intake. We determined the range in deviation of composition of breast milk from the assumed average used in our ward.

**Methods:** Breast milk was analysed once a week in 55 mothers of preterm infants (gestational age < 32 weeks and/or birth weight < 1500 g). Macronutrient breast milk composition was measured in samples of 24 hour collections of breast milk, using the Miris Human Milk Analyzer. The assumed breast milk composition used in our ward is protein 1.56 g/dL, lactose 7.41 g/dL, fat 3.55 g/dL and 67.84 kcal/dL.

**Results:** We found deviation from the assumed breast milk composition for all macronutrients. Deviation was > 20% in 51% of samples for protein, 12% of samples for lactose, 78% of samples for fat and 49% of samples for calories. The Figure shows median breast milk composition with interquartile range and significance of difference from assumed, average composition. Protein content measured in week 1 was significantly higher than the assumed average. In week 2 and 3 there was no significant difference, in week 4 and later the measured protein content was significantly lower than the assumed average. * P < 0.05; ** P < 0.02.

**Image:**

**Conclusion:** The use of a standard, average breast milk composition to determine caloric intake in preterm babies fed by breast milk gives over- or underestimation of true intake in a large proportion of patients. Measuring the composition of breast milk in all babies might improve current feeding practices.

**Disclosure of Interest:** None declared.

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**PO-N-0186**

**Clinical Nutrition**

**STUDY OF PHTHALATE PLASMATIC LEVELS IN CHILDREN ON CYCLIC PARENTERAL NUTRITION**

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**Objectives and Study:** Perfusion materials in PVC such as drip chambers and tubing may contain phthalates including di (2-ethylhexyl) phthalate (DEHP). This is dispersed in the PVC matrix from which the phenomenon of leaching may occur as soon as it comes into contact with lipophilic preparations such as ternary solutions used in parenteral nutrition (PN). However, several studies have questioned the harmlessness of phthalates. Children on prolonged cyclic PN therefore constitute a group at risk of chronic exposure, the severity of which will vary according to the stage of development. The aim of our study was to assess DEHP exposure in children benefitting from cyclic ternary PN by quantifying its plasmatic concentrations at the start and finish of a session. The results obtained will be compared with those obtained from children used as controls and receiving no PN, so as to determine the possible factors responsible for this phenomenon.

**Methods:** The plasmatic concentration of DEHP was determined by high performance liquid chromatography (HPLC) from blood samples taken from 22 children at the start and finish of a 12-hour ternary cyclic PN period. The plasmatic concentrations of DEHP measured at the onset as well as their evolution between start and finish were studied for each

**Image:**
child and were compared with those obtained from 20 control children of comparable age and gender but receiving no PN.

**Results:** DEHP concentrations were not quantifiable in 4 patients (18%) at the start of PN. In one patient (5%), they were quantifiable neither at the start nor at the end of PN. However, for 17 patients (77%), DEHP concentrations were quantifiable at the start of PN and were very variable from one child to another, which indicates that residual levels of DEHP are not totally eliminated between PN sessions (20 to 449 ng/mL, which is an average rate of 104.50 ± 114.00 ng/mL). At the end of PN, plasmatic concentrations of DEHP had significantly but very variably increased in these children (22 to 1420 ng/mL, which is an average rate of 259.70 ± 319.90 ng/mL). They also varied from one session to another in the same patient. At both the start and finish of PN, DEHP concentrations were significantly higher than in the control population not exposed to DEHP ($P = 0.002$). No correlation between DEHP concentrations was revealed as far as lipid levels of the different ternary preparations, perfusion flow-rate, age and gender were concerned.

**Conclusion:** The results show that children on cyclic PN are continually exposed to non-negligible amounts of DEHP. This chronic exposure, poses a public health problem, as is indicated in available results of published research on the toxic effects of phthalates.

**Disclosure of Interest:** None declared.

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**Table:** $z$ scores at baseline and changes between measurements, median (range)

<table>
<thead>
<tr>
<th></th>
<th>Baseline $z$ scores compared to reference</th>
<th>Differences baseline to week 12</th>
<th>Differences week 12 to 24</th>
<th>Differences week 24 to 52</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trabecular density</td>
<td>$-0.8$ (&lt;-2.0; 1.4), $P = 0.193$</td>
<td>$0.3$ (-0.0; 1.0), $P = 0.006$</td>
<td>$-0.3$ (-2.3; 1.0), $P = 0.461$</td>
<td>$0.4$ (-0.3; 1.7), $P = 0.055$</td>
</tr>
<tr>
<td>Cortical Ddensity</td>
<td>$0.9$ (-0.4; 2.2), $P = 0.065$</td>
<td>$-0.4$ (-1.1; 0.5), $P = 0.027$</td>
<td>$-0.4$ (-1.6; 0.6), $P = 0.250$</td>
<td>$0.6$ (-0.3; 0.8), $P = 0.039$</td>
</tr>
<tr>
<td>TotalCSA$^\text{height}$</td>
<td>$0.1$ (-0.9; 0.9), $P = 0.910$</td>
<td>$0.2$ (-0.2; 0.6), $P = 0.014$</td>
<td>$0.3$ (-0.2; 0.6), $P = 0.078$</td>
<td>$0.0$ (-0.5; 3.8), $P = 0.547$</td>
</tr>
<tr>
<td>MuscleCSA$^\text{height}$</td>
<td>$-2.5$ (-3.5; -1.0), $P = 0.002$</td>
<td>$1.0$ (0.6; 1.8), $P = 0.002$</td>
<td>$-0.1$ (-1.1; 0.2), $P = 0.313$</td>
<td>$-0.2$ (-0.7; 0.9), $P = 0.844$</td>
</tr>
</tbody>
</table>

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**PO-N-0187**

**Clinical Nutrition**

**INFLUENCE OF EXCLUSIVE ENTERAL NUTRITION THERAPY ON BONE DENSITY AND GEOMETRY IN NEWLY DIAGNOSED PAEDIATRIC CROHN’S DISEASE PATIENTS**

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**Objectives and Study:** Paediatric Crohn’s disease (CD) patients present with deficits in muscle mass and bone quality at diagnosis. Exclusive enteral nutrition (EEN) induces remission and may have positive effects on muscle and bone. We followed the development of muscle and bone in paediatric CD patients initially treated with EEN within the first year after diagnosis.

**Methods:** Ten patients (7 boys) with newly diagnosed CD were assessed by dynamometric grip strength and peripheral quantitative computed tomography (pQCT) at the forearm before starting an 8 weeks therapy of EEN with a TGF-β enriched casein based formula, and at follow up visits after 12, 24, and 52 weeks. Paediatric Crohn’s Disease Activity Index (PCDAI) was calculated. Trabecular and cortical density, total, cortical, and muscle cross-sectional area (CSA) measured by pQCT were expressed as age- and sex-related $z$-scores, and size-dependent CSA corrected for low height-for-age. Wilcoxon rank sum test was applied.

**Results:** At baseline, patients had a median age of 13.7 years (range 10.6; 17.7), 3 had mild, 5 moderate and 2 severe disease activity. At week 12, 8 patients were in remission (PCDAI <10), 2 had mild disease (10 and 25). Between weeks 12 to 52, 7/10 patients relapsed, 5 of them repeated EEN. No steroids were applied. Low trabecular density $z$-scores at baseline improved significantly until week 12. In parallel, initially high cortical density $z$-scores decreased to normalisation, but increased again in week 24 to 52. Low $z$ scores for MuscleCSA$^\text{height}$ improved until week 12, but remained on a low level compared to reference. The low $z$ scores for grip strength at baseline ($-1.72; (-2.8; 0.8), P = 0.020$) increased in the first year ($1.4 (-0.7; 3.5), P = 0.020$). There were no changes in the $z$ scores for CorticalCSA$^\text{height}$.

**Conclusion:** Low trabecular and high cortical bone density at diagnosis indicate disturbed bone remodelling. MuscleCSA was significantly impaired. Within 3 months after initiation of EEN therapy, bone metabolism and muscle mass significantly improve towards normalisation.

**Disclosure of Interest:** K. Werkstetter: None declared, S. Schatz: None declared, M. Alberer: None declared, B. Filipiak-Pittroff: None declared, S. Koletzko Grant / Research Support from: Nestlé Nutrition, Vevey, Switzerland.

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**PO-N-0188**

**Clinical Nutrition**

**YOUNG ELITE MALE HOCKEY PLAYERS START EXERCISE HYPOHYDRATED**

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Objectives and Study: Appropriate hydration is an important but often overlooked aspect for adequate proper training and competition. Hydration status, during exercise, can be estimated by urinary color and measured by urinary specific gravity and serum or urinary osmolality.

Methods: We included 20 elite male junior field hockey players, born in 1992 or 1993, preparing their participation to the first Youth Olympic Games in Singapore in a study evaluating hydration and its physiologic effects during intensive training in Belgium. We investigated their hydration status before and after training. All players were weighted with the same precision balance by the same investigator. Water loss was calculated with a standard formula by evaluating weight loss, urine loss, metabolic water production and perspiration. Hydration before and after exercise was measured by urinary osmolality.

Results: Mean weight loss was 1.08 % (range 0.3% - 2.6%) body weight. Urine was collected two hours after breakfast, just before training started and immediately after training. Mean urinary osmolality (SD) was 868.3 ± 169.9 mOsm/L before training and 737.4 ± 263.0 mOsm/L after training. Although normal hydration or euhydration is not a constant but a variable state, it is generally defined by an urinary osmolality under 700 mOsm/L. Urinary osmolality between 700 and 900 mOsm/L is considered as slight hypohydration and above 900 mOsm/L as severe hypohydration. In our study, before starting the training, only 1/19 (5.2%) adolescent athlete was normally hydrated, 6/19 (31.6%) were mildly hypohydrated and 12/19 (63.2%) severely hypohydrated. Hydration improved after exercise, but still 12/19 (63.2%) were not in euhydrated state. There was no relation between water loss and hydration as measured by osmolality.

Conclusion: Education about the importance of proper hydration before and during physical exercise will improve athletic performance and decrease health risks related to dehydration. We think that further investigation is needed to elaborate an easy to use hydration test in a field setting with standardized normal values.

Disclosure of Interest: None declared.

PO-N-0189

Clinical Nutrition
NONALCOHOLIC FATTY LIVER DISEASE PREVALENCE AND ASSOCIATED RISK FACTORS IN OVERWEIGHT PEDIATRIC POPULATION
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Objectives and Study: Pediatric nonalcoholic fatty liver disease (NAFLD) is an underdiagnosed condition which resulting significant liver injury including cirrhosis. The aim of the study was to obtain the prevalence of NAFLD and the associated risk factors in an overweight pediatric population in a tertiary European hospital nutrition unit.

Methods: A prospective protocol was used in a consecutive first appointing of children and adolescents with overweight. The protocol included gender, family background, blood pressure (BP), body mass index (BMI), Acantose nigricans, waist circumference, abdominal US and biochemical parameters, such as AST (elevated when ≥34 IU/L), ALT (elevated when >30 IU/L), fasting insulin, glycated hemoglobin (HbA1C), total cholesterol, high-density lipoprotein (HDL) and triglycerides. For this analyze, NAFLD was defined only by US criteria of hepatic steatosis. We used the χ², Mann-Whitney U, Wilcoxon signed-rank test and t test for statistic analyzes.

Results: Of the 411 patients, 47.7% were male. The median age was of 10 years old (range 1 to 17 years) and the median BMI was 25.9 (range 17.9 to 41.9) with 5.1 % having BMI>35. Systolic and diastolic BP were a median of 115 (range 80 to 150) and 64 (range 32 to 98) mmHg, respectively (n = 353). Acantose nigricans was present in 37.6% of the patients (n = 398). At 6 months follow-up, the median BMI decreased to 25.8 (range 17.7 to 39.5), P < 0.001 (n = 269). NAFLD prevalence was found in 80 patients (19.5%). The median waist circumference was 92.2 cm in NAFLD patients versus 84.5 cm without NAFLD defined criteria (n = 388). In the entire population, 122 had elevated ALT (29.7%) (n = 359) and 69 elevated AST (16.8%) (n = 350). In the NAFLD population, 31 had an increased ALT (38.8%) (n = 75), 12 patients had an elevated AST (15.0%) (n = 72). From all analyzed parameters included in the protocol, the NAFLD population was significantly associated with gender, boys with more risk (P = 0.013), and increased levels of biochemical markers such as HbA1C (P = 0.001) (n = 194), C-peptide (P = 0.009) (n = 320), insulin (P = 0.003) (n = 324), AST (P = 0.005) and ALT (P < 0.001). Waist circumference was significantly increased in NAFLD population (P = 0.001). Mother history of type 2 diabetes (n = 400) (P = 0.008), obesity (n = 396) (P = 0.029) and dyslipidemia (n = 398) (P = 0.017) revealed a significant risk factor for NAFLD. The prevalence of NAFLD defined by US and/or elevated AST and/or elevated ALT was 46.2%.

Conclusion: About one-fifth of the population had NAFLD. The male gender, abdominal obesity, disturbance of glucose metabolism (HbA1C, C-peptide and fasting insulin) and mother history of type 2 diabetes, obesity and dyslipidemia revealed as risk factors for NAFLD in this population.

Disclosure of Interest: None declared.

PO-N-0229

Pediatric Nutrition
GLUCOMANNAN IS NOT EFFECTIVE FOR THE TREATMENT OF FUNCTIONAL CONSTIPATION IN CHILDREN: A DOUBLE-BLIND, PLACEBO-CONTROLLED, RANDOMIZED TRIAL
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Objectives and Study: There is uncertainty whether glucomannan (GN) is effective in treating childhood constipation. The aim of the study was to assess the efficacy of GNN as a sole treatment for functional constipation.

Methods: Children aged 3 to 16 years with functional constipation diagnosed according to Rome III criteria were randomly assigned to receive GNN (2.52 g/d) or placebo for 4 weeks. The trial was registered at ClinicalTrials.gov (http://clinicaltrials.gov) number NCT01151878.

Results: Of the 80 children randomized, 72 (90%) completed the study. The primary outcome, treatment success (defined as ≥3 stools per week with no episodes of soiling), was similar in the GNN (n = 56) and placebo (n = 36) groups (56% vs 58%, respectively, relative risk 0.95, 95% CI 0.6 to 1.4). In the GNN group compared with the placebo group, the stool consistency score was higher at week 1 (P < 0.0001), lower at week 3 (P = 0.008), and similar at weeks 2 and 4. Stool frequency was higher only at week 3 (P = 0.007). Abdominal pain episodes were more frequent in the GNN group at week 1 (P = 0.04) and week 4 (P < 0.0001) but were similar at weeks 2 and 3. There was no difference with regard to the frequency of any other secondary outcome or adverse event.

Conclusion: GNN, as dosed in this study, was not more effective than placebo in achieving therapeutic success in constipated children.

Disclosure of Interest: None declared.

PO-N-0231

Pediatric Nutrition

ROLE OF THE CYSTATIN C IN VASCULAR ALTERATIONS ASSOCIATED TO CHILDHOOD OBESITY

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Objectives and Study: Cystatin C is implicated in atherogenesis in mice. Epidemiological studies report positive relationships between serum cystatin C and cardiovascular outcomes in selected at-risk adult populations. Here, we tested the relevance of cystatin C as a biomarker of vascular alterations in severely obese children.

Methods: 219 obese children (140 F; age = 11.7 ± 2.7 y, BMI z-score = 4.4 ± 0.1 SD) and 257 nonobese children (124 F; age = 11.6 ± 0.1 y, BMI z-score = 0.2 ± 0.1 SD) were studied. All children had phenotypic characterization including anthropometry (height, weight, Tanner stage), blood pressure, and biological measurements (lipid profile, fasting insulin and glycemia, serum creatinin, leptin, adiponectin and cystatin C). Noninvasive ultrasonic measurements were performed in obese children to evaluate the mechanical characteristics of the common carotid artery (intima-media thickness (IMT) and incremental elastic modulus (Einc)) and the arterial endothelium function by the changes in brachial artery diameter in response to reactive hyperemia (FMD) and to glyceryl trinitrate (GTNMD).

Results: Serum cystatin C was significantly higher in obese children when compared to controls (0.87 ± 0.16 vs 0.80 ± 0.01, P < 0.0001). Cystatin C was positively correlated to BMI z-score independently of age, sex, Tanner stage and serum creatinin (r = 0.13, P = 0.0062). No significant correlation was found between cystatin C and blood pressure, lipids and fasting insulin after adjustment for age, sex, Tanner stage and serum creatinin. A positive correlation was found between IMT and cystatin C in obese children in univariate analysis after adjustment for age, sex, Tanner stage and serum creatinin (r = 0.23, P = 0.0007). In multivariate analysis with fasting insulin and BMI z-score as independent variable, IMT still correlated to cystatin C (r = 0.1, P = 0.01). Interestingly, Einc, FMD and GTNMD were not correlated to cystatin C.

Conclusion: Higher cystatin C is associated to childhood obesity, probably due to an increased secretion by adipose tissue. Since only IMT was correlated with cystatin C, whereas other arterial function parameters were not, we concluded that this protein is not implicated in early vascular alterations in obese children.

Disclosure of Interest: None declared.

PO-N-0232

Pediatric Nutrition

ASSOCIATIONS BETWEEN VITAMIN D STATUS IN INFANTS AND BLOOD LIPIDS, BODY MASS INDEX, AND WAIST CIRCUMFERENCE

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Objectives and Study: Studies in adults and children indicate that vitamin D deficiency is associated with risk factors of the metabolic syndrome such as adiposity, high fasting glucose and low insulin sensitivity. Most of the data are, however, derived from the study of populations with high prevalence rates of hypovitaminosis D and mainly in studies with adults or school age children. The aim of the present study was to study the relationships between 25-hydroxyvitamin D (25(OH)D) status and blood lipids, insulin, glucose, body mass index (BMI) and waist circumference in infants supplemented with vitamin D.

Methods: In a cross sectional study, 312 infants aged 9 months ± 2 wk were seen at the first examination of the prospective Danish cohort – the SKOT cohort. A blood
sample for examination of plasma 25(OH)D concentration was available from 255 of the infants. Plasma 25(OH)D concentrations were analysed by chemiluminescent immunoassay. Information about breastfeeding patterns and consumption of vitamin D supplements were collected. Associations between plasma 25(OH)D and high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol, triglycerides, insulin, glucose, BMI and waist circumference were analysed.

**Results:** Mean plasma 25(OH)D was $77.2 \pm 22.7$ nmol/L (ranging from 12–151 nmol/L). Two and 24 had 25(OH)D below 25 nmol/L and 50 nmol/L, respectively. At the time of the examination, 97% of the infants received vitamin D supplementation. In univariate analysis adjusted by gender, 25(OH)D was negatively associated with total cholesterol ($P = 0.001$), HDL ($P = 0.003$), LDL ($P = 0.033$), BMI ($P = 0.016$) and waist circumference ($P = 0.001$). If also controlled for season, BMI, length, birth weight and breastfeeding in multivariate analysis, 25(OH)D was negatively associated with HDL ($P = 0.001$), cholesterol ($P = 0.002$) and triglycerides ($P = 0.010$). 25(OH)D was negatively associated with BMI ($P = 0.005$) and waist circumference ($P = 0.002$) in multiple regression analysis controlled for gender, season, breastfeeding, birth weight and length. There were no associations between 25(OH)D and glucose or insulin (all $P > 0.05$) in either univariate or multiple regression.

**Conclusion:** Vitamin D status is negatively associated with blood lipids, BMI and waist circumference even in infants where nearly all received vitamin D supplements and most have 25(OH)D above 50 nmol/L. Whether these findings have long-term health effects remains to be elucidated.

**Disclosure of Interest:** None declared.

**PO-N-0242**

**Pediatric Nutrition**

**THE EFFECTS OF INFANT FORMULA BETA-PALMITATE STRUCTURAL POSITION ON BONE SPEED OF SOUND, ANTHROPOMETRICS AND INFANTILE COLIC: A DOUBLE-BLIND, RANDOMIZED CONTROL TRIAL**

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**Objects and Study:** Palmitate presents about 25% of the fatty acid content in human milk, with 70–75% in the sn-2 position on the glycerol backbone (beta palmitate). In this position, palmitate is not hydrolyzed by pancreatic lipase, and is well absorbed by forming mixed micelles with bile salts. In contrast, palmitic acid in the sn-1 and sn-3 positions, the predominantly fat composition in infant formulas, is hydrolyzed by pancreatic lipase, resulting in free palmitic acid that forms calcium-free fatty acid complex, which are poorly absorbed and may be associated with lower calcium deposition in bones and with abdominal discomfort. The aim of the present study was to compare the effect of 12 weeks feeding of high vs. low beta palmitate formulas on bone Speed of Sound (SOS), infantile colic and stool consistency.

The 12 donor samples was also compared for fat, lactose and protein content, to another new group of 11 samples of milk of mothers of premature infants, analysed with Milkoscan Minos 6 (Foss, Denmark). Statistical analysis was done using SPSS 17.0.

**Results:** Fat and protein content was lower in bank milk than in preterm milk. Eicosapentanoic acid (EPA, 20:5ω3) and docosahexaenoic (DHA, 22:6ω3) acid concentrations were significantly lower in bank milk than in premature milk ($P = 0.002$ and $P < 0.0001$, respectively). Linoleic acid (LA 18:2ω6) was higher in bank milk ($P = 0.001$), while arachidonic acid concentration (AA 20:4ω6) was lower compared to preterm milk ($P < 0.0001$). Saturated fatty acids (SFA) showed lower concentrations in bank milk compared to premature milk except for lauric (12:0) and stearic (18:0) acids. In donor samples no significant changes of the LCPUFA concentrations were observed over time.

**Conclusion:** Our study indicates that the significantly lower concentrations of LCPUFA in bank milk compared to mother’s milk after premature delivery call in question if unfortified bank milk is optimal for the premature infants. Although fat content may vary in bank milk, the mean content was lower than in preterm milk further supporting that bank milk is insufficient to cover the need of the preterm infants.

**Disclosure of Interest:** None declared.

**PO-N-0236**

**Pediatric Nutrition**

**DOES DONOR BREAST MILK CONTAIN SUFFICIENT LONG-CHAIN POLYUNSATURATED FATTY ACIDS (LCPUFA) TO BE CALLED THE OPTIMAL NUTRITION FOR PREMATURES?**

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**Objectives and Study:** Breastfeeding has been the cornerstone of infant nutrition through centuries. Donor milk is therefore used for optimal nutrition to premature not obtaining their own mother’s milk. The objective of this study was to investigate fatty acid concentrations in donor milk (breast milk of mothers delivering term), in comparison with milk from mothers delivering preterm infants.

**Methods:** Twelve breast milk samples from donors taken between 6 and 99 days after term delivery were analysed and compared to 42 samples of breast milk taken 1 week after preterm delivery. The fatty acid concentrations were analysed by capillary gas chromatography and given in mol%.

The fatty acid concentrations were analysed by capillary gas chromatography and given in mol%.

**Results:** Fatty acids (LCPUFA) to be called the sufficient long-chain polyunsaturated fatty acids (LCPUFA) to be called the optimal nutrition for premature are:

- Linoleic acid (LA 18:2ω6)
- Linolenic acid (ALA 18:3ω3)
- Alpha linolenic acid (ALA 18:3ω3)
- Eicosapentanoic acid (EPA 20:5ω3)
- Docosahexaenoic acid (DHA 22:6ω3)
- Docosapentanoic acid (DPA 22:5ω3)
- Docosatetraenoic acid (DPA 22:5ω3)
- Docosahexaenoic acid (DHA 22:6ω3)
- Arachidonic acid (AA 20:4ω6)
- Docosatetraenoic acid (DPA 22:5ω3)

**Conclusion:** The fatty acid concentrations in donor milk are sufficient for optimal nutrition for premature infants.

**Disclosure of Interest:** None declared.
We hypothesized that feeding infants with high beta-palmitate containing formulas, similar to human milk, will enhance bone SOS and reduce infantile colic.

Methods: Eighty-three term, appropriate for gestational age infants (58 formula-fed and 25 breast-fed) were studied following informed consent. Formula fed infants were randomly assigned to receive either formula with high beta-palmitate [43% of the palmitic acid is esterified to the middle position of the glycerol backbone, InFat group (Enzymotec Ltd), n = 30], or formula with standard vegetable oil mix [13% of the palmitic acid is esterified to the middle position of the glycerol backbone, control group, n = 28]. Anthropometric measurements of growth, and bone SOS measured by quantitative ultrasound (Sunlight Omnience Premier) were done at randomization, at 6 and at 12 weeks postnatal age. Before each visit parents filled a three days report on infant feeding, stool characteristics and colic symptoms.

Results: At randomization, gestational age, birth weight and SOS were comparable between the 3 groups. At 12 weeks mean SOS of the InFat group was significantly higher than the mean SOS of the control group [2887 ± 126 vs. 2832 ± 75 m/sec, respectively (P < 0.05)], and comparable to the breast-fed group (2875 ± 85 m/sec). There were no significant differences in weight, length and head circumference between the groups. Infants in the InFat group had less episodes of crying per day compared to infant in the control group (0.3 ± 0.6 vs. 0.8 ± 0.9, respectively, P < 0.05) as well as significant decrease total daily crying time (3.8 ± 8 vs. 23.6 ± 44.8 min/day respectively, P < 0.05).

Conclusion: Feeding with high beta-palmitate formula had beneficial effects on both bone SOS and colic symptoms at 12 wks.


Objectives and Study: An accurate assessment of habitual dietary intake is very important in determining the association between diet and disease and seems to be essential for dietary counseling in obesity. There is some evidence on misreporting of true intake by obese patients but it has never been related to misreporting in children with non-alcoholic fatty liver disease (NAFLD). The aim of our study was to indicate, which method: 3 days record (3dr) or Food Frequency Questionnaire (FFQ) is a better tool of assessment of habitual dietary intake among NAFLD and obese/overweight subjects.

Methods: We investigated 67 NAFLD patients (s), 28 healthy overweight/obese (o) children and 40 healthy controls (c) with normal BMI. Dietary intake was tested in all subjects with 2 methods: 3dr and FFQ. FFQ included 300 items, frequency categories included X times per day/week/month. Standardized album of photographs of products was used. We compared data with both methods in 3 studied groups. We regarded the method to underreport energy intake if energy intake was lower than 70% of normal values of energy intake according to age, gender and physical activity level.

Results: 50% of all 3dr records and 12% of all FFQ were underreported. There were significant differences between 3dr and FFQ in reporting energy expressed as % of normal values in s patients (70.2 ± 24.7 vs. 115.6 ± 74.7, respectively), o patients (76.7 ± 24.9 vs. 119.6 ± 77.2) and c group (79.8 ± 25.5 vs. 168 ± 175.4). Water intake expressed as % of normal values also differed by assessment with two methods in s (57.1 ± 27.3 vs. 101.2 ± 41.1 resp.), o (57.4 ± 21.1 vs. 100.3 ± 71.1) and c group (59 ± 22.9 vs. 86.6 ± 33.4). Sacharose intake was significantly different in s (121.1 ± 6 vs. 147.7 ± 6.3) and o group (12.3 ± 5.1 vs. 15.6 ± 4.5). There were no differences in intakes of protein, fat, carbohydrates and PUFA expressed as % of energy intake and fiber per 1000 kcal tested by both methods in these groups.

Conclusion: Results indicate that patients underreport sugar containing products like sweetened liquids and juices. FFQ seems to be more reliable for dietary assessment in obese children and adolescents and mainly those with fatty liver disease.

Disclosure of Interest: None declared.
Objectives and Study: An economic analysis was undertaken to determine cost-effectiveness of a 100% whey-based partially hydrolyzed infant formula (PHF-W) (NAN-HA, Nestlé S.A, Switzerland) in the prevention of atopic dermatitis (AD) in “at risk” German children compared to standard cow’s milk formula (SF).

Methods: Based on a 12-month time horizon (including 6 months of formula consumption), the model synthesised treatment pathways, resource utilization and costs associated with the management of AD in healthy “at risk” German newborns not exclusively breast-fed. Inputs were retrieved from the literature, official formularies and expert opinion. Treatment pathways considered a medical treatment approach, supplemented in some instances by a change of the formula consumed by affected children. The final outcome was the expected cost per avoided case of AD, yielding an incremental cost per avoided case (ICER) of AD for PHF-W vs. SF. Outcomes were presented from three perspectives: Statutory Health Insurance (SHI), subject’s family and society (SOC). A secondary analysis compared PHF-W to whey-based extensively hydrolyzed formula (EHF) in prevention.

Results: By selecting PHF-W over SF, 10,513 AD cases were expected to be avoided in a birth cohort of 145,858 “at risk” infants. Base case analyses generated an expected ICER of €1,314 from the SHI perspective, savings of €1,392 for the family as well as savings of €78 from the SOC perspective. The cost of formula was the main cost driver from the SHI and SOC perspectives, while time loss was prominent for the family. In a secondary analysis, PHF-W yielded 171.6M savings against EHF-Whey when the latter was assumed to be used in prevention. Univariate sensitivity analyses confirmed the robustness of the model. A series of 10,000 probabilistic sensitivity analyses, based on wide variations of parameter values, estimated cost-savings in approximately 15% of simulations and cost-effectiveness in 85% of simulations. Extending the analysis to 3 years further reduced the expected cost per avoided AD case from the SHI perspective, and increased the savings from the SOC perspective.

Conclusion: Under a range of assumptions, this analysis has established the dominance (i.e., cost saving) of PHF-W over SF from the family and societal perspectives and attractive cost-effectiveness from the SHI perspective in the prevention of AD in Germany. Accordingly, its use should be more widely adopted, and reimbursement should be considered in at-risk infants in Germany.


Objectives and Study: There is a significant increase in the number of paediatric patients with severe intestinal failure who survive with long-term home parenteral nutrition (PN). Although growth is generally acceptable, the effects of long-term PN on body composition (BC) are unknown. The aim of the study was to assess BC in paediatric patients receiving long-term home PN due to severe intestinal failure.

Methods: Total and regional BC were measured using dual x-ray absorptiometry (DXA; GE Lunar Prodigy) in 34 children (44% male) aged 5–20.2 years (median 12.6). BC variables were adjusted for differences in body size by height square. Age and sex specific BC standard deviation scores (SDS) were calculated using reference data from 514 healthy UK children aged 4–21 years. PN duration was 0.7–18 years (median 10.0). 11(32.4%) children received PN >80% of nutritional requirement (TPN), 17 (50%) had <80% (PPN) and 6 children (17.6%) were weaned off PN. Underlying diagnoses were enteropathy (n = 15), short gut (n = 8) and pseudo-obstruction (n = 11).

Results: The mean weight, height, and BMI SDS were −0.8 (SD 1, significantly < zero \(P < 0.001\)), −1.5 (SD 1.5, \(P < 0.001\)), and 0.2 (SD 1, n.s.) respectively. In 13 (38.2%) children height Z-scores were less than −2 SDS. Mean fat mass index (FMI; FM/height\(^2\)), fat free mass index SDS, trunk fat mass index (Trunk FM/height\(^2\)) SDS and limb fat-free mass index SDS were −0.21 (SD 1.1, \(P < 0.001\)), −0.1 (SD 1.2, \(P < 0.001\)), and −0.14 (SD 1.1, n.s.) and 0.8 (SD 1.1, \(P = 0.001\)). Patients with underlying gut inflammation (n = 11) had a significant higher FMI SDS and trunk fat mass index SDS compared to children without mucosal inflammation with mean of 0.3 (1.1, range: −1.4 to 1.7) vs −0.8 (0.8, range: −1.7 to 0.1, \(P = 0.04\)) and 0.4 (1.4, range: −2.4 to 1.8) vs −0.8 (0.8, range: −1.7 to 0.1, \(P = 0.04\)). Children on TPN had significant higher FMI SDS and trunk fat mass index SDS compared to children on PPN or off PN. There were no significant changes according BC and underlying diagnosis or receiving steroids.

Conclusion: Paediatric home PN patients were lighter and shorter than the UK reference. Despite close nutritional status monitoring, they tended towards higher FMI and central fat distribution with a deficit in lean body mass and bone mass. TPN, concurrent gut inflammation and corticosteroid treatment might partly explain these findings.

Disclosure of Interest: None declared.
PO-N-0246

Pediatric Nutrition

BONE HEALTH IN PAEDIATRIC INTESTINAL FAILURE PATIENTS RECEIVING LONG-TERM HOME PARENTERAL NUTRITION

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Objectives and Study: Paediatric patients receiving long-term home parenteral nutrition (PN) may present with low bone mineral density (BMD). It is uncertain whether this reflects small body size or suboptimal bone mineralization. To assess bone health in paediatric patients receiving long-term home PN due to severe intestinal failure.

Methods: Bone mass was measured using dual x-ray absorptiometry (DXA; GE Lunar Prodigy) at the lumbar spine (LS; L2–4) in 45 patients (24 males) aged median 7.7 years (range: 5 to 17.8 y). To assess the effect of body size, bone mineral apparent density (BMAD) SDS were calculated. PN duration was median 5 years (range: 3.2–12.2 y). The underlying diagnosis for IF was short bowel syndrome (SBS) in 12 (27%), mucosal inflammation (MI) in 20 (44%) and motility disorder (MD) in 13 (29%).

Results: Mean weight, height, and BMI standard deviation scores (SDS) were −0.8 (SD 1.3), −1.80 (1.5), and 0.4 (1.3). Height SDS less than −2 was found in 23 (50%) of the children. Patients with MI or gut inflammation were significantly shorter than those without. Mean age-matched LS BMD SDS were −1.7 (SD 1.6) and mean BMAD SDS was −1.4 (SD 1.5) that was independent of primary diagnosis or mucosal inflammation. Overall, 19 patients (42%) had low BMD (SDS < 2.0) and 14 (31%) low BMAD. A cohort of 25 (55%) was studied with scans one and two years apart. The bone mass seemed to remain static with no significant changes in SDS over time. The main predictor of change in bone mass was a change in weight SDS.

Conclusion: Despite close nutritional status monitoring, paediatric patients on long-term PN were shorter than the UK reference and had low bone mass. Especially those with underlying enteropathy and mucosal inflammation were at risk for GF. Small skeletal size contributes to low bone mass but there was evidence of reduced mineralization at the LS after adjusting for size. Bone mass remained static over a time period.

Disclosure of Interest: None declared.

PO-N-0247

Pediatric Nutrition

INCIDENCE OF PULMONARY THROMBOEMBOLISM IN PAEDIATRIC INTESTINAL FAILURE PATIENTS RECEIVING LONG-TERM HOME PARENTERAL NUTRITION

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Objectives and Study: Although parenteral nutrition (PN) is life saving, major life-threatening complications such as sepsis or thrombosis can develop. Pulmonary thromboembolism (TPE) is a well-recognized problem in these children and is associated with a high risk of morbidity and mortality with an incidence around 35%. The aim of the study was to assess the incidence of TPE in paediatric patients receiving long-term home PN due to severe intestinal failure.

Methods: All the VQ scans (Krypton-81m was used for the ventilation scan. The perfusion study was performed with Tc-99m macroaggregates of albumin) performed in the children on PN between January 2003 and August 2010 as surveillance test for TPE were reviewed. 48 consecutive children (24 males and 24 females) were included. The median age at the start of PN was 1.1 years (range 0.1 – 15.2 years). Twenty out of 48 children were on total PN (42%) and 28 (58%) on partial PN.

Results: The 48 children on study had a total number of 170 VQ scans. 10 (6%) episodes of TPE were detected. None of the 48 children on study (19%) had at least one episode of TPE (1 child had 2 different episodes of TPE). In 7 scans positive for TPE the perfusion defect was single and sub-segmental; 1 study demonstrated 2 subsegmental perfusion defects. In 2 scans there were large and bilateral perfusion defects. The 2 patients with large emboli had not been on warfarin before. Two patients with new small subsegmental perfusion defects were already on warfarin, the other 6 patients were not on warfarin. None of these children had clinical symptoms of TPE. The median age at the start of home PN in children with TPE was 3.4 years (range: 0.4 – 15.2 years). The mean age of the children was 14.5 years (range 2.1 – 18.4 years). The mean time interval between the beginning of total PN and the diagnosis of TPE was 6.7 years (range 1.5 – 15.0 years). The underlying diagnosis of the 9 children with TPE was SBS in 4 (44.4%), MD in 3 (33.3%) and MI in 2 children (22.2%). The children with TPE did not show a significant association with the underlying diagnosis, with bowel inflammation, septicaemia, lipid type and thrombophilia screening.

Conclusion: The overall prevalence of TPE in our paediatric patient population requiring CVC for long-term PN was 19%, lower compared to previous studies. An explanation for the lower prevalence of TPE found in our cohort could be the major improvements in constituents of intravenous nutrition such as lipids in recent years. Children on PN with TPE may well be asymptomatic and therefore a surveillance programme with lung scintigraphy is important.

Disclosure of Interest: None declared.
PO-N-0248

Pediatric Nutrition

WHEY-BASED ENTERAL FORMULA AND GASTROINTESTINAL FUNCTION IN CHILDREN WITH CEREBRAL PALSY: A RANDOMISED DOUBLE-BLIND CONTROLLED TRIAL

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Objectives and Study: Children with cerebral palsy (CP) commonly suffer a high prevalence of gastrointestinal (GI) dysfunction including gastro-oesophageal reflux (GOR) and impaired gastric emptying (GE) often leading to complications of enteral feeding and poor feed tolerance. Adjusting nutritional intake (namely protein) may reduce GI dysfunction and potentially improve feed tolerance. Whey based enteral formulae have been associated with improved tolerance through reduced vomiting and accelerated GE. The aim of this study was to determine the effect of whey based enteral formulae (compared to casein) on GE and GE in enterally fed CP children with a history of GOR.

Methods: 13 children (2–18 years) with severe CP and a history of GOR were enrolled. They were randomised to receive a casein based enteral formula (Pediasure, Abbott) for 1 week and either a 50% whey whole protein (WWP) formula (Nutmix Junior, Nestle) or a 100% whey partially hydrolysed (WPH) formula (Peptamen Junior, Nestle) for one week. Acid and nonacid reflux was measured using 24-hour multichannel intraluminal impedance with pH-metry. Gastric half emptying time (GE t½) was measured using the 13C-Na-octanoate breath test. Both tests were performed on day 6–7 of each week. A validated pain checklist and a visual analogue scale (VAS) symptoms questionnaire were used on day 6 of each week. A parent/carer recording sheet was also used to assess GI symptoms throughout the study.

Results: Whey formula overall emptied significantly faster than casein formula (median GE t½ 33.9 [25.3–166.2] min and 56.6 [46–191] min, respectively, p 0.033). The 50% WWP produced a greater improvement in GE t½ (42.1 min faster [–206.4, –22.9]) compared to the 100% WPH (1.2 min faster [–31.1, 32.5], p 0.022). Nine out of 13 children (69%) experienced delayed GE (t½ >90 %ile for age and sex) with the casein formula, which decreased to 4 (31%) with either of the whey-based formulae. pH-impedance measured reflux was unchanged in relation to formula type, however this may relate to all but 1 child having undergone fundoplication with gastrostomy. There was no difference in the severity of reported symptoms for the group during the casein vs whey week, however pain symptoms and symptoms overall were significantly lower in children who received the 50% WWP vs 100% WPH. Median aggregate pain score was 3.0 [2.0–11.0] for the 50% WWP versus 32.7 [11.8–43.4] in the 100% WPH (p 0.014). Median aggregate VAS symptoms score was 0 [0–11.8] for the 50% WWP versus 13.1 [2.5–24.8] for the 100% WPH (p 0.035).

Conclusion: In children with CP and GOR, gastric emptying of whey-based enteral formulae is significantly faster than casein based. A 50% whey whole protein formula appears to empty faster and is better tolerated with less GI symptoms than a 100% whey partially hydrolysed formula.

Disclosure of Interest: None declared.

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Pediatric Nutrition

COMBINATION WITH SECRETORY IMMUNOGLOBULIN A POTENTIATES EFFECT OF PROBIOTICS ON NEONATAL IMMUNE DEVELOPMENT

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Objectives and Study: Secretory IgA (SIgA) naturally binds to commensal bacteria. Interestingly, SIgA, alone or combined with bacteria, has been shown to cross back through the intestinal epithelium and to promote immune responses. During neonatal period, such mechanisms can also take place with SIgA originating from breast milk, contributing to immune development. The aim of the present work is to evaluate whether association of SIgA with probiotics in the form of immune complexes (IC) is able to optimize interaction of the probiotics with mucosal epithelium and, as a consequence, to potentiate immune-related benefits of probiotics.

Methods: In vitro: Probiotics (Bifidobacterium lactis CNCM I-3446) were incubated alone, or as IC with non-specific SIgA, with polarized intestinal epithelial Caco-2 cells. Adhesion of probiotics and responsiveness of the cells were measured. In vivo: (A) Fluorescent probiotics or IC were administered into intestinal loops containing one Peyer’s patch (PP). Fate of probiotics within PP over time was analyzed by confocal microscopy. (B) Germ-free neonates (C3H/HeN mice) were supplemented with probiotics alone or IC, from day 7 to 21 of life. Pups were conventionized concomitantly to start of supplementation to induce natural neonatal gut colonization. At weaning and at day 49, pups were orally immunized with live attenuated Salmonella typhimurium (strain DaroA). At day 63, pups were sacrificed and IgA production (ELISPOTs) in PP and response to immunization were assessed.

Results: Combination of B. lactis with SIgA increased probiotic adhesion by a factor of 4. Production of plgR and TSLP in Caco-2 cells was significantly increased with IC as compared to probiotics alone. Intestinal administration of probiotics showed that bacteria were naturally taken up by dendritic cells in PP, but that the process was speeded up with IC. Early-life supplementation of young mice with B. lactis alone significantly enhanced thenumbers of IgA producing cells in PP of pups 6 weeks after the end of the supplementation period as compared to controls. Noteworthy, feeding
with IC significantly further increased the number of IgA producing cells in comparison to probiotics alone. Blood anti-Salmonella-LPS IgG responses following mucosal immunization were also enhanced in both treatment groups compared to controls. Moreover, the IC-fed group displayed a trend for higher responses compared to probiotics alone.

**Conclusion:** These data demonstrate that association with SIgA in the form of IC is able to potentiate the interaction of probiotics with the intestinal mucosa and the associated immune system early in life.


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**Pediatric Nutrition**

**PREVALENCE TRENDS OF OVERWEIGHT AND OBESITY IN DANISH PRESCHOOL CHILDREN OVER A 10-YEAR PERIOD: A COHORT STUDY IN GENERAL PRACTICE**

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**Objectives and Study:** A steep increase in overweight and obesity in schoolchildren has been shown to occur in several populations. However, sparse knowledge exists in preschool children on the prevalence of overweight and obesity. The objective was to determine trends in prevalence of overweight and obesity in preschool children over a 10-year period and to identify possible predictors of overweight in 5-year-olds. A further objective was to compare study data with 30 year old reference data.

**Methods:** In Denmark all children are offered free health examinations by general practitioners up to the age of 5 years. Height and weight are registered at all examinations. All 162 general practices on the island of Funen, Denmark, were invited to participate in a cohort study based on the anthropometric data from birth and health examinations at 3 and 5 years of age for children born in 1992 and 2001, respectively. The island has a mixture of city and rural areas, representing 9% of the Danish population. Overweight and obese children were identified by the criteria from the International Obesity Task Force including age- and gender-specific BMI cutoff values from 2 to 18 y corresponding to BMI values of 25 and 30 kg/m² at 18 years of age. A comparison of growth data in this study with a 30-year-old Danish reference material was performed using z scores. This reference material was collected from free health examinations in general practice in preschool children as in the present study.

**Results:** Data were obtained from 5580 children from the 2 Funen birth cohorts, representing 48% of the total population. The average BMI and the prevalences of overweight (for girls at 5 y: 10.4–10.9%, for boys at 5 y: 7.5–7.1%) and obesity (for girls at 5 y: 2.9–2.6%, for boys at 5 y: 1.9–2.3%) from birth to 5 years of age did not vary significantly during the 10-year period. No changes in mean birth weight were registered and mean BMI in the group of obese children did not increase. Comparison with 30 year old reference data revealed a minor decline in average BMI in 3-year-old boys born in 1992, while a minor increase in average BMI was observed at 5 years of age in both birth cohorts. Overweight or obesity at 5 years was strongly associated with overweight and obesity at 3 years (OR > 13 in all cases) and with birth weight and gender.

**Conclusion:** In a Danish population-based survey of preschool children a stable prevalence of overweight and obesity was observed over a decade. Mean BMI for obese children did not change during the time period. Still, a strong association was found between overweight and obesity at 3 and 5 years of age, especially if the birth weight is high.

**Disclosure of Interest:** None declared.

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**Pediatric Nutrition**

**IS THERE A LINK BETWEEN SMALL INTESTINAL BACTERIAL OVERGROWTH AND COLONIC MICROBIOTA?**

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**Objectives and Study:** To determine the prevalence of small intestinal bacterial overgrowth (SIBO) in children from two socioeconomic classes and the participation of Lactobacillus spp and Escherichia coli in the colonic microbiota.

**Methods:** We studied 120 children (ages 6 to 10 years) from a slum and 30 children from a private school in Osasco, Brazil. After fasting and the collection of a sample of expired air, 10 g of lactulose in 100 ml of water were administered in the children. Breath samples were collected after 15, 30, 45, 60, 90, 120, 150 and 180 minutes. Hydrogen and methane were analyzed in the Quintron instrument. SIBO was diagnosed if hydrogen level ≥20 ppm and/or the methane level ≥10 ppm above baseline at ≤60 minutes after oral intake of lactulose. Lactobacillus spp and E. coli were quantified in the feces samples by real-time PCR using the ABI 7500 Real-Time PCR System (Applied Biosystems).

**Results:** SIBO occurred in 56.9% (58/102) of children from the slum and 30 children from a private school (< 0.001). The count of Lactobacillus spp (median and percentiles 25 and 75) in the feces of the children in the private school was higher than in children from slum (10.40 × 10⁶ cells/g [1.60 – 39.1 × 10⁶ cells/g] and
1.45 × 10^6 cells/g [0.54 – 3.25 × 10^6 cells/g]; P < 0.001). For E. coli there was not statistical difference between the 2 groups (P = 0.484). The children from slum without SIBO (n = 44) showed higher counting the Lactobacillus spp in relation those with SIBO (2.15 × 10^6 cells/g [0.70 – 5.72 × 10^6 cells/g] and 0.97 × 10^6 cells/g). However, the count of E. coli did not differ between the 2 groups (P = 0.874).

**Conclusion**: The count of Lactobacillus spp was higher in children from primary school. In children from slum the count of Lactobacillus spp were lower in children with SIBO. The count of E. coli did not differ between the 2 groups and there was no association with SIBO in the slum. These results may indicate the association between SIBO and differences in colonic microbiota. This may be consequence of environmental factors.

**Disclosure of Interest**: None declared.

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**Pediatric Nutrition**

**PEPTIDOMICS OF HYDROLYSED INFANT FORMULAS**

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**Objectives and Study:** Prevalence of allergic diseases, including food allergy, has increased in the last few decades in Western countries [1]. Hydrolysed infant formulas (HA IF) have been prescribed by paediatricians for the prevention of cow’s milk allergies in “at-risk” babies. The degradation of native proteins prevents sensitization, an effect which is linked to either passive prevention (avoidance of sensitizing epitopes) or active induction of oral tolerance, or even to both phenomena. The induction of specific oral tolerance by “tolerogenic” peptides derived from milk protein hydrolysis has been described previously in a rat model [2] and represents an effective tool to guide the immune system towards tolerance instead of sensitization. Different tolerance induction levels have been found when partially and extensively hydrolysed formulas were compared, only the former inducing oral tolerance to beta-lactoglobulin [3]. The objective of this work was in-depth characterisation of hypoallergenic infant formulas. Calculation of median peptide mass distribution reveals a powerful tool to assess HA IF and establish a promising link to correlate compositional data with functional results.

**Methods:** The peptide pool of different HA IF was fractionated by size-exclusion chromatography (peptide SEC) and further analysed by LC-MS/MS on a high resolution Orbitrap MS system. Peptide sequences (length > 5aa) were identified by Mascot database searches and individually mapped on major bovine milk proteins.

**Results:** Partially and extensively hydrolysed formulas differ substantially in their peptide SEC profile. The choice of proteolytic enzymes has a significant impact on the peptide size distribution which can vary from peptide masses of several kDa down to 200–500 Da. Typically, more than 1000 peptides can be identified routinely in a HA IF with mass precisions better than 2 ppm, allowing precise peptide sequence alignment to the parent protein(s). Based on sequence information from all identified peptides, enzyme specificity maps for different HA formulas were generated.

**Conclusion:** The combination of size-exclusion profiling and pre-fractionation, combined with high resolution mass spectrometric detection of peptides in highly complex milk protein hydrolysates represents a very efficient approach for qualitative and quantitative characterisation of hypoallergenic infant formulas. Calculation of median peptide mass distribution reveals a powerful tool to assess HA IF and establish a promising link to correlate compositional data with functional results.

**References:**


**PO-N-0256**

**Pediatric Nutrition**

**HUMAN MILK AND BOVINE COLOSTRUM PROTECT AGAINST NECROTIZING ENTEROCOLITIS IN PIGS**

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**Objectives and Study:** Preterm birth and formula feeding predispose to development of necrotizing enterocolitis (NEC) in infants. As mother’s milk is often absent following preterm delivery, artificial milk formula or human donor milk are used as alternatives. We have previously shown that porcine and bovine colostrum provide similar NEC protection in pigs relative to infant formula, but it remains unknown whether human donor milk would exert similar effects in preterm neonates. We hypothesized that both donor human milk and bovine colostrum provide NEC protection of the immature gastrointestinal tract. We used preterm pigs as models for preterm infants.

**Methods:** Caesarean delivered preterm pigs (n = 40) received 2 days of total parenteral nutrition, followed by 2 days of enteral feeding (15 mL/kg/3 h) with bovine colostrum (BC, n = 13), donor human milk (HM, n = 13) or infant formula (IF, n = 14) at isonenergetic levels. Following an in vivo hexose absorption capacity test, pigs were euthanized on day 5 and the gastrointestinal tract was collected to record intestinal NEC-like lesions (clinical scores 1–6, NEC size distribution which can vary from peptide masses of several kDa down to 200–500 Da. Typically, more than 1000 peptides can be identified routinely in a HA IF with mass precisions better than 2 ppm, allowing precise peptide sequence alignment to the parent protein(s). Based on sequence information from all identified peptides, enzyme specificity maps for different HA formulas were generated.

**Conclusion:** The combination of size-exclusion profiling and pre-fractionation, combined with high resolution mass spectrometric detection of peptides in highly complex milk protein hydrolysates represents a very efficient approach for qualitative and quantitative characterisation of hypoallergenic infant formulas. Calculation of median peptide mass distribution reveals a powerful tool to assess HA IF and establish a promising link to correlate compositional data with functional results.

**References:**


PO-N-0262

Pediatric Nutrition
FRUCTOSE MALABSORPTION AS A PROTECTIVE FACTOR FOR CHILD OBESITY: A MULTICENTER STUDY
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Objectives and Study: In the past decades, a dramatic increase in childhood obesity has been reported worldwide in the industrialized countries. Simultaneously, nationwide studies from the USA have shown that since the 1970's, approx. 50% of the per capita consumption of sucrose has been replaced by the monosaccharide fructose [1]. The main source of fructose is beverages sweetened with high fructose corn syrup, frequently consumed by children and adolescents. Thus, fructose can be suspected to play a major role in the pathogenesis of obesity. We hypothesized that a chronic condition leading to decreased absorption of fructose, such as fructose malabsorption (FM), could turn out to be a protective factor in childhood obesity. In a multicenter, retrospective study, we analyzed a pediatric sample for a possible relationship between FM and obesity.

Methods: We collected data from 3 German children's hospitals (Giessen, Marburg, Wiesbaden) of all patients aged 0–18 years in whom a hydrogen breath test with fructose provocation had been performed between 2005–2010. The patients were diagnosed with FM if the concentration of breath hydrogen increased by >20 ppm after a standardized fructose meal. In order to rule out secondary fructose malabsorption, we recorded information on chronic intestinal diseases such as celiac disease and chronic inflammatory bowel disease. Our study was approved by the ethic committee for all 3 hospitals.

Results: A hydrogen breath test had been performed in 642 patients with a suspected diagnosis of FM. 14 patients had to be excluded from the database due to a former or current diagnosis of celiac disease or chronic inflammatory bowel disease. The breath test confirmed the diagnosis of FM in 302 patients (48.1%). Analysis of body mass index (BMI) showed that 27 patients (4.3%) were obese as defined by the current guidelines of the Working Group on Childhood Obesity (BMI exceeding the 97th age- and sex-specific percentile). The proportion of obese patients was significantly lower in the FM group (2.3%) than in the non-FM group (6.5%), P = 0.018. Analysis of subgroups revealed that the protective effect of FM was strongest in children aged 6–11 years (1.2% versus 6.2%). There are a few minor limitations to our study: H2 nonproducers could not be ruled out with certainty because hydrogen breath test with lactulose provocation was not performed routinely. We had no data on potential confounders such as eating behaviours and physical activities.

Conclusion: Fructose malabsorption constitutes a protective factor in the pathogenesis of childhood obesity.

Disclosure of Interest: None declared.

References:

PO-N-0263

Pediatric Nutrition
STUDY TO INVESTIGATE THE POTENTIAL OF PROBIOTICS IN CHILDREN ATTENDING SCHOOL
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Objectives and Study: Functional foods, especially yogurt, are attractive delivery agents for probiotics due to their popularity with parents and children; however, few commercially available products have strong clinical-based evidence. Our primary objective was to determine if consumption of a probiotic-supplemented yogurt-based beverage containing Bifidobacterium animalis subsp. lactis (B. lactis), BB-12, at a high dose (1 × 1010 cfu/100 mL), decreases absences in children 2–4 years attending daycare/school centers.

Methods: We conducted a double-blinded, randomized, placebo-controlled, allocation concealment clinical trial with healthy children between the ages of 2–4 years attending
daycare at least three days per week in the Washington, DC area. Participants consumed 4 ounces of active or control drink daily for 90 consecutive days. The active intervention was a strawberry yogurt-based drink supplemented with BB-12. The placebo contained the 2 cultures commonly found in all yogurts without BB-12 and was indistinguishable from the active beverage.

Results: One hundred and seventy-two children were enrolled. There were no significant differences in the primary outcome, missed days of daycare/school due to illness per 100 days, between the active (2.54 days absent/100 school days) and control groups (2.42 days absent/100 school days) ($P = 0.873$). A subset of less healthy children at baseline (N = 59) showed a significant difference in rates of constipation between the groups, active (2.76 days affected/100 days) and control (0.68 days affected/100 days).

Conclusion: Consumption of a probiotic-supplemented yogurt-based beverage containing BB-12 did not decrease absences due to illnesses in daycare/school for healthy children ages 2–4 years. However, the yogurt was found to be safe and well-tolerated. As there are many probiotic products on the market, we believe it is important that other products be tested independently and in patient-oriented settings.

Disclosure of Interest: T. Tan: None declared, D. Merenstein: None declared, J. Gonzalez: None declared, A. Young Conflict with: former employee - health sciences consulting company conducting safety evaluations for probiotic manufacturers, R. Roberts: None declared, M. Sanders Consultant for: numerous probiotic manufacturers, S. Petterson: None declared.

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Pediatric Nutrition

THE STUDY TO INVESTIGATE THE POTENTIAL BENEFITS OF PROBIOTICS IN YOGURT: A PATIENT-ORIENTED, DOUBLE-BLIND, CLUSTER-RANDOMISED, PLACEBO-CONTROLLED, CLINICAL TRIAL

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Objectives and Study: Probiotic functional foods are growing in popularity, in particular, yogurts fortified with additional probiotics. Our previous studies have found that providing an intervention in a functional food greatly increases compliance, and present parents with a simple alternative to traditional supplements.1–3 This study combined data from two previous randomized controlled clinical trials of probiotic yogurt in settings similar to how probiotics are consumed and used in the U.S.4–7 The primary objective was to determine if consumption of a probiotic-containing yogurt-based drink decreases absences due to illness for children attending daycare.

Methods: Data from the 2 original clinical trials included 354 healthy children ages 1–4 years attending daycare at least three days per week in the Washington, DC metropolitan area. Participants consumed four ounces of an active or control drink for 90 consecutive days. The active intervention was a yogurt-based drink supplemented with Bifidobacterium animalis ssp. lactis BB-12. The placebo was indistinguishable, differing only in absence of the BB-12.

Results: In this analysis, we combined our data and examined a per protocol analysis to better understand the potential usages for BB-12 in the future and help understand what further testing is warranted. Preliminary analysis of outcomes excluding non-protocol from the active group showed no significant differences in missed days of daycare due to illness between the active (2.71 days absent/100 school days; n = 78) and control groups (2.45 days absent/100 school days; n = 168).

Conclusion: Preliminary results show that BB-12 supplemented yogurt did not reduce daycare absences due to illness in healthy children 1–4 years old. Our trials did substantiate that BB-12 fortified yogurt is safe, well tolerated and that this mode of ingestion has high rates of compliance. We are currently conducting further investigations utilizing the drink in different settings and with different patient populations. The need for well-designed, objective studies which assess patient-oriented efficacy and safety outcomes is tremendously important in the field of probiotics.

References:

Disclosure of Interest: T. Tan: None declared, D. Merenstein: None declared, R. Roberts: None declared, K. Herbin Smith: None declared, M. Scriven: None declared, M. E. Sanders Consultant for: numerous probiotic manufacturers, S. Petterson: None declared, J. Gonzalez: None declared, A. Young Conflict with: former employee - health sciences consulting company conducting safety evaluations for probiotic manufacturers.

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