THURSDAY, JUNE 4, 2009
Plenary Session 1: Gastroenterology

OP1-01
CROHN’S DISEASE PRESENTATION IN PEDIATRIC PATIENTS
Presenter: C. Jung. INSERM U843, Paris, France.

Aim: To compare the phenotypic differences between pediatric-onset CD (PCD; <17 years old) and adult-onset CD (ACD) patients in a French multicentric cohort.

Methods: Ninety-five items including location and behavior (Montreal classification), medical treatments and surgery and other complications were retrospectively assessed in 495 CD patients (152 PCD and 343 ACD) recruited between 1993 and 2005 in Lille and Paris, France. The mean follow-up time was 4.59 years (1 to 9 years).

Results: The mean age at diagnosis was 12.6 and 28.4 years old in the PCD and ACD groups, respectively. At the time of diagnosis, terminal ileon location (L1) (P < 0.0001) and strictures (P = 0.002) were less frequent in PCD while weight failure was more common (P < 0.0001). At the end of follow-up, L1 was still less frequent in PCD (P = 0.005) but CD involving the ileo-colon (L3) was more common (P < 0.0001). Perineal disease (PD) was more frequent in PCD (P = 0.005) but they were less often perforating (P = 0.017). A logistic regression model confirmed that PCD is inversely associated with the L1 location (OR = 0.21 [0.09–0.53]); strictures (OR = 0.09 [0.01–0.78]) and perforating PD (OR = 0.19 [0.09–0.39]). Kaplan-Meier survival estimate of the cumulative incidence of surgery was higher in ACD than in PCD (RR = 0.71 [0.51–0.99]). In PCD, multivariate Cox models showed that strictureing complications were associated with an increased risk of surgery (HR = 2.88 [1.54–5.40]) whereas PD and inflammatory behavior (B1) were associated with a decreased risk (respectively, HR = 0.45 [0.24–0.82] and HR = 0.27 [0.09–0.82]). In ACD, Cox models showed that strictureing (HR = 1.95 [1.34–2.84]) and penetrating complications at diagnosis (HR = 3.11 [1.76–5.47]) were associated with an increased risk of surgery whereas B1 behavior and treatment with infliximab were associated with a decreased risk (respectively, HR = 0.26 [0.12–0.55] and HR = 0.48 [0.28–0.74]). Kaplan-Meier survival estimates of the cumulative incidence of the use of corticosteroid, immunosuppressive therapies (azathioprine, methotrexate) and biotherapies did not differ between PCD and ACD groups. Enteral nutrition was more often used in children (P < 0.0001).

Conclusions: PCD are characterized by a more extensive disease after follow-up with delayed ileal lesions. PD is frequent but less often penetrating. The therapeutic strategies and responses to treatments, including surgery, did not significantly differ between PCD and ACD groups except for enteral nutrition which is more used in children.

OP1-02
MICROBIOTA AND GENETIC ADAPTATION IN PEDIATRIC PATIENTS WITH INFLAMMATORY BOWEL DISEASE
Presenter: V. Iebba. Sapienza University of Rome, Rome, Italy.
Co-authors: S. Schippa1, M. Conte1, G. Di Nardo2, M. Proietti Checchi1, V. Mancini2, S. Oliva2, V. Totino1, S. Cucchiara2. 1Sapienza University of Rome, Department of Public Health Sciences Rome, Italy; 2Pediatric Gastroenterology and Liver Unit, Sapienza University of Rome, Rome, Italy.

Background and Aim: Pathogenesis of inflammatory bowel disease (IBD) is an interplay among interacting
variables, i.e., mucosal immunity, the intestinal commensal microbiota, and genetic susceptibility. There is evidence that “dysbiosis” of intestinal microbiota has crucial effects in triggering or maintaining mucosal inflammation in IBD. In ileo-colonic mucosa of pediatric IBD patients, *Bacteroides vulgatus* was found to be decreased, whilst *E. coli* increased (Conte et al, Gut 2006;55:1760–7). Large amounts of mucosa-associated *E. coli* have been observed in both Crohn disease (CD) and ulcerative colitis (UC), while genonomic analysis of mucosa-associated *E. coli* strains showed a close genetic association between strains isolated from CD and UC patients (Schippa et al, *Inflamm Bowel Dis* 2009). Isolated *E. coli* strains also exhibited a significant detection of fimH gene (the adhesin portion of type 1 fimbria) and adhesion levels related to the intestinal location of each disease. In this study we aimed at characterizing the dominant microbiota in IBD pediatric patients and to assess fimH variants in isolated *E. coli* strains.

**Methods:** Endoscopic biopsies from ileum, colon and rectum of 10 CD and 8 UC patients (age range: 4–16 years) and 6 controls underwent total DNA extraction, 16S rDNA PCR amplification and temporal temperature gel electrophoresis (TTGE) separation. Genomic DNA from *E. coli* strains was PCR amplified for fimH gene detection and submitted to TTGE for mutational detection. In silico simulations of FimH protein molecular movement and docking domain under shear-stress were conducted. Multivariate analysis was performed on overall data.

**Results:** TTGE profiles revealed 3 clusters: the first in 80% of CD patients, the second in the 75% of UC patients and the third in 100% of controls. A marked individuality in profiles and significant differences in index of biodiversity were observed. Multivariate analysis showed a significant and predictive separation between groups. TTGE profiles from fimH gene revealed a higher detection of point mutations in adhesin and interdomain region, associable to adhesive-ness, in patients than in controls. *E. coli* strains from UC rectum showed mutations also in pilin region of fimH gene. In silico simulations under shear-stress showed less stability of FimH mutated proteins.

**Conclusions:** In intestinal mucosa of IBD children, microenvironment, reflected by the population structure of resident microbiota, was different between CD, UC and IC. Intestinal gut districts that differ in shear-stress, could select point mutations that arise naturally in *E. coli* strains, thus revealing a different role of CD and UC in selecting genetic variations that are adaptive to a pathologic environment.

**OPI-03**

**DIAGNOSTIC WORK-UP OF IBD PATIENTS IN EUROPE: RESULTS OF A 5-YEAR AUDIT OF EUROKIDS**


Co-authors: J. Escher1, I. Espghan2. 1Erasmus MC-Sophia, Rotterdam, The Netherlands; 2ESPGHAN IBD Working Group, Porto, Portugal.

**Aim:** To evaluate adherence to the Porto criteria for diagnostic work-up of paediatric IBD patients.

**Methods:** The EUROKIDS registry is a web-based interactive registry of newly diagnosed paediatric IBD patients that was initiated in 2004. A European audit of the diagnostic work-up of IBD patients in the years following publication of the Porto criteria was performed. According to these, upper GI-endoscopy, ileocolonoscopy and radiology of the small bowel by small bowel follow through (SBFT) should be performed. During the years, the registry has extended to allow inclusion of patients from 41 centres in 20 countries: Belgium, Croatia, Czech Republic, Denmark, France, Germany, Greece, Hungary, Israel, Italy, Latvia, the Netherlands, Norway, Poland, Portugal, Slovenia, Spain, Sweden, Switzerland, and the United Kingdom.

**Results:** As of January 2009, 2005 patients have been registered, with a diagnosis of CD in 1265 patients (63%), UC in 576 patients (29%), and IC in 146 patients (8%). Throughout years 1–5, upper GI endoscopy is performed in 82% of CD patients, 72% of UC patients and 79% of IC patients and colonoscopy in 95%, 99% and 99% of the CD, UC and IC patients, respectively. Successful inspection of the terminal ileum (TI) is reported in 56% of all IBD patients in year 1, but increases yearly, to 69% in year 5. In CD patients, TI inspection is performed in 56%, 65%, 61%, 65%, and 72% in year 1, 2, 3, 4, and 5, respectively. Main reason reported for not inspecting the TI is “technical problems.” SBFT is performed in 66% of CD patients in year 1, but this percentage decreases to only 10% of the CD patients in year 5. On the other hand, MRI imaging of the small bowel seems to be performed more often in CD patients during these years: 9% in year 1, 11% in year 2, 16% in year 3, 20% in year 4, and in 31% in year 5.

**Conclusions:** Although successful TI inspection is increasing, overall adherence to the full Porto criteria is poor. Overall, only 45% of CD patients have a full work-up, consisting of ileocolonoscopy as well as radiology (SBFT and/or MRI). On the other hand, 89% of CD patients undergo either ileocolonoscopy or radiology (or both). Performance of SBFT is clearly decreasing, while the use of MRI has increased up to 31% in CD patients today. The Porto criteria need to be updated, while endoscopic skills of paediatric gastroenterologists may need to improve.
OP1-04

CELIAC ANTI-TISSUE TRANSGlutaminase AUTOANTIBODIES INTERFERE WITH THE ABILITY OF ALPHA-GLIADIN PEPTIDE 31-43 TO DRIVE INTESTINAL EPITHELIAL CELLS INTO S-PHASE

Presenter: I. Caputo. University of Salerno, Salerno, Italy.

Co-authors: M. Barone2, M. Lepretti1, S. Martucciello1, R. Troncone2, S. Auriacchio2, D. Sblattero1. 1Department of Chemistry, University of Salerno Salerno, Italy; 2Department of Pediatrics & ELFID, University Federico II Napoli, Italy; 3Department of Medical Science, University of Piemonte Orientale Novara.

Methods: We used, for our studies, a recombinant miniantibody (clone 2.8) to tTG from a celiac patient. We investigated whether anti-tTG autoantibodies, also early appearing in celiac mucosa, could interfere with such ability of p31-43.

Results: We found that p31-43 (100 µg/mL) was effective in driving Caco-2 cells into S-phase (48.7% ± 1.5% bromodeoxyuridine incorporation; untreated cells, 36.0% ± 2.0% bromodeoxyuridine incorporation). Clone 2.8 (0.2 µg/mL) reduced the stimulating effect of p31-43 (40.5% ± 0.7% bromodeoxyuridine incorporation). By using monodansylcadaverine, a tTG competitive substrate, or cystamine, a tTG irreversible inhibitor, in the presence of p31-43, no significant inhibition of bromodeoxyuridine incorporation was obtained. After exposure to clone 2.8 (2 µg/mL) we observed a reduction of lissamine-labelled p31-43 uptake by Caco-2 cells (48.7% ± 18.6% of fluorescence intensity; with non specific IgG, 97.1% ± 4.7% of fluorescence intensity). Moreover, we did not observe any effect of monodansylcadaverine and cystamine on lissamine-labelled p31-43 uptake.

Conclusions: Anti-tTG autoantibodies affect the ability of p31-43 to induce Caco-2 cells into S-phase. Such an effect may be due to an inhibition of p31-43 uptake by cells and probably do not involve tTG catalytic activity. In particular, as anti-tTG antibodies interfere with EGF endocytosis, we assume that anti-tTG antibodies can exert their effect by reducing p31-43 endocytosis. Our results suggest that autoantibodies to tTG may have an unexpected protective role towards toxic effects of p31-43.

OP1-05

ALTERED EXPRESSION OF INNATE IMMUNITY GENES IN BIOPSIES FROM DIFFERENT INTESTINAL SITES IN CHILDREN WITH ULCERATIVE COLITIS

Presenter: P. Maria. ENEA, Rome, Italy.

Co-authors: L. Stronati1, A. Negroni2, O. Borrelli2, S. Oliva2, F. Conte2, S. Cucchiara2. 1Section of Toxicology and Biomedical Sciences, ENEA, Rome, Italy; 2Sapienza University of Rome, Pediatric Gastroenterology and Liver Unit, Rome, Italy.

Background and Aim: Ulcerative colitis (UC) is an inflammatory disorder of the colon of unknown etiology. It is suggested that bowel mucosa damage is a result of a dysregulated immune response to multiple mucosal antigens comprised within the constituents. A first mechanism involves the inappropriate regulation of the innate immune response at the intestinal mucosa. NOD2, an intracellular pattern-recognition molecule of the Nod-like receptor family, triggers the innate immune response to intestinal microbiota through the activation of NF-κB. The first step of the NF-κB signalling cascade is the NOD2 oligomerization and the binding to the serine-threonine kinase RIP2 (receptor-interacting serine-threonine kinase 2). While studies on mucosal expression of NOD2 and other innate immune genes are not uncommonly reported in Crohn disease, they are very rare in UC. We investigated NOD2 and RIP2 mRNA expression in the inflamed and non inflamed tissues of active UC in comparison to a control group. Moreover, as NOD2-mediated NF-κB signalling produces several pro-inflammatory cytokines, gene expression of TNF-alpha and IL-1beta were analysed to confirm previous data (Gastroenterology 2008;135:1764–89), suggesting that these Th1 cytokines are up-regulated in UC, despite this is thought to be a typical Th2-mediated disease. Interestingly, the mRNA analysis was performed in both colonic and ileal districts of patients to assess whether an altered

expression of the innate immune genes was also evidenced in uninvolved UC areas.

**Methods:** 15 children affected by active UC (9 extensive pancolitis and a backwash ileitis, 6 left-sided colitis and no backwash ileitis) (age range 4–16 years) entered into the study. 10 age-matched children with functional abdominal pains and no signs of inflammatory diseases served as controls. Real time PCR was performed for mRNA analyses. All the experiments were repeated 3 times. Comparison of the groups was performed by the Mann-Whitney U test (significance at \( P < 0.05 \)).

**Results:** In UC children, NOD2, RIP2, IL-1beta, TNF-alpha mRNA expressions were significantly increased in inflamed colonic biopsies as compared to uninflamed colonic mucosa and to healthy controls (\( P < 0.01 \)). On the other hand, the same genes were strongly upregulated (\( P < 0.01 \)) in the ileal biopsies of children with both pancolitis or left-sided colitis.

**Conclusions:** Intestinal mucosal genes regulating the first steps of the innate response to microbiota are over-expressed in the inflamed colon of UC children with either pancolitis or left-sided colitis. Intriguingly, these genes are also upregulated in the ileum of UC patients. Thus, we propose that they could be considered as molecular markers of inflammation in intestinal sites classically uninvolved in UC. Thus, we propose that intestinal sites classically uninvolved in UC are immunologically activated at molecular level.

**OPI-06**

**APICAL RECYCLING ENDOSOME-ASSOCIATED MYOSIN VB IS MUTATED IN MICROVILLUS INCLUSION DISEASE AND IS INVOLVED IN INTESTINAL BRUSH BORDER DEVELOPMENT**


Co-authors: S. Ijzendoorn¹, E. Rings¹, ¹University Medical Centre, Groningen, The Netherlands.

**Background and Aim:** Microvillus inclusion disease is a rare and fatal congenital enteropathy, presenting with intractable secretory diarrhea shortly after birth. The complete inability to absorb nutrients from intestinal lumen demands total parenteral nutrition, and, eventually, transplantation of the small intestine. MID characteristics varies among patients and generally comprises of villous atrophy and crypt hyperplasia, and, at the cellular level, by the apical brush border atrophy, accumulation of apical proteins, lysosomes and microvilli-like inclusions in the apical cytoplasm of intestinal absorptive cells. Previously we have shown that MID enterocytes display abnormal expression of apical recyling endosomal markers, i.e. Rab11a, FIP-1 (RCP), FIP-5 (Rip11), resulting in a defective apical recycling system in MID. In this study, we aimed to identify the genetic cause and functional consequences that underlie the microvillus inclusion disease.

**Methods:** We screened the genomic DNA of three patients diagnosed with microvillus inclusion disease, their siblings and parents. Biopsies of small intestine from MID and control patients were used to analyze the organization of organelles and localization of proteins involved in intracellular trafficking of brush border proteins.

**Results:** In all MID patients together we have identified 2 substitutions, 1 deletion, and 2 protein truncating mutations in the *myosin 5B* gene. The *MYO5B* encodes for an actin filament-binding molecular motor protein that interacts with the small GTPase Rab11a, a marker of recycling endosomes, and thus facilitates the intracellular trafficking of apical proteins towards the apical membrane. We also found aberrant expression and subcellular distribution of myosin Vb protein and other key proteins that interact with myosin Vb and/or control apical recycling endosome-mediated protein trafficking.

**Conclusions:** The endosomal system that ensures the recycling of brush border proteins, with myosin Vb as a critical regulator, is required to develop and maintain functional apical cell surface in human enterocytes, and perturbations in this can be causally linked to microvillus inclusion disease. Mutations occurring at different positions of *MYO5B* gene (this study, Nat Genet 2008;40: 1163-5, Am J Med Genet A 2008;146A:3117-9) and thus affecting different functional regions of MYO5B protein could explain the diversity of phenotypes present in MID patients. The identification of mutations in MYO5B as the cause for MID brings a major advance in setting the reliable diagnosis, enables the genetic counseling and prenatal screening, as well as paves the way for developing alternative therapeutic strategies.

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**Parallel Session: Gastroenterology**

**G1-01**

**BETA-LACTOLOBULIN SPECIFIC MEMORY RESPONSE IN THE PERIPHERAL BLOOD OF CHILDREN WITH ACTIVE AND OUTGROWN COW’S MILK ALLERGY**

Presenter: C. Gianfrani. Institute of Food Sciences-CNR, Avellino, Italy.

Co-authors: I. Voca¹, R. Berni Canani², S. Ruotolo², A. Camarca², M. Tardi², R. Troncone², C. Gianfrani¹.

1University Medical Centre, Groningen, The Netherlands.
Background and Aim: The great majority of the studies that have analyzed immune response in children affected by allergy to cow’s milk proteins (CMA) have used allergen-specific T-cell clones or T-cell lines, that could result in switched cell phenotype and function. In this study, we have investigated the profile of recall responses to beta-lactoglobulin (bLG) in peripheral blood mononuclear cells of children with IgE-mediated or non IgE-mediated CMA, and of children who outgrew allergy.

Methods: 46 children were enrolled in this study. Of these, 14 were healthy controls (age 39.28, range 6–81 months), 11 had an IgE-mediated CMA (age 40.45, range 10–66 months), 12 had a non-IgE-mediated CMA (age 27.08, range 6–89 months), and 9 became tolerant to IgE-mediated CMA (age 49.7, range 19–138 months). PBMCs were isolated from blood and assayed for response to bLG and bovine serum albumin (BSA), as negative control protein. Production of IL-13, IL-10, IL-4, and interferon (IFN)-γ were assessed by ELISPOT and/or ELISA; cell proliferation was evaluated by incorporation of 3H thymidine.

Results: Immune responses to bLG, but not to BSA, were observed in all groups analyzed, including healthy controls. Three distinct profiles of response were obtained: children with IgE-mediated CMA had a significantly increased level of proliferation (mean ± SD of stimulation index(SI): 6.9 ± 5.6) and of IL-13 (mean ± SD: 1157 ± 909 pg/mL), and reduced IL-10 (mean ± SD of IL-10-spot forming units/2 × 10^5 cells (SFU): 912 ± 510), compared to healthy subjects (3.4 ± 2.7 SI; 355 ± 396 pg/mL; 1272 ± 623 SFU, for proliferation, IL-13 and IL-10, respectively P < 0.05); children with a non-IgE-mediated CMA had an increased, although not statistically significant, production of IFN-γ (33.7 ± 54.6 SFU) and significant reduction of IL-13 (192 ± 362 pg/mL), compared to control subjects (9.5 ± 9.5 SFU, P < 0.05) and to IgE-allergic patients (0.6 ± 0.8 SFU, P < 0.05); by contrast PBMC responses of tolerant patients were characterized by reduced IL-13 (716 ± 1091 pg/mL) and proliferation (3.6 ± 3.4 SI), compared to patients with active CMA. Noteworthy, IL-4 was undetectable in all patients even in the presence of antibodies neutralizing the IL-4 receptor.

Conclusions: Milk proteins-specific, cellular immune responses can be recalled in peripheral blood of cow’s milk allergic patients, as well as of normal and tolerant children. A Th2-like response with IL-13 production and proliferation is dominant in IgE-mediated CMA patients; by contrast a Th1-skewed profile with an IFN-γ production is present in non-IgE-mediated allergic and in those children who outgrew IgE-allergy. Healthy donors are characterized by massive IL-10-response that could be the key mediator of tolerance to food allergens.

G1-02

GASTROINTESTINAL SYMPTOMS, QUALITY OF LIFE, AND BONE MINERAL DENSITY IN MILD ENTEROPATHY COELIAC DISEASE: A PROSPECTIVE, CONTROLLED CLINICAL TRIAL

Presenter: K. Kurppa. Tampere University Hospital, Tampere, Finland.

Co-authors: M. Melti1, P. Saavalainen2, J. Partanen3, P. Collin1, K. Kaukinen1, K. Kaukinen1, 1University of Tampere and University Hospital, Tampere, Finland; 2University of Helsinki, Helsinki, Finland; 3Research and Development, Finnish Red Cross Blood Service, Helsinki, Finland.

Background and Aim: The diagnostic criteria of coeliac disease require small bowel mucosal villous atrophy with crypt hyperplasia. However, we have recently shown that patients having positive endomysial antibodies (EmA) but only mucosal inflammation suffer from gluten-dependent disorder similar to those having celiac disease with villous atrophy, when measured by histological, serological and clinical parameters (Kurppa et al. Gastroenterology, 2008; Nov 24 [Epub ahead of print]). However, a life-long gluten-free diet may be restrictive and difficult to maintain; therefore the benefits and disadvantages of the treatment must be balanced. The purpose of the study was to evaluate the gastrointestinal symptoms, quality of life and possible complications in mild enteropathy coeliac patients and the effect of an intervention with a gluten-free diet.

Methods: A prospective clinical trial was carried out in 73 consecutive adults with positive EmA. Subjects with normal villi, but small-bowel mucosal inflammation with or without crypt hyperplasia comprised the mild enteropathy group (n = 27). EmA positive subjects who had classical small-bowel mucosal villous atrophy and crypt hyperplasia served as coeliac control group (n = 46). A gluten-free diet was initiated to all study participants and after one year on diet the baseline evaluations comprising the measurements of gastrointestinal symptoms (GSRS), quality of life (PGWB), and bone mineral density were repeated. A total of 110 subjects without known coeliac disease served as noncoeliac control group.

Results: At the baseline, the gastrointestinal symptoms did not differ markedly between the mild enteropathy and coeliac control groups. However, patients in both groups had significantly more gastrointestinal symptoms compared with the noncoeliac control group. Furthermore, 11 out of 20 (55%) subjects in mild enteropathy group had...
either osteopenia or osteoporosis at baseline. With a gluten-free diet the symptoms and depression alleviated and bone mineral density increased.

**Conclusions:** Patients with mild enteropathy and EmA may suffer from gluten-dependent symptoms or even the complications of coeliac disease. The benefit of dietary treatment in these patients is inevitable, regardless of the degree of enteropathy. Our results further confirm that these patients should be included in the current diagnostic criteria of coeliac disease.

**G1-03**

**T-CELL REGULATION OF NEUTROPHIL INFILTRATE AT THE EARLY STAGES OF A MURINE COLITIS MODEL**

**Presenter:** P. Van Lierop. *Erasmus MC-Sophia Children’s Hospital, Rotterdam, The Netherlands*

**Background and Aim:** T-cells are a main target for anti-inflammatory drugs in inflammatory bowel disease. As the innate immune system is equally implicated in the pathogenesis of these diseases, T-cell suppressors may not only inhibit T-cell-dependent production of pro-inflammatory mediators but also affect innate immune cell function. Specifically, these drugs may impair innate immune cell recruitment and activation through inhibition of T-cells or act independent of T-cell modulation. We explored the extent of immune modulation by the T-cell inhibitor tacrolimus in a murine colitis model.

**Methods:** We assessed the effects of tacrolimus on TNBS colitis in wild type and RAG2-deficient mice. Severity of colitis was assessed by means of histological scores and weight loss. We further characterized the inflammation using immunohistochemistry and by analysis of isolated intestinal leukocytes at various stages of disease.

**Results:** Tacrolimus treated wild-type mice were less sensitive to colitis and had fewer activated intestinal T-cells. Inhibition of T-cell function was associated with strongly diminished recruitment of infiltrating neutrophils in the colon at the early stages of this model. In agreement, immunohistochemistry demonstrated that tacrolimus inhibited production of neutrophil chemoattractants CXCL1 and CXCL2. In T-cell deficient mice, tacrolimus did not affect the severity of TNBS colitis or numbers of intestinal neutrophils.

**Conclusions:** Both the innate and the adaptive mucosal immune system contribute to TNBS colitis. Tacrolimus suppresses colitis directly through inhibition of T-cell activation and by suppression of T-cell-mediated recruitment of neutrophils.

**G1-04**

**SLC26A3 MUTATIONS IN CHILDREN AFFECTED BY CONGENITAL CHLORIDE DIARRHEA**

**Presenter:** G. Terrin. *University Federico II of Naples, Naples, Italy*

**Background and Aim:** Congenital chloride diarrhea (CLD) is an inherited disorder of intestinal electrolyte transport transmitted by autosomal recessive manner caused by a defect in SLC26A3/DRA gene that codifies for Cl-/HCO3- exchanger. Patients affected by this disorder present intestinal Cl- malabsorption that induces severe life-threatening chronic diarrhea with neonatal onset. Early identification of this disorder is essential because an early introduction of appropriate therapy could be lifesaving for these patients. Data on the prevalence and on molecular genetics of CLD are missing in many countries. We investigated the genotype and the genotype-phenotype correlations of children from different countries affected by CLD.

**Methods:** Using automated direct sequencing (310 Abi Prism, PE, Transgenomic), we screened the whole coding region of SLC26A3 gene in 12 patients affected by CLD enrolled by the European Consortium for Congenital Diarrheal Diseases (ECCDD); 2 unrelated from southern Italy; 3 (2 siblings) from Morocco; 1 from Zimbabwe; 2 from Tunisia; 4 from USA. Main clinical characteristics of each child were recorded (number of bowel movements per day; total fecal volume per day; stool consistency according the following score: 1: formed; 2: loose; 3: semiliquid; 4: liquid; urgency or incontinence; average doses of Cl- as mmol/kg in the substitution therapy).

**Results:** Molecular analysis revealed 2 novel missense mutations and a large homozygous novel deletion in patients from southern Italy, a novel frameshift in 1 patient and a known Arabic homozygous mutation in
siblings patients from Morocco, 4 novel homozygous mutations in 1 patient from Zimbabwe, and a novel missense and a point deletion in the patients from USA and Tunisia. Majority of mutations were identified in STAS-like domain of the SLC26A3 gene. No clear correlation between mutations and clinical characteristics was found.

Conclusions: Molecular analysis can contribute to rapid and specific diagnosis of CLD and can be used to investigate the disease prevalence in different ethnic groups. We confirm the strong genetic heterogeneity of CLD in ethnic groups where the disease is sporadic, and conflicting genotype-phenotype correlations in this condition. The complex genotype observed in patient from Zimbabwe, born to unrelated parents bearing to two different ethnic groups of southern Africa, led to the hypothesis that such mutations could spread in such ethnic groups due to some yet unidentified protective effect.

Parallel Session: Nutrition

N1-01

A MIXTURE OF SHORT-CHAIN GALACTO-Oligosaccharides AND LONG-CHAIN FRUCTO-Oligosaccharides AND A PROBIOTIC STRAIN SUPPRESSES THE ALLERGIC SENSITIZATION AGAINST WHEY PROTEIN IN MICE

Presenter: B. Schouten. Division of Pharmacology and Pathophysiology, UIPS, Utrecht University, Utrecht, The Netherlands.

Co-authors: B. Van Esch1, G. Hofman1, S. Van Doorn2, A. Nauta2, G. Boehm3, J. Garssen1, L. Willemse1, L. Knippels2, 1Division of Pharmacology and Pathophysiology, UIPS, Utrecht University, Utrecht, The Netherlands; 2Danone Research, Centre for Specialised Nutrition, Wageningen, The Netherlands; 3Danone Research, Centre for Specialised Nutrition, Friedrichsdorf, Germany.

Background and Aim: Cow’s milk allergy (CMA) is the most common food allergy in children. So far, the optimal strategy for prevention and treatment are under discussion. The purpose of this study was to examine whether dietary supplementation with a specific prebiotic mixture consisting of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides in a ratio of 9:1 (Immunofortis) and/or a specific probiotic strain, Bifidobacterium breve M-16V, could reduce allergic sensitization in a murine model of orally induced IgE-mediated CMA.

Methods: Three-week-old female C3H/HeOuJ mice were fed diets containing 2% of the prebiotic mixture, the B. breve M-16V (2 × 10^8 CFU/g), or a combination of both. Mice were orally sensitized with whey protein for 5 times during weekly intervals. The acute allergic skin response was determined by measuring ear swelling. Antigen-induced anaphylaxis was scored. Furthermore whey-specific serum immunoglobulins and mouse mast cell protease (mMCP-1) were determined.

Results: The studied prebiotics (67.4 ± 7.2 μm, P<0.05), B. breve M-16V (83.1 ± 8.1 μm, P<0.01) and the combination (29.9 ± 6.4 μm, P<0.01) effectively suppressed the allergic skin response as compared to whey sensitized mice fed control diet (117.6 ± 8.6 μm) (Fig. 1). The anaphylactic reaction was less severe in mice fed the probiotic diets (P<0.01). The whey-specific IgE and IgG1 responses were not affected, however IgG2a was increased in prebiotic (2332 ± 776 AU, P<0.05), B. breve M-16V (3209 ± 11104 AU, P<0.01) and synbiotic (3047 ± 621 AU, P<0.01) fed animals. mMCP-1 concentrations, reflecting mucosal mast cell degranulation, were reduced by the synbiotic diet (4.0 ± 0.9 vs 17 ± 4.5 ng/mL), but not by the prebiotics or the probiotic strain alone.

Conclusions: Dietary supplementation with the studied prebiotic mixture and B. breve M-16V reduces the clinical allergic response in a murine model of IgE-mediated hypersensitivity that closely mimics the human situation. This model shows the potential for dietary intervention with pro-, pre-, and synbiotics in reducing the allergic response.
N1-02

CAN A POSTNATAL HIGH-PROTEIN FORMULA MODIFY THE INTESTINAL TRANSCRIPTOME IN A PORCINE MODEL OF INTRAUTERINE GROWTH RESTRICTION?

Presenter: R. D’inca. INRA, Saint-Gilles, France.
Co-authors: A. Jamin1, A. Jamin1, I. Le Huëou-Luron1.
1INRA, UMR 1079, Rennes, France.

Background and Aim: Intrauterine growth restricted (IUGR) neonates have higher mortality and morbidity during the first days of life, including intestinal immaturity associated with higher risks of intestinal diseases. They are often fed high-protein formula to ensure catch-up growth. However, the impact of such formulas on intestinal functions is not known. We aimed to evaluate the effects of a high protein formula on intestinal gene expression of IUGR piglets using a wide-scale approach.

Methods: Sibling IUGR piglets (birth weight around the 10th percentile, 0.91 ± 0.11 kg) were bottle-fed from 2 days of age (d2) with an adequate protein formula (AP, equivalent to sow milk) or progressively bottle-fed a high protein formula (HP, 35% of protein between d2 and d7 then 50% until d28). At euthanasia at d7 or at d28, distal ileum was sampled. RNA was prepared for transcriptomic analysis using 20K oligonucleotides microarrays (University of Arizona, USA). Differential analysis taking into account animal pairings was performed using the software package R. Genes were considered differentially expressed in HP vs AP formula-fed piglets when P < 0.01. They were classified using Gene Ontology (GO) terms.

Results: At d7, the 189 differentially expressed genes in HP piglets belonged to 5 main GO biological processes that were macromolecule (13%), signal transduction (14%), transport (7%), and catabolism (4%). Some of these genes were associated to metabolic or cardiovascular diseases (downregulation of ADAD1 and ABCA1 and upregulation of SOD3) and increased intestinal cells turnover (downregulation of FUBX7 and TFDP2 and upregulation of DUSP16 and DIS3). Upregulation of CAR6, CCL22, and IRF1 genes suggests an increased inflammation probably mediated by NF-κB pathway in HP intestine. At d28 identical GO biological processes represented by 215 differentially expressed genes were modified. Both a higher cells turnover and an increased inflammation with modifications in genes expression of the NF-κB pathway were still observed in HP piglets’ intestine. Last Wnt signalling cascade (upregulation of HERPUD1 and VPS35) was stimulated.

Conclusions: A high-protein formula given during the early postnatal period modified intestinal transcriptome. It increased the renewal of intestinal cells. HP formula-induced NF-κB stimulation may enhance the intestinal inflammation we previously described in IUGR piglets. Last, stimulation of Wnt pathway raises questions about the effects of HP formula on regulation of early development in the intestine. Long-term consequences of HP diet are being investigated.

N1-03

INFLUENCE OF LIFESTYLE AND GUT MICROBIOTA IN WEIGHT MANAGEMENT

Presenter: Y. Sanz. Institute of Agrochemistry and Food Technology (IATA), Spanish National Research Council (CSIC), Burjassot-Valencia, Spain.
Co-authors: A. Santacruz1, M. Collado1, A. Marcos2, J. Warnberg3, A. Martí3, M. Martin-Matillas3, C. Campoy4, L. Moreno5, O. Veiga6, C. Redondo-Figuero7, J. Garagorry8, C. Azcona9, M. Delgado9, M. Garcia-Fuented, Y. Sanz1. 1Institute of Agrochemistry and Food Technology (IATA), Spanish National Research Council (CSIC), Valencia, Spain; 2Instituto del Frio (CSIC), Madrid, Spain; 3Departamento de Fisiología y Nutrición, Universidad de Navarra, Pamplona, Spain; 4Departamento de Pediatría, Facultad de Medicina, Universidad de Granada, Granada, Spain; 5EU Ciencias de la Salud, Universidad de Zaragoza, Zaragoza, Spain; 6Departamento de Educación Física, Deporte y Movimiento Humano, Universidad Autónoma de Madrid, Madrid, Spain; 7Departamento de Ciencias Médicas y Quirúrgicas, Universidad de Cantabria, Santander, Spain; 8Departamento de Pediatría, Radiología y Medicina Física, Universidad de Zaragoza, Zaragoza, Spain; 9Facultad de Ciencias de la Actividad Física y Deporte, Universidad de Granada, Granada, Spain.

Aim: To determine the influence of a lifestyle intervention to treat obesity on the gut microbiota and body weight of overweight adolescents.

Methods: Thirty-six adolescents (14–15 years), classified as overweight according to the International Obesity Task Force body mass index (BMI) criteria, were submitted to a treatment program consisting of a calorie-restricted diet (10%–40%) and increased physical activity (15–23 kcal/kg body weight/wk) over 10 weeks. Gut bacterial groups were analyzed by quantitative real-time PCR before and after the intervention.

Results: A group of subjects (n = 23) experienced more than 4.0 kg weight loss and showed significant BMI (P = 0.030) and BMI z score (P = 0.035) reductions after the intervention, while the other group (n = 13) showed less than 2.0 kg weight loss. No significant differences in dietary intake were found between both groups. In the high weight loss group, Bacteroides and Lactobacillus counts increased (P = 0.015 and P = 0.002, respectively), whereas Clostridium cocoides and B. longum counts...
N1-04

FERMENTED INFANT FORMULA WITHOUT LIVE BACTERIA TENDS TO MIMIC EFFECT OF BREAST-FEEDING ON INTESTINAL MICROBIOTA IN WEANING BABIES

Presenter: M. Romond. Faculté des Sciences Pharmaceutiques et Biologiques, Lille, France.
Co-authors: C. Cauchie1, I. Dessaint1, C. Aubert-Jacquin2, N. Kalache3. Faculté des Sciences Pharmaceutiques et Biologiques, Laboratoire de Bactériologie-Virologie EA 3610 (Université Lille 2), Lille, France; 2Clinique de Pédiatrie St Antoine, Hôpital St Vincent de Paul, Lille, France.

Aim: To assess the impact of a fermented infant formula without live bacteria, i.e., containing heat-killed B. breve C50 and S. thermophilus 065 (FIF-HKBBST) on intestinal microbiota (IM) in weaning infants.

Methods: A prospective multicenter, randomized, double-blind controlled clinical trial was carried out between April 2006 and April 2007 in exclusively breast-fed infants from birth until weaning period. At the beginning of the weaning period, infants were randomly assigned to receive either a FIF-HKBBST (study formula) or a standard formula (control) and compared to a reference of exclusively breast-fed infants. Microbiological and clinical investigations were reported at baseline (V1), 1 (V2), and 3 months (V3) after consumption of the studied formulae. IM was assessed using culture-dependent techniques.

Results: A total of 91 infants were enrolled: 30 received a FIF-HKBBST (study group), 30 received a standard formula (control group), and 31 were exclusively breast-fed (reference group). At V1, infants had a median postnatal age of 8 weeks [Interquartile (Iq): 8–9 weeks] and a median weight of 5150 g [Iq: 4720–5600 g]. Infants' anthropometric data were similar in the 3 studied groups. Weaning period was similar in study and control groups: it started at a median age of 9 weeks [Iq: 8–10 weeks] and stopped at a median age of 14 weeks [Iq: 11–16 weeks]. In the 3 studied groups, solid food introduction started at the same time (median age of 18 weeks [Iq: 18–21 weeks]). The study and reference groups' IM showed stable Bifidobacteria and Enterobacteria total IM ratios between V1-V3 (1.2% vs –1.3% and 0% vs –0.2%, respectively). In contrast, Bifidobacteriatotal IM ratio decreased (~12.5%) and Enterobacteriatotal IM ratio increased (8.3%) in control group. Between V1-V3 Bifidobacteria/total IM and Enterobacteria/total IM ratios were not significantly different in the study and reference groups, whereas they were significantly lower (P=0.005) and higher (P=0.019) in control group as compared to reference group, respectively. At V3, faecal consistency was not significantly different in study and reference groups, whereas a significant difference was reported between control and reference groups (P=0.002).

Conclusions: The use of a fermented infant formula without live bacteria tends to mimic effect of breast-feeding on intestinal microbiota in weaning infants.

FRIDAY, JUNE 5, 2009
Plenary Session 2: Hepatology

OP2-01

GIANT CELL HEPATITIS ASSOCIATED TO AUTOIMMUNE HAEMOLYTIC ANEMIA: A 25-YEAR RETROSPECTIVE, MULTICENTRIC STUDY

Presenter: M. Sciveres. ISMETT, University of Pittsburgh Medical Center Italy, Palermo, Italy.
Co-authors: G. Maggiore1, L. Gori1, L. Pacifico2, J. Choulou3, O. Bernard3. 1Dipartimento di Pediatria, Università di Pisa, Pisa, Italy; 2Dipartimento di Pediatria, Università Roma, Italy; 3Centre Hospitalier de Pau, Pau, France.

Background: Giant cell hepatitis (GCH) associated with autoimmune haemolytic anaemia (AHA) is a rare, severe disease of early childhood with unknown pathogenesis, probably related to autoimmune phenomena. After the first description in 1982, only a few cases have been sporadically reported. Medium and long-term course of this condition lay completely unexplored. Here we present the first long-term, large series of patients.

Methods: We retrospectively reviewed all medical records of children presenting a GCH associated with
AHA referred in the last 20 years to the Pediatric Hepatology Unit of Bicêtre Hospital and to the Pediatric Hepatology and Gastroenterology Unit of the University of Pisa. A total of 17 patients (10 Bicêtre and 7 Pisa) were studied.

**Results:** Patients were 9 males and 8 females (M:F ratio 1.12:1). Median age at onset was 6 months (2.5–17), 10/17 patients were younger than 6 months. AHA in 5/17 cases preceded GCH by 1 up to 10 months. 6/17 patients experienced acute liver failure (ALF), 3 at onset and 3 during a relapse. 11/17 children presented with severe anemia, requiring blood transfusion (Hb < 7 g/dL), at onset and 2 later, during a relapse. 4 patients presented also autoimmune thrombocytopenia (Evans syndrome). At diagnosis, mean ALT serum activity was 50 × N (times upper the normal limit) [range: 9–190], mean GGT activity was 2 × N [N-5], mean bilirubin 13.5 mg/dl [±12]. Prothrombin time was < 50% in 3/15. Hypergammaglobulinemia was present in all, but 6 patients and major leucocytes were 0.99 × 10⁹/L [± 0.36]. Autoantibodies were found in 6 children, isolated anti-nuclear (ANA) in 3, isolated anti-smooth muscle (SMA) in 2 and ANA/SSA in 1. Liver histology showed diffuse giant cell transformation in all. Treatment associating prednisone (PDN) and azathioprine (AZA) was used as first-line therapy in 14 patients and resulted in treatment failure in 1, in partial response in 2 and complete remission in 8. Cyclosporine A (CsA) was used, associated to PDN and AZA or cyclophosphamide (CFM), as first-line therapy, in 3 cases and as second-line treatment in 6 cases. CsA administration was successful in 5 cases, partial efficacy in 2, and showed no effect in 2 patients. Orthotopic liver transplantation (OLT) was performed in 2 patients. One patient died 1 month later of multivisceral failure and the other survived to early disease recurrence, treated with PDN and AZA, and is alive after 8 years. AHA was usually responsive to steroid treatment, but heavily corticosteroid-dependent, leading to perform splenectomy in 4 cases. After a median follow-up of 15 years, 12 patients are alive without OLT (70%). All finally reached stable remission, even if experiencing several relapse when tapering treatment. In 7 patients treatment discontinuation was attempted after a median duration of 6 years and 6 months. All are free of disease after a median follow-up after therapy stop of 10 years.

**Conclusions:** GCH with AHA is a severe, often life-threatening disease requiring heavy immunosuppressive treatment associating several immunosuppressive drugs. The short-term course appears uncertain and death due to ALF or septic shock is possible. The medium term evolution is generally challenged by possible relapse of GCH and/or AHA in the tapering phase of treatment. The long-term prognosis seems to be better, and in a considerable proportion of patients, conversely to classic autoimmune hepatitis, it is possible to stop treatment without disease recurrence.

**OP2-02**

**AGE- AND ETIOLOGY-RELATED OUTCOME OF ACUTE LIVER FAILURE IN PAEDIATRIC AGE GROUP**

Presenter: N. Shanmugam. Paediatric Liver Centre, King's College Hospital, London, UK.
Co-authors: S. Jayaseelan, A. Dhawan.

**Aim:** To determine the effect of age, etiology, and liver transplantation on outcome of acute liver failure (ALF) in the pediatric age group.

**Methods:** Retrospective review of the case records of patients presented with ALF (defined as, INR > 2 not correctable by I.V. vitamin K, along with biochemical evidence of liver dysfunction). The patients were classified into neonates (≤ 28 days), infants (≤ 1 year), children (≤ 12 years) and adolescents (≤ 17 years) and the etiology, management and outcome were analysed.

**Results:** 215 patients (105 males) which includes 42 neonates, 23 infants, 95 children, and 55 adolescents, were diagnosed with ALF at a median (range) age of 11 (1–28) days, 138 (32–327) days, 4.5 (1.1–11.4) years, and 14.7 (12.4–17) years, respectively. The overall etiology of ALF were indeterminate 68 (32%), drug-induced 51 (24%), viral 23 (11%), neonatal haemochromatosis 17 (8%), metabolic 15 (7%), autoimmune 13(6%), Wilson’s 10 (4%), and miscellaneous 18(8%). Medical management included supportive and disease specific, but patients with INR > 4 with liver-specific disease were listed for liver transplantation. Of 102 patients listed, 82 underwent liver transplantation with a median (range) of 2 (1–62) days from the time of listing and 20 were not transplanted, as either they died while waiting for organ or removed from list as their liver recovered. At one year, 92 children were alive without transplant, 18 died posttransplant and 9 required retransplantation. Survival analysis with regard to etiology showed no significant difference in outcome in liver transplanted patients (P < 0.54), while survival remained etiology dependent in other groups (listed but not transplanted (P < 0.01) and nonlisted patients (P < 0.007)). This implies liver transplantation alters the natural course of illness and improves survival.

**Conclusions:** Etiological diagnosis could be made in 68% of patients. Transplant-free survival was etiology dependent while etiology has no effect on posttransplant survival. Acute liver failure in neonates carries high mortality and mortality decreases with age.
IN VIVO PROTON MAGNETIC RESONANCE SPECTROSCOPY CHANGES IN CHILDREN WITH EXTRAHEPATIC PORTAL VEIN OBSTRUCTION

Presenter: A. Srivastava. Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India.
Co-authors: S. Yachha1, R. Lal1, J. Agarwal1, S. Yadav1, A. Srivastava2, M. Thomas4, R. Gupta2. 1Department of Pediatric Gastroenterology, SGPGIMS, Lucknow, India; 2Department of Radiology, SGPGIMS, Lucknow, India; 3Department of Surgical Gastroenterology, SGPGIMS, Lucknow, India; 4Department of Radiological Sciences, UCLA School of Medicine Los Angeles, California, USA.

Aim: The aim of this study was to compare the brain metabolites in children with extrahepatic portal vein obstruction (EHPVO) and controls, using in vivo proton magnetic resonance spectroscopy (PMRS), and to look for any correlation between brain metabolites and neuropsychological tests (NPT) (Cross-Cultural Res 1999; 33:3–25).

Methods: PMRS, conventional MRI, and NPT were done in 19 EHPVO (age 11.2 ± 3.3 years) and 14 age- and sex-matched healthy controls. Arterial blood ammonia level was estimated simultaneously. Student t test was used to evaluate differences in metabolite ratios between patients and controls. Bivariate analysis of correlation was done to study the relation between the metabolite ratios and NPT.

Results: EHPVO patients exhibited significantly increased Glx/Cr ratio (mean ± SD 2.5 ± 0.2) compared to controls (2.1 ± 0.4, P = 0.002). No significant difference was observed in ratio of NAA/Cr, Cho/Cr, and mI/Cr between patients and controls (Fig. 1). Subjects with EHPVO had normal liver function tests and increased arterial blood ammonia level (142.2 ± 42.5 μmol/L, normal 60–80 μmol/L). NPT evaluating visual-motor coordination and spatial orientation had a significant negative correlation with arterial ammonia and Glx/Cr ratio (Fig. 2).

Conclusions: Abnormal in vivo PMRS derived metabolite ratio was seen in subjects with EHPVO. The Glx/Cr ratio was increased, which is similar to that found in MHE due to cirrhosis. Decrease in ratio of mI/Cr and Cho/Cr, typical of cirrhosis (Am J Neuroradiol. 2006; 27:1019–26) was not observed in EHPVO. This may be explained by portal-systemic shunting without liver parenchymal abnormality. It also suggests that mI and Cho changes are primarily associated with liver dysfunction rather than to the presence of HE.


FIG 2. Correlation between NPT score and Glx/Cr ratio.
OP2-04

EUROWILSON: THE FIRST PROSPECTIVE STUDY OF WILSON'S DISEASE IN EUROPE

Presenter: S. Tanner. EuroWilson, Sheffield, UK.
Co-authors: A. Dhawan¹, P. Socha², G. Loudianos³, S. Lszl⁴, S. Parker⁵, A. Vegnente⁶, R. Houwen⁷, I. Sarles⁸, J. Deutsch⁹. Paediatric Liver Centre, King's College Hospital, London, UK; Children's Memorial Health Institute, Warsaw, Poland; University of Cagliari, Cagliari, Sardinia, Italy; Semmelweis University, Budapest, Hungary; Orphan Europe, Paris, France; University of Naples “Federico II”, Naples, Italy; University Medical Centre, Utrecht, The Netherlands; Université de la Méditerranée, Marseilles, France; Medizinische Universität Graz, Graz, Austria.

Aims and Methods: To establish a European database of Wilson disease and assess feasibility of paediatric clinical trials. Patients diagnosed between January 1, 2005 and November 30, 2008 were entered using a Web-based secure protocol. A previously validated score was used to verify diagnoses.

Results: 353 cases (176 female), aged 0.25–56.1 (mean 20.4, median 17.6) years from 321 families were entered. Apparent incidence (as cases/million population/35 months) was higher in central Europe (eg, Poland 2.78, Austria 2.53) than western (eg, France 0.56, UK 0.48, Spain 0.32). Patients homozygous for the common mutation H1069Q were older (27.8 ± 12.2 years, n = 65) than the others (19.3 ± 12.5 years). Patients aged <18 years (N = 181, female = 88) had a more homogeneous geographic spread. Country by country variations in the percentage of paediatric cases probably demonstrates variable adult case ascertainment, eg, Poland 18%, UK 86%, 2 non-EU countries, Turkey and India, contributed 27 and 16 paediatric cases, respectively. The mean (median) BMI SDS in 81 cases whose height was measured was 1.8 (2.06). The median diagnostic score was 6 (range 4–10); KF rings were present in 50; caeruloplasmin was measured in 172, and was >0.18 g/L in 34; urine copper was measured in 161, and a penicillamine challenge was done in 172, and was >250 µg/g; 2 mutations were identified in 82, of whom 26 were homozygotes (9 for H1069Q). Severity of liver disease varied: 5% had hepatic encephalopathy, 32% had severe and 18% mild liver disease, 38% abnormal LFTs only, and only 7% had no hepatic abnormalities. Neurological abnormalities were present in 21%, and were severe in 5%, with no correlation with severity of liver disease. There was a wide variety of initial treatments and doses.

Conclusions: This first prospective study of WD demonstrates: (1) the higher incidence in eastern Europe; (2) diagnosis remains difficult because lack of sensitivity in each diagnostic parameter and selective use of tests but is aided by a scoring system; (3) relatively high BMI SDS at diagnosis; (4) wide phenotypic and genotypic variation; (5) in patients <18 years liver failure is rare, but 93% cases show some hepatic abnormality, and 21% have some neurological abnormality; (6) inclusion of cases from non-EU countries will be necessary to mount paediatric RCTs.

OP2-05

LIVER TRANSPLANTATION FOR MITOCHONDRIAL CYTOPATHIES IN CHILDREN: A SINGLE-CENTRE EXPERIENCE

Presenter: R. Vara. King’s College Hospital, School of Medicine, King’s College London, London, UK.
Co-authors: J. Raiman¹, M. Champion¹, B. Portman¹, J. Poulton², N. Heaton¹, G. Mieli-Vergani¹, N. Hadzic¹. ¹King’s College Hospital, School of Medicine, London, UK; ²Nuffield Department of Obstetrics and Gynaecology, University of Oxford, Oxford, UK.

Aim and Methods: To review the outcome of liver transplantation (LT) in children with mitochondrial cytopathy (MC) in a retrospective study of the outcome of all patients diagnosed with MC and received LT between 1987 and 2008.

Results: Overall 29 children with a diagnosis of MC established on tissue or mutational analysis were identified. Two were not listed due to preexisting neurological involvement, both died. One was listed with normal muscle biopsy, a diagnosis of mitochondrial depletion syndrome on the liver tissue postmortem was made, she died awaiting LT. Seven patients (24%, 6 male) were transplanted, median age at presentation 1.8 years (0.24–2.24). Three patients, all presenting with ALF, died less than 2 years post-LT secondary to neurological deterioration. Two had normal CNS imaging before surgery. In all cases diagnosis was retrospective. In patient 7, emergency living related LT was performed before CNS imaging was possible, he died 6 weeks later. In patient 3, CNS imaging could not be obtained before emergency LT, MC was diagnosed on the explanted liver. In patient 4, complex IV deficiency was detected only in liver tissue; muscle enzymeology and CNS imaging were normal. Patient 1 had known complex IV deficiency, decompensation of chronic liver disease warranted LT, after thorough neurological assessments were normal. Patient 2 had LT due to progressive liver disease, complex IV deficiency was made retrospectively after presentation with seizures (Table 1).

Conclusions: MCs with liver involvement in children are rare and heterogeneous disorders. LT is often required as a life saving treatment, but diagnosis is frequently only...
made retrospectively. Children with ALF due to MC have poor prognosis, while those with chronic presentation in the absence of neurological involvement should be assessed on an individual basis. Prompt clinical screening tests for MCs are urgently needed.

**OP2-06**

MULTICENTER TRIAL OF PEGINTERFERON ALFA-2A AND RIBAVIRIN FOR PAEDIATRIC CHRONIC HEPATITIS C

Presenter: E. Sokal. Université Catholique de Louvain, Cliniques St Luc, Brussels, Belgium.

Co-authors: A. Bourgois¹, X. Stephenne¹, T. Silveira², G. Porta³, D. Gardovska⁴, B. Fischer⁵, D. Kelly⁶. ¹Université Catholique de Louvain, Cliniques St Luc, Brussels, Belgium; ²Hospital de clinicas de Porto Alegre, Porto Alegre, Brazil; ³Hospital das clinicas da facultade de medicina da universidade de Sao Paulo, Sao Paolo, Brazil; ⁴Children university hospital, Riga, Latvia; ⁵Huddinge university hospital, Karolinska institute, Stockholm, Sweden; ⁶Birmingham children’s hospital, Birmingham, UK.

**Aim:** This open-label multicenter study aimed to prospectively evaluate the safety and efficacy of a genotype-based Pegylated Interferon alfa-2a/Ribavirin (Pegasys/Copegus, Roche) therapy in treatment-naïve hepatitis C virus (HCV)-infected children.

**Methods:** 65 treatment naive children (aged 6–18 years) with positive HCV serology and quantifiable HCV RNA (>600 IU/mL), were assigned to study medication consisting of 100 μg/m² bsa of peginterferon alfa-2a once weekly and ribavirin (15 mg/kg/day). Genotypes 2 and 3 patients (group 1) received treatment for 24 weeks, and genotypes 1, 4, 5, and 6 patients (group 2) for 48 weeks. End of treatment response (ETR) and sustained virologic response (SVR) were defined as normal transaminases and negative HCV RNA at the end of treatment and at 24 weeks follow-up, respectively.

**Results:** 30 of 65 patients had acquired infection by maternal transmissions, 15 by blood transfusion, 6 by medical procedure, and 14 undetermined. Basal ALT values were normal in 30/65. Ten of 18 (55%) patients from group 1 had a screening viral load >500 000 IU/mL as compared to 13/47 (27.6%) in group 2 (P < 0.05). 34 of 64 patients had no fibrosis on histology (8/18 in group 1). Nine patients, all from the group, stopped prematurely the treatment, 1 for serious adverse event (SAE) (acute hepatitis), and 8 because of no virologic response at week 24. One patient received 48 weeks despite having a week 24 viral load of 695 IU/mL. Peginterferon alfa-2a and ribavirin dose was adjusted in 15 patients, 11 for neutropenia, and 3 patients, for anemia, respectively. No significant weight loss was observed at the end of treatment (−0.3 ± 0.9 vs −0.4 ± 0.9 SD), with no influence on height growth (baseline vs follow-up height SD: −0.4 ± 1.0 vs −0.5 ± 1.1). SAE included acute hepatitis (n = 1), pulmonary hypertension (patient with previous haematological disease), urinary tract infection (n = 1), and thyreotoxicosis (n = 1). Other adverse events included flu-like symptoms and fatigue (42/65), fever (34/65), headache (33/65), vomiting (12/65), abdominal pain (27/65), thyroid disease (7/65), and dermatitis (27/65). Early viral response at week 12 (2 log drop in HCV RNA) was observed in 15/16 patients (group 1) and in 27/46 (group 2) (P < 0.05). ETR was achieved in 100% of patients in group 1 (18/18) and in 58.7% (27/46) in group 2 (P < 0.001). SVR was maintained in 16/17 patients in group 1 and in 27/46 in group 2 (P < 0.01).

**Conclusions:** In paediatric patients with chronic hepatitis C, once-weekly peginterferon alfa-2a plus ribavirin produced SVR in 68% of treated patients, reaching 94% for genotypes 2 and 3. All genotypes 1, 4, 5, and 6 patients with ETR (58.7%) maintained response at follow-up.

<table>
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<th>Age at presentation, y</th>
<th>Consanguinity</th>
<th>Presentation</th>
<th>CNS involvement</th>
<th>Time to LT, y</th>
<th>MC diagnosis (muscle/liver)</th>
<th>Outcome</th>
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<td>N</td>
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<td>2.63</td>
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<td>N</td>
<td>ALF</td>
<td>Y</td>
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<td>II/III (L)</td>
<td>Alive</td>
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<td>Y</td>
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</table>

**TABLE 1.**

Parallel Session: Gastroenterology

G2-01

BIFIDOBACTERIA COUNTERACT THE PROINFLAMMATORY RESPONSE OF INTESTINAL EPITHELIAL CELLS TO GLIADIN-DERIVED PEPTIDES

Presenter: Y. Sanz. Institute of Agrochemistry and Food Technology (IATA), Spanish National Research Council (CSIC, Burjassot-Valencia, Spain).
Co-authors: M. Laparra1, Y. Sanz1, 1Institute of Agrochemistry and Food Technology (IATA), Spanish National Research Council (CSIC), Valencia, Spain.

Aim: To evaluate the capacity of different Bifidobacterium strains to counteract the inflammatory potential of gliadin-derived peptides in intestinal epithelial (Caco-2) cells.

Methods: Gliadins (a,b, v, and g) were subjected to a simulated gastrointestinal digestion (pepsin at pH 3, pancreatin-bile at pH 6) in a bicameral system, and incubated in the presence or absence of cell suspensions (10^8 cfu/mL) of Bifidobacterium animalis IATA-A2, B. bifidum IATA-ES1, and B. bifidum IATA-ES2. The produced gliadin-derived peptides were analysed by reverse phase-HPLC chromatography connected on line to an electrospray system and a quadrupole ion trap mass spectrometer. The production of pro-inflammatory markers (NF-kB p65/p50 and cytokines) was determined by ELISA.

Results: The molecular mass of most of the gliadin peptides from samples not inoculated with bifidobacteria ranged from 2032.4 to 2470.0 Da, and some of them exhibited toxic amino acid sequences, such as a/b-Gld [158–164] and a/b-Gld [222–241]. The molecular mass of gliadin peptides from bifidobacteria-inoculated samples was smaller, ranging from 803.4 to 1154.8 Da, and some of them were not detectable. Gliadin peptides from samples noninoculated with bifidobacteria induced a marked inflammatory response (P < 0.05) in Caco-2 cells. NF-kB expression was increased to 35.4% in gliadin-treated Caco-2 cells in comparison with controls. TNF-a production was 857.2 ± 185.2 pg/mL in gliadin-treated samples and of 230.6 ± 57.4 pg/mL in controls, and IL-1b production was 617.6 ± 98.6 ng/mL in gliadin-treated samples and of 227.2 ± 51.0 ng/mL in controls. This pro-inflammatory response was reduced by co-incubation of gliadin peptides with bifidobacteria to a different extent. NF-kB expression was significantly reduced (P < 0.05) in relation to controls to 13.0 ± 0.7, 14.1 ± 5.2 and 17.2 ± 2.2% by incubation with B. bifidum IATA-ES2, B. animalis IATA-A2 and B. longum IATA-ES1, respectively. In addition, TNF-a production was significantly reduced (P < 0.05) to values of 301.6 ± 3.9, 429.1 ± 85.9 and 616.9 ± 53.7 after incubation with B. longum IATA-ES1, B. animalis IATA-A2 and B. bifidum IATA-ES2, respectively.

Conclusions: The results indicate that Bifidobacterium strains can counteract the pro-inflammatory effects of gliadin peptides on intestinal epithelial cells (Caco-2 cells), and that these effects are partly related to the ability of these bacteria to modify the peptide pattern generated after the gastrointestinal digestion of gliadins.

G2-02

COOPERATION BETWEEN IL15 R ALFA AND EGFR IS RESPONSIBLE FOR GLIADIN PEPTIDE-INDUCED PROLIFERATION IN CACO2 CELLS

Presenter: M. Barone. University of Naples, Federico II, Naples, Italy.
Co-authors: M. Barone1, D. Zanzi1, M. Nanayakkara1, S. Santagata1, G. Lania1, L. Iaffaldano1, R. Kosovo1, M. Ten Eikeider1, R. Troncone1, S. Auricchio1, 1University of Naples, Federico II, Naples, Italy.

Background and Aim: We previously observed that A-gliadin peptide P31-43 induces proliferative effects similar to epidermal growth factor (EGF) both in cultured cell lines and enterocytes from celiac disease (CD) patients. The effect is mediated by delayed EGF degradation and prolonged EGF receptor (EGFR) activation in endocytic vesicles due to P31-43-mediated interference with endocytic maturation. To study P31-43 effects on IL15 induction and the cooperation in signal transduction between a cytokine receptor (IL15-R alfa) and a receptor tyrosine kinase (EGFR).

Methods: Semiquantitative and real time PCR investigated P31-43 effects on IL15 mRNA levels. Protein levels and distribution was analyzed by FACS, ELISA, and immunofluorescence. Stat5, IL-15 receptor alfa (IL15Ra) and EGFR activation has been examined by WB, BrdU (bromodeoxiuridine) analyzed proliferation.

Results: In Caco2 cells IL 15 protein was found increased after P31-43 treatment only on the cell surface together with markers of recycling vesicles, such as transferrin receptor and Lamp2, implying a P31-43-mediated interference with IL15 vesicular trafficking. On the cell surface IL15 is linked to the receptor, its increase is not dependent on new protein synthesis and functions as a growth factor for CTLL 2 cells. Stat5, IL15Ra, and EGFR are activated after P31-43 treatment. P31-43 induces IL-15R alfa/EGFR complex, and both
EGFR silencing mRNA and IL15 blocking antibody can prevent P31-43 induced proliferation in Caco2 cells.

**Conclusions:** P31-43 induces enhanced presentation of IL15 in trans to the neighboring cells, interfering with its vesicular trafficking. Justacrine signaling of the IL15/IL15-receptor-alfa contributes both to cell proliferation and activation of innate immunity. Cooperation between II15 R alfa and EGFR is responsible for gliadin peptide induced proliferation in Caco2 cells.

**Co-authors:** F. Sauvat1, F. Joly2, J. Hugot3, C. Talbote1, France.

**Presenter:** U. Halac.

**DISCUSSION FOR TRANSPLANTATION PARENTERAL NUTRITION AND EARLY IMPROVED PROGNOSIS WITH EXPERT MICROTILLUS INCLUSION DISEASE:**

**Background and Aim:** Microvillous inclusion disease (MVID) is a severe congenital disorder of the intestinal epithelial cell, responsible for neonatal intestinal failure, and previously described as mostly lethal in the first year of life. In this retrospective study, we report an improved prognosis, but also the specific complications due to huge water and electrolytes losses, in order to emphasize the importance of an early specialized care and discussion of small bowel transplantation (SBTx).

**Methods:** From 1995 to 2008, 21 patients (7 girls, 2 siblings) with MVID were followed up, for a median of 5 years (birth to 23 years). All of them received a parenteral nutrition (PN), up to 200–250 mL/kg, in 8 of them 24 hours/day. SBTx was performed in 13. Growth, neurological development, liver and renal functions, and bone disease were recorded.

**Results:** Half of the children were from consanguineous families (12/21) from the Mediterranean area (19/21). Before or without SBTx, growth failure was common (mean height, −2.5 SD), as well as developmental delay (12/21), liver (19/21 with fibrosis), kidney (4/21 with clearance <70 mL/min/1.73 m²), and bone (6/21 with osteoporosis) diseases. Thirteen children underwent SBTx, isolated SBTx in 9, with the liver in 4, at a median age of 3.5 years (1–11.5 years). Median follow-up after SBTx is 4 years (3 months–14 years). Four underwent a removal of the graft, and 2 a re-transplantation. Five children died, 3 without, 2 after SBTx. Patient survival rate was 63% without SBTx, 85% after SBTx. After SBTx a catch-up growth was observed in 4 children. Renal function did not worsen despite tacro-limus. Six children needed special care for developmentnal delay. Neither neurological nor renal disease was observed in 7/8 children on continuous PN.

**Conclusions:** PN in MVID is difficult to manage, and needs a real expertise. Neurological and renal abnormalities are mostly due to episodes of dehydration or electrolyte imbalance, better prevented with continuous PN. SBTx is also a difficult treatment, but remains the only hope for these children to improve their quality of life and long-term prognosis, before the development of irreversible complications.

**G2-03**

**MICROVILLOUS INCLUSION DISEASE: AN IMPROVED PROGNOSIS WITH EXPERT PARENTERAL NUTRITION AND EARLY DISCUSSION FOR TRANSPLANTATION**

**Presenter:** U. Halac. *Necker-Enfants malades, Paris, France.*

**Co-authors:** F. Sauvat1, F. Joly2, J. Hugot3, C. Talbote1, V. Colomb1, F. Ruemmele1, O. Goulet1.1 *Necker-Enfants Malades, Paris, France.*

**Methods:** From 1995 to 2008, 21 patients (7 girls, 2 siblings) with MVID were followed up, for a median of 5 years (birth to 23 years). All of them received a parenteral nutrition (PN), up to 200–250 mL/kg, in 8 of them 24 hours/day. SBTx was performed in 13. Growth, neurological development, liver and renal functions, and bone disease were recorded.

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**G2-04**

**IN VITRO TH1/TH2, AND TH3/TR1 (REGULATORY) CYTOKINE SECRETION IN PERIPHERAL BLOOD MONONUCLEAR CELLS IN RESPONSE TO STIMULATION WITH COW’S MILK PROTEINS IN PRETERM INFANTS WITH NEC AND SEPSIS**

**Presenter:** A. Adel. *Chelsea and Westminster Hospital, London, UK.*

**Co-authors:** P. Hayes2, S. Chuang1, J. Fell1, J. Fell1.1 *Chelsea and Westminster Hospital, London, England; 2Imperial College, London, England.*

**Aim:** To explore evidence of an alteration in the balance of proinflammatory and regulatory cytokine responses (Th1/Th2/Th3/Th1) in the blood to in vitro stimulation with cow’s milk antigens in infants with NEC, preterm controls, and preterm infants with systemic sepsis (non-NEC).

**Methods:** 14 newborn infants with Bell’s staging 2–3 NEC [median postconceptional age (PCA); 32.5 weeks (range 29–36)], 14 age- and gestation-matched controls (CON) [median PCA 32.5 weeks (29–36)] and 10 preterm infants with blood culture positive sepsis (SEP) [median PCA 28.5 weeks (26–35)] were studied. Spontaneous and antigen: casein and beta lactoglobulin (cow’s milk proteins: CMP) or mitogen elicited interferon-gamma (IFN), IL-4, IL-10, and transforming growth factor-beta responses were enumerated using single-cell enzyme-linked immunospot (ELISPOT) assay in peripheral blood mononuclear cells (PBMC). Prior to assessment 0/14 NEC, 7/14 CON, and 1/10 SEP infants had been exclusively breast-fed, the rest had received some formula milk (less than 50% formula for 10/14 NEC, 4/14 CON, and 9/10 SEP). Median C-reactive protein levels for NEC 182 mg/L (IQR 123–231), SEP 104 mg/L (range 70–250), and CON 36 mg/L (IQR 28–48).

**Results:** In NEC infants, compared to controls, there was a significant increase in the baseline number of PBMC cytokine secreting cells, vigorous mitogen responses (10-fold increase) for IFN, IL-4, and IL-10 (P<0.002), strong responses to beta lactoglobulin (10-fold increase) for IFN,
IL-4, and IL-10 ($P=0.002$) and a somewhat smaller (5-fold) casein responses ($P=0.002$). In NEC, TGF-beta secreting cells were fewer in number although there were small responses to mitogen and milk proteins. In general cytokine responses in SEP cases were smaller (2- to 5-fold) than for NEC, with minimal TGF-beta response to milk proteins.

**Conclusions:** We have thus demonstrated significant CMP sensitisation in NEC, at least in the systemic compartment (IFN, IL-4, IL-10). The TGF-beta (regulatory) cytokine response to CMP was less a pronounced.

The ‘intermediate’ responses in septic infants may reflect generalised immune upregulation in sepsis, although loss of mucosal integrity and thus antigen translocation across the mucosal barrier could also account for the antigen-specific immune activation in these cases.

**Parallel Session: Hepatology**

**H1-01**

**CO-CULTURE WITH MESENCHYMAL STEM CELLS RESULTS IN IMPROVED VIABILITY AND FUNCTION OF HUMAN HEPATOCYTES: POTENTIALLY A NEW STRATEGY FOR THE CELLULAR THERAPY OF LIVER DISEASE**

Presenter: E. Fitzpatrick. King's College Hospital, London, UK.

Co-authors: J. Waelzel1, C. Philippeos1, S. Lehec1, J. Puppi1, R. Hughes1, R. Mitry1, A. Dhawan1

**Background and Aim:** Mesenchymal stem cell (MSC) transplantation has been shown to improve survival in animal models of liver disease and in pilot clinical studies of liver failure. It is not known whether this therapeutic effect is due to the potential of MSC to differentiate into hepatocytes in the appropriate microenvironment or to their immunomodulatory effects. The aim of this project is to determine the effect of MSC on human hepatocyte viability and function in vitro.

**Methods:** Human hepatocytes (HC) were isolated from livers donated but unsuitable for transplantation and from liver resection specimens using a collagenase perfusion technique. Hepatocytes were cultured under standard conditions on collagen coated plates. A human MSC line derived from adipose tissue was used. Co-culture was performed using direct cell-cell contact (ratio from 1:1 to 10:1 MSC:HC) and indirectly using a porous transwell membrane. Viability of hepatocytes was assessed by trypan blue, flow cytometry (propidium iodide/Annexin V assay) and real time PCR. Albumin production was measured by ELISA. PCR was used to detect the expression of liver-specific genes in MSC following co-culture to identify transdifferentiation. MSC and HC were also co-cultured in alginate beads to determine if MSC had any effect on viability and function of HC in this format.

**Results:** A 20% increase in the viability of hepatocytes indirectly co-cultured with MSC compared to control was detected using flow cytometry. This was confirmed by PCR showing a 3-fold downregulation in expression of caspase 3 in the co-cultured group. Hepatocyte albumin production in direct co-culture with MSC increased to 180% ± SE 23% ($P < 0.05$) of control values at a ratio of 2:1 on day 2 and to 480% ± SE 121% ($P < 0.01$) at a ratio of 10:1 on day 4. This effect was not seen in cells which were indirectly co-cultured. Partial transdifferentiation of MSC into hepatocytes may have occurred in indirect co-culture as PCR revealed albumin expression by MSC. Increased albumin production to 180% and 310% of control values was seen in hepatocytes which were co-encapsulated with MSC, at day 7 and day 11, respectively.

**Conclusions:** An improvement in viability was seen in hepatocytes indirectly co-cultured with MSC, direct contact was needed between hepatocytes and MSC to demonstrate an improvement in albumin production. Co-encapsulation with MSC improved long-term hep atocyte function. The use of MSC to improve the function and viability of hepatocytes may be beneficial in the cellular therapy of liver disease.

**H1-02**

**TREATMENT OF CD20+ POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDERS BY RESPONSE-ADAPTED SEQUENTIAL RITUXIMAB AND CHEMOTHERAPY: INTERIM RESULTS OF TRIAL PED-PTLD 2005 PILOT**

Presenter: B. Maecker-Kolhoff. Hannover Medical School, Hannover, Germany.

Co-authors: B. Meissner1, C. Kebelmann-Betzing2, G. Henze2, C. Klein1. 1Hannover Medical School, Hannover, Germany; 2Charité University Medicine, Berlin, Germany.

**Background and Aim:** Epstein-Barr virus (EBV) associated posttransplant lymphoproliferative disorders (PTLD) have been recognized as severe side effects after organ transplantation. So far, no standard diagnostic and therapeutic approaches have been defined. The aim of this study is to evaluate a standardized diagnostic procedure and response-adapted treatment protocol for...
pediatric patients with CD20-positive PTLD after solid organ transplantation.

**Methods:** We report an interim result of the prospective multicenter trial Ped-PTLD 2005 Pilot. After biopsy and central pathology review pediatric patients with histologically confirmed CD20+ PTLD were treated by weekly infusions of anti-CD20-antibody (rituximab). Initial treatment response was assessed after 3 weeks by ultrasound, MRT-, CT-, and/or PET-scan imaging. Patients with at least a partial remission (>25% reduction in tumor volume) received 3 further rituximab infusions on a protracted schedule. All others were stratified to receive a moderate chemotherapy regimen (mCOMP; vincrisin, cyclophosphamide, low dose methotrexate and prednisone). During treatment, EBV copy number in peripheral blood was monitored by PCR, and patients’ EBV-specific T cells were evaluated using interferon-γ ELISPOT analysis.

**Results:** 23 patients have been enrolled so far, among them four liver transplant recipients. 17 patients were treated with CD20-antibody alone, one of whom relapsed. 6 patients received additional mCOMP chemotherapy, which led to complete remission in 4 patients, including 2 patients with chromosomal translocations involving the C-MYC locus. 2 patients were nonresponsive and went to receive chemotherapy according to NHL/BFM protocol. One patient died of bowel perforation during treatment, while treatment was well tolerated in all other patients. Overall survival is 90% at 2 years with an event-free survival of 75%. After 3 rituximab doses, rituximab responders showed significantly lower EBV copy number in peripheral blood (P=0.008) than nonresponders.

**Conclusions:** Early evaluation of treatment response may identify patients in which rituximab monotherapy is sufficient to control PTLD. Complete remission is achieved by moderate chemotherapy in patients not responding to rituximab including Burkitt-like PTLD. These results support the need for standardized, interdisciplinary approaches for this severe transplant complication.

**H1-03**

**LIVER TRANSPLANTATION IN HEPATOCELLULAR CARCINOIMA IN CHILDREN WITH UNDERLYING CHRONIC LIVER DISEASE**


Co-authors: M. Samyn¹, A. Quaglia¹, D. Lewis¹, P. Kane¹, G. Mieli-Vergani¹, N. Heaton¹, M. Rela¹.

¹Institute of Liver Studies, London, UK.

**Background and Aim:** Hepatocellular carcinoma (HCC) is the second most common primary malignant liver tumour in childhood and often found in the context of an underlying chronic liver disease (CLD). Complete resection of the tumour offers the only chance of survival since the response to chemotherapy is poor. Liver transplantation (LT) has been reported as a therapeutic option, however numbers are small. The aim of the study was to report our experience at King’s College Hospital with LT in HCC in children with underlying CLD.

**Methods:** Review of clinical records, histopathology and radiology of children with CLD and HCC who underwent LT.

**Results:** Nineteen children with CLD were diagnosed with HCC between 1991 and 2008. Five had metastatic disease and 1 unresectable disease and were treated with chemotherapy/immunotherapy and died at a median time of 0.2 years after diagnosis. One child underwent chemotherapy, embolisation, followed by an extended hepatectomy and is alive 1.7 years after surgery. LT was carried out in 12 children (7 male) between 1997 and 2008, at a median age of 2.2 years (range 1.2–17.8 years). Underlying liver disease was: liver disease (6), liver disease (1), biliary atresia (5), PFIC (4) and autoimmune liver disease, alpha-1-antitrypsin deficiency and parenteral nutrition-related liver disease in 1 each. In 3 HCC was an incidental finding on histology of the explanted liver. Median values for serum bilirubin and alpha-fetoprotein were 160 μmol/L (range 35–676) and 191 KU/L (range <2–23,355), respectively. Tumour size on radiology varied between 0.5 and 9 cm with portal vein thrombus in 1 and lymphadenopathy in 2. Three children received a whole graft and 1 transplant was living-related in a child with no suspicion of HCC prior to LT. Histology on the explant showed vascular invasion in 6 (macroscopic (1) microscopic (5)), in chemotherapy prior to LT was given and 1 child with macroscopic vascular invasion received 1 course post LT. All children are alive without recurrence at a median time of 3.3 years (0.5–11.1 years). There has been no graft loss. Two children were outside Milan criteria, 2 outside UCSF criteria, and all were within Hangzhou criteria.

**Conclusions:** In contrast to previous reports we show that LT without adjuvant chemotherapy is successful in pediatric HCC on the background of CLD. Six monthly screening for HCC in children with CLD using ultrasound scan and alpha-fetoprotein is advisable for early recognition and to avoid progression to extrahepatic disease, which remains a contraindication for LT.
Background and Aim: Natural killer T (NKT) cells are a unique subset of T lymphocytes that express T cell receptors (TCR) and NK1.1, a marker typically associated with natural killer (NK) cells. Most NKT cells in mice express TCR composed of an invariant Va14Ja18 chain that recognizes antigenic glycolipids presented in association with nonclassical MHC class Ib molecules: CD1d. These invariant (i)NKT cells have been implicated in numerous diseases including autoimmune hepatitis and have been shown recently to suppress cholestatic liver injury (CLI). Kupffer cells have been described to ameliorate cholestatic liver injury in an interleukin-6 dependent manner. The aim of this study was to delineate the role of iNKT cells in the early development of cholestasis and to decipher the interaction with Kupffer cells. As a widely established animal model of CLI, ligation of the common bile duct injury (BDL). This effect was omitted if mice were rendered neutrophil deficient, relative to wild-type, mice as judged by plasma alanine aminotransferase (ALT) levels were diminished and comparable in wild-type and NKT-KO mice rendered neutrophil-deficient prior to BDL, as well as in NKT-KO mice that received adoptively transferred NKT-cells prior to BDL.

Conclusions: These data document the role of iNKT cells in attenuating neutrophil-dependent CLI and show their Kupffer-cell dependent activation.

Co-authors: C. Cheng2, S. Gehring4, S. Wirth1, L. Brossay3, S. Gregory2, 1HELIOS, Klinikum Wuppertal, Witten-Herdecke University, Wuppertal, Germany; 2Department of Medicine, Rhode Island Hospital and the Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA; 3Department of Molecular Microbiology and Immunology, Division of Biology and Medicine, Brown University; 4University of Mainz, Mainz, Germany.
variants located in the KIAA1109/Tert/IL2/IL21 region and in TNFSF14 gene, might suggest gene-gene interactions between these two genomic regions.

PG1-02
INCREASED HEAT SHOCK PROTEIN 72 EXPRESSION IN COELIAC CHILDREN
Presenter: B. Szebeni. Semmelweis University and Hungarian Academy of Sciences, Budapest, Hungary.
Co-authors: E. Sziksz, A. Vannay, G. Veres, A. Dezső, A. Öndö, I. Korponay-Szabó, A. Szabó, T. Tulassy, A. Arató. Research Group for Pediatrics and Nephrology, Semmelweis University and Hungarian Academy of Sciences, Budapest, Hungary; First Department of Pediatrics, Semmelweis University, Budapest, Hungary; Szentágothai Knowledge Centre, Semmelweis University, Budapest, Hungary; University of Debrecen, Department of Pediatrics, Debrecen, Hungary.

Background and Aim: Heat shock protein (HSP) 72 can be released from cells during the response to stress and injury. Extracellular HSP72 activates monocytes, macrophages and dendritic cells and upregulates the expression of proinflammatory cytokines by binding Toll-like receptor (TLR) 2 and TLR4. Recently we have found higher TLR2 and TLR4 protein levels in the duodenal mucosa of children with untreated coeliac disease (CD) and children with treated CD compared to controls. Since the role of HSP72 in CD is unknown, our aim was to characterise the expression of HSP72 in duodenal biopsy samples taken from children with CD and from controls.

Methods: Duodenal biopsy specimens were collected from 16 children with untreated CD [median age (range): 6.7 (3.7–13.9)], 9 children with treated CD [median age (range): 6.7 (4.9–12.7)] and 10 controls [median age (range): 8 (1.7–13)]. The mRNA expression of HSP72 was determined by real-time reverse transcription-polymerase chain reaction (RT-PCR). HSP72 protein levels were determined by Western blot.

Results: We found higher HSP72 mRNA and protein levels in the duodenal mucosa of children with untreated CD as well as children with treated CD compared to controls (P < 0.05). In the duodenal mucosa of children with treated CD, HSP72 mRNA expression was decreased and HSP72 protein levels were lower than in children with untreated CD (P < 0.05).

Conclusions: Our results of increased expression of HSP72 in untreated CD and decreased expression in treated CD suggest that this heat shock protein should mediate cellular protection against gliadin induced cytotoxicity. HSP72 may act as a “danger signal” via TLR2 and TLR4 to the innate immune system, so the distressed cells can warn neighboring cells of potential injury.

PG1-03
ALTERNATIVE CEREALS IN CD DIET
Presenter: M. Barone. University of Naples, FedericoII, Naples, Italy.
Co-authors: M. Maglio, B. Colicchio, M. Nanayakkara, L. Gazz, R. Troncone, S. Auricchio, M. Barone.

Background and Aim: The search for cereals that can improve the quality of CD diet is a real need both to render more acceptable the diet and to improve its nutritional quality. Alternative cereals to be introduced into CD diet should be tested for their effects on both adaptive and innate/proliferative response (Barone et al. Gut 2007). We have developed assays to investigate the proliferative/innate immunity activation. The aim of this study was to investigate the toxicity of prolamin derived from two oats varieties (Avena potenza and Avena gentziana) and four accessions of ancient wheat Triticum monococcum (ID331, 3882, 3221, Monlis).

Methods: We evaluated by BrdU incorporation crypt epithelial cell proliferation in small intestinal biopsies from CD patients on a gluten containing diet cultured for 24 h with 0.5 mg/mL of a peptic-tryptic (PT) digest from various oats or T. monococcum, PT digest from bread wheat (T aestivum Sagittario) was used as positive control. In addition, we analyzed ERK2 phosphorylation by Western blot in CaCo2 cells after 30 min stimulation with the same cereals.

Results: PT digest from three varieties of T. monococcum (ID331, 3882, 3221) as well as PT from Avena genziana did not induce significant increase of crypt epithelial cell proliferation in small intestinal biopsies after 24 h of incubation. Monococcum monlis and Avena potenza, instead, induced a very significant increase of crypt proliferation. Increased ERK2 phosphorylation was observed in Caco2 cells after 30 min incubation with A. potenza. The three varieties of T. monococcum (ID331, 3822, 3221) did not induce ERK2 phosphorylation.

Conclusions: Not all monococcus accessions and Avena varieties have the same in vitro effects on the celiac mucosa. Therefore the toxicity for celiac patients of these various cereals should now be tested in vivo.

PG1-04
PASSIVE TRANSFER MODEL OF COELIAC AUTOIMMUNITY IN NEWBORNS FROM MOTHERS WITH COELIAC DISEASE AND TRANSGlutaminase ANTIBODIES
Background and Aim: It is still unclear how much role antibodies against transglutaminase 2 (TG2) play in the intestinal and extraintestinal manifestations of coeliac disease, because no experimental model exists where humoral and cellular immune pathology could be assessed separately. The placenta is rich in TG2 and coeliac disease is frequently associated with obstetric problems and low birth weight in the offspring. We investigated whether maternal antibodies appear and cause pathology in the newborns.

Methods: Placenta, umbilical cord and serum specimens were investigated for TG2-specific antibodies at 8 deliveries when the mother had biopsy-proven coeliac disease. Umbilical cord vein endothelial cells (HUVEC) were prepared and studied in culture. The tissue specimens also were analysed for TG2 by monoclonal anti-TG2 antibodies recognising various extracellular and intracellular TG2 epitopes.

Results: Four mothers were positive for anti-TG2 antibodies, one of them was IgA deficient with high IgG class serum anti-TG2. Extensive IgA class anti-TG2 antibody depositions were present within all maternal tissues of the placenta in all 3 IgA competent coeliac mothers with active disease. Furthermore, chorionic villi had less secondary branches and vascular arches. IgA also bound to surface TG2 of chorionic villous structures, but did not reach foetal blood. Also IgM was found in the placenta as a line at the mother/infant interfaces. Umbilical cords were negative for both IgA and IgM, but contained deposited IgG, and IgG anti-TG2 antibodies appeared in the blood of the babies. In the case of the IgA deficient coeliac mother, IgG anti-TG2 was bound in endomysial pattern to umbilical cord TG2 and reached high concentrations in the serum of the newborn. This baby was small for date, had hyperviscosity, low blood sugar levels and liver damage, but recovered spontaneously and lost maternal antibodies at the age of 9 months. HUVECs exposed in utero to anti-TG2 antibodies had reduced cell survival and showed changes in their shape and adhesion compared to HUVECs obtained from the babies of antibody-negative coeliac mothers.

Conclusions: TG2-specific antibodies in the placenta may play a role in the obstetric complications of coeliac disease. Coeliac IgA does not penetrate into the baby, but passive transfer of maternal IgG anti-TG2 antibodies may cause pathology in the newborn also in the absence of T-cell reactions. It remains to be further investigated whether the observed structural changes in the chorionic villi share similarities with jejunal villous atrophy.

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Methods: A population-based incident case-referent study performed during the epidemic. Included were celiac disease cases diagnosed before 2 years of age and fulfilling ESPGHAN 1990 criteria. Cases (n = 475) and referents (n = 950), matched for age, sex and area of residence, received a questionnaire returned by 96% and 90%, respectively. Information was received on common childhood infections, socioeconomic status, and infant feeding, i.e., breast-feeding, age at introduction of dietary gluten and the amount consumed per day two weeks later. Complete data and a matched set with one case and one or two referents were available for 373 (79%) cases and 581 (61%) referents. Bivariate and multivariate conditional logistic regressions were performed.

Results: Children with three or more infectious episodes before six months of age had increased celiac disease risk before two years of age (OR 1.5, 95% CI 1.1–2.0), after adjusting for infant feeding and socioeconomic status. The risk remained also when disregarding episodes of symptomatic gastroenteritis. Belonging to a low socioeconomic group, compared to high-medium, remained an independent risk factor also in the final model. The celiac disease risk increased further if infants, in addition to several infectious episodes, were introduced to gluten in large amounts, compared to small or medium amounts (adjusted OR 2.4, 95% CI 1.5–3.7).

Conclusions: In this incident case-referent study we found that children with three or more infectious episodes early in life had increased celiac disease risk. Thus, infectious episodes might have a causal role in celiac disease development. It is unclear whether the infectious panorama changed or not over the celiac disease epidemic period, but still our results suggest that early infections indirectly might have contributed. The reason is a synergistic effect in-between early infectious episodes and amount of gluten during introduction, where
the latter changed concurrently with the beginning and ending of the epidemic. Therefore, in addition to abrupt introduction of gluten without ongoing breast-feeding, also early infections might contribute to celiac disease risk, and thereby further explain the Swedish epidemic. Our finding has implications for both increased understanding of celiac disease etiology and for exploring the possibility of primary prevention.

PG1-06

IL-17A: A NEW INFLAMMATORY MEDIATOR IN COELIAC DISEASE

Presenter: M. Barba. University of Rome La Sapienza, Rome, Italy.
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Background and Aim: Coeliac disease (CD) is a complex autoimmune and inflammatory intestinal disorder, characterized by progressive lesions of small intestinal mucosa on a gluten-containing diet. The pathogenetic mechanism is mainly determined by gliadin dendritic cell presentation to intraepithelial CD4+ lymphocytes which release different cytokines, such as IFN-gamma, IL–10, IL–2 and IL–4, responsible of the inflammatory onset. Accumulating evidence underlies the role of IL–17A, the more characterized member of a new proinflammatory cytokine family, during the onset of many inflammatory and autoimmune diseases. This cytokine is mainly produced by Th17 cells, a subset of CD4 T helper cells and can trigger inflammatory and degenerative responses. The aim of our study was to investigate whether different lymphocyte subsets purified from peripheral blood or from distal duodenum biopsy of 12 CD pediatric patients and 5 gastrointestinal controls were able to produce IL–17A.

Methods: Lymphocytes from peripheral blood were isolated by Lymphoprep gradient centrifugation whereas lymphocytes derived from distal duodenum biopsy were extracted by a mechanical smashing performed using 70micron cell strainer. In order to assess IL–17A production from the cells mentioned above we stimulated cells with PMA and Ionomycine for 8h and we checked the intracellular cytokine expression by FACS analysis on different lymphocytes subsets (CD4+CD3+, CD8+CD3+, Tgamma delta, CD56+CD3–).

Results: Our experiments show that only CD3+ lymphocytes extracted from intestinal mucosa of CD patients significantly produce IL–17A and IFN-gamma, while CD3+ lymphocytes purified from peripheral blood of CD patients as well that purified from gastrointestinal controls express non significant levels of IL17A. We individuate that CD4+CD3+ and CD8+CD3+ lymphocytes subsets are the main source of IL–17A in CD pediatric patients.

Conclusions: Our results suggest that the release of IL–17A from CD+ and CD8+ lymphocytes extracted from distal duodenum biopsy may contribute to the pathogenesis of CD through the regulation and the amplification of the inflammatory response.

PG1-07

PREVALENCE OF THYROID AUTOIMMUNITY IN CELIAC DISEASE CHILDREN–DIAGNOSED AND ON GLUTEN FREE DIET VERSUS SCREENING DETECTED UNTREATED CASES, AND ON AGE- AND SEX-MATCHED CONTROLS

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Background and Aim: Celiac disease (CD) is associated with increased prevalence of thyroid autoimmunity as well as other autoimmune diseases. Studies have reported a correlation between duration of untreated CD and risk for other autoimmune diseases, suggesting that early diagnosis and treatment might reduce the risk to develop concomitant autoimmune diseases. In this study our aim was to investigate the prevalence of thyroid autoimmunity in 3 groups of 12-year-old children: i) symptomatic CD with early diagnose and treatment, ii) screening-detected untreated CD, and iii) children without CD.

Methods: Blood samples were collected from 7207 Swedish 12-year-old children within a nationwide school based CD screening study performed in 2005–2006. All blood samples were analysed for anti-human tissue transglutaminase [tTG] by Celikey, and total s-IgA. In IgA-deficient children Celikey IgG was also evaluated. When tTG values were below, but close to cut off, endomysial antibodies were also analysed. A small intestinal biopsy was recommended for all children with elevated serological markers. Previously diagnosed CD was reported. Criteria for CD diagnosis were villous
atrophy or a combination of increased intraepithelial lymphocytes and symptoms compatible with CD. A random sex-stratified sample of 1151 controls was selected. Blood samples from all CD cases and controls were analyzed for autoantibodies against thyroid peroxidase (TPOAb).

**Results:** In total 207 CD cases were revealed; 62 were symptomatic cases diagnosed and treated with gluten free diet, and another 145 were detected and biopsy confirmed within the study. Among CD children 6.3% (13/207) had elevated titres of TPOAb, compared to 2.7% (31/1151) of the controls. Stratifying on CD cases previously symptomatic and therefore diagnosed and treated, and the now screening detected and untreated cases, the prevalence of TPOAb positivity was 8.1% (5/62) and of 5.5% (8/145), respectively.

**Conclusions:** This nationwide screening study for children with CD indicated an increased prevalence of thyroid autoimmunity among children with CD as compared to controls. However we could neither verify results from earlier studies showing a significant association between CD and thyroid autoimmunity, nor confirm the proposed hypothesis that duration of untreated CD increases the risk for development of concomitant autoimmune disease. Thus, our findings challenge a simple relation between early initiation of CD treatment and risk for thyroid autoimmunity.

**PG1-08**

**ANTIBODIES AGAINST DEAMIDATED GLIADIN PEPTIDES IN DIAGNOSING AND MONITORING COELIAC DISEASE IN SELECTIVE IGA DEFICIENCY**

*Presenter:* I. Korponay-Szabo. *University of Debrecen, Debrecen, Hungary.*

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**Background and Aim:** IgG class antibodies against endomysium (EMA) and type-2 transglutaminase (TG2) are good serologic markers of coeliac disease (CD) in subjects with selective IgA deficiency (Adef), but they remain often long time positive after introducing the gluten-free diet. This makes noninvasive clinical and dietary evaluation of Adef coeliac patients challenging. We investigated whether measuring serum antibodies against deamidated gliadin peptides (DGP) would earlier show treatment effect by recession of seropositivity.

**Methods:** DGP antibodies were measured from serum samples of 77 biopsy-proven untreated Adef CD patients (median age 6.9 years, range 1–73) and 44 Adef controls (median age 2.1 years, range 1–16) by a commercial kit with a combined IgA and IgG secondary conjugate. Follow-up samples were available from 64 of the Adef CD patients. Serum samples were collected at 3, 6–9 and 12 months and on a long-term (longer than 3 years) gluten-free diet and EMA/TG2 antibodies were prospectively measured. DGP antibodies were detected from the stored samples. Antibody levels and kinetics were compared in follow-up samples in 18 sib pairs (all children) where one sib had IgAdef CD while the other had CD with normal serum IgA level. Diet compliance was assessed by a structured questionnaire.

**Results:** From the untreated Adef CD patients, 75 (97.4%) had circulating IgG class EMA or TG2 antibodies while all 77 were initially positive for DGP antibodies. DGP antibodies were positive in two controls (specificity 95.4%). During long term diet, 25/64 (39%) Adef CD patients achieved eventually negative EMA/TG2 results and 73% achieved negative DGP antibody results. Whereas all but one Adef CD had still had high or moderately elevated EMA or TG2 antibodies after one year on diet, DGP antibodies already decreased in 41% of Adef CD patients below the cut-off of clear positivity. In CD sib pairs where similar dietary compliance could be assumed, the IgA competent subjects attained significantly earlier seronegativity for EMA/TG2 antibodies than the Adef CD patients, while such a difference was not observed for DGP antibody positivity.

**Conclusions:** DGP antibodies are highly sensitive and specific for CD also in subjects with selective IgA deficiency. In the first 1–3 years of treatment after gluten exclusion, DGP antibodies indicate better the adherence to the gluten-free diet than EMA/TG2 antibodies in Adef CD patients.

**PG1-09**

**THE SEARCH FOR INTESTINAL DEPOSITS OF ANTI-TISSUE TRANSGLUTAMINASE IGA IN THE DIAGNOSTIC APPROACH TO PEDIATRIC COELIAC DISEASE**

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**Background and Aim:** The majority of patients with celiac disease (CD) have IgA anti-tissue-transglutaminase antibodies (anti-TG2) that are produced and deposited at intestinal level (Korponay-Szabo Gut...
Methods: The study involved 226 patients consecutively enrolled from January 2004 to September 2008 (108 males and 118 females, median age 6.3 years, range 6 months–17.10 years) who underwent duodenal biopsy for the suspicion of CD. Our population was divided into 4 groups. The first group (group A) included 87 celiac patients with intestinal mucosal damage type M3b-c (according to Marsh classification modified by Oberhuber) of which 76 (87%) had high levels of anti-TG2. The second group (group B) was made up of 59 patients with potential CD, subjects with positive serology for CD but architecturally normal intestinal mucosa (M0,M1). The third group (group C) included 12 celiac patients on a gluten free diet with normal intestinal mucosa who were negative for anti-TG2. Finally, the last group (group D) included 68 control patients without CD of which 47 (69%) with normal mucosa (M0,M1) while 21 (31%) with mucosa classified as M3a; all of them were negative for anti-TG2. All the jejunal sections were evaluated for the presence of intestinal deposits of IgA anti-TG2 by double immunofluorescence.

Results: Deposits of IgA anti-TG2 were present in 84%, 73%, 17% and 15% of patients from group A, B, C and D, respectively. Overall the sensitivity was 79% (compared to 92% of serum antiTG2) and a specificity of 85% (vs 100%). The PPV for CD was of 92% (vs 100%), while the NPV was of 66% (vs 86%). Correlation with serum anti-TG2 was 84%.

Conclusions: The detection of intestinal deposits of IgA anti-TG2 represents a useful diagnostic tool. Further research is needed to evaluate in potential CD to what extent they are predictive of evolution to villous atrophy.

PG1-11
SEVERE INTRAHEPATIC CHOLESTASIS IN MICROVILLOUS INCLUSION DISEASE
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**Background:** Microvillous inclusion disease (MVID) is a congenital disorder of the enterocyte, responsible for an intractable neonatal diarrhoea. A few patients presented with a severe cholestatic liver disease, beginning either in the first months of life, or only after small bowel transplantation (SBTx). This suggests a role for the myosin Vb, the cytoplasmic motor protein deficient in MVID, in the physiology of bile synthesis.

**Patients:** Among the 24 children with MVID seen in our unit from 1995, 9 received an isolated SBTx. Five of them (2 girls) developed a severe cholestatic pruritus, 3 already before and 2 only after SBTx (age at last follow-up 2 to 13 y).

**Results:** The clinical symptoms in the 5 patients were a permanent and severe pruritus, an intermittent jaundice, and a mild hepatomegaly. The biology showed a normal GGT activity, an elevation of conjugated bilirubin, a high serum level of bile acids, and mildly elevated transaminases. Liver histology showed a lobular cholestasis, was not cholestatic in the pruritus-free patient. In 3 children (P1, 2, 3), this developed from the first months of life, and in 2 (P4, 5), only 3 months after SBTx. Before SBTx, pruritus was poorly responsive to medical treatment. After SBTx, an external biliary drainage in P5 relieved the pruritus; he underwent an ileal exclusion, and has no more cholestasis. Ileal exclusion was less efficient in P3, who needs rifampicin for pruritus, and has a mildly elevated bilirubin. P2 has no ileal exclusion, an intermittent jaundice, and a partially controlled pruritus with UDCA and rifampicin. The graft was later removed in P1 and P4 for rejection; the pruritus resolved in P4. They both wait for a second SBTx with the liver; histology showed cirrhosis in P1, severe fibrosis in P4. Genetic study of the myosin Vb gene in 4/5 children showed different mutations in all of them.

**Conclusions:** These patients’ liver disease is very similar to progressive familial intrahepatic cholestasis (PFIC), and different from the parenteral nutrition-associated one, where pruritus is not an usual feature. This points to a role of myosin Vb not only in the enterocyte, but also in the hepatocyte, maybe through an interaction with bile acid transporters. Functional studies are needed to understand the patients’ different clinical features. The symptomatic treatment is difficult, and the future uncertain.

**PG1-12**

**VALIDATION OF A NEW POINT-OF-CARE TEST FOR A UNIVERSAL SCREENING OF CELIAC DISEASE**

**Presenter:** F. Benkebil. Pediatric University Hospital (HUG), Geneva, Switzerland.

**Co-authors:** C. Combescure, V. Aubert, C. Salomon, D. Belli, M. Tempia-Caliera, HUG, Pediatric Gastroenterology Unit, Geneva, Switzerland; 2 Laboratory of Immunology, CHUV, Lausanne.

**Aim:** A prospective study is being conducted to evaluate the clinical efficacy of a new Point-of-Care (PoC) test for screening celiac disease (CD) in at risk populations, including IgA deficient patients.

**Methods:** Patients are enrolled from the gastroenterology consult unit at the Pediatric Department of The University Hospital of Geneva. Local ethical committee approval has been granted as well as signed informed consent from patients prior to enrollment. Inclusion criterion was patient undergoing blood test for either suspected or confirmed CD. A multi-analyses PoC test screening for IgA/IgG anti-tTG and total IgA is run in all patients using 4 different biological samples (whole blood, heparin, venous blood, EDTA blood and serum) in very small volumes (20 μL). In parallel, laboratory reference testing is conducted for IgA and IgG anti-tTG on enzyme linked immunosorbent (ELISA) assays and total serum IgA determination. A total of 61 patients have been included in the study of whom 36% are celiac (7 untreated and 15 under gluten-free diet) and 1.6% is IgA deficient (1 nonceliac patient). All celiac diagnoses were confirmed with positive intestinal biopsies. Technical performance of the PoC test (linearity, interference, precision, correlation and agreement) and diagnosis accuracy (sensitivity and specificity) were compared to the standard laboratory ELISA references.

**Results:** The PoC test was found to be user-friendly in a ward setting taking an overall of 15 minutes to be performed. Its results correlate well with the laboratory values and biopsy data for all of the 4 biological samples tested. 45 samples (97.8%) were PoC/ELISA concordant (positive or negative). 1 sample was not concordant (false negative). It appeared that the sample was drawn from a celiac patient under bad diet compliance with values of anti-tTG at the cut-off levels. No false positive results were observed with the healthy control group. The specificity and sensitivity of the PoC test were 100% (95%CI 59–100) and 97.4% (95%CI 86.5–99.9), respectively, for the combined celiac/IgA deficiency markers with a test accuracy of 97.8% (95%CI 88.5–99.9).

**Conclusions:** The use of this PoC test is found to be highly accurate in detecting untreated celiac patients. It can be easily performed during the course of a consultation and can accommodate different types of biological samples making a reliable alternative to laboratory assays for screening celiac disease in all at-risk populations.
PG1-13

BIOLOGICAL AND IMMUNOLOGICAL ACTIVITIES OF SERUM ANTI-TISSUE TRANSGLUTAMINASE ANTIBODIES FROM NONCELIAL SUBJECTS

Presenter: S. Quaglia, IRCCS Burlo Garofolo Trieste, University of Trieste, Trieste, Italy.
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Background and Aim: The evaluation of anti-tissue transglutaminase antibodies (anti-tTG Abs) was advocated in the diagnostic work-up of celiac disease (CD). However, positive anti-tTG Abs results have recently been described also in patients affected by other disorders. In this study we evaluated the immunological characteristics and the biological activities of the anti-tTG derived from children with infectious disease in comparison with those of the untreated celiacs’ anti-tTG Abs.

Methods: Anti-tTG Abs were isolated by an affinity chromatography from sera of both patients with infectious disease tested positive for anti-tTG Abs and untreated CD control subjects. We evaluated the tissue transglutaminase (tTG) fragments recognition of these antibodies by an Elisa assay: we used one tTG fragment well recognized by sera from CD patient (fragment 11; amino acids 1–376; 45 KDa) and one not recognized (fragment 8; amino acids 269–687; 60 KDa). The avidity of anti-tTG Abs was measured by elution of low avidity antibodies with urea (5M) before detection. Using rhodamine-conjugated phalloidin staining, we evaluated whether anti-tTG Abs cause cytoskeletal changes in Caco-2. We also monitored cell levels of bromodeoxyuridine incorporation to determine if anti-tTG Abs were able to induce epithelial mucosal cells into S phase and to study the inhibitory effects towards the catalytic activity of the tTG and modulating both the cytoskeleton rearrangement and the cell-cycle of Caco-2 cells in vitro. They also present strong avidity to their target. There is only a mismatch in the fragment recognition: anti-tTG Abs both from infectious disease patients and celiac disease patients recognized full tTG protein and fragment 11 but only anti-tTG Abs from celiac patients had a statistically significant diminution in the recognition of fragment 8.

Conclusions: Some studies support the hypothesis that anti-tTG Abs are involved in the pathogenesis of CD. For the first time we studied the immunological features and the biological activities of anti-tTG Abs from nonceliac patients and we demonstrated that they have properties comparable with those of CD patients’ anti-tTG Abs. We can hypothesize, therefore, that these anti-tTG Abs in nonceliac patients could be harmful, at least in the spanning time of the auto-antibodies production.

PG1-14

HYPOXIA INDUCIBLE FACTOR 1 ALPHA (HIF-1α) EXPRESSION IN COELIAC DISEASE

Presenter: E. Sziksz, Semmelweis University, Budapest, Hungary.
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Background and Aim: In our previous study we have revealed higher TLR4 protein levels in the duodenal mucosa of children with coeliac disease (CD). Recently it was found that Toll-like receptor 4 (TLR4) downstream signalling leads to the accumulation of a hypoxia inducible factor-1 alpha (HIF-1α), which is important for TLR4-dependent expression of proinflammatory cytokines. Since the role of HIF-1α in CD is unclear, our aim was to depict the alteration of this hypoxia regulated transcription factor expression in this disease.

Methods: Duodenal biopsy specimens were collected from 16 children with untreated CD [median age (range): 6.7 (3.7–13.9)], 9 children with treated CD [median age (range): 6.7 (4.9–12.7)] and 10 controls [median age (range): 8 (1.7–13)]. HIF-1α protein levels were determined by Western blot.

Results: We found about 3-fold elevation of HIF-1α protein levels in the duodenal mucosa of children with untreated CD compared to controls (P < 0.05). In the duodenal mucosa of children with treated CD, HIF-1α protein level was approximately 2-fold lower than in children with untreated CD (P < 0.05).
Conclusions: Our results suggest that increased TLR4 expression in CD should upregulate HIF-1α expression, which can modulate inflammation and/or apoptosis in this disease.

PG1-15

IS COELLIAC DISEASE OVERREPRESENTED IN PATIENTS WITH CONSTIPATION?


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Background and Aim: As coeliac disease (CD) is sometimes seen in patients with constipation it has been suggested that serological screening might be warranted in this patient group, especially when the usual treatment of constipation fails. However it is not known whether CD is indeed overrepresented in patients with constipation, which is a frequent condition, affecting 5–10% of the general population. Aim of the study was therefore to find the incidence of coeliac disease in children with constipation who were referred to the paediatrician by a primary care physician. At the same time patients were screened for hypothyroidism and hypercalcemia, as experts have suggested that these conditions too might have a causative relation with constipation.

Methods: Between October 2006 and October 2008 prospectively 370 consecutive patients with constipation (clinically scored with the Rome III criteria, age between 1 and 18 years of age, gluten ingestion of at least 3 months) were included and screened for total serum IgA, IgA-human tissue transglutaminase (Celkey tTG Elisa kit, Pharmacia and Upjohn Diagnostics, Freiburg, Germany), serum calcium, TSH (thyroid stimulating hormone). All patients with an abnormal IgA, TSH, or a low serum IgA, underwent a small intestinal biopsy.

Results: Seven of the 370 patients with constipation had biopsy proven coeliac disease. This is significantly higher (P < 0.001) than the 1:198 incidence of CD in the Netherlands, as recently determined in a survey of 6127 schoolchildren. An additional 2 patients had Hashimoto disease. There were no patients with hypercalcemia.

Conclusions: Coeliac disease is significantly overrepresented in patients with constipation. All patients with constipation referred from a primary care physician to a paediatrician who fulfill the Rome III criteria should therefore be screened for coeliac disease.

PG1-16

DIAGNOSTIC CHARACTERISTICS OF GIVEN CAPSULE ENDOSCOPY IN DIAGNOSIS OF CELLIAC DISEASE: A META-ANALYSIS


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Background and Aim: In the view of small sample sizes of the studies published so far, the value of VCE in diagnosing CD is yet to be determined. The aim of this work is to systemically determine the overall diagnostic characteristics of VCE in diagnosing uncomplicated celiac disease compared to the gold standard using meta-analysis.

Methods: An extensive literature search was performed looking for prospective controlled trials, with investigators blinded to results of the pathology of small bowel biopsies. Two independent authors performed data extraction and assessment of the methodological quality of each trial. Diagnostic characteristics of each trial were collected and pooled sensitivity, specificity, likelihood ratios and diagnostic odds ratios were computed. Description of complications and costs was included if reported.

Results: A total of 3 studies met the inclusion criteria (n = 107; 63 with CD and 44 without). The overall pooled VCE sensitivity was 83% (95% CI = 71–90%), specificity 98% (95% CI = 88–99.6%). These high sensitivities and specificities lead to very favorable likelihood ratios for capsule endoscopy. A patient’s odds of having the disease were nearly 35 times higher after testing positive with capsule endoscopy (LR+ve: 34.5; 95% CI: 16.7–43.5) while patients’ odds of disease were nearly 5 times smaller after a negative test result (LR-ve: 0.22; 95% CI: 0.01–0.64). These results were generally homogeneous across studies (I² = 0%). No major complications were reported. The costs were mentioned only in one study.

Conclusions: The overall diagnostic characteristics of VCE when used to diagnose celiac disease, though good with an experienced eye, could not justify routine use of VCE as an alternative to pathology of small bowel biopsies. More studies are needed with proper cost-benefit analysis.
Background and Aim: Ghrelin is a newly discovered hormone that has effects on nutrient intake and GH release, consequently on physical development and growth. Ghrelin is predominately produced in the stomach, but new findings indicate that the intestinal wall is an important source of the hormone. In patients with short bowel syndrome the reduction of the intestinal tissue resulted in a decrease of circulating ghrelin levels. Celiac disease (CD), a gluten-sensitive enteropathy, is characterized by chronic inflammation of small intestine, malabsorption and impaired nutritional status. Since in CD the intestinal mucosa atrophy is main founding the alterations in duodenal ghrelin-positive cells population can be expected. The aim of the study was to evaluate the density of ghrelin-positive cells in duodenum of CD children and its relationship with BMI and clinical presentation.

Methods: The study included 31 consecutive patients with newly diagnosed CD (9 boys), age 10.06 ± 3.44 years (4–17 years), body mass index (BMI) 15.87 kg/m² ± 2.86. The control group consisted of 21 children (6 boys), age 11.52 ± 4.06 years (6–17 years), BMI 16.78 ± 2.63 kg/m², diagnosed because of growth retardation, anemia or abdominal pain. The exclusion criteria were gastrointesti- nal surgery and concomitant diseases. All of the patients underwent endoscopy with biopsies samples taken from distal duodenum and gastric corpus. The immunohistochemistry was done using monoclonal rabbit anti-human antibody (Phoenix Pharmaceutical), the immunoreactive cells were counted in light microscope and results were expressed as a number of cells/mm².

Results: The number of ghrelin positive cells in duodenum was significantly higher in children with CD than in controls (14.82 ± 11.12 vs 5.69 ± 5.02 P = 0.0013) and not correlated with the number of these cells in stomach (r = 0.2752). The density of ghrelin-positive cells in duodenum did not correlate with severity of villous atrophy, clinical presentation, laboratory markers or age. No correlation between BMI and ghrelin-positive cells number in duodenum have been found in CD patients and in controls (respectively, r = 0.9787 and r = 0.8942).

Conclusions: In duodenum of children with celiac disease the number of ghrelin-positive cells is increased despite of mucosal atrophy. The changes are not dependent on degree of mucosal lesion or nutritional status. This suggests that the population of ghrelin-positive cells in duodenum not simply reflects an altered mucosal morphology or failure to thrive but is under the influence of other conditions. Since ghrelin has also anti-inflammatory effects increased local ghrelin production may be one of the defense mechanism in CD in gut mucosa.

Background and Aim: Endomysial (EmA) and tissue transglutaminase antibodies (tTG-ab) are widely applied in the screening of coeliac disease. Although known to be specific for coeliac disease with overt villous atrophy, the antibodies may lack sensitivity when the small-bowel mucosa is still morphologically normal. Previously used antigliadin antibodies lack sufficient specificity and sensitivity for coeliac disease and their use is no longer recommended. However, they may be the first antibody to appear in the sera of coeliac patients. The sensitivity of antibodies for mild enteropathy coeliac disease was 75% for DGP, 82% for EmA and 61% for tTG-ab, respectively. The combined sensitivity for coeliac disease with overt villous atrophy, the antibodies to deamidated gliadin peptides (DGP) has been shown to be a promising method for coeliac disease with overt villous atrophy (Scand J Gastroenterol. 2007;42:1428–32). The purpose of this study was to evaluate whether DGP is an accurate method for the diagnostic work-up of mild enteropathy coeliac disease.

Methods: The study group comprised 44 cases with coeliac disease with overt villous atrophy, and after a year with a gluten-free diet. The control groups contained 20 comparable subjects with overt villous atrophy and 39 subjects who evinced no signs of coeliac disease. The control subjects were DGP-positive; therefore the specificity for coeliac disease and their use is no longer recommended. However, they may be the first antibody to appear in the sera of coeliac patients. Serological tests for DGP, EmA and tTG-ab were performed to all participants at the baseline and after a year with a gluten-free diet.

Results: The sensitivity of antibodies for mild enteropathy disease was 75% for DGP, 82% for EmA and 61% for tTG-ab, respectively. The combined sensitivity for EmA and DGP was 91%. The sensitivity for coeliac disease with villous atrophy was 95% for DGP and 100% for both EmA and tTG-ab. Two HLADQ2-positive control subjects were DGP-positive; therefore the specificity was 95% for DGP and 100% for both EmA and tTG-ab. During the gluten-free diet all three antibodies
decreased similarly in subjects with mild enteropathy or coeliac disease with overt villous atrophy.

Conclusions: The results showed that DGP is a sensitive and specific test for the screening and follow-up of mild enteropathy coeliac disease. Although the specificity of DGP was slightly lower compared with the other antibodies, it is possible that two antibody-positive patients in the control group will develop coeliac disease in the future. On the grounds of the results, DGP and EmA combined is the most sensitive method for the detection of mild enteropathy coeliac disease.

PG1-19

IMPROVEMENT OF SEROLOGICAL SCREENING TECHNIQUES FOR CHILDHOOD COELIAC DISEASE

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Background and Aim: This study evaluated whether (1) different serological techniques and optimisation of standard cut offs would allow for better discrimination between children with and without villous atrophy, (2) false positives could be reduced by evaluation of the persistency of positive serology over time.

Methods: Dutch children of 2–4 years old (n = 6127) were screened serologically for coeliac disease with anti-endomysium (EmA) tests in 1997–1998. Small bowel biopsies were performed in 57 EmA positive children; 31 children had villous atrophy and the remaining 26, all HLA-DQ2/8, had no histological abnormalities. Of the biopsied children, the original screening samples were retrospectively evaluated for EmA, antibodies to tissue transglutaminase (tTGA; guinea pig and human recombinant), deamminated gliadin peptides (anti-DGP) and a combination of DGP with tTGA (anti-DGP/tTGA). The positive antibody persistency for EmA and tTGA was evaluated between the moment of screening and biopsy, with a time interval of 6 (± 2) months.

Results: At the moment of screening, children with villous atrophy had significantly higher EmA titres than the children with a normal small bowel biopsy (P < 0.005). By increasing the cut off for EmA positivity to a titre of >1:50, the number of false positives was halved, however 2 children with villous atrophy would not have been detected. All tTGA assays had comparable values for accuracy, sensitivity, specificity and positive predictive value (PPV), and, interestingly, these outcomes were essentially similar to the anti-DGP and anti-DGP/tTGA assays. Increasing the cut off points in all these assays never resulted in full discrimination between the children with and without villous atrophy, although the accuracy could be improved from 59–75% to 79%–87.5%. At the time of biopsy, all children with villous atrophy were still EmA positive and 87% were tTGA positive, whereas of the children with normal small bowel biopsy, only 50% were EmA and 20% tTGA positive. The PPV could be significantly improved (P = 0.02) by changing the original test strategy (EmA positive on one occasion) to tTGA positive at 2 occasions.

Conclusions: Coeliac antibodies can be transiently presented in a substantial part of healthy, genetically predisposed children. This unavoidably reveals seropositive children without small bowel abnormalities in serological mass screening programmes. We have shown that unnecessarily performed biopsies could be prevented by adjusting the standard cutoffs from the EmA, tTGA and anti-DGP/tTGA test kits and by evaluating the persistency of seropositivity in time. Importantly, however, all these achievements were at the cost of sensitivity.

PG1-20

MULTIDIMENSIONAL STUDIES IN POTENTIALLY PATHOLOGICAL NETWORKS FOR THE IDENTIFICATION OF CD SUSCEPTIBILITY LOCI


Co-authors: I. Santin1, A. Martin-Pagola1, G. Gutierrez1, I. Irastorza2, J. Vitoria2, J. Bilbao1. 1Immunogenetics Laboratory, Hospital de Cruces, Barakaldo, Spain; 2Pediatric Gastroenterology Unit, Hospital de Cruces, Barakaldo, Spain.

Background and Aim: Celiac disease is a complex multifactorial process resulting from multiple events that interact with each other. Combined genetic variation at several loci is probably responsible for the dysregulation of many biological processes that ultimately lead to the disease.

Methods: Comparing expression profiling results of biopsies from active and treated patients (long-term effects of gliadin), and of biopsies from gluten-free diet treated patients incubated in vitro with or without gliadin (acute effects) we have described the alterations provoked by gliadin on the intestinal mucosa of CD patients at a network level. Using bioinformatics tools we have selected rSNPs of the differentially expressed genes in the networks to test them for association with the disease.
Results: Integration of the significantly altered transcripts identified in the long-term and acute experiments into potentially pathogenic networks, suggests important dysfunction of processes related to cell-cell communication, intracellular matrix, and cell cycle and apoptosis. Single SNP association studies in genes from ubiquitin-proteasome system showed some weak associations with the disease. We used a two-step strategy to identify SNP interactions and found stronger associations with the disease.

Conclusions: Analysis of expression profiling results at the network level provides a more accurate picture of the events that lead to the disease helping in the selection of novel functional candidates. Multidimensional studies integrating SNPs from genes in an altered network could be more powerful than single-gene association studies in the search of genetic susceptibility.

PG1-21

DETECTION OF GLUTEN-REACTIVE T-CELLS IN PERIPHERAL BLOOD AFTER GLUTEN CHALLENGE IS A REPRODUCIBLE DIAGNOSTIC TOOL IN TREATED CELIAC ADOLESCENTS

Presenter: C. Gianfrani. Institute of Food Sciences-CNR, Avellino, Italy.
Co-authors: A. Camarca1, R. Di Mase2, G. Terrone2, S. Auricchio2, L. Greco2, C. Gianfrani1, R. Troncone3.
1ISA-CNR Avellino Italy; 2Department of Pediatrics & ELFID, University of Naples, Federico II, Naples, Italy.

Background and Aim: It has been shown that following consumption of wheat-containing food, gluten-reactive T-cells secreting IFN-γ transiently circulate from the gut to peripheral blood in adult celiac patients on gluten-free diet. Since peripheral blood offers a more accessible biological sample for immunological studies compared to intestinal mucosa, we have studied this phenomenon in treated adolescents and have assessed its reproducibility.

Methods: Ten patients (mean age 18 yrs, range 14–21) on gluten-free diet for at least 5 years and negative CD serology were given 200 g of wheat bread for 3 days. Peripheral blood mononuclear cells, obtained the day before and six days after the gluten challenge started, were freshly analyzed for gluten-reactivity by IFN-γ ELISPOT assay. Next, after 2–4 months since the first challenge, 8 of 10 patients underwent another gluten challenge with the same experimental procedure.

Results: In the first challenge the frequency of IFN-γ secreting cells (IFN-γ-SFC) in response to gluten (denaturated peptic-tryptic digestion of gliadin) significantly increased in peripheral blood a day 6 compared to day 0: median of IFN-γ-SFC/4×10^5: 40 (range 6–175) and 17 (range 1–83) at day 6 and 0, respectively (P < 0.004 by paired t test), with a median fold-increase of 2.0 (range 0.1–26). In the second challenge, although a higher number of gluten-reactive cells were detectable at day 0 compared to the first challenge: 32 (range 1–543), their frequency significantly increased after the challenge: 185 (range 10–922), with a fold-increase of 3.4 (range 1–233, P = 0.0001). Interestingly, the higher frequency of gluten-reactive cells detected at day 0 of the second challenge (t = −2066, P = 0.045), negatively correlated with the days elapsed between the first and the second challenge (P = 0.006).

Conclusions: We reported that following gluten challenge, the frequencies of gluten-reactive cells significantly increased in blood of treated adolescent patients. Similar profile of response, but with a memory-like effect, was obtained in the same cohort of subjects upon a re-exposure of gluten after 2–4 months. Reproducibility in a single patient at different times is crucial to implement this test for monitoring the efficacy of intervention strategies.

PG1-22

PRIMARY INTESTINAL LYMPHANGECTASIA: A FAMILIAL DISEASE

Presenter: A. Ali. King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia.
Co-authors: H. Halaby1, Z. Rahbeeni1, S. Shabib1, H. Nazer1. 1King Faisal Specialist Hospital, Riyadh, Saudi Arabia.

Aim: To review the clinical, biochemical, radiological and histological data of children with intestinal lymphangectasia (IL) in 5 families.

Methods: Retrospective chart review of familial IL patients.

Results: 11 children (6 males) with a mean age of 8.4 years in 5 families. First degree consanguinity was documented in 60% (3/5) of their parents. All patients had edema, growth failure occurred in 7, recurrent chest infection in 5, recurrent chylothorax which needed pleuroctomy in 2, diarrhea in 5, hypocalcemic seizure in 2, recurrent cellulitis in 4 and 6 had associated congenital lymphedema. All patients had hypoalbuminemia (mean serum albumin of 17 g/l) whereas hypogammaglobulinemia, lymphopenia and hypocalcemia were seen in 63.6%, 72.7% and 36% of cases, respectively. Upper GI series were performed in 6 children, which showed thickening of the jejunal folds and nodular, or punctate lucencies in the mucosa of the small bowel. Oudenal biopsies were done in all the cases, 8 showed wide villi, dilated lacteals and enlarged submucosal lymphatic, 2 of them had more
than one biopsy to reach the diagnosis. The other 3 showed nonspecific inflammation with partial villous atrophy. 

Conclusions: Primary IL is a familial disease. It is inherited as an autosomal recessive disease. Further genetic studies are needed.

PG1-23

CELIAC DISEASE AND RISK OF URINARY STONE—A POPULATION-BASED STUDY
Presenter: J. Ludvigsson. Örebro University Hospital, Örebro, Sweden.

Aim: To examine the risk of urinary stone in patients with celiac disease (CD) from a general population cohort.

Methods: Using Swedish national registers we identified 14,255 individuals with a diagnosis of CD (1964–2003) and 69,216 reference individuals matched for age, sex, calendar year and county of residence at the time of diagnosis. Cox regression estimated the hazard ratios (HRs) for a subsequent diagnosis of urinary stone. We restricted analyses to individuals with more than one year of follow-up and no diagnosis of urinary stone prior to, or within one year after study entry. Conditional logistic regression estimated the association of urinary stone with undiagnosed CD.

Results: CD was associated with an increased risk of subsequent urinary stone (HR = 1.4; 95% CI = 1.1–1.8; P = 0.019; based on 76 positive events in individuals with CD vs 298 positive events in reference individuals). This risk increase was restricted to individuals diagnosed with CD in adulthood (1.5; 1.1–2.0; P = 0.005). Among the 9,363 individuals with CD diagnosed 0–15 years, there was no increased risk of future urinary stone (0.9; 0.5–1.7; P = 0.816). Urinary stone was statistically significantly associated with undiagnosed CD (odds ratio (OR) = 1.5; 95% CI 1.2–1.9; P < 0.001).

Conclusions: This study found a small risk increase for urinary stone in CD, both before and after diagnosis of CD. This risk increase was not seen in children with CD.

**TABLE 2. Adjusted ratio rate [and 95% CI] of SO_{48} between treatments**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>LGG</th>
<th>Bismuth</th>
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<tbody>
<tr>
<td>LGG</td>
<td>1.06 (0.87–1.30)</td>
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<tr>
<td>Bismuth</td>
<td>0.86** (0.72–0.99)</td>
<td>0.80 (0.60–1.07)</td>
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</tr>
<tr>
<td>Racecadotril</td>
<td>0.54*** (0.40–0.72)</td>
<td>0.51* (0.36–0.73)</td>
<td>0.63* (0.44–0.89)</td>
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* P < 0.01.
** P < 0.001.
Conclusions: This is the first IPD meta-analysis comparing the efficacy of adjuvant treatments in AD. Stool output was significantly reduced by racecadotril as compared to placebo,*Lactobacillus* LGG and bismuth. Our meta-analysis, limited to 3 RCT due to our need of IPD and strict comparability between studies for MTC, requires to be confirmed on a wider basis in collecting all the existing RCTs.

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**Poster Session 2**

**Gastroenterology: Inflammatory Bowel Disease**

PG2-01

**1001 INFUSIONS OF INFlixIMAB IN CHILDREN WITH INFLAMMATORY BOWEL DISEASE: A 10-YEAR STUDY FROM THE ITALIAN SOCIETY OF PEDIATRIC GASTROENTEROLOGY, HEPATOLOGY, AND NUTRITION**

Presenter: M. Paganelli. *Sapienza University of Rome, Roma, Italy.


1Sapienza University of Rome, Rome, Italy; 2University of Brescia, Brescia, Italy; 3University of Padua, Padua, Italy; 4University of Palermo, Palermo, Italy; 5University of Florence, Florence, Italy; 6Spirito Santo Hospital, Pescara, Italy.

**Aim:** To evaluate efficacy and safety of infliximab in children with inflammatory bowel disease (IBD).

**Methods:** Data collection from the Italian Registry of Biologic Therapy in Pediatric IBD was conducted. The Registry contains data from chart review of all pediatric patients with Crohn disease (CD), ulcerative colitis (UC) and indeterminate colitis (IC) seen in the 17 leading Italian pediatric gastroenterology units between June 1999 and January 2009.

**Results:** 146 patients (114 with CD, 27 with UC and 5 with IC) from 17 pediatric gastroenterology units in Italy have been investigated, for a total of 1001 infusions of infliximab (median number of infusions: 5, range 1–35). 57.9% of children with CD were passed to infliximab because not responsive to conventional therapy (74.1% for UC), 8.7% because affected by a steroid-dependent illness (22.2% UC) and 29.8% with a fistulizing disease. Mean age at first infusion was 13.6 ± 3 years, while mean duration of disease was 2.9 ± 2.7 years. PCDAI at first infusion was 30.7 ± 16.1. PUCAI 27.9 ± 16.4. According to clinical scores, 13.8% patients with CD were in remission (0% UC), 36.8% had a mild disease (71.4% UC) and 49.4% had a moderate/severe disease (UC: 28.6% moderate, 0% severe). PCDAI improvement after first infusion was −18.2 ± 16.1 (*P* < 0.05). Fifty-five (48.2%) patients with CD received at least 5 doses, with at least 6 months follow up. At each infusion (0, 2, 6, 14, 22) PCDAI was significantly lower than PCDAI at the previous infusion (*P* < .05). PCDAI at week 30 was 8.9 ± 10.2. PCDAI improvement after 6 months of therapy was −20.4 ± 16.1 (*P* < 0.01). Seventy-two patients with CD were on azathioprine at first dose (63.2% of patients, 1.8 ± 0.5 mg/kg/day), 4 on 6-mercaptopurine (0.3%, 0.3 ± 0.1 mg/kg/week) and 39 were receiving corticosteroids (34.2%, 1 ± 0.9 mg/kg/day of prednisone). At week 30, 33 patients were on azathioprine (51.6%, 1.6 ± 0.6 mg/kg/day), 3 on 6-mercaptopurine (4.7%, 0.3 ± 0.2 mg/kg/week), and only 6 were receiving corticosteroids (9.4%, 0.5 ± 0.3 mg/kg/day). A total of 70 infusions reactions were registered in 34 patients (4.2% of infusions, 23.3% of patients). In 5 cases the infusion had to be stopped, while in 3 cases the infusion had to be suspended and started again after 1 hour. Adverse events following the infusion were registered in 17 patients (11.6%; 35 infusions, 3.5%).

**Conclusions:** Infliximab proved to be efficacious in inducing and maintaining remission in children with CD and in reducing the number of patients receiving corticosteroids. It also proved to be safe in children with both CD and UC. Adverse events are mild or moderate and only rarely lead to stop the therapy.

PG2-02

**CORRELATION BETWEEN CROHN DISEASE (CD) CHILDREN WHO ACHIEVE CLINICAL REMISSION ON EXCLUSIVE ENTERAL NUTRITION (EEN) AND REDUCTION IN FAECAL CALPROTECTIN CONCENTRATION**

Presenter: K. Gerasimidis. *University of Glasgow, Glasgow, UK.

Co-authors: K. Gerasimidis 1, C. Nikolaou 1, C. Edwards 1, P. McGrogan 2.

1Human Nutrition Section, Developmental Medicine, University of Glasgow, Yorkhill Hospitals, Glasgow, UK; 2Paediatric Gastroenterology, Hepatology and Nutrition, Yorkhill Hospitals, Glasgow, UK.

**Aim:** To assess changes of faecal calprotectin concentration in CD children on treatment with EEN and correlation with clinical activity index and systemic markers of inflammation.
Methods: 4 serial stool samples (within 4 hours of defaecation) were collected from CD children with clinically active disease (PCDAI >10) over their 8 week course (0, 15, 30, 60 days) on EEN with a polymeric liquid diet (Modulen, Nestle). All samples from each patient were analysed in duplicate on the same ELISA plate (PhiCal, Norway). Systemic markers of disease activity (CRP, ESR, serum albumin) were measured at the beginning and end of treatment.

Results: 16 CD children (12 newly diagnosed; 7 girls; 11.8 ± 2.3 years) and 10 healthy controls (5 girls; 10.6 ± 2.1 years) participated. 13 children had disease involving the upper and lower digestive tract. 14 children completed at least 6 weeks on EEN. For 15 children PCDAI decreased compared to baseline and 7 children achieved complete clinical remission (PCDAI ≤10). Faecal calprotectin levels were negatively associated with serum albumin (r = −0.62, P = 0.001) and positively with ESR (r = 0.56, P = 0.001), CRP (r = 0.48, P = 0.002) and PCDAI (r = 0.49, P = 0.001). In only 1 child did the calprotectin level reached normal levels (<50 mg/kg) by the end of EEN and 3 children had values of around 100mg/kg. All healthy controls had calprotectin values within the normal range (<50 mg/kg). Only in those children who entered clinical remission (PCDAI <10) did faecal calprotectin concentration decrease significantly (Fig. 1) where mean faecal calprotectin was reduced during EEN and was significantly lower than baseline after 30 (P < 0.01) and 60 days of treatment (P < 0.0001). Mean calprotectin concentration was reduced by 921 mg/kg (95% CI: −1568 to −274) and 1541 mg/kg (95% CI: −2187 to −894) after 30 and 60 days on EEN, respectively. Faecal calprotectin did not change in patients who did not achieve clinical remission (PCDAI >10) (Fig. 2). No patient on concomitant medication (n = 7) achieved clinical remission (PCDAI >10) and no significant change in faecal calprotectin levels were noted in these patients.

Conclusions: Induction of clinical remission was accompanied by reduction in faecal calprotectin levels although in most patients levels remained elevated at the end of EEN. This may indicate that improvement in clinical activity precedes resolution of intestinal inflammation. Future studies should address whether calprotectin levels at the end of EEN can predict the time of a subsequent relapse. If this is the case then, monitoring faecal calprotectin concentration along with changes in clinical activity may be a more appropriate clinical marker for indicating the time of treatment termination.

Mr Konstantinos Gerasimidis is a recipient of a scholarship from the Greek State Scholarship Foundation and the Hellenic Association of Gastroenterology and Nutrition.

PG2-03

INTESTINAL FLORA DIRECTS INFILTRATE COMPOSITION AND DISEASE SEVERITY IN A NOVEL ZEBRAFISH COLITIS MODEL

Presenter: S. Brugman. Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands.

Co-authors: K. Liu1, D. Lindenbergh-Kortleve1, J. Samson1, G. Furuta2, S. Renshaw3, R. Willemsen4, E. Niwenhuis1. 1Laboratory of Pediatric Gastroenterology, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands; 2Section of Gastroenterology, Hepatology and Nutrition, The Children’s Hospital Aurora, Colorado, USA; 3MRC Centre for Developmental and Biomedical Genetics, University of Sheffield, Sheffield, UK; 4Department of Clinical Genetics, Erasmus Medical Center Rotterdam, Rotterdam, The Netherlands.

Background and Aim: In order to obtain a more controllable model that will enable us to study the conserved
cross-talk between cells of the innate (mucosal) immune system and the bacterial flora in health and under inflammatory conditions, we developed a model for colitis in the zebrafish.

Methods: Colitis was induced by intrarectal administration of the hapten oxazolone in adult wild type and transgenic (mpo::EGFP, all neutrophils are EGFP-positive) zebrafish in the presence or absence of antibiotics. Intestinal inflammation was evaluated by histology, flow cytometry, and cytokine profiling through quantitative real time PCR. Next, shifts in the intestinal flora composition due to antibiotic treatment were assessed by 16S rRNA PCR, cloning and sequencing of intestinal content.

Results: Zebrafish oxazolone colitis is flora-dependent and characterized by an influx of granulocytes, epithelial damage, Goblet cell depletion and increased expression of IL-1β, TNF-α, and IL-10. Vancomycin treatment diminished the intestinal microbial load and resulted in a bacterial composition dominated by Fusobacteria. Vancomycin conferred strong protection from colitis associated with reduced percentages of infiltrating neutrophils. In contrast to vancomycin, exposure to colistin sulphate resulted in a predominance of gamma-proteobacteria in the intestine that correlated with reduced infiltration of eosinophils and lymphocytes and no significant reduction in colitis.

Conclusions: Components of the intestinal microbiota drive zebrafish oxazolone colitis and directly affect the severity of disease and composition of the intestinal infiltrate.

PG2-04

EFFICACY AND TOLERANCE OF AZATHIOPRINE IN PEDIATRIC CROHN’S DISEASE


Co-authors: A. Rubio1, J. Svahn1, J. Schmitz1, C. Talbot1, O. Goulet1, F. Ruemmele1. Hôpital Necker Enfants Malades, Paris, France.

Background and Aim: Azathioprine is considered to be the gold standard in the treatment of moderate to severe pediatric Crohn’s disease (CD). Markowitz and colleagues recently demonstrated that early introduction of azathioprine allows to weaken patients from steroids and to maintain remission over 1 year in 90% of patients. In the present study we wanted to analyze efficacy to maintain remission of azathioprine in a homogenous single center cohort of pediatric CD patients.

Methods: 71 pediatric CD patients (male/female 46/25) were retrospectively evaluated for the efficacy of azathioprine (AZA) to maintain remission at 6, 12, 18, and 24 months of follow-up. All children had active disease with a PCDAI >10. Induction therapy consisted of enteral nutrition (72%) and steroid therapy (28%). Remission was defined as a PCDAI <10 and patients had to be free of steroids or receive <0.3 mg/kg of prednisone or equivalent. Patients who required anti-TNF medication or other immunomodulators were considered to experience a relapse and were excluded from the following analyses, as well as patients who needed surgery. Since 2003, TPMT activity (and genotype) was determined routinely in all patients. AZA doses were in the range of 2.5–3.5 mg/kg.

Results: At 6 months, 34/65 patients (52%) were in complete and steroid-free remission, whereas 31 patients still had active disease (PCDAI >10). In three patients AZA maintenance therapy was not started due to low TPMT activity, and in a further three patients, AZA therapy was stopped secondary to pancreatitis. At follow-up visits of 12, 18 and 24 months, remission rates were 24/65 patients (37%), 16/65 patients (25%) and 14/65 patients (22%), respectively. Overall tolerance of AZA was excellent, no neutropenia was noted in patients with normal TPMT activity. However, in three patients severe bacterial or viral infections were documented and AZA medication was temporarily stopped.

Conclusions: AZA treatment is markedly less efficacious in maintaining remission in pediatric CD patients as recently suggested. However, the majority of patients who acquire and maintain complete steroid-free remission at 12 months remain in prolonged remission beyond the first year of treatment. Overall tolerance of AZA in this cohort was excellent and no severe adverse reactions directly related to AZA medication were observed.

PG2-05

WIRELESS CAPSULE ENDOSCOPY AND SMALL INTESTINE CONTRAST ULTRASONOGRAPHY (SICUS) IN THE DIAGNOSTIC ASSESSMENT OF SMALL BOWEL IN PEDIATRIC PATIENTS WITH CROHN DISEASE

Presenter: E. Romeo. Sapienza University, Rome, Italy.

Co-authors: N. Pallotta1, F. Viola1, F. Civitelli1, G. Di Nardo1, S. Oliva1, M. Carabotti2, G. Vincoli2, V. Mancini3, F. Conte3, F. Nuti4, G. Maiella1, E. Corazzari1, S. Cucchiara2. Pediatric Gastroenterology and Liver Unit, Sapienza University, Rome, Italy; Clinical Science Department, Sapienza University, Rome, Italy.

Background and Aim: Wireless capsule endoscopy (WCE) and small intestine contrast ultrasonography (SICUS) can noninvasively assess the small bowel. However, in patients with known or suspected small bowel
pathology the presence of strictures contraindicates WCE examination. In adults SICUS performed after ingestion of 375 mL of macrogol solution, enables to visualize and to measure wall thickness and lumen diameter at any level of the small bowel (Lancet 1999;353[9157]:985–6). SICUS accurately assesses presence, number, extension, and sites of Crohn’s disease (CD) lesions identifiable as increased wall thickness (>3 mm) and strictures as reduced luminal diameter (<1 cm) (Inflamm Bowel Dis 2005;11:146–53). We evaluated the clinical usefulness of the two radiation free, noninvasive procedures SICUS and WCE in assessing mural and endoluminal disease in pediatric patients with known or suspected small bowel disease.

Methods: Sixteen patients (4 F; median age 16 years; range 9–20 yrs) were consecutively enrolled: 2 patients with suspected CD; 14 patients with a diagnosis of CD, of which 2 had previous ileo-colonic resection for strictures. Before WCE, (Given M2A capsule, Given Imaging) SICUS (Tosbee equipment, Toshiba, Japan, with 5 MHz linear and 3.5 MHz convex array transducers) was performed by a sonologist unaware of other radiological and endoscopic findings, after the ingestion of 125–500 mL of macrogol solution. The day before the WCE examination patients received an intestinal preparation with 3 L of polyethylene-glycol solution.

Results: SICUS was performed in 15 patients; 1 patient refused. Five patients had one or more small bowel strictures detected at SICUS and WCE study could not be performed. SICUS and WCE detected distal and terminal ileum lesions in 10 patients with diagnosis of CD, confirmed at standard ileo-colonoscopy. In the 2 patients with ileocolonic resection both techniques were able to individuate CD recurrence at the ileocolonic anastomosis. In 3 patients WCE also showed jejunal and proximal ileum erosions not detectable at SICUS.

Conclusions: These findings suggest: (1) the opportunity to perform SICUS before WCE to exclude the presence of small bowel strictures; (2) the usefulness of noninvasive SICUS and WCE techniques in the assessment of both wall and luminal small bowel lesions, respectively; (3) the possibility to avoid radiation exposure in detecting SB disease. In conclusion WCE and SICUS in pediatric patients may be used as noninvasive complementary techniques for the assessment of small bowel diseases.

PG2-06

ROLE OF METHOTREXATE IN PEDIATRIC ULCERATIVE COLITIS

Presenter: M. Aloi. Sapienza University of Rome, Rome, Italy.

Co-authors: F. Viola1, F. Conte1, N. Cavallari1, O. Iacono1, F. Civitelli1, F. Nuti1, S. Oliva1, V. Pannone1, M. Barbato1, S. Cucchiara1. 1Sapienza University of Rome, Rome, Italy.

Aim: We wished to describe our experience in using methotrexate (MTX) in children with ulcerative colitis (UC) followed up at a tertiary referral center for pediatric gastroenterology.

Methods: Twenty-four patients (50% male; age range: 8.3–19.8 years; median 13.9 years) with UC treated with MTX were identified by the departmental database. Case records were reviewed for site of disease, age at diagnosis, duration of disease before the introduction of MTX, indication to MTX use, side effects of therapy, Pediatric Ulcerative Colitis Activity Index (PUCAI) and corticosteroid (CS) use were evaluated baseline as well as 3, 6 and 12 months.

Results: Ulcerative colitis was diagnosed at a mean age of 10 ± 4.3 years. Pancolitis was detected in 18 of 24 patients (75%), left-sided colitis in 4 (16%) and proctitis in 2 (8%). MTX was introduced 23.4 ± 9.9 months after diagnosis. Indications to MTX therapy were a nonresponse to or a relapse under azathioprine (AZA) (15), AZA intolerance/toxicity (7) or spondyloarthropathy (2). The mean dose of MTX for patient was 13.7 ± 3.6 mg/ m²/week. Clinical remission was achieved in 18 of 24 patients (75%); 55% of patients at 3 months, 13% at 6 months and 9% at 12 months. Among them, 4 (22%) relapsed after 10 ± 2 months. Mean PUCAI was 49.5 ± 23.3 baseline, 32.9 ± 21.9 at 3 months (P < 0.05), 29.5 ± 22.8 at 6 months and 29.4 ± 25.9 at 12 months (NS). At the beginning of MTX treatment 10 of 24 patients (41%) were treated with CS, 8 of whom (80%) discontinued CS by 6 months. At 12 months, 25% of patients needed CS for relapsing of the disease. The dose of MTX (≥ 15 mg/week) was associated with the induction of remission (P < 0.02). Adverse reactions were observed in 8 patients (33%) (liver enzyme elevation in 1; fever in 3; nausea in 3), in 2 of these (8%) discontinuation of the drug was required (1 due to intractable nausea, 1 because of recurrent fever).

Conclusions: Our data suggest that in children with active UC MTX therapy is effective in obtaining and maintaining remission as well in reducing CS exposure. Thus, MTX could be considered in UC patients who escaped or did not tolerate thiopurines. Nevertheless large, controlled trials are warranted to better define its role in pediatric UC.

PG2-07

THROMBIN GENERATION IN PEDIATRIC PATIENTS WITH INFLAMMATORY BOWEL DISEASE

Presenter: A. Deutschmann. Medical University of Graz, Graz, Austria.
Co-authors: H. Bernhard\textsuperscript{1}, M. Novak\textsuperscript{1}, B. Leschnik\textsuperscript{1}, A. Hauer\textsuperscript{1}, H. Haidl\textsuperscript{1}, A. Rosenkranz\textsuperscript{1}, W. Muntean\textsuperscript{1}.
\textsuperscript{1}Department of Pediatrics, Graz, Austria.

**Background and Aim:** In adults, inflammatory bowel disease (IBD) is associated with an increased risk of thromboembolic complications. This might be caused by increased levels of hemostatic parameters like factor V, VII, VIII and fibrinogen, caused by the chronic inflammatory process. On the other hand, the pathogenesis of IBD is not really clear and a high thrombin activity might contribute to disease progression. Sensitive markers of activation of coagulation such as prothrombin fragment 1+2 (F1+2) and thrombin-antithrombin complex (TAT) are high in IBD. This study was conducted to investigate the role of hypercoagulation in inflammatory condition during course (active and quiescent state) of IBD in pediatric patients. Therefore, we measured thrombin generation by means of calibrated automated thrombography (CAT) to detect this hypercoagulable condition in children with IBD. Results of CAT shows a high inter-individual variability, but measures rather constant over time in one person. We wanted to see whether children with IBD have a higher thrombin generation.

**Methods:** Plasma samples were collected of 22 pediatric patients with IBD and of 60 healthy controls. Age ranged from 10 to 19 years. Thrombin generation was measured by means of CAT. The disease activity was estimated, using the Pediatric Crohn’s Disease Activity Index (PCDAI). We investigated F1+F2, TAT, tissue factor pathway inhibitor (TFPI) and fibrinogen. Statistical analysis were performed by using Mann-Whitney $U$ test and Pearson’s correlation and $P$ values less than 0.05 were considered to be significant.

**Results:** There was a significant increase of endogenous thrombin potential (ETP), lag time and time to peak (TTP) in patients with IBD, while peak showed no difference to healthy controls. ETP and F1+F2 in children with IBD also showed a significant correlation with PCDAI and fibrinogen.

**Conclusions:** Our study shows that IBD in children is associated with high thrombin generation, but this seems to be caused mainly by the inflammatory process than by an individual disposition.

**PG2-08**

**WIRELESS CAPSULE ENDOSCOPY (WCE) IS HELPFUL IN THE DIAGNOSTIC WORK-UP FOR PEDIATRIC INFLAMMATORY BOWEL DISEASE (IBD) PATIENTS**

**Presenter:** V. Mancini. Sapienza University of Rome, Rome, Italy.

**Co-authors:** G. Di Nardo\textsuperscript{1}, A. Staiano\textsuperscript{2}, G. Lombardi\textsuperscript{3}, S. Oliva\textsuperscript{1}, E. Romeo\textsuperscript{1}, N. Cavallari\textsuperscript{1}, E. Miele\textsuperscript{2}, F. Conte\textsuperscript{1}, S. Cucchiara\textsuperscript{1}, Sapienza University of Rome, Pediatric Gastroenterology & Liver Unit, Rome, Italy; \textsuperscript{2}Federico II University of Naples, Pediatric Gastroenterology Unit, Naples, Italy; \textsuperscript{3}Pescara Hospital, Pediatric Gastroenterology Unit, Pescara, Italy.

**Background and Aim:** WCE has gained wide acceptance in gastrointestinal (GI) endoscopy by revealing lesions of the small bowel (SB) not seen with other imaging tools. The usefulness of WCE in the diagnostic work-up of pediatric IBD is rarely reported. We determined the diagnostic yield of WCE for children with IBD, either with a definite diagnosis, ie, Crohn’s disease (CD), ulcerative colitis (UC), as well as for children with suspected or unclassified IBD (IBDU).

**Methods:** Seventy patients, mean age (SD) 13.5(4.37), recruited in 3 Italian pediatric gastroenterology centres underwent PillCam SB2 WCE (Given Imaging) examination after an overnight fast and a mini-bowel preparation with not more than 1 L of PEG 4000 oral solution, administered in 200 mL increments every 10 minutes in the early morning of the examination. Indications included: (1) determination of SB involvement in 31 CD patients and in 18 UC patients; (2) work-up of suspected IBD (14 patients); (3) diagnostic definition in 7 patients with IBDU. WCE findings were classified as diagnostic (> 3 ulcerations, also in incomplete studies); suspicious (5 ulcerations or erosions); nonspecific or normal. Incomplete studies were defined as the capsule not reaching the cecum. All patients previously underwent upper and lower GI endoscopy and IBD was diagnosed according to widely agreed criteria (J Pediatr Gastroenterol Nutr 2007;44:653).

**Results:** WCE disclosed typical SB lesions in 20 out of 31 CD patients (64.5%) and allowed to relate their abnormal clinical and biochemical features to the SB involvement. In 3/18 (16.6%) patients previously diagnosed as UC, typical ileal lesions of CD were detected and diagnosis was switched on CD, in the remaining 15 UC patients (83.3%) SB was unaffected or showed nonspecific lesions and UC diagnosis was confirmed. Of the 14 patients with suspected IBD and in whom upper and lower GI endoscopy had not revealed specific features, WCE disclosed CD lesions in the SB in 9 (64.2%), whereas other entities were detected in 5 (35.7%) (polyps, vascular malformations, drug-induced lesions). Of the 7 IBDU patients, WCE was diagnostic for CD in 5 (71.4%), whereas IBD remained unclassified in 2 (28.6%).

**Conclusions:** In children investigated for IBD, WCE is a very helpful tool for defining a diagnosis of IBD (if traditional methods are inconclusive) and for discriminating between the two classical forms (CD, UC) of IBD. Of great clinical value is the fact that WCE may definitely classify IBDU. These results have important clinical and therapeutic implications.
PG2-09

PROINFLAMMATORY DNA METHYLATION CHANGES IN MURINE COLONIC MUCOSA DURING POSTWEANING DEVELOPMENT

Presenter: R. Kellermayer. Baylor College of Medicine, Houston, Texas, USA.
Co-authors: A. Balasa1, R. Himes1, S. Mirza1, N. Tatevian2, L. Shen1, R. Waterland1. 1Baylor College of Medicine, Houston, TX, USA; 2University of Texas, Houston, TX, USA; 3M.D. Anderson Cancer Center, Houston, TX, USA.

Aim: To evaluate the range of genome wide, functionally relevant DNA methylation changes in the colonic mucosa during childhood and adolescence in mice as a model.

Methods: DNA from colonic mucosal scrapings of 30-day- and 90-day-old C57BL/6J mice was isolated. Two independent methylation specific amplification microarrays were performed with colonic mucosal DNA, each comparing 90-day vs 30-day. Concordant hits with arrays were performed with colonic mucosal DNA, each independent methylation specific amplification microarray.

Results: 49 genes showed a 2-fold decrease in methylation. While 15 genes showed a 2-fold increase in methylation.

Conclusions: Significant gene related DNA methylation changes occur during childhood/puberty in mouse colonic mucosa. Associated changes in gene expression correlate with an age-dependent increase in colitis susceptibility. Similar changes may occur in human colonic mucosa. Abnormalities in these processes may lead to disease (eg, inflammatory bowel diseases), a possibility requiring further investigation.

PG2-10

EFFICACY OF FRACTIONATED ORAL VERSUS CONTINUOUS ENTERAL NUTRITIONAL THERAPY IN PEDIATRIC CROHN’S DISEASE


Background and Aim: Nutritional therapy has an established role as induction therapy in pediatric Crohn’s disease (CD). However, compliance is the main difficulty in total and exclusive enteral nutrition, and it may be greatly influenced by the administration route. This study aimed to compare the efficiency of oral route versus continuous nasogastric feeding to induce remission in children with newly diagnosed CD.

Methods: The medical records of 85 patients with active severe CD treated by exclusive nutritional therapy (Modulen IBD) by oral or continuous enteral route via a nasogastric tube were retrospectively reviewed. Comparative analyses of remission rates, changes in anthropometry, Pediatric Crohn’s Disease Activity Index (PCDAI), Harvey-Bradshaw index (HB), laboratory indices and compliance rates were performed.

Results: At the 8-weeks endpoint visit, 23/32 patients achieved remission in the oral group (72% on intention-to-treat analysis) and 45/53 patients (85%) in the enteral nutrition group (P=0.151). All patients showed a significant decrease in disease severity assessed by PCDAI (P<0.0001) and HB (P<0.0001) scores and significant improvements in anthropometric measures and inflammatory serum indices. No significant difference was evidenced whether Modulen IBD was administered orally or by continuous enteral feeding, apart from weight gain which was greater in the continuous enteral group (P=0.041). However, the induction of remission was faster in the continuous enteral group: at four weeks of exclusive nutritional therapy, PCDAI and HB scores were lower in the continuous enteral group (P=0.01 and 0.009, respectively). The compliance rates (87 and 88%) were similar in either group, with noncompliant children failing to achieve complete remission.

Conclusions: The use of fractionated oral nutritional therapy is as efficacious as continuous enteral administration via a nasogastric tube to induce remission in children with newly diagnosed CD. Compliance rates were similar between both groups; however, only patients who maintained exclusive nutritional therapy were able to maintain remission. Therefore, the efficacy of nutritional therapy is not dependent on the mode of administration but on the effect of being given exclusively.
Co-authors: E. Ecochard 1, C. Martinez-Vinson 1, J. Hugo 1, V. Degas 1, M. Balleau-Hé 1, J. Viala 1, E. Jacqz-Aigrain 2, J. Cézard 1. 1 Gastroentérologie, Mucoviscidose et Nutrition pédiatrique, Hôpital Robert Debré, Paris, France; 2 Pharmacologie, Hôpital Robert Debré, Paris, France.

Background and Aim: Infliximab is a monoclonal antibody against tumor necrosis factor-alpha. It has widely demonstrated its' efficacy in the treatment of adult and pediatric inflammatory bowel disease. Nevertheless, several risks are associated with this treatment. The aim of this study was to assess long-term clinical adverse events of infliximab in a pediatric population.

Methods: We retrospectively analysed the clinical serious adverse effects occurring in a pediatric population of 73 patients, treated with infliximab for an inflammatory bowel disease, over a period of 7.5 years (December 1999 to May 2007), in a tertiary care center.

Results: 73 patients (average age: 14.1 years) received a total of 887 infliximab infusions (median: 10 per patient), with a median follow-up of 23 months. 90% of patients received concomitantly immunosuppressive therapy (azathioprine, 6-mercaptopurine, methotrexate) and/or steroid (86% of patients). Serious adverse effects occurred in 33% of patients, that mean in 5% of infusions. Acute and delayed infusion reactions occurred in 5 of the 73 patients (6.8%), respectively in each group. Some adverse effects had never been described before in children (infliximab-induced systemic lupus erythematosus: 1 case; seizures: 1 case). A severe toxic drug-induced eruption was noted in one patient. 16/73 patients (22%) developed 27 severe infections, corresponding to 3% of infusions. Among these were noted: 1 catheter-related sepsis, 8 gastrointestinal infections (of which 1 CMV enterocolitis), 14 skin infections including 2 opportunistic infections (zona), 2 respiratory tract infections of which one pulmonary tuberculosis, 1 neck abscess, and 1 hepatitis B. These adverse events lead to treatment interruption in 10 patients (13.7%). None was lethal.

Conclusions: This study, original through the length of follow-up and the important number of infusions per patient, shows a good overall long-term tolerance of infliximab. Severe adverse events, notably infections, were more frequent than in adults. This suggests that infliximab therapy should be reserved to severe pediatric inflammatory bowel diseases.

PG2-12

CHANGES IN INCIDENCE AND MANAGEMENT OF INFLAMMATORY BOWEL DISEASES (1997–2007) IN SOUTHWEST UK

Presenter: S. Hosdurga. Bristol Royal Hospital for Children, Bristol, UK.

Co-authors: C. Spray 1, C. Gardner 1, B. Sandhu 1. 1 Bristol Royal Hospital for Children, Bristol, UK.

Background and Aim: A 1997 national prospective survey of childhood inflammatory bowel disease (IBD) in the UK provided the best international prospective data of a large IBD cohort. It documented a national incidence of 5.2/10^5, symptomatology, disease distribution, seasonality, ethnic differences, variations in diagnostic methodology and time interval from symptoms to diagnosis (Lancet 2001;357:1093–4; Arch Dis Child 2003;88:995–1000). 33 children were from the southwest (SW) region. It is not possible to repeat the national study because the numbers are too large so a regional study has...
been undertaken in the SW where endoscopy and initial management in children with suspected IBD is carried out in a single regional centre and since 1990 a regional prospective database of all children 0–16 years with newly diagnosed IBD has been maintained. The aim of the study was to document current incidence and note any change since 1997, document whether children are being diagnosed in a way concordant with ESPGHAN’s Porto criterion, and document whether children were receiving appropriate treatment and support in line with best practice (BSPGHAN Guidelines on Management of IBD, 2008).

Methods: Data were collected from 13 paediatric centres within hospitals in the SW region using International Diagnosis Codes (ICD-10) to identify newly diagnosed cases of IBD (Crohn, ulcerative colitis, and indeterminate colitis). Patient details were correlated with the database to obtain information on incidence and a clinical questionnaire was developed to obtain information on management.

Results: All 13 centres responded. There were a total of 47 newly diagnosed children with IBD during 2007 compared with 33 in 1997. 44/47 were diagnosed at the regional centre and were on the database; 3/47; all from 1 centre were not. The figure on page E37 shows number of cases of IBD by disease type over the ten year period. The data on disease distribution, symptomatology, diagnostic methodology, treatment and concordance with ESPGHAN and BSPGHAN guidance will be presented.

Conclusions: Incidence of IBD increased from 1997 to 2003 but has since stabilised. Most of the increase was in Crohn disease but there has been a more recent increase in UC. There has been some change in management.

PG2-13
LOCALIZATION OF THE DISEASE ACTIVITY IN NEWLY DIAGNOSED CHILDREN WITH INFLAMMATORY BOWEL DISEASE IN POLAND: A PROSPECTIVE, 2-YEAR STUDY, 2002–2004
Presenter: K. Karolewska-Bochenek. Medical University of Warsaw, Warsaw, Poland.


Methods: Patient records from 24 pediatric gastroenterology centers which service the whole population of Poland were prospectively collected between December 2002 and December 2004. IBD diagnosis was based on clinical, radiological, endoscopic and histological features. Records of all newly diagnosed IBD individuals 0–18 years old were mailed by the diagnosing physicians to the coordinating center.

Results: There were 491 new IBD patients. Children had various combinations of rectoscopy, sigmoidoscopy, colonoscopy, upper GI endoscopy, barium enema, and barium follow through. Complete diagnostic work-up (upper GI endoscopy, barium follow through, colonoscopy) was performed in 102 (20.77%) of all IBD patients. Of those, 53 had Crohn disease (CD), 26 had ulcerative colitis (UC) and 23 had indeterminate colitis (IC). Those patients’ records were further analyzed to determine the localization of the disease (Table 3). For those with CD the most commonly affected sites were ileum, ascending colon and rectum. 25% had gastroduodenal activity, 3.9% had isolated small bowel disease and 10% had isolated colonic disease. Majority had both colonic and small bowel disease. Of the patients with ulcerative colitis 50% presented pancolitis, 16.7% had left side colitis and 4.2% had isolated proctitis. The disease distribution of indeterminate colitis overlaps both that of CD and ulcerative colitis.

Conclusions: Only one-half of the patients with UC had pancolitis, a rate lower than in other recent studies from the US and western Europe. Among the CD patients, we saw more ileocolonic disease than isolated involvement (ileal or colonic), different from other studies in Western countries.

<table>
<thead>
<tr>
<th>Localization</th>
<th>CD, %</th>
<th>UC, %</th>
<th>IC, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouth</td>
<td>3.8</td>
<td>0.0</td>
<td>4.3</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>3.8</td>
<td>4.2</td>
<td>8.7</td>
</tr>
<tr>
<td>Stomach and duodenum</td>
<td>25.0</td>
<td>12.5</td>
<td>26.1</td>
</tr>
<tr>
<td>Jejunum</td>
<td>12.2</td>
<td>0.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Ileum</td>
<td>68.6</td>
<td>13.6</td>
<td>22.2</td>
</tr>
<tr>
<td>Ascending colon</td>
<td>66.7</td>
<td>50.0</td>
<td>55.0</td>
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<tr>
<td>Transverse colon</td>
<td>47.1</td>
<td>76.9</td>
<td>59.1</td>
</tr>
<tr>
<td>Descending colon</td>
<td>48.1</td>
<td>92.3</td>
<td>63.6</td>
</tr>
<tr>
<td>Sigmoid</td>
<td>57.7</td>
<td>88.5</td>
<td>78.3</td>
</tr>
<tr>
<td>Rectum</td>
<td>66.7</td>
<td>84.6</td>
<td>87.0</td>
</tr>
<tr>
<td>Perianal</td>
<td>26.0</td>
<td>7.7</td>
<td>8.7</td>
</tr>
</tbody>
</table>

PG2-14
TO REVIEW THE INCIDENCE OF FOOD-RELATED SYMPTOMS AFTER EXCLUSIVE ENTERAL FEED THERAPY IN CROHN DISEASE
Presenter: S. Hill. Great Ormond Street Hospital for Children, London, UK.


Aim: To review the risk of developing food-related symptoms in children with Crohn disease after nutritional
therapy with either exclusively an amino acid or protein hydrolysate based feed and to assess the need for introducing each food in turn.

**Methods:** All 49 children (24 male, 25 female) aged 5–16 years with histologically proven Crohn disease who were treated with liquid enteral feed up to September 2005 in our gastroenterology department were studied. Response to treatment was assessed using the Paediatric Crohn’s Disease Activity Index (PCDAI) before and after treatment. Hospital medical case notes and dietician records were reviewed. Foods were introduced to the diet one at a time and any gastrointestinal symptoms that developed after reintroduction of an individual food were recorded. A personal or family history of atopy and the child’s total IgE level and positive specific IgE levels for foods were also recorded.

**Results:** Sixteen of 49 children or 33% developed gastrointestinal symptoms on introducing one or more of cow’s milk, egg, wheat, potato soya, banana or shellfish. Other foods were tolerated. The main symptom was abdominal pain. Total PCDAI score was 1350 before treatment (mean 27) and 282 (mean 5.7) after treatment. 13 patients had raised total IgE and three raised specific IgE to milk. Seven (or 44%) of these children were symptomatic. Sixteen children were atopic and 8 of them (or 50%) developed food-related symptoms. Symptoms occurred in children with ileal and/or colonic Crohn.

**Conclusions:** Food-related abdominal pain that resolved when the offending food was removed from the diet again was common, affecting 33% of Crohn children reintroducing diet after a period of liquid enteral feed. Children with an atopic history had a higher incidence of food-related symptoms than those without. Abdominal pain may be associated with disease recurrence which could be related to the offending food, causing the disease to relapse.

**PG2-15**

**EFFICACY AND SAFETY OF ADA LIMUMAB IN PEDIATRIC CROHN DISEASE: A PROSPECTIVE, OPEN-LABEL, SINGLE-CENTRE TRIAL**

Presenter: F. Civitelli, Sapienza University of Rome, Rome, Italy.

**Background and Aim:** Adalimumab (ADA) (Humira, Abbott), a fully human IGG, anti-TNFα monoclonal antibody, is effective in adult patients with Crohn disease (CD), regardless of previous experience with infliximab (IFX) therapy. There are only few data on its use in pediatric CD. We prospectively evaluated efficacy and safety of ADA in children with moderate to severe CD.

**Methods:** Twenty-three CD patients, median age 16.1 years (range 9–20), naïve to (9 patients) or who had failed prior IFX therapy (14 patients) received ADA. The induction schedule at week (wk) 0 and 2 was 160 mg/80 mg in 13 patients, 80 mg/40 mg in 8, followed, respectively, by 80 mg or 40 mg every other week (eow) during the 44 wks maintenance phase. Two patients received an induction dose of 120 mg/80 mg, followed by 80 mg eow. Primary outcomes were: clinical remission (PCDAI score ≤10), clinical response (decrease in PCDAI score of ≥50% versus baseline), safety at wk 2, 4, 12, 24, and 48. ITT analysis including all patients who received at least 1 dose of ADA was performed.

**Results:** Mean disease duration at the beginning of ADA course was 52.2 ± 42.5 months. Fourteen patients had previously received IFX (9.2 ± 8.2 doses), discontinued due to loss of efficacy (11) or intolerance (3). At baseline 13 patients were receiving immunomodulators; only 2 at wk 48. Mean oral corticosteroids dose (mg/kg/day) was 0.9 ± 0.2 mg at wk 0, 0.07 ± 0.1 at wk 48 (P < 0.001). The mean PCDAI, ESR, and CRP values significantly decreased throughout the trial (Table 4). Rates of remission at wk 2, 4, 12, 24 and 48 were 36.3%; 44.8%; 50%; 62.5%; 68.8%; 60.8%; 30.5%; 50% and 65.2%. Rates of response were, respectively: 54.5%; 34.8%; 39.1%; 40.9%; 30.4%. Along the maintenance phase 5/8 patients receiving 40 mg eow had to increase ADA dose to 80 mg eow in order to maintain clinical remission; 6/13 patients receiving 80 mg eow were able to shift to 40 mg eow without CD exacerbation. Only 2 patients presented a serious adverse event requiring temporary cessation of ADA: abdominal abscess (1), severe cutaneous infection (1).

**TABLE 4. PCDAI, ESR, and CRP values**

<table>
<thead>
<tr>
<th>Wk</th>
<th>PCDAI</th>
<th>ESR, mm/hour</th>
<th>CRP, mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>36.5 ± 5.7</td>
<td>54 ± 36</td>
<td>31.2 ± 24*</td>
</tr>
<tr>
<td>2</td>
<td>14.7 ± 5.05*</td>
<td>22 ± 11.1**</td>
<td>6.5 ± 5.8*</td>
</tr>
<tr>
<td>4</td>
<td>11.5 ± 2.38*</td>
<td>27.9 ± 19**</td>
<td>7.3 ± 6.2*</td>
</tr>
<tr>
<td>12</td>
<td>16.6 ± 4.7*</td>
<td>27.0 ± 14**</td>
<td>11.2 ± 5.6*</td>
</tr>
<tr>
<td>24</td>
<td>13.7 ± 6.4*</td>
<td>20 ± 14.8**</td>
<td>8.8 ± 4.8*</td>
</tr>
<tr>
<td>48</td>
<td>9.9 ± 2.7*</td>
<td>13.1 ± 4.4**</td>
<td>2.7 ± 1.3*</td>
</tr>
</tbody>
</table>

* P < 0.01 vs baseline.

** co-authors:** F. Viola1, F. Nuti1, S. Oliva1, G. Di Nardo1, V. Labalestra1, F. Pannone1, F. Conte1, M. Barbato1, M. Alo1, O. Borrelli1, S. Cucchiara1. 1Pediatric Gastroenterology and Liver Unit, Sapienza University, Rome, Italy.
Conclusions: Our data suggest that ADA is effective and well tolerated in rapidly inducing and maintaining clinical remission in children with refractory CD, irrespective of prior IFX therapy. ADA maintenance dose can easily and efficiently be modulated over time according to disease activity.

PG2-16

IS PAEDIATRIC-ONSET CROHN DISEASE MORE SEVERE?
Co-authors: J. Girardet¹, S. Viola¹, B. Dubern¹, P. Tounian¹, P. Seksik², J. Cosnes². ¹Armand Trousseau Hospital, Pediatric Gastroenterology and Nutrition Unit, Paris, France; ²Saint Antoine hospital, Gastroenterology Unit, Paris, France.

Background and Aim: Limited information is available on the characteristics of longstanding Crohn disease with onset in childhood or adolescence. The aim of our study is to describe and compare the severity of the disease between paediatric-onset Crohn disease patients and adult-onset.

Methods: 206 patients (92 female) with paediatric-onset Crohn disease (before 16 years) were compared to 412 patients (184 female) with disease diagnosed as adults. Patients were matched on sex, date of diagnosis and initial location of disease. The two groups were compared retrospectively on disease history, complications and surgery. Annual disease activity and treatment were assessed prospectively between 1995 and 2007 in both groups.

Results: Paediatric patients had a lower BMI and final height than the adult ones (P < 0.01) but weight was significantly reduced in males. There was no difference between the paediatric and adult groups regarding the risk for developing stricturing disease (at 10 years, 21.8% versus 24.7%), perforating disease (25.9% versus 27% at 10 years) and perianal disease (21.8% and 24.7%, respectively at 10 years). Duration of steroid therapy was equal between the two groups but the number of paediatric patients requiring steroid was higher. Use of immunosuppressive therapy was more frequent and more important in the paediatric group (54% patients treated at 10 years of disease versus 45% in the adult group, P < 0.01). The interval between time of diagnosis and first surgery was not statistically different between the two groups. The risk at 30 years to have undergone extensive intestinal resection was 48% in the paediatric group and 14% in the adult group (P < 0.01). The risk of definitive stoma occurred at an earlier age in paediatric patients. After 25 years of disease evolution, 17% of paediatric patients had a definitive stoma and 10% of adults (P < 0.01). Prospective analysis between 1995 and 2007 showed a greater disease activity in the paediatric group (752 patient-years (37%) vs 1181 (31%); P < 0.01).

Conclusions: Paediatric-onset disease is more severe than adult-onset disease. When followed for an extended period of time, paediatric patients had the same disease complexity but do so at a younger age than patients with adult-onset Crohn disease.

PG2-17

LUNG MEMBRANE DIFFUSION AND CAPILLARY BLOOD VOLUME IN CROHN’S DISEASE: A PILOT STUDY
Presenter: T. Lamireau. Hôpital des Enfants, Bordeaux, France.
Co-authors: M. Choukroun¹, G. Masse¹, L. Rebouissoux¹, S. Bui¹, H. Clouzeau¹, V. De Ledinghen². ¹Hopital des Enfants, Bordeaux, France; ²Hopital Haut Leveque, Pessac.

Background and Aim: Perturbation of alveolo-capillary diffusion is frequent in Crohn’s disease. It is usually attributed to the presence of interstitial pneumonitis, but it could also be explain by changes in the capillary bed. The aim of this study was to assess capillary blood volume in Crohn’s disease.

Methods: Lung function tests were performed in 10 patients with Crohn’s disease. Forced vital capacity (FVC), forced expiratory volume in 1 sec (FEV₁), functional residual capacity (FRC), residual volume (RV), and total lung capacity (TLC) were measured by body plethysmography (Sensor Medics 6200). Diffusing capacity of carbon monoxide (DLCO) and its constituents, i.e. membrane diffusion capacity (Dm) and pulmonary capillary blood volume (Vc) were measured by the method of simultaneous transfer of carbon monoxide and nitric oxide. Results are expressed as percentage of normal values and compared to the Pediatric Crohn’s Disease Activity Index (PCDAI).

Results: Patients (7 M, 3 F), aged 13.5 years, were investigated at the time of diagnosis in 4 children or after a 22 month-duration (1–42 months) of disease course in 6 children who were treated with 5-aminosalicylate (n = 1), corticosteroids (n = 3) and/or azathioprine (n = 4). Median PCDAI score was 35 (range: 10–50). Two children had dyspnea during sport, and one had asthma. Capillary blood gazes (Pao2, PacO2), FVC, FEV₁, FRC, RV, and TLC were normal in all infants. DLCO was decreased (<80%) in 1/10 children (median: 92%, range: 79%–117%), Dm in 2/10 children (median: 93%, range: 69%–103%), and Vc in all children (median: 46%, range: 42%–74%). Vc was inversely correlated to PCDAI.
Conclusions: It is suggested that changes in alveolo-capillary diffusion observed in Crohn’s disease could be due to a decrease of the pulmonary capillary bed. These changes are likely related to the disease activity.

PG2-18

IMMUNOHISTOCHEMICAL MARKERS OF SMALL BOWEL INFLAMMATION IN CHILDREN WITH ULCERATIVE COLITIS

Presenter: L. Quaglietta. University of Naples, Naples, Italy.

Co-authors: E. Giannetti1, C. Aquino1, F. Paparo1, E. Miele1, C. Friano1, R. Troncone1, A. Staiano1. 1Department of Pediatrics, University of Naples, Naples, Italy.

Background and Aim: We recently demonstrated that abnormal cellulose/mannitol small intestinal permeability study is a predictive marker of early relapse in children with ulcerative colitis (UC). The purpose of this study was to evaluate in the jejunum of children with UC, by immunohistochemistry, subtle inflammatory changes, even in presence of normal histopathology.

Methods: From February to December 2008, 18 pediatric patients (M:F 8:10, mean age: 126 months, age range: 30–191 months) with new diagnosis of UC, based on accepted historical, endoscopic, histological and/or radiological criteria, were evaluated. The Pediatric Ulcerative Colitis Activity Index (PUCAI) was used to measure disease activity. All patients underwent upper gastrointestinal (GI) endoscopy and duodenal cryostat sections were stained for CD3+ and gamma/delta + T cells and CD25+ mononuclear cells. Twenty-four children with functional dyspepsia, who underwent upper GI endoscopy, represented the control group.

Results: Although UC patients and controls did not show any jejunal endoscopic lesions, in 3/18 (16%) UC children the histological evidence of mild inflammation of jejum was noted. As a group UC children presented a significant higher density of lamina propria CD25 + cells compared to controls (mean ± SEM: 9.4/mm² ± 1.7 vs 2.9 ± 0.2; P < 0.0001). Among these children, 3/12 (75%) presented a PUCAI value ≥20 (moderate disease activity) and 9/12 (69%) patients showed a PUCAI value ≥35 (severe disease activity). No significant differences were noted as regard CD3+ and gamma/delta + T cells between study population and controls.

Conclusions: The majority of UC children present inflammatory signs at level of the jejunal mucosa even in the absence of gross histopathological changes. The extent of upper GI tract involvement is wider than previously thought. Upper GI tract inflammation is related to the activity of the disease and is probably a predictive marker of early relapse.

PG2-19

DOES INFLIXIMAB FAVOURABLY AFFECT GROWTH AND BODY COMPOSITION IN PAEDIATRIC CROHN DISEASE?

Presenter: R. Hill. University of Queensland, Herston, Australia.

Co-authors: I. Grange2, L. Ee1, G. Withers1, P. Lewindon1, F. Connor1, G. Gleghorn1, P. Davies1. 1University of Queensland, Herston, Australia; 2Institut Polytechnique LaSalle Beauvais, Beauvais, France.

Aim: To determine if infliximab therapy improves growth, weight, body mass index (BMI) and nutritional status in children with Crohn disease (CD).

Methods: In our laboratory, measurements of growth and nutritional status are routinely carried out (6 monthly) on children with CD. Data are presented from a subset of these children (n = 7) who commenced infliximab therapy following their baseline assessments. All children were dosed with infliximab according to body weight and standard protocols. Height and weight were measured and BMI was calculated. Total body potassium (TBK) was used to determine nutritional status. Data were converted to z-scores and changes were assessed in relation to the number of doses of infliximab received and the duration of exposure (6, 12, 18 and 24 months). Relationships were analysed using Pearson’s correlation and ANOVA was used to determine differences.

Results: The number of infliximab doses per child ranged from 2 to 13 (mean ± SD = 6.6 ± 3.3). The duration of data available ranged from 6 to 24 months. At baseline, the mean (±SD) age, height, weight, BMI and TBK of the children were 13.5(±2.4) years, 150.2(±14.3) cm, 43.0(±11.2) kg, 18.7(±2.1) kg/m², and 74.4(±20.2) g, respectively. The mean z scores for height, weight, BMI and TBK were −1.17(±1.0), −0.79(±1.09), −0.16(±0.97) and −0.84(±0.91), respectively. The mean change in z scores from baseline were positive (height = 0.28(±0.38), weight = 0.37(±0.54), BMI = 0.25(±0.64) and TBK = 0.29(±0.81)), however, no significant differences were found between baseline measurements and subsequent time points. The change in height z score was significantly positively correlated with the number of doses of infliximab (r = 0.62, P = 0.00) and the duration of exposure (r = 0.59, P = 0.00). However, a partial correlation between height z score change and number of doses, controlling for duration, was not significant. No significant relations were found for change in weight, BMI and nutritional status z scores.

Conclusions: Change in height z score increased with increasing number of doses of infliximab and increased duration of exposure; however, the lack of significance found after controlling for duration suggests that the positive change in height z score may simply be a function of normal growth over time, irrespective of

the influence of infliximab therapy. A larger cohort is necessary to confirm these findings. In conclusion, while treatment with infliximab is evidenced in the literature to improve disease activity, it does not appear to influence growth and nutritional status in paediatric patients.

PG2-20

FAILED CLINICAL RESPONSE TO POLYMERIC FEEDS IN PATIENTS WITH NEWLY DIAGNOSED SEVERE PAEDIATRIC CROHN DISEASE: A SINGLE-CENTRE EXPERIENCE

Presenter: F. Kiparissi. Great Ormond Street Hospital, London, UK
Co-authors: S. Macdonald¹, M. Elawad¹, K. Lindley².
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Aim: To assess clinical response to either polymeric or elemental feeds as sole treatment amongst children with newly diagnosed severe Crohn disease referred to a single tertiary gastroenterology centre over a 2-year period.

Methods: Retrospective review of 20 case notes of 10 sequential patients started on elemental feeds (E028 Extra), median age 12 years (y) 10 months (m) (range 11y1m to 17y5m, 7 male) and 10 sequential patients started on polymeric feeds (Modulen IBD), median age 15 y (range 9y6m to 17y2m, 6 male).

Results: In both groups all patients had severe Crohn disease diagnosed by histology and aided by clinical history and laboratory pathology including raised inflammatory markers. Disease distribution was similar between the 2 groups. 10/10 patients started on EO28 had panenteropathy. 9/10 patients started on Modulen had panenteropathy. 1/10 had colitis only. All patients were started on azathioprine at diagnosis plus a 6-weeks course of exclusive elemental or polymeric feeds. The majority of patients (52%, 18/22 episodes) took their feed orally. Only 2/12 receiving EO28 (including 2 who failed on Modulen) and 2/10 receiving Modulen required nasogastric feeding. In the EO28 group 6/10 (60%) of patients responded with clinical remission, 2 (20%) were referred for surgery for stricture (n = 1) and RIF mass (n = 1) and 2 (20%) were given additional infliximab for fistulating Crohn disease. In the Modulen group 3/10 (30%) of patients responded with clinical remission, 2 (20%) were referred for surgery for strictures, 2 (20%) were changed to elemental feeds with adjunctive medical therapy (prednisolone, methotrexate) as there was no clinical response to Modulen and 4 (40%) were started on oral prednisolone.

Conclusions: Our study suggests that although polymeric feeds are considered more palatable and cheaper, they appear to be less potent in achieving remission in paediatric patients with newly diagnosed severe Crohn disease.

PG2-21

BONE MINERAL DENSITY IN INFLAMMATORY BOWEL DISEASES IN CHILDREN

Presenter: M. Szumera. Medical University of Gdańsk, Gdańsk, Poland.
Co-authors: A. Jankowska¹, M. Góra-Gębka¹, B. Kamińska¹. ¹University of Medicine, Gdańsk, Poland.

Aim: A determination of the bone mineral density in children treated from the inflammatory bowel diseases.

Methods: 67 patients were included: 42 with ulcerative colitis (UC) and 25 with Crohn disease (CD). The duration of illness was from 2.0–26.0 months. The glucocorticoid therapy was applied in 78.9% of patients with the duration time from 4–680 days. The cumulative doses of glucocorticoids ranged from 160 mg to 12,600 mg. Bone mineral density (BMD) and \( z \) score were established.

Results: BMD values varied from 0.531 to 1.301 g/cm. \( z \) score values varied from 1.0 to –5.1 SD. Bone mineral disturbances occurred in 67.4% of children and it was equally osteoporosis and osteopenia in 33.7% of cases. In ulcerative colitis osteopenia was predominant (25.8%), while in Crohn disease osteoporosis occurred more often (23.8%). There was no significant correlation among the duration time of the disease and BMD and \( z \) score. The statistically significant differences were found in the duration of steroid therapy and \( z \) score. No association was found between cumulative dose of steroids and \( z \) score. No significant differences were found in BMD and \( z \) score of lumbar spine in ulcerative colitis and Crohn disease patients.

Conclusions: Bone mineral disturbances are frequent complication of inflammatory bowel diseases in children. The association between the duration of steroid therapy and bone mineral density was confirmed. No significant differences were found in bone mineral density in UC and CD group.

PG2-22

FIRST 2-YEAR ANALYSIS OF PROSPECTIVELY DIAGNOSED PATIENTS WITH INFLAMMATORY BOWEL DISEASE (IBD) IN THE HUNGARIAN PAEDIATRIC IBD REGISTRY (HUPIR)

Presenter: G. Veres. Semmelweis University, Budapest, Hungary.
Background and Aim: On behalf of the Hungarian Pediatric Gastroenterology Society, a prospective registry of pediatric inflammatory bowel disease (IBD) was launched from 1 January 2007 with the cooperation of 27 institutes (clinics, hospitals, outpatient departments) ensuring the coverage of the whole country. The survey data has been forwarded online to the center of the European Pediatric IBD Registry (Rotterdam). In recent epidemiological studies from Europe and North America the reported incidence of childhood Crohn disease (CD) is 3–4 cases per 100,000 per year, and of ulcerative colitis (UC) 2–3 cases per 100,000 per year. So far the incidence of pediatric IBD in Hungary is unknown.

Methods: The participating institutes are requested to fill out a questionnaire (78 parameters) about every newly diagnosed IBD patients younger than 18 years. The questionnaire is about epidemiological and anthropometrical data, main symptoms, diagnostic procedures (endoscopy, CT, MRI), and the detailed results of histological and imaging procedures.

Results: Between 1 January 2007 and 31 December 2008, 262 newly diagnosed cases of IBD were prospectively identified: 164 cases of CD, 82 cases of UC, and 16 cases of indeterminate colitis. As a result the incidence of childhood CD was 6.45 cases per 100,000 per year, with the incidence of CD being 4.05 cases per 100,000 per year, the incidence of UC 2.05 cases per 100,000 per year and the incidence of indeterminate colitis 0.35 cases per 100,000 per year. The mean age at diagnosis was 13 years (range: 1.5–18 year). There was a male preponderance in CD (CD/UC = 1.65), in contrast, sex ratio in UC patients were equal. Positive family history of IBD was registered in 7.6% of patients and 9.6% of patients with CD were reported to have a fistula. Ileoscopy rate was only 52% technical problem was the most common reason for the lack of ileal intubation. Oesophago-gastro-duodenoscopy was performed in 53% of all cases. In 38% were MRI or CT scan made for the detailed verification of the disease.

Conclusions: The incidences reported in the first 2 years of HUPIR are similar to the European and North American data. The dominance of CD proved to be also consistent with other studies. Almost 10% of the patients with CD had fistula. Ileal intubation and oesophago-gastro-duodenoscopy were performed in the half of the cases, and this rate should be improved in the future.

PG2-23

AGE AT FIRST MANIFESTATION AND DIAGNOSIS OF IBD IN SWITZERLAND

Presenter: C. Braegger. University Children’s Hospital, Zurich, Switzerland.

Co-authors: P. Ballaben2, D. Rogler1, V. Pitter2, M. Friedt1. 1University Children’s Hospital, Zurich, Switzerland; 2Institute of Social and Preventive Medicine, CHUV, Lausanne, Switzerland.

Background and Aim: Increasing numbers of paediatric and adolescent patients with Crohn’s disease (CD) and ulcerative colitis (UC) are reported. Aim of our study was to test the hypothesis that this observation is a consequence of a shift towards younger ages at first manifestation and diagnosis during the last decades.

Methods: The first 1240 paediatric and adult patients recruited from a population-based cohort in Switzerland (Swiss IBD cohort study/SIBDCS) were included in this study. The SIBDCS is prospectively collecting data on a large sample of IBD patients across Switzerland through physician and patient questionnaires. Patients were stratified according to diagnosis (CD: N = 732, UC: N = 476, indeterminate colitis (IC): N = 32), as well as age at first manifestation and diagnosis related to calendar year of first manifestation and diagnosis, respectively. Both questionnaires were extracted from the Enrolment Physician Questionnaires (EPQ) as well as the Patient Enrolment Questionnaires (PEQ). Both questionnaires were analysed separately. Linear regressions of age at first manifestation, respectively at diagnosis, on calendar year of first manifestation, respectively of diagnosis, were performed. Negative regression slopes would confirm our hypothesis. Analyses were performed separately for each diagnosis and each questionnaire.

Results: All regression coefficients (slopes) for CD and UC were significantly positive, ie, age at first manifestation and age at diagnosis have increased with time (coefficients ranged between 0.20 and 0.57). Only the coefficients of the IC analyses were statistically not significantly different from zero.

Conclusions: These results do not support the hypothesis that first manifestation and diagnosis of both CD and UC patients in Switzerland occur today at younger ages. In the contrary, the results of the SIBDCS show that there is a significant trend for both first manifestation and diagnosis occurring at older ages today compared to the last decades. The results for IC are statistically not significant, probably because of the low number of patients. However, there is also in IC patients a trend towards increasing age parallel to higher calendar years. We conclude that the observation of increasing numbers of paediatric and adolescent patients with IBD is not
caused by a trend towards younger ages. It might rather be a consequence of increasing incidence of these conditions.

PG2-24

TREATMENT OPTIONS AND THERAPEUTIC OUTCOMES FOR POLYPECTOMY IN PAEDIATRIC PEUTZ-JEGHER’S SYNDROME; RESULTS FROM THE LARGEST EUROPEAN COHORT

Presenter: E. Giles. Chelsea and Westminster Hospital, London, UK.
Co-authors: J. Fell1, M. Samarasinghe2, C. Fraser2, M. Haddad1, W. Hyer2 1Chelsea and Westminster Hospital, London, UK; 2Northwick Park Hospital & St Mark’s Hospital, London, UK.

Background and Aim: It has been shown that approximately 70% of children with Peutz-Jegher’s Syndrome (PJS) will have a laparotomy by 18 years (Hinds et al, J Pediatr Gastroenterol Nutr 2004:39:219–20). This study explores the indications and operative course of patients who underwent surgery for gastrointestinal complications of PJS.

Methods: The St Mark’s Polyposis Registry holds the largest paediatric database of PJS children in Europe. All patients who underwent surgery for PJS from 1996 to 2008 were reviewed. Ethical approval was not applicable for this review.

Results: Eleven patients were identified who underwent 15 laparotomies. Age at first laparotomy ranged from 1 to 15 years (median 9). Follow-up period was from 1 to 10 years (median 4). Indications for first intervention were intussusception (3), obstructive symptoms (5), and bleeding (3, including 1 acute life-threatening bleed). Four of the 11 patients underwent attempted endoscopic polypectomy for symptoms listed above, but all went on to surgery. Three patients perforated, despite the procedure being performed by an expert in therapeutic endoscopy. In the fourth case the polyp was too large, and required removal by laparotomy. Four laparotomies were performed without intraoperative enteroscopy (2 due to emergency surgery, 2 for other reasons). When no enteroscopy was performed, the small bowel was explored by hand, but in one case this missed a large polyp later seen on MRI. Preoperative imaging included ultrasound, CT, MRI, barium studies and capsule endoscopy. Of the 4 barium studies, there were 2 false positive results. One of the capsule endoscopies gave a false negative result. Findings at surgery ranged from a single polyp (5 patients) to multiple (2 to 14) polyps. The polyps were from stomach to sigmoid colon, although predominantly in the small bowel.

Conclusions: This is the largest case series of paediatric Peutz-Jegher patients undergoing laparotomy. Barium studies and capsule endoscopy gave false-positive and false-negative results, respectively, which illustrates the difficulties of screening this group. Patients who underwent endoscopic polypectomy by experienced endoscopists with extra precautions are still at high risk of perforation. This experience suggests that should paediatric patients require polypectomy for symptoms, they should undergo planned laparotomy with intraoperative enteroscopy.

PG3-01

PAEDIATRIC OESOPHAGEAL HIGH-RESOLUTION MANOMETRY: UTILITY OF A STANDARDIZED PROTOCOL AND SIZE-ADJUSTED MOTILITY PARAMETERS

Presenter: H. Goldani. Hospital de Clinicas de Porto Alegre-UFGRS, Porto Alegre-RS, Brazil.
Co-authors: A. Staiano1, N. Thapar2, K. Lindley2. 1University Federico II, Naples, Italy; 2Great Ormond Street Hospital and Institute of Child Health, London, UK.

Background and Aim: Oesophageal high resolution manometry (OHRM) is rapidly evolving from a research tool to a routine investigation in adult clinical practice. This study proposes and validates a standardized protocol of OHRM for use in paediatric clinical practice.

Methods: Twenty-three paediatric patients underwent unsedated OHRM (MMS, Netherlands). Indications for OHRM were dysphagia, feeding difficulty or pre-fundoplication assessment. Two 20-channel customized water perfused silicone catheters, outside diameter 3.8 mm (MuiScientific, Ontario, CA) were used. The microlumina were perfused with a pneumohydraulic perfusion system at a water perfusion rate 0.15 mL/min. Catheters had 1 distal gastric channel, 5 channels 0.5 cm apart for esophageal and 14 proximal channels either 1cm (for children <5 years) or 2 cm apart (for children >5 years). Single wet swallows, multiple rapid swallows, and solid swallows were systematically studied. Data were analyzed using MMS software version v8.11.

Results: Median age was 9 years (range 6 months–15 years). The oesophageal motor findings were normal peristalsis (n = 9), peristaltic dysfunction (n = 11), achalectasia (n = 3) and spasm on solid food (n = 2). Distal
contractile integral (DCI) was calculated by multiplying the mean pressure in the space-time box by the length and duration of the space-time box. DCI adjusted for oesophageal length (DCIa) of patients with peristaltic dysfunction was significantly lower than patients without peristaltic dysfunction ($P < 0.001$). On multiple rapid swallows (MRS), aperistalsis with lack of oesophageo-gastric junction (OGJ) relaxation was seen in achalasia patients, and aperistalsis with complete OGJ relaxation was seen in patients with severe peristaltic dysfunction. On solid food, oesophageal spasm associated with bolus impaction was seen.

**Conclusions:** OHRM is diagnostically useful for oesophageal motility assessment in the paediatric clinical setting which can be performed unsedated even in infants. MRS and solid swallowing should be included as a routine part of the investigation because they improve the diagnosis of motility disorders in children with dysphagia.

**PG3-02**

**IS THERE A ROLE FOR NEW HELICOBACTER PYLORI VIRULENCE MARKERS IN PEDIATRIC PEPTIC ULCER DISEASE?**

**Presenter:** M. Oleastro. *Instituto Nacional de Saúde Dr Ricardo Jorge, Lisboa, Portugal.*

**Co-authors:** J. Cabral$^1$, P. Magalhãs Ramalho$^2$, P. Sande Lemos$^3$, A. Santos$^4$, L. Monteiro$^5$, A. Lopes$^6$. $^1$Unidade de Gastroenterologia Infantil, Centro Hospitalar Lisboa, Lisboa, Portugal; $^2$Unidade de Gastroenterologia Pediátrica, Hospital de Santa Maria, Lisboa, Portugal; $^3$Servicio de Pediatría, Hospital Fernando Fonseca, Lisboa, Portugal; $^4$Departamento de Doenças Infecciosas, Instituto Nacional Saúde Dr Ricardo Jorge, Lisboa, Portugal.

**Background and Aim:** Although the development of *H pylori*-associated peptic ulcer disease is recognized as a relatively rare event in children, its occurrence at young age may suggest a pathogenic role for the implicated strains. The genotype profiling of *H pylori* strains isolated from children with peptic ulcer disease has been scarcely characterized so far. Our recent identification of a novel *H pylori* putative virulence marker (*J Infect Dis* 2008;198:1379–87), prompted us to compare the *H pylori* genotype profile of strains isolated from children presenting with peptic ulcer as compared to non-ulcer gastritis, using an extensive panel of *H pylori* virulence markers.

**Methods:** A total of 97 *H pylori* strains were obtained from Portuguese children, during a 9-year period; 44 subjects had peptic ulcer disease (60% male, mean age 12.3 ± 3.6 years), of which 41 (93.2%) duodenal ulcer (DU) and 3 (6.8%) gastric ulcer (GU); 53 subjects had nonulcer gastritis (56.6% male, mean age 9.1 ± 3.6 years). *H pylori* genotyping was performed by PCR and sequencing.

**Results:** In this cohort, the prevalence of specific *H pylori* virulence genotypes was significantly higher in ulcer-associated strains than in nonulcer gastritis strains: cagA (79.5% vs 17%), vacA s1 (79.5% vs 18.9%), oipA (75.0% vs 32.1%), hopQ (59.1% vs 28.3%) and homB (81.8% vs 34%), with the exception of sabA, which was significantly associated with nonulcer gastritis (22.6% vs 56.6%). Concerning other *H pylori* virulence-associated genes, such as babA, hopZ and dupA, no significant difference was observed between the two clinical samples.

**Conclusions:** *H pylori* strains recovered from children with peptic ulcer, namely duodenal ulcer, have shown a distinctive genotype virulence pattern, as compared to nonulcer gastritis strains, suggesting a potential pathogenic role for new markers, such as homB. Moreover, these results are in contrast with previous adult studies in the same geographic background, where only cagA was associated with peptic ulcer. Globally, our data suggest that in some populations, the severity of *H pylori*-associated disease in younger subjects may be closely related to the virulence of the strain, irrespectively of the contribution of host and/or environmental factors, which play a major role in the adult. Furthermore, the present study emphasizes the relevance of including a diversity of clinical isolates for the research of novel virulence genes.

**PG3-03**

**SEQUENTIAL THERAPY VERSUS STANDARD TRIPLE THERAPIES FOR HELICOBACTER PYLORI INFECTION**

**Presenter:** P. Bonnemis. *Queen Fabiola Children’s University Hospital, Brussels, Belgium.*

**Co-authors:** N. Kalach$^1$, G. Oderda$^3$, L. Muyshincl$^1$, S. Assaad$^1$, L. Waroquier$^1$, S. Cadreau$^1$, M. Scaillon$^1$. $^1$Queen Fabiola Children’s University Hospital, Brussels, Belgium; $^2$Department of Pediatrics, Saint Antoine Paediatric Clinic, Lille, France; $^3$Pediatric Department, University of Piemonte Orientale, Novara, Italy.

**Background and Aim:** To assess the eradication rate of *Helicobacter pylori* (*H pylori*) infection in children using a sequential treatment regimen compared with the classical omeprazole-containing triple therapy. The secondary objective was to evaluate the impact of the antimicrobial susceptibility of *H pylori* strains on the eradication rates.

**Methods:** Prospective, open-label study, multi-center study. Children with nonulcer dyspeptic manifestations undergoing upper GI endoscopy were included if *H*
pylori infection was proven by histology and culture. Children having received proton pump inhibitor, H₂-blockers or antibiotics during the 4 weeks preceding endoscopy were excluded. Children received randomly either a 10-day sequential treatment comprising omeprazole with amoxicillin (AMO) for 5 days and omeprazole, clarithromycin (CLA) and metronidazole (MET) for the remaining 5 days or a 7-days treatment, comprising omeprazole with AMO and CLA in case of H pylori strains susceptible to CLA or MET in case of resistance to CLA. H pylori eradication was assessed by 13C urea breath test (UBT) at least 8 weeks after completion of the treatment, considered successful if the UBT was negative.

Results: From October 2007 to September 2008, 98 children were included (58 female/42 male, median age 11 years, range 1.5 to 17). Age and sex distribution was similar between groups but resistance rate was higher in the sequential group. Eradication was achieved in 74 children out of 88 who returned for a follow-up test. The intention-to-treat eradication rate (ITT) was thus 76% (sequential 41/55 = 75%, triple therapy 33/43 = 77%) and the per-protocol cure rate (PP) 84% (sequential 41/49 = 84%, triple therapy 33/39 = 85%). In case of CLA resistance, ITT eradication rate was 9/14 (sequential 7/12 = 58%, triple therapy 2/2) and PP 9/13 (sequential 7/11 = 64%, triple therapy 2/2). In case of MET resistance, ITT eradication rate was 13/16 (sequential 9/13 = 69%, triple therapy 4/5) and PP 13/15 (sequential 9/10 = 90%, triple therapy 4/5). Both treatments were well tolerated.

Conclusions: Sequential treatment seems highly effective for eradicating H pylori, with similar or higher eradication rate than with triple therapy prescribed in accordance with antimicrobial susceptibility testing. However, in case of CLA resistance the cure rate is decreased. Sequential treatment may be used as a first line therapy if antimicrobial susceptibility testing is not available in population with CLA resistance not exceeding 20%.

PG3-04
PREVALENCE AND MANAGEMENT OF GASTROESOPHAGEAL REFLUX (GOR) IN CHILDREN AND ADOLESCENTS IN FRANCE: RESULTS OF A CROSS-SECTIONAL STUDY
Presenter: G. Frederic. CHU Lille, Lille, France.
Co-authors: L. Martigne1, P. Delaage2, F. Thomas2, P. Barthelemy2, F. Gottrand1, 1CHU Lille, Lille, France; 2AstraZeneca, Rueil-Malmaison, France.

Background and Aim: GOR manifestations and frequency in childhood are poorly known. The aim of this study was to determine GOR prevalence, symptoms and treatment in patients aged from 0 to 17 years (y) old, in France.

Methods: The study was carried out by TNS-Healthcare on a sample of 404 general practitioners (GP) and 180 paediatricians (PAED), representatives for French medical population. All patients, aged from 0 to 17 y, consulting spontaneously over two periods of three consecutive days, were listed. For each child presenting with GOR, doctor filled out a questionnaire.

Results: 10,394 children were listed (GP = 5,143, PAED = 5,251). GOR prevalence was 15.1% (n = 776) for children seen by GP (0–23 mo = 28.7% (n = 449) to 2–11 y = 7.9% (n = 176), 12–17 y = 11.0% (n = 151)). Prevalence was 15.1% (n = 793) for patients seen by PAED (0–23 mo = 22.7% (n = 706), 2–11 y = 4.0% (n = 74), 12–17 y = 5.1% (n = 13)). Extrapolating to French population, GOR prevalence in children younger than 18 y is 10.3% (0–23 mo = 24.4%, 2–11 y = 7.2%, 12–17 y = 10.7%). For 0 to 23 mo infants, most frequent GOR symptoms are regurgitation (85%), crying (45%), feeding difficulties (42%) and stridor (10%). The most common examination is pH-metry (13%) and medical manipulations (NMM) include bottle volume reducing or meal thickening (85%) and dorsal positioning (83%). 79% are prescribed a medical treatment (antacids/alginates (AA) = 71%, antibiotics (AE) = 64%, PPI = 20%) and 24% are referred to another doctor (paediatric gastroenterologist (PGE) = 49%, general paediatrician = 37%). For 2 to 11 y children, typical GOR symptoms are heartburn (37%), regurgitation (36%) and vomiting (32%); atypical GOR symptoms are chronic cough (68%), ENT-symptoms (35%) and asthma (24%). Most frequent examinations are pH-metry (24%) and upper GI-endoscopy (18%). Recommended NMM are head of bed raising (62%) and food avoiding (56%); 90% are prescribed a medical treatment (AA = 61%, AE = 49%, PPI = 37%). 43% are referred to another doctor (PGE = 48%, gastroenterologist (GE) = 25%). For 12 to 17y patients, most common GOR symptoms are heartburn (86%) and regurgitation (33%); 33% present a chronic cough. The most frequent examination is upper GI-endoscopy (30%); NMM are food avoiding (73%) and head of bed raising (52%). 96% are prescribed a medical treatment (AA = 54%, AE = 25%, PPI = 64%); 34% are referred to another doctor (GE = 72%).

Conclusions: This study shows that 10.3% of French children present GOR. Prevalence is high in infants (24.4%), lower in children aged from 2 to 11 y (7.2%) and increases again to 10.7% in adolescents. Clinical presentation and treatment vary depending on age.

PG3-05
THE PREVALENCE AND ROLE OF RDXA AND FRYA GENES IN RESISTANCE OF METRONIDAZOLE IN HELICOBACTER PYLORI FROM PEDIATRIC PATIENTS IN CHINA
Presenter: C. Jie. Children Hospital of Zhejiang University School of Medicine, Hang Zhou, China.
Aim: To investigate the prevalence of MTZ resistance in *Helicobacter pylori* isolates isolated from pediatric patients and to explore its mechanism.

Methods: 127 *H pylori* isolates were separated from gastric antrum biopsy of local pediatric patients with *H pylori*-associated gastritis or peptic ulcer. All the susceptibility of *H pylori* for MTZ was determined by two-fold agar dilution method and simultaneous E-test method. Then 9 random-selected wild MTZ-sensitive isolates (MTZ\(^S\)), 5 random-selected wild MTZ\(^R\) isolates and 9 acquired MTZ\(^R\) isolates (from in vitro induction resistance of MTZ, with MIC = 64 mg/L for 1 and MIC \(\geq 256\) mg/L for 8 isolates, respectively). All the genome DNA of the *H pylori* isolates were extracted and enrolled in PCR of the *rdxA* and *frxA* genes, while genome DNA of wild MTZ\(^S\) and the corresponding acquired MTZ\(^R\) isolates enrolled in genetic fingerprinting by randomly amplified polymorphic DNA based on PCR (RAPD-PCR) as quality control, all the amplified fragments of the two genes were sent for sequencing analysis to detect the MIC50 at 0.5 mg/L vs 16 mg/L, the MIC 90 stable at 128 mg/L, respectively. According to two-fold agar dilution method, the sensitivity of E-test method is (57/127), the ranges of MICs from \(0.125 < \text{MIC} < 1\) and \(\text{MIC} = 64\) mg/L for 1 and MIC \(\geq 256\) mg/L for 8 isolates, respectively. All the genome DNA of the *H pylori* isolates were extracted and enrolled in PCR of the *rdxA* and *frxA* genes, while genome DNA of wild MTZ\(^S\) and the corresponding acquired MTZ\(^R\) isolates enrolled in genetic fingerprinting by randomly amplified polymorphic DNA based on PCR (RAPD-PCR) as quality control, all the amplified fragments of the two genes were sent for sequencing analysis to detect the mutations compared by using program DNA MAN 5.2.2 from NCBI, H pylori with the reported sequence from National Center of Biotechnology Information (NCBI).

Conclusions: (1) The prevalence of resistance of MTZ in *H pylori* isolates from wild MTZR, and 12 points of 31 in gene, in detail. All types of mutations in *rdxA* and *frxA* genes, while genome DNA of wild MTZ\(^S\) and the corresponding acquired MTZ\(^R\) isolates enrolled in genetic fingerprinting by randomly amplified polymorphic DNA based on PCR (RAPD-PCR) as quality control, all the amplified fragments of the two genes were sent for sequencing analysis to detect the mutations compared by using program DNA MAN 5.2.2 from NCBI.

PG3-06

IMPROVED PERFORMANCE OF A NOVEL RAPID ONE-STEP MONOCLONAL CHROMATOGRAPHIC IMMUNOASSAY FOR DETECTION OF *HELICOBACTER PYLORI ANTIGEN IN STOOL IN CHILDREN*

Presenter: C. Prell. Dr. von Haumersches Kinderspital, Munich, Germany.

Co-authors: S. Osterrieder\(^1\), A. Schwarzer\(^1\), C. Lottspeich\(^2\), H. Rüsmann\(^2\), G. Ossiander\(^1\), S. Koletzko\(^1\).

\(^{1}\)Dr. von Haumersches Kinderspital, Ludwig-Maximilians-University, Munich, Germany; \(^{2}\)Max von Pettenkofer Institute for Medical Microbiology, Munich, Germany.

**Background and Aim:** Recently we reported on the performance of a one step monoclonal chromatographic immunoassay for detection of *H pylori* antigen in stool (RAPID Hp St\(\text{ART}^\text{TM}\), DakoCytomation, Cambridge, UK) in symptomatic children compared to the results of a well-established monoclonal EIA using the same antigen (Amplified IDEIA\(^\text{TM}\), Hp St\(\text{ART}^\text{TM}\), DakoCytomation, Cambridge, UK). Evaluation against biopsy based diagnostic methods showed a poor sensitivity, but a good specificity. Discordant results between the 2 observers occurred in 11.2% of the tests (Eur J Clin Microbiol Infect Dis 2007;26:475–80). Here we report the results of an improved version of this office based test.

**Methods:** Coded stool samples (n = 243) from symptomatic children (0.3–18 years) were tested with the monoclonal immunoassay (rapid test) and the monoclonal EIA. The monoclonal immunoassays were read by 2 independent investigators (C.P., S.O.). The monoclonal EIA performed by a third person (C.L.). Both tests were compared to the gold standard: Prior therapy (PG3-06)

MTZ resistance mechanisms other than mutations of the *rdxA* and/or *frxA* genes.

Co-authors: H. Hu\(^1\), J. Yu\(^1\), J. Chen\(^1\). \(^{1}\)Children’s Hospital of Zhejiang University School of Medicine, Hangzhou, China.

**Results:** The prevalence rates of MTZ\(^R\) were 44.89% (57/127), the ranges of MICs from \(0.125 < \text{MIC} < 1\) and \(\text{MIC} = 64\) mg/L for 1 and MIC \(\geq 256\) mg/L for 8 isolates, respectively. All the genome DNA of the *H pylori* isolates were extracted and enrolled in PCR of the *rdxA* and *frxA* genes, while genome DNA of wild MTZ\(^S\) and the corresponding acquired MTZ\(^R\) isolates enrolled in genetic fingerprinting by randomly amplified polymorphic DNA based on PCR (RAPD-PCR) as quality control, all the amplified fragments of the two genes were sent for sequencing analysis to detect the mutations compared by using program DNA MAN 5.2.2 from NCBI, H pylori with the reported sequence from National Center of Biotechnology Information (NCBI).

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specificity were 91.0% and 93.4%, respectively, with no differences between results prior and posttreatment. The monoclonal EIA showed an excellent performance with a sensitivity of 94.7% and a specificity of 97.6%.

**Conclusions:** The improved monoclonal chromatographic immunoassay seems to be an excellent alternative in situations were the monoclonal EIA or the 13C-UBT are not available or difficult to perform, especially in very young or handicapped children. Results are available within 10 minutes. Very weak lines should be considered as positive test result.

**PG3-07**

**PRIMARY ANTIBIOTIC RESISTANCE OF HELICOBACTER PYLORI STRAINS ISOLATED FROM PORTUGUESE CHILDREN: A PROSPECTIVE MULTICENTRE STUDY**

**Presenter:** M. Oleastro. **Instituto Nacional de Saude Dr Ricardo Jorge, Lisboa, Portugal.**

**Co-authors:** J. Cabral¹, P. Magalhães Ramalho², P. Sande Lemos¹, J. Benoliel¹, A. Santos⁴, L. Monteiro⁴, A. Lopes², ¹Unidade de Gastrenterologia Infantil, Centro Hospitalar Lisboa, Lisboa, Portugal; ²Unidade de Gastrenterologia Pediátrica, Hospital de Santa Maria, Lisboa, Portugal; ³Serviço de Pediatria, Hospital Fernando Fonseca, Lisboa, Portugal; ⁴Departamento de Doenças Infecciosas, Instituto Nacional Saúde Dr Ricardo Jorge, Lisboa, Portugal.

**Aim:** To prospectively assess the evolution pattern of resistance to antibiotics in *H pylori* strains isolated from Portuguese children over the last 9 years (2000–08).

**Methods:** During a 9-year period (2000–08), a total of 940 *H pylori* strains were isolated from Portuguese children attending the three pediatric gastroenterology units in the Lisbon area for upper gastrointestinal symptoms (51.2% males; age distribution: 16% <6 years, 46.1% 6–11 years and 37.9% >11 years). Antibiotic susceptibility testing to clarithromycin, metronidazole, tetracycline, ciprofloxacin and amoxicillin, was performed by E-test and disk diffusion. For all cases, testing was carried out before the first treatment.

**Results:** Overall, the *H pylori* primary resistance rate was: 34.0% to clarithromycin, 13.5% to metronidazole and 4.2% to ciprofloxacin. Simultaneous resistance to two of these antibiotics occurred in 6.8% of the isolates. Resistance to tetracycline and amoxicillin was not observed. *H pylori* antibiotic resistance rate was not associated to gender or to children age. When considering 3-year periods, 2000–02 (n = 191), 2003–05 (n = 320) and 2006–08 (n = 429), no significant temporal trend was noticed for clarithromycin and metronidazole resistance, while resistance rate to ciprofloxacin has been significantly increasing over time (P = 0.009). The same temporal trend has been observed for the double-resistant strains (P = 0.038).

**Conclusions:** According to our study concerning resistance of *H pylori* strains isolated from Portuguese children, the overall antibiotic resistance persists high. These results are particularly relevant concerning clarithromycin resistance, thus reflecting the recognized overuse of macrolides in children in southern European countries. Additionally, the increasing detection of ciprofloxacin-resistant and double-resistant strains should deserve specific attention and surveillance, as it may compromise the efficacy of second line *H pylori* eradication treatment schemas at this age group, in a population with a high prevalence of *H pylori* infection, as ours.

**PG3-08**

**GASTROESOPHAGEAL REFUX (ACID AND NONACID) AND UNEXPLAINED CHRONIC COUGH IN CHILDREN: SYMPTOM ASSOCIATION ANALYSIS USING OBJECTIVE COUGH DETECTION**

**Presenter:** Y. Vandenplas. **UZ Brussel Kinderen, Brussels, Belgium.**

**Co-authors:** K. Blondeau¹, B. Hauser³, D. Thierry³, M. Anne³, D. Iris³, D. Lieven², S. Daniel¹, V. Yvan³, ¹Center for Gastroenterological Research K.U.Leuven, Belgium; ²Division of Respiratory Medicine, University Hospital Gasthuisberg, K.U.Leuven, Belgium; ³UZ Brussel Kinderen, VUB Brussels, Belgium.

**Aim:** To assess the exact time relation between reflux and cough in a group of children and infants with unexplained chronic cough.

**Methods:** 26 children with cough of unknown origin were studied “off” PPI with ambulatory impedance-pH-monitoring (Sandhill Sci). Esophagogastric manometry was used for recognition of a typical pressure pattern of cough. Reflux was independently assessed with impedance-pH monitoring and defined as acid (pH <4) or weakly acidic (pH 4 – 6.5). Acid only events were defined as a pH drop <4 for greater than 5 sec without typical impedance reflux pattern. Cough was considered “induced by” reflux, if it started in a 2 min period after a reflux event. A symptom association probability (SAP) was calculated for each type of reflux in every patient and considered positive if >95%. Cough induced reflux if a cough burst preceded reflux in a 30-sec period.

**Results:** Impedance-pH recording detected 26 (20–52) reflux episodes/patient, of which 49% acid and 51% weakly acidic. Acid-only events were found in 16 patients (11–3). 13/26 children had an increased esophageal acid exposure. Esophagogastric pressure recordings detected
Background and Aim: Accidental caustic substance ingestion and Research Hospital, Istanbul, Turkey. The role of steroids in preventing corrosive-induced strictures is controversial. Our aim is to study the influence of high doses of steroids to prevent the most serious complication of caustic burns; esophageal strictures.

Methods: Eighty-two children with a mean age of 49.24 \( \pm \) 31.65 months with grade 2b esophageal burns (esophagogastroscopy was performed within 24–48 hours) due to accidental caustic substance ingestion are included between the dates of September 2005–February 2008 in our study. Forty-two children (study group) were received methylprednisolone (1 g/m²/day) plus ranitidine and ceftriaxone and total parenteral nutrition. Forty-one children (control group) were prescribed that protocol without methylprednisolone. Methylprednisolone treatment was received for 3 days in the study group. Control esophagogastroscopy was performed at the end of 10th day. Esophagogastroscopy was repeated to search stricture development for some patients whose clinic and endoscopic findings did not improve. Upper gastrointestinal system series with barium meal was performed, if necessary, at the end of third week. Development of stricture was compared between two groups.

Results: Stricture development was found in 4 patients (10.8%) in the study group and in 12 patients (30%) in the control group. The difference was statistically significant \( (P < 0.05) \). We stopped methylprednisolone treatment only in one patient in study group because of chicken pox rash.

Conclusions: High doses of methylprednisolone in the management of grade 2b esophageal burns seems to improve prognosis and may reduce stricture development.

PG3-09

HIGH DOSES OF METHYLPREDNISOLONE IN THE MANAGEMENT OF ACCIDENTAL CAUSTIC ESOPHAGEAL BURNS IN CHILDREN

Presenter: M. Usta. Istanbul University Cerrahpasa Medical Faculty, Istanbul, Turkey.

Co-authors: T. Erkan, F. Cullu Cokugras, Z. Onal, M. Gulcan, N. Urganci, T. Kutlu. **Department of Pediatric Gastroenterology, Hepatology and Nutrition, Istanbul University, Istanbul, Turkey; Department of Pediatric Gastroenterology Hepatology and Nutrition, Yeditepe University Medical Faculty, Istanbul, Turkey; Department of Pediatric Gastroenterology, Hepatology and Nutrition, Siisti Eftal Education and Research Hospital, Istanbul, Turkey.**

Background and Aim: Accidental caustic substance ingestion in childhood is still a big problem in developing countries and several management protocols were proposed to prevent esophageal strictures. The role of steroids in preventing corrosive-induced strictures is controversial. Our aim is to study the influence of high doses of steroids to prevent the most serious complication of caustic burns; esophageal strictures.

Methods: Eighty-two children with a mean age of 49.24 \( \pm \) 31.65 months with grade 2b esophageal burns (esophagogastroscopy was performed within 24–48 hours) due to accidental caustic substance ingestion are included between the dates of September 2005–February 2008 in our study. Forty-two children (study group) were received methylprednisolone (1 g/m²/day) plus ranitidine and ceftriaxone and total parenteral nutrition. Forty-one children (control group) were prescribed that protocol without methylprednisolone. Methylprednisolone treatment was received for 3 days in the study group. Control esophagogastroscopy was performed at the end of 10th day. Esophagogastroscopy was repeated to search stricture development for some patients whose clinic and endoscopic findings did not improve. Upper gastrointestinal system series with barium meal was performed, if necessary, at the end of third week. Development of stricture was compared between two groups.

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Conclusions: High doses of methylprednisolone in the management of grade 2b esophageal burns seems to improve prognosis and may reduce stricture development.

PG3-10

DUMPING SYNDROME FOLLOWING NISSEN FUNDOPICATION IN CHILDREN


Co-authors: T. Bosmans, I. Hoffman. **University Hospital Leuven, Leuven, Belgium.**

Background and Aim: Dumping syndrome is a well-recognized complication of gastric surgery in adults. In children, there have been increasing numbers of case reports following Nissen fundoplication, with a current prevalence of 10 to 30%. The aim of this study is to describe prevalence, symptoms, diagnosis, treatment and outcome of 190 children with antireflux surgery over a follow-up period of 13 years.

Methods: We observed children who underwent Nissen fundoplication between January 1995 and August 2008. All surgical interventions were performed by three thoracic surgeons in our University Hospital. Children with symptoms suggestive of dumping syndrome were included and underwent further investigations. Presenting symptoms of dumping syndrome were pallor, diaphoresis, lethargy, nausea, failure to thrive, watery diarrhea and abdominal pain. The diagnosis was confirmed on an oral glucose tolerance test with ingestion of 1.75 g/kg glucose orally and measurements of plasma glucose levels after 0, 30, 60, 120, and 180 minutes. Hyperglycemia was defined as any glucose level above 166, 151, 130 and 122 mg/dL capillary blood at 30, 60, 120, and 180 minutes, respectively. Hypoglycemia was defined as capillary blood glucose below 50 mg/dL at any
time during the test. The caloric intake and subdivision in protein, carbohydrates and fat intake was calculated. The standard treatment was diet, consisting of a balanced intake of 10–12% protein, 45% carbohydrates and 45% fat.

**Results:** Hundred ninety patients (122 boys, mean age 6.7 years) underwent anti-reflux surgery. Twenty patients (14 boys, mean age 4.9 years) or 11% had symptoms suggestive of dumping syndrome. The most common postprandial symptoms were abdominal pain (11/20), diarrhea (10/20), nausea (9/20), pallor (9/20). The mean interval to reporting of dumping symptoms was 24 months (range 2–168 months). An oral glucose tolerance test was performed in 18 patients. Sixteen children had abnormal results. Hypoglycemia (minimum 28 mg/dL) was found in 2 patients, 2 patients had a positive test due to combined hypo- and hyperglycemic values, 12 patients had significant hyperglycemia (maximum 486 mg/dL) during the test. Two tests were normal but the children nevertheless had symptoms suggestive of dumping during the test. Measurements of caloric intake were performed in 18 patients. The mean caloric intake was 1336 kcal daily, consisting of average 14% protein (min 11%, max 19%), 49% carbohydrates (min 38%, max 60%) and 38% fat (min 29%, max 46%). All except 1 child were successfully treated with anti-dumping diet. One patient had good clinical improvement on Acarbose (3.7 mg/kg/day).

**Conclusions:** Dumping syndrome is a complication of antireflux surgery, with a prevalence of 10% in our study. Following fundoplication, strict follow-up is mandatory, because growth and quality of life are compromised in children with dumping syndrome. Abdominal pain, diarrhea, nausea and pallor are the most presenting symptoms. An oral glucose tolerance test is a simple and inexpensive diagnostic. The diet has proven to be a very effective form of treatment in children.

**PG3-11**

**EFFECT OF THE “MULTICARE AR-BED” IN 3 WEEKS TO 3-MONTH-OLD INFANTS ON REGURGITATION, ASSOCIATED SYMPTOMS AND ACID REFLUX**

**Presenter:** Y. Vandenplas, UZ Brussels, Brussels, Belgium.

**Co-authors:** T. Devreker1, S. Verheyden2, J. Franckx2, B. Hauser1. 1UZ Brussel Kinderen, Brussels, Belgium; 2Onze Lieve Vrouweziekenhuis Aalst, Campus Asse, Asse, Belgium.

**Aim:** To evaluate the efficacy of a 40° supine body- position on infant regurgitation, reflux-associated symptoms and acid reflux.

**Methods:** 30 consecutive infants presenting with frequent regurgitation and reflux-associated symptoms occurring mainly during feeding were evaluated in the “Multicare AR-Bed” (Pios, Belgium; Web site http://www.multicare.be/index.html). The Infant Gastroesophageal Reflux Questionnaire-Revised (I-GERQ-R) and an esophageal pH monitoring were performed at inclusion and after one week.

**Results:** Eight (27%) parents stopped the intervention within the first 48 hours because of increasing discomfort of the baby. The data in 22/30 infants (73%) could be evaluated after one week. Prior to inclusion, there had been symptomatic failure of at least one dietary change in all non-breast-fed infants (extensive hydrolysate, AR-formula). At least one trial of medication (domperidone, alginat, H2 receptor antagonist, proton pump inhibitor) had failed in 20/30 patients (66%); medication was stopped at least 3 days prior to inclusion. According to the diary kept by the parents, the incidence of regurgitation at baseline (mean 6.56) decreased after one week by more than 50% (mean 2.59) (P < 0.001). The I-GERQ-R score was above 16 and was above 10 after 1 week and did not decrease in 3/22 (14%) infants after 1 week in the “MC-AR Bed”. As a consequence, the “MC-AR bed” did not improve symptoms in 11/30 (37%) infants, including the 8 parents that stopped within 48 hours. The I-GERQ normalized in 8/22 (37%) infants, and in 11/22 (50%) there was a clinical meaningful drop of at least 5 points. The majority of the parents were very satisfied by the result of the intervention; also the physicians evaluated the intervention positively. The bed was used for a mean duration of 12.91 ± 3.06 hours (median 13; range 7–18 hours) per day during the first week. All pH monitoring parameters decreased significantly in the 50% of bed-using infants whose parents approved the follow-up monitoring: the total reflux index (% time pH was <4.0 in the esophagus), the reflux index during the periods the baby was awake and the number of long lasting reflux episodes. The mean duration of use of the “Multicare AR-Bed” was 3.2 months.

**Conclusions:** The 40° supine sleeping position reduces regurgitation, acid reflux and reflux-related infant’s distress.

**PG3-12**

**LONG-TERM EFFICACY AND SAFETY OF THE LOCAL APPLICATION OF MITOMYCIN C TO REFRACTORY ESOPHAGEAL STRICTURES IN CHILDREN**

**Presenter:** F. Gottrand. Department of Pediatrics; Reference Centre for Congenital and Malformative Oesophageal Diseases, Lille, France.

**Co-authors:** S. Coopman1, L. Michaud1, P. Fayoux2, A. Delattre2, R. Sfeir2, D. Turck1, F. Gottrand1. 1Department of Pediatrics, Reference Centre for Congenital and Malformative Oesophageal Diseases, Jeanne de Flandre
Background and Aim: Mitomycin C (MC) is an antiproliferative agent that has been used successfully as local treatment in ophthalmologic procedures to prevent scar formation, for laryngeal and tracheal stenosis, and more recently to prevent recurrence of caustic esophageal stricture in children. The aim of this study was to assess the efficacy and safety of the local application of MC to refractory esophageal strictures in children.

Methods: We performed a cross sectional study in 6 children (medium age: 7 years, range: 5–9.5) who received at least one local application of MC for refractory (that recurred at least 4 dilatation session) esophageal stricture (caustic stenosis n = 3, esophageal atresia n = 3). Clinical evaluation, radiological control and esophagoscopy with multilevel esophageal biopsies at the site of stenosis were performed.

Results: Mean follow-up after MC application was 4.3 years (range: 3.3–4.7 years). Frequency of dilatation decreased from 0.32 per child per year before MC application to 0.04 per child per year after the procedure (P < 0.05). Three patients needed a second application of MC (respectively, 15 days, 3 months and 13 months after the first one), while only one patient needed a third application (12 months after the first one). Digestive symptoms improved, especially concerning food impaction (P = 0.04). Only 2/6 patients still presented food impaction at the last follow-up. None of the 6 children presented esophageal stenosis at endoscopy or x-ray at last follow-up. Biopsy revealed gastric metaplasia at the site of stenosis in 2 cases. There was neither dysplasia nor Barrett’s esophagus at any sites of biopsies.

Conclusions: Our study shows that mitomycin C is beneficial several years after application in children presenting recurrent esophageal stenosis and that no complication was observed. However, the risk–benefit ratio of this treatment should be weighed considering possible long-term complication (especially neoplastic). Such patients require long-term follow-up.

PG3-13

DELAYED GASTRIC EMPTYING IN CHILDREN WITH POSTINFECTIONOUS FUNCTIONAL GASTROINTESTINAL DISORDERS (FGIDs)


Co-authors: P. Quitadamo¹, P. Masi¹, P. Coccorullo¹, A. Rocco², A. Staiano¹.¹Department of Pediatrics, University of Naples “Federico II,” Naples, Italy; ²Department of Gastroenterology, University of Naples “Federico II,” Naples, Italy.

Background and Aim: Infectious gastroenteritis is a significant risk factor for development of FGIDs. Postviral gastroparesis, described as delayed gastric emptying (GE) measured by scintigraphy, early satiety and postprandial emesis, has been reported in children. The 13C-Octanoic Acid Breath Test (13C-OABT) is an alternative noninvasive method to evaluate GE, using a natural nonradioactive isotope. The aims of this study were to evaluate: (1) the prevalence of postinfectious FGIDs (according to the Rome III criteria) in children after acute bacterial or viral gastroenteritis; (2) whether delayed GE, studied by 13C-OABT, could be predictive of postinfectious FGIDs development.

Methods: From April to December 2008, all children presenting with a positive stool culture were enrolled 2 months after the viral or bacterial acute gastroenteritis. Each exposed child was matched with a healthy age and sex matched control subject who came to the hospital for a well-child visit within 4 weeks from the index case. At time of enrolment, children over 10 years old and parents of children under 10 years of age filled out a standard questionnaire relating to gastrointestinal symptoms according to the Rome III criteria. 8 children underwent 13C-OABT after a solid standard meal.

Results: Eleven exposed children (M:F = 8:3, mean age 62 months, age range 43–118 months) and eleven healthy controls were recruited. Eight out of 11 patients had positive stool culture for salmonella (72.8%) and 3/11 (27.2%) for rotavirus. Two months after the acute gastroenteritis, 3 out of 11 exposed children (27.2%) fulfilled Rome III criteria for irritable bowel syndrome, 1 child (9%) for functional abdominal pain. Among the control group, no subject developed FGIDs within two months from enrolment. Seven out of 11 exposed children and 3/11 controls showed a delayed GE when evaluated by 13C-OABT (respectively, 63.6% vs 27.2%; P<0.09).

Conclusions: Our findings confirm that postinfectious FGIDs occur also in children. In addition, our data seem to indicate a strong relation between infectious gastroenteritis and delayed GE. Larger prospective studies are needed in order to propose the delayed GE as biological predictive marker of postinfectious FGIDs.

PG3-14

EFFECTIVE TREATMENT OF UNCONJUGATED HYPERBILIRUBINEMIA WITH THE LAXATIVE POLYETHYLENE GLYCOL IN GUNN RATS

Presenter: F. Cuperus. Pediatric Gastroenterology, Department of Pediatrics, Center for Liver, Digestive,
and Metabolic Diseases, University Medical Center Groningen, Groningen, The Netherlands.
Co-authors: A. Iemhoff, R. Havinga, H. Verkade, Pediatric Gastroenterology, Department of Pediatrics, Center for Liver, Digestive, and Metabolic Diseases, University Medical Center Groningen, Groningen, The Netherlands.

Background and Aim: Patients with Crigler-Najjar disease suffer from permanent, unconjugated hyperbilirubinemia and rely on daily phototherapy to prevent bilirubin-induced brain damage. Phototherapy, however, becomes less effective with age and has a profound impact on social life. Several clinical conditions that delay the gastrointestinal transit are associated with increased concentrations of plasma unconjugated bilirubin (UCB). We investigated whether direct pharmacological acceleration of the gastrointestinal transit decreases plasma UCB concentrations in hyperbilirubinemic Gunn rats, and thus could provide a novel treatment strategy for severe unconjugated hyperbilirubinemia.

Methods: Gunn rats received, for various time periods, oral polyethylene glycol 4000 (PEG) to accelerate the gastrointestinal transit, or oral loperamide to delay the gastrointestinal transit. Other Gunn rats were treated with phototherapy for 3 weeks, followed by phototherapy combined with oral PEG treatment for another 3 weeks. Gastrointestinal transit time and UCB concentrations in plasma and feces were determined at regular intervals.

Results: Within 36 hours, PEG administration accelerated the gastrointestinal transit by 45% and simultaneously decreased plasma UCB concentrations by 23% (each \( P < 0.001 \)). The decrease in plasma UCB concentrations coincided with an increase in fecal UCB excretion (+153%, \( P < 0.05 \)). PEG administration for 2 weeks accelerated the gastrointestinal transit by 36% (\( P < 0.001 \)) and resulted in a sustained decrease in plasma UCB concentrations (−41%, \( P < 0.001 \)). Loperamide administration for 1 week delayed the gastrointestinal transit by 57% and elevated plasma UCB concentrations by 30% (each \( P < 0.001 \)). Dose-response experiments showed a strong, positive correlation between the gastrointestinal transit time and plasma UCB concentrations (\( r = 0.87, P < 0.001 \)). The combination of PEG treatment and phototherapy decreased plasma UCB concentrations by 65% (\( P < 0.001 \)) and was more effective than phototherapy alone (\( P < 0.01 \)), which decreased plasma UCB concentrations by 47%; compared with baseline (\( P < 0.001 \)).

Conclusions: Acceleration of the gastrointestinal transit by PEG rapidly and sustainably decreases unconjugated hyperbilirubinemia in Gunn rats. The mechanism involves increased fecal UCB disposal. The combination of phototherapy with PEG was superior to either treatment alone. Present results show that pharmacological acceleration of the gastrointestinal transit time could be a feasible strategy to treat patients with severe unconjugated hyperbilirubinemia.

PG3-15

INCIDENCE, MANAGEMENT AND PROTECTIVE FACTORS OF INTUSSUSCEPTION IN CHILDREN: RESULTS FROM A NATIONWIDE GERMAN SURVEILLANCE

Presenter: J. Andreas. HELIOS Children’s Hospital Wuppertal, Wuppertal, Germany.
Co-authors: A. Jenke, A. Weckelmann, M. Zilbauer, U. Heininger, H. Trampisch, S. Wirth, HELIOS Children’s Hospital, Witten/Herdecke University, Wuppertal, Germany; Universitäts-Kinderklinik beider Basel, Basel, Switzerland; Ruhr-Universitäts Bochum, Bochum, Germany.

Background and Aim: Intussusception (IS) is a common pediatric gastroenterologic problem in children under 4 years of age. Nevertheless data regarding exact incidence, optimal conservative management and possible risk factors are rare. With respect to the newly introduced rotavirus vaccines these data are even more important.

Methods: In order to provide clear epidemiological and therapeutic data about IS a prospective multicentre nationwide surveillance was initiated through the “German surveillance unit of rare pediatric diseases” (ESPED) between Jan. 1, 2006 and Dec. 31, 2007. Data concerning cases of IS was obtained by a follow-up IS questionnaire that was filled by the reporting hospitals. To define IS the Brighton Collaboration Group (BCG) Criteria were applied. In addition an unbiased estimation of underreporting was obtained by a separate collection of all IS cases in a random sample of 40 clinics considering the ICD code in the clinic register and the medical record.

Results: During the surveillance period 1232 cases have been reported nationwide fulfilling the BCG Criteria for definite IS. 53.5% of the children were under 2 years of age. The incidence for the age group between 0 and 24 months was calculated to be 52.2/100,000 life birth/year after correction for underreporting. The most commonly used conservative treatment was reduction by ultrasound/ fluid based methods (54.8%), followed by contrast solution (27.1%) and air under x-ray control (10.9%). In terms of preventing secondary surgery reduction by air under x-ray control was significantly more effective than ultrasound/fuid (OR 2.02) or contrast solution/x-ray based methods (OR 3.34). The success of conservative treatment did not depend on the specialisation of the
PG3-16

EFFECT OF MEDIUM-CHAIN TRIGLYCERIDE-ENRICHED FORMULA ON GASTROESOPHAGEAL REFLUX AND GASTRIC EMPTYING TIME IN TERM INFANTS

Presenter: A. Schwarzer. Dr. von Hauners Children Hospital, Ludwig-Maximilian-University, Munich, Germany. Co-authors: S. Kritas1, L. Mccall2, S. Koletzko1, G. Davidson2, T. Omar2. 1Dr. von Hauners Children Hospital, Ludwig-Maximilian-University, Munich, Germany; 2Universite´ Paris Descartes, Faculte´ Necker, INSERM U793, Paris, France.

Background and Aim: The relation between delayed gastric emptying (GE) and increased gastroesophageal reflux (GER) in infants is unclear, nevertheless strategies that improve GE have been utilised in infants with GER disease. The aim of this study was to measure GER and GE in infants fed a medium chain triglyceride (MCT)-enriched formula which we hypothesized would empty more rapidly from the stomach than the standard long chain triglyceride (LCT)-containing infant formula.

Methods: Ten infants (6 male, age 1.6–7.7 months and weight 4.6–6.7 kg) referred for investigation of GER were enrolled. Using a randomized, double-blind, crossover study design, infants were fed an MCT-enriched formula (Caprilon, SHS International, MCT 75% of total fat) and a standard LCT containing formula (S-26 Newborn, Wyeth, MCT <10% of total fat) over two consecutive days. GER episodes were recorded using a pH-impedance probe which remained in place for 48 h and GE of both formulas measured using the 13C-Na octanoate breath test performed on the morning of each study day. Symptoms of cough, regurgitation and irritability/crying were recorded using event markers.

Results: The half GE time of standard infant formula was significantly faster than the MCT enriched formula median (IQR) GE1/2 38.1 min (21.3, 63.9) vs 71.9 min (41.3, 164.6) (P = 0.0002). Over the 24 h of formula administration, there were no significant differences in esophageal acid exposure (median % time pH <4.5% vs 5.7% for standard vs MCT enriched, respectively, P = 0.9), the frequency of bolus GER episodes (median 32 vs 31 acid GER, P = 0.5 and 44 vs 51 weakly acidic GER, P = 0.2) or the frequency of symptom episodes (median 20 vs 23 cough, P = 0.7, 8 vs 8 regurgitation, P = 1.0 and 32 vs 46 crying, P = 0.1).

Conclusions: Contrary to our hypothesis, GE of MCT enriched formula was significantly slower than standard LCT containing infant formula suggesting that components, other than the MCT content, may have a profound influence on mechanisms that regulate GE. Despite the marked difference in GE, no change in the rate of GER or typical GER symptoms was observed. This suggests that feed content-based modulation of GE has little impact on GER and it is therefore unlikely to produce a therapeutic benefit in infants with GER disease.

PG3-17

MICROVILLUS INCLUSION DISEASE RESULTS FROM LOSS OF MYOSIN VB FUNCTION: A CELLULAR DISEASE MODEL

Presenter: T. Muller (1). Innsbruck Medical University, Innsbruck, Austria. Co-authors: F. Ruemmele2, N. Schiefermeier3, H. Ebner4, S. Lechner5, K. Pfaller2, P. Heinz-Erian1, F. Lacaille6, O. Goulet2, M. Hess1, A. Janecke5, L. Huber7. 1Department of Pediatrics I, Innsbruck Medical University, Innsbruck, Austria; 2Universite´ Paris Descartes, Faculte´ Necker, INSERM U793, Paris, France; 3Division of Histology and Embryology, Innsbruck Medical University, Innsbruck, Austria; 4Division of Cell Biology, Innsbruck Medical University, Innsbruck, Austria; 5Division of Histology and Embryology, Innsbruck Medical University, Innsbruck, Austria; 6Division of Clinical Genetics, Innsbruck Medical University, Innsbruck, Austria; 7Assistance Publique-Hopitaux de Paris, Necker-Enfants Malades Hospital, Paris, France.

Background and Aim: Autosomal recessive microvillus inclusion disease (MVID) is characterized by an intractable diarrhea starting within the first few weeks of life, a lack of microvilli on the surface of mature enterocytes and occurrence of intracellular vacuoles lined by microvilli (microvillus inclusions). Very recently, it has been shown that mutations in MYOSB, encoding the uncon-
ventional type Vb myosin motor protein, caused MVID in a cohort of patients with early-onset MVID. MYO5B mutations were associated with disrupted epithelial cell polarity implicating MYO5B in the regulation of intracellular protein trafficking. To independently validate the significance of MYO5B in the pathogenesis of MVID, we investigated whether siRNA-mediated knockdown of MYO5B in CaCo-2 intestinal epithelial cells mimics the cellular phenotype of MVID.

**Methods:** Immunofluorescence, Western blotting and immunoelectron microscopy were applied to analyze the effects of MYO5B siRNA knock-down in Caco-2 intestinal epithelial cells.

**Results:** Characteristic microvillus inclusions as well as shortening, erosion and deformation of cell surface microvilli were induced in myosin Vb-silenced Caco-2 cells.

**Conclusions:** MYO5B knock-down recapitulates most of the cellular phenotype in vitro, thus independently showing loss of MYO5B function as the cause of MVID. MYO5B may mobilize recycling endosomes and apical proteins necessary for brush border maintenance.

**PG3-18**

**HISTAMINE INTOLERANCE IN PEDIATRIC GASTROENTEROLOGICAL PRACTICE—DIAGNOSIS, OCCURRENCE, AND RESPONSE TO HISTAMINE-FREE DIET**

**Presenter:** K. Hoffmann. *Medical University Graz, Graz, Austria.*

**Co-authors:** E. Gruber1, J. Jahnel1, A. Deutschmann1, A. Hauer1. 1University Clinic of Paediatrics and Adolescent Medicine, Department of General Paediatrics, Medical University Graz, Graz, Austria.

**Background and Aim:** The growing demand by parents to have children tested for food intolerances places an increasing burden on gastroenterological outpatient clinics. Patients with histamine intolerance (HIT) develop symptoms after ingesting foods with high histamine content. Gastrointestinal (GI) symptoms include abdominal pain and diarrhea. Little is known about the role of HIT in children presenting with abdominal pain.

**Methods:** Children with abdominal pain without alarm symptoms for organic disease were included. HIT was suspected when symptoms were linked to ingestion of histamine-rich foods at initial presentation. Plasma diaminoxidase (DAO) and histamine levels as well as urine histamine levels were measured. If low DAO levels were found (<10 U/mL) patients were instructed how to avoid histamine-rich foods by a nutritionist. Parents and patients were advised to adhere to the diet for at least four weeks. The effect of the diet was evaluated using a simple symptom score (0–10, ranging from no symptoms to maximal symptoms). Double-blinded placebo controlled (DBPC) histamine challenge was performed in a subset of patients.

**Results:**Within 26 months we measured low DAO levels in 31/394 (8%) children who initially presented with abdominal pain (median 8 [7–19] years, 55% boys). 22/31 (71%) children had elevated plasma histamine levels (median 0.19 [0.1–2.75] μg/mL) and 14/31 (45%) had elevated urine histamine levels (median 33.9 [6.5–634] μg/mL). DAO levels did not correlate with serum or urine histamine levels (r2 0.38 and 0.28, n.s.). In the final survey 2/31 patients could not be reached, 5 did not adhere to the diet, and in 8 patients other explanations for abdominal pain were found (lactose-, fructose malabsorption, chronic appendicitis, dysmenorrhea). 16/31 children adhered to the histamine-free diet. 14 of these 16 patients reported marked improvement of their abdominal symptoms. Mean symptom scores of the 16 patients were 8.2 ± 0.3 and 2.5 ± 0.7 before and after histamine-free diet, respectively (P < 0.001, paired t test). In a subset of patients the diagnosis was further confirmed by DBPC histamine challenge.

**Conclusions:** In approximately 4% of children with abdominal pain HIT could be diagnosed. In these patients histamine-free diet led to a significant improvement of symptoms. Low DAO levels alone were not diagnostic for HIT since only half of these patients profited from histamine-free diet. In our patients plasma and urine histamine levels did not contribute to the diagnosis. HIT can present with GI symptoms in children. A histamine-free diet seems to be most helpful for diagnosis.

**PG3-19**

**MUTATIONS IN SPINT2 CAUSE A SYNDROMIC FORM OF CONGENITAL SODIUM DIARRHEA**

**Presenter:** P. Heinz-Erian. *Medical University, Innsbruck, Austria.*

**Co-authors:** T. Mueller1, M. Vujic2, M. Krantz2, I. Booth3, C. Holmberg3, C. Wijmenga4, G. Grigenioiene5,6, C. Kneepkens7, V. Siur8, S. Rosipal9, M. Mistrik10, M. Kappler11, F. Gottrand12, H. Zoller13, A. Janecke14, 1Dept Pediatrics, Innsbruck, Austria; 2Dept Clinical Genetics, Gothenburg, Sweden; 3Dept Pediatrics, Gothenburg, Sweden; 4Dept Pediatric Gastroenterology, Birmingham, UK; 5Dept Pediatrics, Helsinki, Finland; 6Dept Genetics, Groningen, The Netherlands; 7Dept Pediatrics, Karolinska Institute, Stockholm, Sweden; 8Dept Pediatrics, Amsterdam, The Netherlands; 9Childrens Hospital, London, Canada; 10Pediatric Center, Cardiovascular Medicine, Poprad-Velka, Slovakia; 11Dept Medical Genetics, Spisska Nova Ves, Slovakia.

Background: Autosomal recessively inherited congenital sodium diarrhea (CSD) is characterized by perinatal onset of persistent watery diarrhea with high fecal sodium excretion. Defective sodium/proton exchange has been reported in a subset of cases but the molecular basis of the disease has not been elucidated.

Methods: Data from a first large series of patients (n=24) with a clinical diagnosis of CSD congenital diarrhea (fecal osmolality <320 mosm/kg, fecal Na >70 mmol/L, plasma Na <130 mmol/L, urinary Na <20 mmol/L, plasma pH <7.30, fecal pH >7.5, necessary Na replacement >6 mmol/kg/24 hr, and exclusion of specific light and/or electron microscopic alterations of epithelial cell morphology indicating microvillus inclusion disease or tufting enteropathy) were reviewed. The positional candidate approach using SNP arrays was applied to identify a CSD gene in 1 extended kindred. Mutation analysis was performed in further 16 CSD families. We assayed the observed mutations in vitro to assess their functional consequences.

Results: Two forms of CSD were distinguished clinically and genetically: (1) syndromic CSD (16 patients) with a variety of associated malformations/dysmorphological features, and (2) classic CSD (8 patients). In the first group a total of 5 distinct, splicing and missense mutations were identified in SPINT2, encoding a Kunitz-type serine protease inhibitor, in all available patient samples. No mutations were found in the second group. SPINT2 mutations were associated with loss of protein synthesis or failure to inhibit the serine protease trypsin.

Conclusions: We have delineated CSD associated with congenital malformation/dysmorphisms as a distinct disease entity caused by SPINT2 loss-of-function mutations. SPINT2 mutations might lead to an excess of yet unknown serine protease activity in CSD enterocytes.

PG3-20

CYCLIC VOMITING SYNDROME (CVS): 10-YEAR EXPERIENCE IN A REFERRAL CENTER

Presenter: S. Martinazzi. Children’s Hospital, Brescia, Italy.

Co-authors: F. Gottardi1, C. Monfredini1, F. Andreoli1, A. Ravelli1, A. Ravelli2. 2CISP-International Committee for People’s Development, Rome, Italy.

Background and Aim: CVS is a functional GI disorder characterized by recurrent attacks of unremitting nausea and vomiting separated by periods of well being. Our aim was to describe the features of CVS in a population of pediatric patients referred to our center, thereby helping gastroenterologists to recognize this condition.

Methods: Retrospective analysis of all our patients diagnosed as having CVS according to Rome criteria between 1997 and 2008.

Results: CVS was diagnosed in 86 patients. In 6/86 an organic disorder (2 Helicobacter pylori gastritis, 2-eosinophilic esophagitis, 1 uretero-pelvic junction obstruction, 1 brain tumor) was found, whereas known gastrointestinal, neurological, urinary and metabolic abnormalities were excluded by appropriate investigation in 80/86. F:M ratio was 1:2.1 and age of onset was 2–6 years in 34% and 6–10 years 26%. Attacks occurred at between 4 and 10 a.m. in 53% of patients. Vomiting was severe, with a mean 7.2 emeses/hour and 28 emeses/day, and was usually associated with nausea (73%) and profound lethargy (58%). Other GI symptoms such as abdominal pain (52%) and diarrhea (19%) were commonly reported, as were migraine-like symptoms such as headache (40%) and photophobia (27%). Several patients showed autonomic arousal, i.e., pallor (65%), hypersalivation (27%), fever (18%) and tachycardia (13%). Prodromic symptoms were reported by 84% of patients. On average, episodes lasted 39 hours, recurring 10 times per year and were separated by symptoms-free intervals of 3.5 months. Hospitalization was usually required in most patients. Triggers were identified in 40% of cases, as positive or negative stress (60%), intercurrent infection (26%), or menses (14%). Forty-five percent of patients had a positive family history of migraine, usually along the matrilineage. Erosive esophagitis was reported in 21% and Mallory-Weiss tear in 3.5% patients. Diagnosis of CVS was made >3 years after the onset of symptoms in 50% of the patients, and after 1–3 years in 33% of them. Clinical improvement or complete symptom remission were achieved, respectively, in 53% and 22% of patients, without significant side effects, using prophylactic drugs such as cyproheptadine, pizotifen and amitryptiline.

Conclusions: CVS mostly affects preschool and school-age children, and a thorough clinical assessment is needed to rule out possible albeit uncommon underlying organic disorders. Although it has a rather severe and fairly distinctive pattern of occurrence, CVS is still largely unknown and the diagnosis is considerably delayed in most cases. An appropriate prophylactic therapy using antimigraine drugs can achieve long-term remission or significant clinical improvement in the majority of patients.

PG3-21

ENTEROPATHY—A NEW FINDING IN CYSTIC FIBROSIS

Presenter: M. Wilschanski. Hadassah Medical Organization, Jerusalem, Israel.
Background and Aim: Treatment with pancreatic enzymes fails to completely correct malabsorption and gastrointestinal symptoms in cystic fibrosis (CF) patients. The aim of this study was to examine the small intestine of CF patients without overt evidence of gastrointestinal disease using capsule endoscopy (CE).

Methods: Patients with CF without history of previous intestinal surgery, meconium ileus as neonates or distal intestinal obstruction syndrome received the agile patency capsule and, depending on the result of that procedure, then underwent standard CE using the PillCam SB capsule (Given Imaging, Yoqneam, Israel). A stool specimen was taken on the same day as the CE for determination of the calprotectin level.

Results: 42 CF patients aged 10–36 years were included. 30 had pancreatic insufficiency. One patient failed to excrete the patency capsule after 36 hours and was withdrawn from the study. Pulmonary function was mild to moderate with FEV1 68.5 ± 16% predicted. Review of the CE videos showed that most patients had varying degrees of diffuse areas of inflammatory findings in the small bowel including edema, erythema, mucosal breaks and frank ulcerations. There were no adverse events. Fecal calprotectin levels were markedly high with a mean of 253 ± 97 μg/g (normal <50) in the PI patients but normal in the PS patients.

Conclusions: Small bowel mucosal pathology may be detected at CE in most CF patients. The high fecal calprotectin levels found are suggestive of mucosal inflammation which may correlate with the CE findings. Further study is required to examine the possible relationship of these mucosal lesions which may be part of a newly identified enteropathy associated with CF, with the persistent intestinal malabsorption and abdominal pain in many of these patients.

PG3-22

GUT INFLAMMATION IN CHILDREN WITH JUVENILE IDIOPATHIC ARTHRITIS

on immunosuppressive therapy. Our data did not confirm a direct correlation between autoantibodies and specific mucosal disease; however, a larger study might be needed to address this with particular emphasis on gut autoantibodies.

PG3-23

HOW TO DEVELOP YOUR MANAGED CLINICAL NETWORK USING TELEMEDICINE. THE SCOTTISH PAEDIATRIC GASTROENTEROLOGY, HEPATOLOGY AND NUTRITION GROUP (SPGHANG) EXPERIENCE

Presenter: A. Barclay. University of Glasgow, Glasgow, UK.

Co-authors: P. Gillett1, D. Goudie2, W. Bissett3, P. McGrogan4, 1Department of Paediatric Gastroenterology, Royal Hospital for Sick Children, Edinburgh, UK; 2Department of Paediatrics, Raigmore Hospital, Inverness, UK; 3Department of Paediatric Gastroenterology, Royal Aberdeen Childrens Hospital, Aberdeen, UK; 4Department of Paediatric Gastroenterology, Royal Hospital for Sick Children, Yorkhill, Glasgow, UK.

Background and Aim: Emphasis has been placed on the development of Regional and National MCN working models for Paediatric Gastroenterology, Hepatology and nutrition (PGHAN) in the UK. The geographic distribution of paediatric services within Scotland limits the ability of such groups to develop effective working relationships for MCN development. In 2005 SPGHANG piloted the introduction of bi-monthly telemedicine education sessions to help address these issues. We assess the development of the SPGHANG telemedicine sessions over the first 3 years, with reference to increasing numbers in the group, formalising educational value and expanding the remit of sessions.

Methods: Initial case discussions between physicians in the three lead centres were redesigned in 2006 with the introduction of multidisciplinary presentations and encouragement for presentation from the district general hospital setting. Formal accreditation was introduced by harmonising sessions with RCPCP guidelines on CPD with virtual CPD registration forms, central storage of attendance and session details. Session format was expanded to include, journal club, original research, guideline development and discussion of national planning documentation. The quality of sessions was improved with the development of a code of conduct. Appraisal was measured by number of centres participating in sessions, a review of the session diary, SPGHANG membership, annual feedback forms and attendance at the SPGHANG annual winter meeting. Time expended was estimated from work diary review. Costs were calculated on NHS Greater Glasgow SpR 5 pay-scale (£23.06/€24.50/h).

Results: SPGHANG telemedicine has expanded its network to include 11 participating paediatric centres with over 50 health professionals. The majority of annual feedback rated sessions as either good or very good. It has been requested that sessions be increased from bimonthly to 12 times per year. Responders also suggested “real-time” discussion of problem cases or the teaching of practical skills such as accessing central venous catheters or “virtual” endoscopy. Sessions have successfully led to the implementation of local guidelines, as well as developing regional guidance for intestinal failure. Attendance at the SPGHANG annual winter meeting has increased by greater than 100% from 2005 to 2007. Total organisational time was 16 h annually at a cost of £368.96 (€392.50).

Conclusions: The use of telemedicine has developed a high quality regular accreditable CPD for professionals who would otherwise have to travel excessive distances to receive this. Additional benefits of to service development include development of MCN practice and harmonisation of SPGHANG’s aspirations for future service development. Telemedicine has helped to cultivate relationships between allied health professionals and forge a group identity for SPGHANG. Future planners of PGHAN services should note the cost effectiveness of tele-education, but should budget time and costs into proposed network developments.

PG3-24

ASSESSMENT OF THE INTEREST OF GRAVITY SCORE IN ACUTE PANCREATITIS OF CHILDREN

Presenter: A. Fabre. APHM, Marseille, France.

Co-authors: P. Petit1, J. Gaudart1, E. Mas2, J. Vial2, J. Olives2, J. Sarles1. 1APHM, Marseille, France; 2Hopital des Enfants, Toulouse, France.

Aim: To assess the severity of acute pancreatitis, several scores are currently used for the adult population, but such a tool is lacking for paediatric patients.

Methods: We retrospectively collected data from children with acute pancreatitis admitted between January 2003 and December 2007 in two French hospitals. We evaluated the severity according to the criteria of the Atlanta symposium. For each patient we calculated the score of Ranson, Glasgow modified, DeBanto and Balthazar. The statistical analysis was performed with Wilcoxon and Fisher tests and receiver operator curve.

Results: We have collected data from 48 patients (23 boys and 25 girls), median age of 133 months (24.5–233.5). The main causes were: traumatic (23%), idiopathic...
Computed tomography is yet to be evaluated. Informations with an easier use. The place of the communications was considered as mild cases. No death occurred in either group. The two groups were similar for age, weight, and the number of collected parameters (Ranson, Glasgow modified, DeBanto). The area under the ROC curve was 0.699 (IC 0.508–0.891, $P = 0.054$) for the DeBanto score, 0.846 (IC 0.69–1, $P = 0.001$) for the Ranson score, and 0.774 (IC 0.584–0.964, $P = 0.08$) for the Glasgow score. The sensitivity was, respectively, 61.54%, 53.85% and 53.8% for Ranson, Glasgow and DeBanto and specificity 88.57%, 91.18% and 80% (but with a threshold of 2). CT scan images of 17 patients were graduated according to Balthazar’s CTSI score. The area under the ROC curve was 0.898 (CI 0.73–1, $P = 0.011$) and for a threshold of 4 sensitivity was 80% and specificity 85.71%. We evaluated a simplified score of 4 blood parameters: potassium, urea and glucose levels on admission and calcium level 24 hours afterwards. In this case the ROC curve was 0.719 (IC 0.519–0.920, $P = 0.034$). This score has not yet been validated.

Conclusions: Either the Ranson score or Glasgow modified score discriminated between mild and severe pancreatitis with a good specificity but a low sensitivity. The use of a paediatric score provides no supplementary informations with an easier use. The place of the computed tomography is yet to be evaluated.

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**Poster Session 1**

**Hepatology**

**PH1-01**

**10 YEARS’ FOLLOW-UP OF PORTAL VEIN THROMBOSIS IN CHILDREN MANAGED CONSERVATIVELY**

Presenter: L. D’antiga, University of Padova, Padova, Italy.

Co-authors: L. Riello 1, C. Chillemi 1, S. Riva 2, M. Sciveres 2, M. Spada 2, L. Zancan 1, 1Paediatric Dept, Padova, Italy; 2ISMETT, Palermo, Italy.

**Background and Aim:** Noncirrhotic portal vein thrombosis (PVT) is one of the main causes of portal hypertension in children. Bleeding episodes are the most dangerous complication. The management can be conservative or surgical but since the long-term prognosis of PVT is still not well defined, there are controversies regarding the indications to choose a surgical approach or to manage it conservatively. We aimed to describe the clinical course and outcome of children followed at 2 Italian centres during the last decade.

**Methods:** We reviewed the notes of 31 patients presenting over the last 13 years. We collected data on blood count, liver function tests (AST, ALT, total bilirubin, INR), endoscopic description of esophageal varices and bleeding episodes at diagnosis and at 1, 2, 5, 10 years’ follow up. After diagnosis all children were followed yearly by upper GI endoscopy and started on beta-blockers in case of development of varices at risk of bleeding (grade 3 or grade 2 with red halo), aiming at a decrease of 20% of the heart rate. Endoscopic treatment of varices (sclerotherapy or banding ligation) was performed in case of failed response to beta-blockers or after a bleeding episode. Varices eradication was performed in three sessions carried out approximately 20 days apart.

**Results:** We observed a progressive development of thrombocytopenia, leukopenia and coagulopathy. Varices at risk of bleeding tend to disappear. Half of patients have no bleeding episodes during the follow-up. Nevertheless one third of patients experiences repeated episodes of bleeding refractory to varices treatment. Further effort should be made to find predictors of severe and repeated bleeding in this subgroup of patients for whom a surgical approach might be beneficial.

**PH1-02**

**THROMBOPHILIC GENETIC FACTORS: ARE THEY PREDISPOSING TO PORTAL VEIN THROMBOSIS IN CHILDREN?**

Presenter: P. Vajro, Università degli Studi di Napoli Federico II, Napoli, Italy.

Co-authors: M. Caropreso 1, R. Campanile 3, S. Maddaluno 1, C. Veropalumbo 1, C. Piscopo 2, D. Nicolina 1, I. Raffaele 1, C. Giuseppe 2, I. Achille 1, Dipartimento di Pediatrica Università di Napoli Federico II, Napoli, Italy; 2CEINGE Università di Napoli Federico II, Napoli, Italy; 3Chirurgia Pediatrica AORN Santobono, Napoli, Italy.
Background and Aim: The most common single cause of portal hypertension in children is portal vein thrombosis (PVT). It is associated with a history of umbilical venous catheterization (UVC) in almost half of the cases whereas in the remaining cases it is idiopathic. Because the role of genetic prothrombotic risk factors in pediatric PVT is still controversial we studied mutations of Factor V Leiden (FVL), Prothrombin (PTHR) and V617F Janus kinase 2 (JAK2) in all PVT patients. DNA was extracted from peripheral blood leukocytes. C677T methylentetrahydrofolate reductase (MTHFR) mutation was evaluated only in subjects with hyperhomocysteinemia.

Results: Twelve of 19 patients (63.1%) underwent UVC in the neonatal period. None had family history of venous thromboembolic events. Heterozygous G1691A FVL mutation and G20210A PTHR mutation were found in 2/19 (10.5%) and 2/19 (10.5%) patients, respectively. None carried JAK2 V617F mutation, while only one (5.2%) hyperhomocysteinemic patient presented a homozygous F677T MTHR mutation. Four (1 FVL; 2 PTHR; 1 MTHFR) out of 12 (33.3%) patients with PVT and history of UVC and only one (FVL) out of 7 patients (14.3%) with idiopathic PVT had a genetic thrombophilic risk.

Conclusions: A congenital prothrombotic condition observed in 26% of our children suggests that thrombophilic mutations may be involved in pediatric PVT. Preexisting stress of portal vein by catheterism might facilitate establishment of PVT in genetically predisposed individuals.

Background: In small children with intestinal insufficiency, liver disease may develop early and rapidly, impairing the use or adaptation of the small bowel, thus precipitating the child toward early liver and/or small bowel transplantation (L-SBT). We report here how a child sent for emergency L-SBT was weaned off parenteral nutrition (PN) without L-SBT.

Methods: This girl was born preterm with gastroschisis. Surgery was performed on first day without resection. Oral and enteral nutrition were impossible, due to dysmotility, sepsis episodes, and gastroesophageal reflux (GOR). Ileostomy was performed, in order to reduce bacterial translocation. She was totally PN-dependent, and developed cirrhosis with jaundice (bilirubin 160 µM), liver insufficiency (clotting factors 45%), and stomal bleeding. She was sent to us for evaluation for L-SBT at 16 months of age. Height was 68 cm, weight 7.5 kg (-3 SD). A Transjugular Intrahepatic Porto-Systemic Shunt (TIPSS) was performed. Bleeding stopped immediately; jaundice resolved in 2 months, liver function was normal in 4 months. The GOR was bypassed using a gastrojejunal tube feeding in association with baclofen. Enteral nutrition could be increased up to weaning of PN. The ileostomy was closed and the child sent home at the age of 2. A catch-up growth could be observed.

Results: In this child, the absorptive function of the small bowel was considered as sufficient if not impaired by portal hypertension. The TIPSS was not an aggressive procedure in this fragile baby, and allowed to break the vicious cycle of PN-dependence and worsening of liver function. It is deemed technically difficult in small children, and was here performed by an experienced pediatric radiologist.

Conclusions: This technique should be developed in large centers that care for small children with intestinal insufficiency and liver failure. In selected children, it could avoid the need for L-SBT at all, or postpone it by relieving severe symptoms of portal hypertension.
Background and Aim: IL-1F7b is a novel homologue of the IL-1 cytokine family discovered by computational cloning. We demonstrated that IL-1F7b shares critical amino acid residues with IL-18 and binds to the IL-18-binding protein enhancing its ability to inhibit IL-18-induced interferon-γ. IL-1F7b was also shown to bind to the IL-18Ra; however, no IL-18Ra-dependent agonistic or antagonistic function was discovered. We recently reported that after LPS-stimulation IL-1F7b translocates to the nucleus and reduces the expression of proinflammatory cytokines. The aim of this study was to investigate the role of IL-1F7b in ConA-induced hepatitis as an IL-18-dependent inflammatory liver disease.

Methods: Human IL-1F7b cDNA was cloned into the expression plasmid pTarget which contains a constitutively active CMV-promotor. 6 weeks old female C57BL/6J mice were rapidly injected with either 20 μg of empty pTarget or pTarget-IL-1F7b in 2 mL of Ringer’s solution into the tail vein (“hydrodynamic injection”). The plasmid pLuc was co-injected at a ratio of 1:20 for in vivo transfection control. After 48 h the mice were injected with ConA 20 mg into the tail vein to induce hepatitis. 2 h after ConA-injection a blood sample was taken for cytokine measurement. 24 hrs after ConA-injection the mice were anesthetized and injected with luciferin to monitor in vivo luciferase-activity for transfection control. Another blood sample was obtained and the mice were sacrificed. The liver was stored for histology and cytokine analysis.

Results: DNA-injected mice expressed high levels of luciferase-activity predominantly in the liver. Transgene IL-1F7b was detected in the liver lysate of mice injected with IL-1F7b plasmid by western blotting. 2 h after ConA-injection significantly reduced serum levels for IL-1α, IL-6, IL-5 and IL-9 were measured in IL-1F7b-expressing mice. IL-6 (P = 0.009) was reduced in the liver lysate 24 hrs after ConA-injection. All mice developed severe acute hepatitis as shown by histology. However, no difference in the histological score was observed between the two groups. In parallel, no difference in serum ALT was detected.

Conclusions: In vivo expression of human IL-1F7b in mice reduces local and systemic inflammation in ConA-induced hepatitis. This observation supports the in vitro-generated hypothesis of IL-1F7b acting as an anti-inflammatory cytokine. Patchy expression of IL-1F7b-protein after tail vein injection might explain the lack of reduced histological severity score despite reduced IL-6 in within the liver.

PH1-05

STUDY OF THE USE OF ICG FOR RAPID ASSESSMENT OF ISOLATED HUMAN HEPATOCYTE FUNCTION

Presenter: D. Ho. King’s College Hospital, London, UK.
Co-authors: A. Dhawan1, R. Hughes1, S. Lehec1, J. Pappl1, C. Phillipeos1, P. Lee2, R. Mitry1. Institute of Liver Studies, King’s College Hospital, London, UK; 2Department of Surgery, National Taiwan University Hospital, Taipei, Taiwan.

Background and Aim: Hepatocyte transplantation is a promising alternative to liver transplantation in children with liver metabolic disorders and acute liver failure. Currently, no rapid assays are available to assess the function of fresh hepatocytes prior to transplantation into the patient. The aim of this study was to investigate whether the uptake and release of indocyanine green (ICG) by hepatocytes could be used as a test of hepatocyte function.

Methods: Human hepatocytes (1 × 10⁶ cells) isolated from unused donor livers were incubated at 37°C for 30 min with ICG (0–2 mg/mL) in William’s medium E containing 10% FCS, prednisolone, insulin and t-glutamine in both cell suspension and culture on collagen-coated plates. Cells were then incubated in medium without ICG for 3 h. The supernatants were collected at 1, 2, and 3 h for measurement of ICG release using a plate reader (OD 820 nm). Viability of cells was determined by trypan blue exclusion. MTT (mitochondrial activity) and SRB (cell attachment) assays were carried out at 18 h after incubation with ICG. Taurine (20 mM) was added to the hepatocyte suspensions in some experiments.

Results: ICG was taken up and secreted by hepatocytes. In hepatocytes incubated with concentrations of ICG above 1.0 mg/mL, ICG had a detrimental effect on hepatocytes with cell death observed. There was a slight increase in mitochondrial activity (MTT OD readings: 0.053 vs 0.045) and cell attachment (SRB OD readings: 0.67 vs 0.59) for plated hepatocytes compared to controls after 18 h incubation post-ICG uptake. ICG release peaked at 1–2 h in both cell suspension and in culture and then declined slightly at 3 h. The pattern of ICG release appeared to be related to cell viability. Higher ICG concentrations caused more detachment of plated cells. Addition of taurine to plated hepatocytes gave better release of ICG and helped hepatocytes attach better compared to controls at all ICG concentrations (SRB OD readings 1.360 ± 0.083 vs. 0.908 ± 0.159, P = 0.011 at 1.0 mg/mL).

Conclusions: Further refinement of this ICG test is needed in order to develop a rapid assay for assessment of isolated human hepatocyte function.
Background and Aim: Primary sclerosing cholangitis (PSC) is a chronic, fibrosing liver disease of unknown etiology; a range of immune abnormalities including lymphocytic portal tract infiltration are suggestive of an immune-mediated pathogenesis. Furthermore, associations with inflammatory bowel disease suggest a systemic immune alteration. Our objective was to characterize lymphocyte subpopulation in different tissues in a mouse model of sclerosing cholangitis.

Methods:
- **Multidrug resistance gene (Mdr2) (Abcb4) knockout mice (Mdr2<sup>-/-</sup>) and wild-type controls (Mdr2<sup>+/+</sup>) were compared at 6 and 26 weeks of age.**
- By detection of specific CD antigens we analyzed following types of lymphocytes by 3-color flow cytometry (FACS) in peripheral blood (PB), bone marrow (BM), liver and spleen: leucocytes (CD45+), B cells (CD19+), T cells (CD3+), T helper cells (CD3+/4+), T suppressor cells (CD3+/8+), and NK cells (CD3+/90+).

Results:
- At the age of 6 weeks, Mdr2<sup>-/-</sup> had reduced amounts of T cells in PB (48 vs 53% in Mdr2<sup>+/+</sup>; all P < 0.05) mainly due to reduced T helper cells (35 vs 38% of all lymphocytes in Mdr2<sup>+/+</sup>). In contrast to PB, the T cell count in liver was higher in Mdr2<sup>-/-</sup> (6 vs 4% of all liver cells in Mdr2<sup>+/+</sup>). However, at the age of 26 weeks, T helper cells massively increased in Mdr2<sup>-/-</sup> (13 vs 1% at 6 weeks) and to a lesser extent in Mdr2<sup>-/-</sup> (7 vs 2% at 6 weeks). In this period the number of T suppressor cells in the liver increased more in Mdr2<sup>-/-</sup> (1% at week 6 and 4% in week 26) than in Mdr2<sup>-/-</sup> (1 and 2%; P < 0.05). No differences in T cell number between the two genotypes in BM and spleen were observed. In all investigated tissues – except in the spleen – higher levels of B cells in Mdr2<sup>-/-</sup> were observed at week 6 (PB 21 vs 15%; BM 12 vs 8%; liver 5 vs 2%; spleen 40% both). NK cells were in all probes below a 0.5% level.

Conclusions: Alterations in immune cell composition in advanced liver disease in Mdr2<sup>-/-</sup> may be useful for examining the role of T cell subpopulations in the progression of chronic cholestatic diseases such as sclerosing cholangitis.

**PHI-07**

Human hepatocyte Labelling with 99mTc-GSA, a Potential Non-invasive Technique for Tracking Transplanted Cells

Background and Aim: The fate of hepatocytes after transplantation in children with liver-based metabolic disorders is not well studied. The aim of the study was to investigate the possibility of labelling human hepatocytes in vitro using 99m<sup>Tc</sup>-technetium-labelled galactosylserum albumin (99m<sup>Tc</sup>-GSA), a clinically used liver scanning agent, and to evaluate its effects on cell function and metabolic activity.

Methods: Human hepatocytes were isolated from liver resections using collagenase perfusion. The asialoglycoprotein receptor which binds 99m<sup>Tc</sup>-GSA for uptake into the cell was characterised using immunohistochemistry and RT-PCR. Freeze-dried GSA (Nihon Medi-Physics Co, Ltd, Japan) was radiolabelled with 99m<sup>Tc</sup> (30MBq/mg GSA). Hepatocytes were incubated in suspension in culture medium containing 99m<sup>Tc</sup>-GSA or culture medium alone (control) for 30 minutes at either 4°C or 37°C. Viability was assessed using trypan blue. Immediately after cell labelling, hepatocytes were incubated at 37°C, and release of 99m<sup>Tc</sup>-GSA into the medium was measured using a gamma counter at 0, 10, 20, 40, 60 and 120 min. Labelled cells were cultured overnight at 37°C in 95% O<sub>2</sub>:5% CO<sub>2</sub> and overall metabolic activity was assessed using MTS and cell attachment with sulphorhodamine B (SRB) assays.

Results: The isolated human hepatocytes were positive for the asialoglycoprotein receptor using both immunohistochemistry and RT-PCR. Labelling of hepatocytes with 99m<sup>Tc</sup>-GSA at 4°C reduced cell viability (mean difference with control in viability 1.50%, ±SE 2.47%, P < 0.05), which was not seen at 37°C (mean difference in viability 1.61%, ±SE 4.27%). Cell metabolic activity and attach-
ment were not significantly affected by the labelling process, with mean change in MTS postlabelling (4°C 7.85% ± SE 5.73; 37°C 10.17% ± SE 4.37) and change in SRB postlabelling (4°C −1.80% ± SE 7.52; 37°C 9.47% ± SE 18.37). Incorporation of 99mTc-GSA into hepatocytes was significantly higher at 37°C compared to 4°C (mean increase 29.63% ± SE2.86, P < 0.05). The time course experiments on 99mTc-GSA release during incubation at 37°C did not show any significant differences between cells labelled at 4°C and 37°C over 120 min. The mean release of radioisotope after two hours was 17.77% ± SE 0.21 at 4°C and 14.66% ± SE 0.73 at 37°C, respectively.

Conclusions: Cell labelling with 99mTc-GSA at 37°C did not significantly affect cell viability or metabolic function and gave a higher incorporation of radioactive ligand. Time course studies suggest that 99mTc-GSA may have potential for detection beyond 2 hours posttransplantation. Scintigraphy of human hepatocytes labelled with 99mTc-GSA could possibly be used to track cells and monitor engraftment after hepatocyte transplantation.

PHI-08

MECHANISMS OF HEPATOCELLULAR DAMAGE WITH RELATION TO CLINICAL PRESENTATION OF WILSON’S DISEASE

Presenter: A. Mustafa. King’s College Hospital, London, UK.

Co-authors: R. Mitry1, M. Hussain1, A. Dhawan1.
1King’s College Hospital, London, UK.

Background and Aim: Wilson’s disease (WD) a disorder of copper metabolism, has variable presentations. Recently in animal models antiapoptotic agents have been proposed to be of benefit. However, the mode of cell death in various presentations is not well defined. The aim is to study the mechanisms of hepatocyte death, in children with WD with different clinical presentations; acute liver failure (ALF), chronic liver disease (CLD) or those diagnosed on family screening (FS).

Methods: The study group comprised 39 children (22 boys) who were diagnosed with WD and had serum and plasma samples available, obtained within the first week of diagnosis. Samples were analyzed by ELISA for the following biomarkers of apoptosis; TNF-related apoptosis-inducing ligand (TRAIL), M30 epitope of cytokeratin 18 (CK 18), and its M65 epitope a marker of necrosis and were compared to healthy controls.

Results: The median age at presentation was 10.4 year (range 2.62–16.3). The clinical presentation was ALF in 10, CLD in 12, and 17 were in the FS group. The geometric means of measured biomarkers presented in Table 5. The necrosis marker M65 was significantly higher in those presented with ALF when compared to both control (P = 0.001) and FS (P < 0.001), but not CLD. The mean of M30, the apoptosis marker, was significantly higher in ALF when compared to control (P = 0.025), CLD (P = 0.035) and FS (P < 0.001). TRAIL, a marker of apoptosis of the extrinsic pathway had significantly lower means in both ALF and CLD when compared to control (P = 0.009 and P = 0.002, respectively).

Conclusions: Apoptosis-mediated cell death is the predominant mode of cell injury in all the presentations; however, in ALF necrosis appears to be a significant contributor.

TABLE 5. Geometric means of measured biomarkers

<table>
<thead>
<tr>
<th>Type of presentation</th>
<th>M65, U/L</th>
<th>M30, U/L</th>
<th>TRAIL, pg/mL</th>
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<tr>
<td>Control</td>
<td>639.88</td>
<td>677.13</td>
<td>89.58</td>
</tr>
<tr>
<td>ALF</td>
<td>2581.69</td>
<td>1262.62</td>
<td>37.44</td>
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<tr>
<td>CLD</td>
<td>1391.85</td>
<td>583.39</td>
<td>36.92</td>
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<tr>
<td>FS</td>
<td>364.03</td>
<td>164.82</td>
<td>73.16</td>
</tr>
</tbody>
</table>

PHI-09

EVALUATION OF THE EFFICACY OF D-PENICILLAMINE THERAPY IN CHILDREN WITH WILSON DISEASE

Presenter: A. Zubovich. Institute of Nutrition, RAMS, Moscow, Russian Federation.

Co-authors: T. Strokova1, B. Kaganov1, E. Pavlovskaya1.
1Institute of Nutrition, RAMS, Moscow, Russian Federation.

Aim: To evaluate the efficacy of prolonged d-penicillamine therapy in children with Wilson’s disease.

Methods: We examined 53 children with Wilson’s disease aged from 6 to 17 years old (mean age was 12 years old). Boys 25 (47%), girls 28 (53%). Hepatic form of the disease was diagnosed in 41 (77%) children, mixed form, in 12 (23%) children. All children received d-penicillamine therapy, mean dose was 17.87 mg/kg/day. We evaluated changes of liver size, serum transaminases level (ALT, AST) and urinary excretion of copper during prolonged course of therapy with d-penicillamine (cuprelin).

Results: Before the start of treatment in 21 (39.6%) children liver size was increased (0.5–2 cm from costal arch), include 3 (14%) children with mixed form of Wilson’s disease. During treatment liver size was reduced in all children, in 15 (28%) children liver size became normal. Mean serum ALT level before the start of therapy was significantly higher in those presented with ALF when compared to control (P = 0.001) and FS (P < 0.001), but not CLD. The mean of M30, the apoptosis marker, was significantly higher in ALF when compared to control (P = 0.025), CLD (P = 0.035) and FS (P < 0.001). TRAIL, a marker of apoptosis of the extrinsic pathway had significantly lower means in both ALF and CLD when compared to control (P = 0.009 and P = 0.002, respectively). There was a statistically significant positive correlation between M30 level and the following markers AST, GGT, bilirubin, and INR. Similar correlations found between Levels of M65 and the same markers. There was a negative correlation between TRAIL and both GGT and bilirubin.

Conclusions: Apoptosis-mediated cell death is the predominant mode of cell injury in all the presentations; however, in ALF necrosis appears to be a significant contributor.
was 112.8 UI/L (normal 5–40), in 6 months of therapy 44 UI/L., during the next 5 years it varied from minimum (23.7 UI/L) to maximum (76.6 UI/L), mean level was 44.8 UI/L. Mean serum AST level before start of therapy was 79.74 UI/L (normal 5–42), in 6 months 42.14 UI/L, in 5 years 31 UI/L. Urinary excretion of copper before start of therapy was 286 μg/day (normal to 50 μg/day), in D-penicillamine loading test 1961 μg/day (normal 600–800). In 6 months of therapy this level was 971.5 μg/day, in 1 year 965 μg/day, in 2 years 685.4 μg/day, in 5 years 524 μg/day. Thus, in a year mean level of urinary excretion of copper decreased to high limit of the norm, and after that, during 5-year period of observation was not higher than 700 μg/day.

**Conclusions:**

In all children during D-penicillamine therapy copper level was reduced; serum transaminases level and urinary excretion of copper were decreased.

**Methods:**

A retrospective analysis of the data of 291 obese (body mass index higher than 97% percentile) children and adolescents attending the liver or obesity outpatient clinics during the last 6 years (2003–2008) has been carried out. Standard anthropometric and laboratory parameters, virology, haptoglobin, alpha-1-antitrypsin and serum caeruloplasmin were analysed. Penicillamine challenge tests were performed in 22 patients, and 14 liver biopsies in patients with elevated transaminases despite a trial of weight reduction (with exception of emergency cases). Genetic analysis and slit lamp examination was performed.

**Results:**

41 (14.08 %) patients had elevated transaminases. Average caeruloplasmin (normal range 0.220–0.605 g/L) value was 0.263 g/L (0.139 – 0.388 g/L); 9/41 (22%) subjects had values below the reference range. 22 patients had a penicillamine challenge test, with mean values of 19 μg, 698 μg, 802 μg and 819 μg on days 1–4.

13 children had values in the intermediate range from 800–1590 μg. 2 tests had values above 1590 μg. Liver histology showed steatosis in 8, steatohepatitis in 4 and cirrhosis in 2 patients, respectively. We discovered 2 cases of Wilson disease in this group by following a multistep approach. Additionally 3 further asymptomatic homozygous relatives with Wilson disease and 4 heterozygous carriers were discovered. Realizing the problems in diagnosing Wilson disease we present an algorithm to systematically search for the disease in obese children with elevated liver enzymes. In contrast to current paediatric literature we cannot confirm the observation of elevated acute phase proteins (C-reactive protein, caeruloplasmin and alpha-1-antitrypsin) in obese children and adolescents.

**Conclusions:**

Currently, there are insufficient data to predict the incidence of Wilson disease in obese children with liver disease. Our data suggest a higher incidence than being estimated at present. All children or adolescents with liver disease should be intensively investigated towards Wilson disease. Early diagnosis should be aimed to provide early therapy prior to development of irreversible organic damage.

**PHI-11**

**HBV AND/OR HCV CO-INFECTION IN CHILDREN WITH LIVER DISEASES IN POLAND**

**Presentation:**

M. Woynarowski. *Children’s Health Memorial Institute, Warsaw, Poland.*

Co-authors: M. Woźniak, W. Chlebcewicz-Szuba, T. Chmurska-Motyka, A. Gorczyca, B. Korczowski, A. Krzywicka, D. Lebensztejn, A. Liberek, E. Majda-Stanislawski, M. Rokita, E. Strawińska, S. Wiecel, I. Zaleska, A. Zdanowska-Ruskan, J. Socha. *Children’s Health Memorial Institute, Warsaw, Poland; Regional Hospital, Szczecin; Regional Pediatric Hospital, Warsaw, Poland; John Paul II Hospital, Krakow; University Hospital, Rzeszow; Silesian Medical University, Zabrze; Medical University, Białystok; Medical University, Gdansk; Medical University, Lodz; Regional Infectious Hospital, Torun; Silesian Medical University, Katowice; Medical University, Wroclaw; Regional Hospital, Olsztyn.

**Background and Aim:**

It is known that frequency of liver problems varies among different populations. The epidemiology of viral and nonviral liver diseases in Polish children has not been studied. The collaborative group for pediatric liver diseases (PEGAZ) was established to describe the pediatric hepatology problems in Poland. The aim of the current study was to collect the snap shot data about the number of HBV and/or HCV infections in Polish children and adolescents.

**Methods:**

The retrospective cohort study was performed in 11 centers. The objective was to establish the number of patients with liver diseases (viral and nonviral) and their incidence. In Poland, the number of HBV and/or HCV infections was recorded, from 2003 to 2008.

**Results:**

A total of 575 patients (396 girls, 179 boys) were included. The mean age was 13.8 years (range 1–18). The majority of patients were aged 10–13 years (40%). The prevalence of HBV and/or HCV co-infections was 8.9%. HBV infection was present in 6.1% of patients, and HCV infection in 3.6%.

**Conclusions:**

The prevalence of HBV and/or HCV co-infections in children with liver diseases in Poland is lower than in other European countries. The study provides important information for the implementation of vaccination programs and screening for liver diseases in children.
co-infection in children with nonviral liver diseases in 2008.

**Methods:** The questionnaire survey was sent to 13 PEGAZ group member hospitals. The questionnaire specified 10 nonviral liver disease diagnoses. The hospitals were asked to record the total number of children treated within the last 12 months, the number of patients with each liver disease and the number of children positive for HBV and/or HCV infection. Total number of 36,000 records of children aged from 1 month to 18 years treated at the study centers was reviewed.

**Results:** 2956 (8.2%) children were treated due to liver problems at the centers participating in the survey. Isolated viral infection without other underlying liver disease was recorded in 1351 (45.7%) children (HBV 996, HCV 305 and HBV+HCV 50). Nonviral liver disease was present in 1605 (54.3%) children (Gilbert syndrome 596, children after LTx 230, cholestasis 201, autoimmune hepatitis 183, Wilson disease 117, A-1-ATD 113, cholelithiasis 83, liver and kidney polycystic disease 31, biliary tract cyst 27 and PSC 20). 98 (6.1%) of children with liver disease had hepatotropic virus co-infection (HBV 37, HCV 51 and HBV+HCV 10). The highest rates of co-infection were noted in children after LTx, 16.9% (HBV 3.9%, HCV 10.8% and HBV+HCV 2.2%), AIH 13.4% (HBV 8.0%, HCV 3.7% and HBV+HCV 1.6%) and A-1-ATD 8.8% (HBV 4.4%, HCV 2.6% and HBV+HCV 1.8%). Lower rates of HBV and/or HCV co-infections were noted in patients with cholelithiasis 3.6%, cholestasis 3.4%, Wilson disease 1.7 and Gilbert syndrome 1.5%. Single cases of HBV and/or HCV co-infections were noted in patients with liver-kidney polycystic disease, biliary tract cyst and PSC but the small total numbers of patients with these diagnoses do not justify calculating the percent rate of co-infection.

**Conclusions:** Our data show that HBV and/or HCV viral infections remain the most common reason for liver problems in Polish pediatric population. The risk of HBV and/or HCV co-infection rate in children after LTx or in children with AIH is higher then in patients with other liver diseases.

**PH1-12**

**PEDIATRIC LIVER TRANSPLANTATION FROM ANTIHBc POSITIVE DONOR: OUTCOME, EFFICACY OF LAMUVIDINE PROPHYLAXIS**

**Presenter:** H. Yuksekkaya. *Ege University School of Medicine, Izmir, Turkey.*

**Co-authors:** H. Yuksekkaya, C. Arikan, M. Cakir, M. Baran, S. Aydogdu, M. Zeytun, M. Kilic, M. Kilic. *Ege University Organ Transplantation and Research Center, Liver Unit, Izmir, Turkey.*

**Method:** To detect the incidence of HBV infection in recipients who received HbcAb-positive donor livers and effect of lamivudine prophylaxis.

**Aim:** To detect the incidence of HBV infection in recipients who received HbcAb-positive donor livers and effect of lamivudine prophylaxis.

**Method:** Between 20/4/99 and 6/11/2008, 138 patients underwent LT and 31 anti-HBs+. HbsAg+ patients received an hepatic allograft from a donor positive for anti-HBc. Lamivudine profilaxis was administered to consecutive 25 patients who transplanted after 2003. Of the 31 patients, 3 were anti-HBs+, anti-HBc+, before transplant, 1 was anti-HBs-, anti-HBc- and 27 were anti-HBs+, anti-HBc-. Serum samples from the donor and recipient were tested for HbcAb, HBV DNA, and hepatitis B surface antibody. De novo HBV infection was defined as positive HBsAg in serum.

**Results:** 7 of 31 (22.5%) recipients developed de novo HBV infections compared with 0 of 107 (0%) recipients who received anti-HBc-negative donor livers (P < 0.0001). The 6 patient who did not received lamivudine profilaxis became HbsAg+ at median 4 months (2–28). Of the 6 recipients, 3 are alive (2 of them anti-HBs+ HbsAg- after 10 months lamivudine) and 3 (50%) were died with an average follow-up of 1 year (range 14–36 months) due to hepatitis B related graft dysfunction. Recipient with allograft dysfunction has significantly elevated viremia levels compared with the other de novo hepatitis B recipients (P < 0.003). One of 25 recipients who received lamivudine at the posttransplant first day and continued indefinitely, developed de novo HBV infection at posttransplant 16th months. He received IFN-α for 6 months without any problem. Median follow-up was 32.8 months (range, 6 to 48 months) in lamivudine profilaxis group. No side effects of lamivudine therapy were reported by any of the patients.

**Conclusions:** Lamivudine well tolerated and seems effective prevent de novo HBV infection in HbsAg+ pediatric recipients of hepatic allografts from anti-HBc donors.

**PH1-13**

**HEPATITIS C VIRUS INFECTION IN HEALTHY EGYPTIAN CHILDREN: PREVALENCE AND RISK FACTORS**

**Presenter:** S. Barakat. *Alexandria University, Faculty of Medicine, Alexandria, Egypt.*

**Co-authors:** S. Hafez, N. Al-Bashir. *University of Alexandria, Faculty of Medicine, Alexandria, Egypt.*

**Aim:** To estimate the prevalence and risk factors of hepatitis C virus (HCV) infection among Egyptian healthy children.

**Methods:** A representative multistratified random sample of 500 children 5–15 years from 7 educational zones in Alexandria was included in the study. The
sample size was calculated using NCSS program according to the previous reports of HCV seroprevalence 9 among rural Egyptian children. A questionnaire solicited information regarding risk factors for infection, and blood samples were tested for HCV antibodies using ELISA test. Sera that are repeatedly reactive by ELISA were further confirmed by HCV-RNA-PCR.

Results: HCV seroprevalence was 5.8% with HCV viraemia in 75% of studied children. The Prevalence of anti-HCV antibodies increased with age, from 0% in those of 6–7 years old to 16% in those of 15 years old. Risk factors analysis for acquiring HCV infection showed a significant association between history of previous blood transfusion, intravenous injections, surgical intervention, dental maneuvers, frequent injection (≥10), circumcision for boys by informal health care providers, low socio-economic class, rural areas residence and anti-HCV seropositivity. Multivariate logistic regression analysis shows that blood transfusion was the most important risk factor, followed by surgical procedures, dental treatment, and increasing age.

Conclusions: HCV seropositive rate of 5.8% among healthy Egyptian children is 10-fold or more than that reported in other countries. Preventive strategies are mandatory to stop the spread of HCV infection.

PHI-14

VERTICALLY ACQUIRED HEPATITIS C VIRUS INFECTION: CAUSE FOR OPTIMISM

Presenter: S. Davison. Leeds Teaching Hospitals NHS Trust, Leeds, UK.
Co-authors: B. Alneaimy1, S. Rajwal1, P. Mcclean1. 1Leeds Teaching Hospitals NHS Trust, Leeds, UK.

Background and Aim: Hepatitis C virus (HCV) infection is a major health issue in the UK, being a leading cause of cirrhosis and hepatocellular carcinoma and a common indication for liver transplantation. Strategies to encourage testing in adults have been adopted, to facilitate referral for timely management. IVDU is a major risk factor, thus compliance with follow up may be difficult to achieve. Children with HCV infection may present with complications in early adulthood if unrecognised and untreated. We describe the clinical course, management and outcome of children with vertically acquired HCV infection referred to a single centre.

Methods: A retrospective data collection from case notes of children confirmed to be HCV RNA positive born to HCV-infected women was performed. Maternal HCV risk factors were ascertained. Routine management included monitoring of blood for LFT and HCV RNA monthly, with shared care provided by DGH or Regional Centre. Since 2007 selected patients have received combination therapy (Rx) with weekly pegylated interferon and daily oral ribavirin.

Results: Thirty-two children (M:15; F:17) were identified. Age at diagnosis was 1–96 months (median 14). Maternal risk factors were IVDU (21), blood Tx (1), not ascertained (3) and no identifiable risk (7). Ethnic origin was white in 24 (20 IVDU), mixed race in 2 (1 IVDU) and Asian in 6. HCV genotype was 1a/1b in 11, 3a in 16, not established in 5. Follow-up (FU) was 4–131 months (median 31). Two attended once only and were lost to FU. Five children did not attend appointments more than once (2–8 times) but have subsequently re-attended. All children are currently asymptomatic. Serum ALT was persistently abnormal in 5 and intermittently abnormal in 23 children, max ALT was 1.1–8.4 (median 2.8) x ULN. Spontaneous HCV RNA clearance occurred in 4/32 (12.5%) at age 11–30 months (median 17 months). To date, 9 children (all genotype 3a) have completed Rx, which has been well tolerated. Of these, 8/9 became HCV RNA negative at end of treatment and to date 4/4 have sustained viral response (RNA negative 6 months post-Rx).

Conclusions: Vertical HCV infection may resolve in at least 12% of affected children. Of those who remain HCV RNA positive, most (60%) are infected with genotype 3a which typically responds well to treatment. Compliance with follow up may be facilitated by a shared care approach.

PHI-15

LEARN MORE IN PREVENTING INFANTS’ HEPATITIS A: THE PREVALENCE OF HEPATITIS A VIRUS ANTIBODY IN PORTUGUESE PREGNANT WOMEN POPULATION

Presenter: H. Antunes. Gastroenterology, Hepatology and Nutrition Unit, Paediatrics Department, S. Marcos Hospital and Life and Health Sciences Research Institute [ICVS], School of Health Sciences, University of Minho, Braga, Portugal.
Co-authors: F. Neiva1, A. Estrada2. 1Gastroenterology, Hepatology and Nutrition Unit, Paediatrics Department, S. Marcos Hospital, Braga, Portugal; 2Clinical Pathology Department, S. Marcos Hospital, Braga, Portugal.

Background and Aim: The hepatitis A virus (HAV) antibody prevalence in a population determines the HAV endemicity. The Centers for Disease Control and Prevention classified Portugal as a low endemicity country. The HAV vaccine is recommended after 12 months of life. HAV antibody from the mother prevents HAV hepatitis in infants. The aim of the study was to determine the prevalence of anti-HAV in a Portuguese pregnant
Methods: In a tertiary hospital from northern Portugal, S. Marcos Hospital (SMH), Braga, we recall serum from PPWP between 2005 and 2006. The pregnant women gave written consent to use the serum for this reason when they needed to performed blood tests for another reason. The total antibody anti-HAV analysis was done through chemiluminescence’s assay method in Vitros ECI equipment from Ortho Clinical Diagnostics. The SMH Ethical Committee approved this study.

Results: The median age of the PPWP was 29 years (minimum: 14 years; maximum: 44 years). The HAV antibody was determinate in 669 samples. Four serums were in grey zone in the test used and were excluded. The HAV antibody was positive in 79.4% (95% confidence interval [CI], 76.3–82.5%). The prevalence was 35.7% (95% CI, 23.2–48.3%) in PPWP less than 20 years; 72.6% (95% CI, 67.3–77.8%) in PPWP between 20–29 years, and 92.5% (95% CI, 89.6–95.3%) in PPWP with 30 years or more. In total PPWP population, 20.6% (95% CI, 17.5–23.7%) did not have HAV antibody to give to their children.

Conclusions: This study shows the first results of HAV prevalence in a PPWP population. The results of the young pregnant women put their infants at risk of having hepatitis A in the majority of the cases. If the vaccine recommendations persist in low HAV endemicity countries the infants could be a concern in outbreaks and travel to higher HAV endemicity countries.

PHI-16

REDUCED LIVER CELL DEATH USING ALGINATE SCAFFOLD AFTER ACUTE HEPATIC FAILURE IN MICE

Presenter: E. Shteyer. Hadassah-Hebrew University Medical Center, Jerusalem, Israel.


1Pediatric Department, Hadassah Hebrew University Medical Center, Jerusalem, Israel; 2Liver Unit, Hadassah Hebrew University Medical Center, Jerusalem, Israel; 3Department of Biotechnology Engineering, Ben-Gurion University, Beer Sheva, Israel.

Background and Aim: Macroporous alginate scaffold is a biocompatible matrix, which promotes the growth, differentiation and long-term cultivation of primary hepatocytes in culture. Seeding primary hepatocytes within alginate scaffolds induced their self-assembly into spheroids with elevated hepatocellular functions. Our aim was to examine the ability of alginate scaffolds implanted after subtotal hepatectomy to support and save remaining liver cells from damage during acute hepatic failure.

Methods: 87% partial hepatectomy was performed in three groups of C57BL/6 mice, followed by implantation of the alginate scaffolds. Mice survival was compared with mice implanted with macroporous collagen scaffolds or with untreated heptatectomized mice (control groups). To elucidate the mechanism of the therapeutic effect, in a repeat study, the mice were sacrificed 3, 6, 24, 48 hours and 6 days following hepatectomy and scaffold implantation. Liver morphology, viability and damage were examined by H&E, serum albumin, AST, ALT and caspase-3. Liver cell proliferation was examined by BrdU staining. Localization of the alginate scaffolds was detected by HRP staining of distinct biotin-labeled alginate scaffolds.

Results: Alginate scaffold significantly increased animal survival to 60% vs. 10% in the control groups (log rank = 0.004). In contrast, the collagen scaffold had no effect on survival. Mice with untreated alginate scaffolds manifested normal AST and ALT serum levels as compared with the 2–20 fold-increase in control groups (P < 0.0001), along with an increase in albumin levels to 25g/L vs 10 in the controls (P < 0.001). A nearly normal histology was observed in alginate scaffold-implanted group vs. significant necrosis in the controls. In all time points, caspase-3 staining showed significantly more apoptotic cells in mice without scaffold treatment compared to the alginate scaffold-treated group (P < 0.005). BrdU positive cells were significantly higher 3 and 6 hours after hepatectomy in the alginate scaffold-treated group. Biotin-alginate scaffolds were well integrated within the liver tissue. To assess the importance of the structure of the scaffold nano-pores alginate scaffolds were design and implanted in the same manner as the macroporous scaffolds. These experiments showed the same effect on survival and necrosis.

Conclusions: The alginate scaffolds can reconstitute the liver by providing a replacement matrix for the remaining liver cells, thus reducing cell necrosis and apoptosis and leading to enhanced animal survival after acute liver failure. The alginate scaffolds improved the hepatic synthetic functions and promoted regeneration early after PH. Alginate scaffolds may serve as novel liver support matrix in acute or chronic liver failure.

PHI-17

MECHANISMS OF HEPATOCYTOCELLULAR DEATH IN ACUTE LIVER FAILURE OF INDETERMINATE AETIOLOGY IN CHILDREN

Presenter: A. Mustafa. King’s College Hospital, London, UK.
Background and Aim: Indeterminate aetiology is the most common diagnostic entity ascribed to children with acute liver failure (ALF). Better understanding of the mode of cell death, apoptosis/necrosis in these patients by measuring by-products of cytokine cascades could help to elucidate the predominant pathway of cell death. The aim of the study was to study mechanisms of hepatocyte death in children with ALF of indeterminate aetiology (IE).

Methods: The study group comprised of 56 children (32 boys), diagnosed with ALF and had available serum and plasma samples obtained in the first week of diagnosis. Samples were analyzed by ELISA for the following biomarkers of apoptosis; Fas ligand (FasL), TNF-related apoptosis-inducing ligand (TRAIL), cytochrome c (cytc) and M30 epitope of cytokeratin 18 (CK 18), and its M65 epitope a marker of necrosis, and were compared to 14 healthy controls.

Results: The median age was 8.2 years (range 0–15.9). Biomarkers were studied in 28 children of Indeterminate aetiology and compared with viral infection in 10, metabolic disorders in 18 and normal control in 14. The geometric means (95% CI) of the measured biomarkers is presented in Table 6. FasL the apoptotic marker of the extrinsic pathway was significantly higher in those presented with IE and viral infection compared to controls (P < 0.001 and 0.003, respectively) and not the metabolic group. TRAIL, the apoptosis marker of the extrinsic pathway had significantly lower means in NANE, Infection and metabolic (P = 0.0015) when compared to controls. The necrosis marker M65 and the apoptosis marker M30 both were significantly higher in IE group (P = 0.01 and 0.003, respectively) compared to controls. There was no difference in the levels of cytochrome c, apoptosis marker of intrinsic pathway between all the study groups. There was a statistically significant positive correlation between M30 and both FasL and M65. TRAIL has a significant positive correlation with cytochrome c.

Conclusions: Cell death in acute liver failure of indeterminate aetiology is secondary to necrosis and apoptosis. Apoptosis is mediated by extrinsic pathway predominately. However, the mediator of the extrinsic pathway is FasL not TRAIL.

PHI-18

INFECTIOUS COMPLICATIONS IN PAEDIATRIC ACUTE LIVER FAILURE: A SINGLE-CENTRE EXPERIENCE

Presenter: N. Shanmugam. Paediatric Liver Centre, King’s College Hospital, London, UK.
Co-authors: G. Godbole, A. Dhawan, A. Verma. Paediatric Liver Centre, King’s College Hospital, London, UK.

Aim: To determine the incidence of infectious complications (IC) and their effect on outcome in children with ALF and in children who received liver transplantation (LT).

Methods: Retrospective review of the case records of children presenting with ALF. All patients had surveillance of cultures from all sterile body fluids every week or more often if clinically indicated. All received antibiotic and antifungal prophylaxis and high-dose acyclovir in neonates. Biochemical parameters of liver dysfunction, renal dysfunction, duration of hospital stay and patient outcome were compared between patients with IC and noninfectious groups overall and in children with LT.

Results: 145 children (69 male), median (range) age of 4.22 (1 day–16 y) years were studied. The aetiology of ALF included paracetamol overdose in 26 patients (18%), viral infections in 18 (13%), metabolic causes in 13 (9%), indeterminate in 42 (29%), autoimmune in and neonatal hemochromatosis in 8 (5% each) and others in 30 (21%). 47/145 patients had proven IC (32%). 18 episodes of bacteraemia were observed in 13 patients, the most common organism was Enterococci spp. LRTI was seen in 12 patients and most common organism was Pseudomonas spp. UTI was also seen in 12; Candida albicans contributed to more than half of the UTI cases. Other infections included gastroenteritis (5), intrabdominal infections (5), wound infections (3) and line site infections (3). IC occurred in patients after a median (range) duration of 14 days (0–54 days) of admission. 8 (5%) had IC at admission. Median (range) duration of

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Control</th>
<th>NANE</th>
<th>Infection</th>
<th>Metabolic</th>
</tr>
</thead>
<tbody>
<tr>
<td>FasL (pg/mL)</td>
<td>63.4 (54.8–73.3)</td>
<td>173.4 (126.5–237.7)</td>
<td>216.8 (158.2–296.9)</td>
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<td>Cytc (ng/mL)</td>
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<td>1.3 (0.8–2.1)</td>
<td>1.4 (0.4–4.7)</td>
<td>1.3 (0.61–2.8)</td>
</tr>
<tr>
<td>M65 (U/L)</td>
<td>639.9 (465.3–880.01)</td>
<td>3817.6 (2591.6–5623.4)</td>
<td>2003.5 (942.4–4259.2)</td>
<td>2374.9 (1444.9–3903.6)</td>
</tr>
<tr>
<td>M30 (U/L)</td>
<td>677.1 (516.7–887.4)</td>
<td>2151.1 (1611.2–2871.8)</td>
<td>1536.8 (799.7–2953.2)</td>
<td>1126.7 (698.9–1816.1)</td>
</tr>
<tr>
<td>TRAIL (pg/mL)</td>
<td>89.6 (61.4–130.6)</td>
<td>47.3 (37.5–59.6)</td>
<td>54.9 (24.1–125.3)</td>
<td>34.1 (26.5–43.9)</td>
</tr>
</tbody>
</table>
hospital stay in patients with IC 35 (4–201) days was significantly higher than those without IC 11 (1–14) day, \( P < 0.0001 \). The duration of ventilation was also significantly higher in the group with IC (10 days) as compared to noninfectious group (5 days); \( P < 0.01 \). Overall mortality was 16% (23) of which 5% (8) were from IC group, 10% (15) from non infectious group, the difference was not statistically significant. The cause of death was culture proven sepsis in 6, clinical suspicion of sepsis/SIRS in 11, multi organ failure in 5 and graft failure in one. 45% (21) in IC group had LT compared with only 26% (26) in non infectious group (\( P < 0.03 \)). Patients with IC and underwent LT had a longer duration of hospital stay median 8 days (\( P < 0.03 \)) and ventilation 5 days (\( P < 0.03 \)) as compared to those transplanted without IC, 4 days. There was no significant difference in mortality of the 2 transplanted groups.

**Conclusions:** Culture-proven sepsis was not associated with increased mortality in children who did or did not receive liver transplantation; however, this group had prolonged duration of ventilation and hospital stay. More stringent surveillance should be used in whom the duration of hospital stay is more than 2 weeks.

**PHI-19**

**IMMUNE SUPPRESSION AUGMENTS THERAPEUTIC EFFICACY OF AAV-MEDIATED LIVER GENE TRANSFER IN A RAT MODEL OF MUCOPOLYSACCHARIDOSIS VI**

**Presenter:** C. Strisciuglio. *University of Naples “Federico II,” Naples, Italy.

**Co-authors:** C. Strisciuglio\(^1\), G. Cotugno\(^2\), A. Tessitore\(^2\), A. Capalbo\(^2\), P. Annunziata\(^2\), A. Faella\(^2\), M. Di Tommaso\(^2\), E. De Leonibus\(^2\), L. Alo\(^3\), A. Auricchio\(^3\).

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Liver is an ideal target for gene delivery of therapeutic proteins as it is the site of many inherited and acquired diseases and can be engineered by gene transfer into a “factory” for efficient secretion of soluble proteins for treatment of systemic diseases. To this extent, adeno-associated viral (AAV) vectors are efficient tools for long-term gene transfer in vivo to liver of animal models, and have good safety profiles. The safety and efficacy of AAV vectors is being evaluated in human liver. Mucopolysaccharidosis VI (MPS VI) is a lysosomal storage disorder caused by deficient activity of arylsulfatase B (ARSB), and characterized by skeletal and joint anomalies, hepatosplenomegaly, cardiac valve thickening and corneal clouding in the absence of central nervous system involvement. We have previously reported that AAV vectors mediate efficient ARSB gene transfer to liver of MPS VI animals; nevertheless below normal ARSB levels were achieved in MPS VI rats harboring a null ARSB mutation and presenting neutralizing immune responses to ARSB following gene transfer. In this study we have evaluated if the combination of immune-suppression with ARSB gene transfer allows to avoid the humoral immune responses to ARSB observed following gene transfer alone. Several, albeit not all, animals in each group reached levels of circulating ARSB up to 80% of normal for 6 months after treatment, which was associated with reduction of the humoral immune response. This resulted in clearance of lysosomal storage in liver as well as in other organs. Studies are in progress to establish which immune-suppressive regimen is more efficacious.

The combination of immune-suppression with liver gene transfer may increase therapeutic efficacy in those conditions where gene transfer alone elicits humoral responses to the transgene product.

**PHI-20**

**A NOVEL MUTATION OF AMINOLEVULINATE SYNTHASE 2 (ALAS-S) GENE IN A PATIENT WITH SIDEROBLASTIC ANEMIA AND HEMOCHROMATOSIS: SUCCESSFULLY TREATMENT WITH DEFERASIROX AND PYRIDOXINE**

**Presenter:** H. Yuksekay, Selcuk University, Meram Medicine Faculty, Konya, Turkey.

**Co-authors:** U. Caliskan\(^1\), H. Esen\(^1\), A. Berdeli\(^1\), Y. Paksoy\(^1\). \(^1\)Selcuk University, Konya, Turkey.

**Background:** Mutations in erythrocyte specific aminolevulinate synthase-2 (ALAS-2) are usually associated with sideroblastic anemia and iron overload. We describe a patient with sideroblastic anemia and hemochromatosis who had a new mutation in exon–7 region of the ALAS2 gene and with therapy pyridoxine and deferasirox.

**Case report:** A 14-year-old boy, who was admitted to our clinic with weakness and paleness for 1 month. In the history, he had closure of patent ductus arteriosus successfully by cardiac catheterization 4 years ago. In the examination, weight and length 25th percentile, had liver and spleen 2 cm palpable from costal margin, tachycardia and paleness. Blood tests; hemoglobin 4.9 g/dL, 308% saturated transferrin, ferritin: 1850 ng/mL, vitamin B12: 132 pg/mL (190–980). Liver and renal function tests were normal, peripheral blood
SEXUAL IMMATURE PREVENTS MANIFESTATION OF LOW PHOSPHOLIPID-ASSOCIATED CHOLELITHIASIS SYNDROME

Presenter: J. Bronsky, Second Faculty of Medicine, Charles University, and University Hospital Motol, Prague, Czech Republic.

Co-authors: M. Hrebicek1, T. Jirasek2, J. Sperl3, M. Przybyla4, I. Bouckova1, V. Simajstrla4, J. Horak5, M. Jirsa3, J. Nevoral6. 1Institute of Inherited Metabolic Diseases, First Faculty of Medicine, Charles University, Prague, Czech Republic; 2Department of Pathology, Third Faculty of Medicine, Charles University, Prague, Czech Republic; 3Institute for Clinical and Experimental Medicine, Prague, Czech Republic; 4Bormed Private Health Centre, Ostrava; 5First Department of Internal Medicine, Third Faculty of Medicine, Charles University, Prague, Czech Republic; 6Department of Pediatrics, Second Faculty of Medicine, Charles University, and University Hospital Motol, Prague, Czech Republic.

Background and Aim: Low phospholipid-associated cholelithiasis syndrome has been defined as symptomatic and recurring cholelithiasis in patients aged below 40 years associated with mutations in the ABCB4 gene encoding the canalicular phospholipid export pump. In our study we investigated the contribution of ABCB4 mutations to etiology of pediatric gallstones.

Methods: Mutational analysis of ABCB4 has been performed in 23 selected pediatric subjects (age 3–17 years) with idiopathic cholesterol gallstones and family history of gallstones in first-degree relatives, and in 5 families of young females with suspect low phospholipid-associated cholelithiasis.

Results: No clearly pathogenic variant of ABCB4 has been found in any pediatric subject with idiopathic cholesterol gallstones whereas none of the 15 heterozygotes for pathogenic mutations in ABCB4 developed gallstones in childhood.

Conclusions: We suggest that hormonal changes accompanying sexual maturity are a necessary condition for development of low phospholipid-associated gallstones and propose two corrections of the clinical criteria for low phospholipid-associated cholelithiasis syndrome: The age range should be restricted to age between middle adolescence and 40 years at the onset of symptoms and the criterion of clinical history of intrahepatic cholestasis of pregnancy should be extended to clinical history of intrahepatic cholestasis induced either by pregnancy, hormones or other yet unknown factors. Supported by grants IGA MZ NR/9079-3 and VZ 64203.

THE COURSE OF AUTOIMMUNE HEPATITIS IN CHILDREN—POLISH COLLABORATIVE STUDY GROUP FOR AUTOIMMUNE HEPATITIS (PEGAZ) POPULATION-BASED SURVEY

Presenter: M. Woźniak. Children’s Health Memorial Institute, Warsaw, Poland.

Co-authors: M. Woynarowski1, D. Lebensztejn2, L. Zaleska3, A. Gorczyca4, S. Skomra5, J. Porębska6, W. Chlebecwicz-Szuba7, A. Mierzejewska-Rudnicka8, B. Kudybowicz9, S. Więcek10, A. Liberek11, E. Smukalska12, B. Iwańczak3, E. Strawinska13, B. Korczowski14, M. Wozniak. 1Institute of Inherited Metabolic Diseases, First Faculty of Medicine, Charles University, Prague, Czech Republic; 2Department of Pathology, Third Faculty of Medicine, Charles University, Prague, Czech Republic; 3Institute for Clinical and Experimental Medicine, Prague, Czech Republic; 4Bormed Private Health Centre, Ostrava; 5First Department of Internal Medicine, Third Faculty of Medicine, Charles University, Prague, Czech Republic; 6Department of Pediatrics, Second Faculty of Medicine, Charles University, and University Hospital Motol, Prague, Czech Republic.

Background and Aim: The collaborative group PEGAZ was established to describe the pediatric hepatology problems in Poland. The group has collected the data about the epidemiology of AIH in Polish children, health care facilities and standards of care for AIH. These data were presented on WCPGHAN in 2008. The aim of this study was to collect the data about the course of AIH in Polish children.

Methods: The questionnaire survey was sent to 18 PEGAZ group members. The questionnaire asked about the rate of patients with abnormalities in laboratory parameters at the diagnosis and at the moment of the survey.
Results: Between 1990 and 2007 AIH was diagnosed in 442 children (M–29%, F–71% mean age 11 years). The observation period varied from few months to 12 years. After the diagnosis of AIH all children received prednisone or prednisone+azathioprine therapy. At the moment of diagnosis AIH presented as acute hepatitis in 40% and as deterioration of chronic liver disease in 36% of subjects. ALT activity was increased in all patients and 43% of them had ALT grater than 5xULN. Bilirubin was increased in 73% and 18% had bilirubin >5 mg%. Prothrombin time was abnormal in 42% of patients and 5% had INR >2. IgG and gammaglobulins were increased in 92 and 89% of patients and the results were above 1.5 xULN in more than half of children. All children had necroinflammatory changes in initial liver biopsy and 57% had grading 3 or 4. Liver fibrosis was present in 97%. 147 patients reached adulthood, 11 underwent LTx and 6 patients died. 278 children are still treated at pediatric centers and 255 of them were available for evaluation. At the moment of the survey the laboratory results improved. ALT activity was abnormal in 54% but only 8% of patients had ALT grater than 5xULN. Bilirubin was abnormal in 19% children but only 5% had bilirubin >5 mg%. Prothrombine time was abnormal in 20% and only 1% of subjects had INR >2. IgG and gammaglobulins were abnormal in 21% and 22% of patients respectively. Liver stiffness values decreased (median: 22.9) in all 4 patients with biliary atresia in whom Kasai procedure failed. In autoimmune hepatitis patients, liver stiffness values decreased in 3 patients (median: 3 kPa, range: 2.2–20.9) as transaminase values normalized. In Wilson disease, transaminase values normalized and liver stiffness values decreased in 3 patients (median: 3 kPa, range: 1.2–5.3) and remained normal in the other one.

Conclusions: Fibroscan could be a useful tool in the follow-up of treatment efficacy in liver diseases.

PH1-24

FAMILY HISTORY OF HEPATOBLASTOMA OR HEPATOCARCINOMA IN CHILDREN WITH LIVER DISEASE IN A GENETIC ISOLATE: A CLUE FOR A RECESSIVE TRAIT

Presenter: M. Girard, Necker Hospital, Paris, France.
Co-authors: M. Girard1, B. Aulagne2, F. Lacaille3, F. Sauvat4, L. Brugière5, I. Earts6, J. Michel7, V. Verkarre8, M. Fabre9, S. Lyonnet10, A. Henrion-Caude11, Department Genetics, Inserm U781, Department Pediatric Gastroenterology, Necker Hospital, Paris, France; Department Genetics, Inserm U781, Department Surgery, Felix Guyon Hospital, Reunion Island, Paris, France; Department Pediatric Gastroenterology, Necker Hospital Paris, Paris, France; Department Pediatric Surgery, Necker Hospital Paris, Paris, France; Department Pediatric Oncology, Gustave Roussy Institute, Villejuif, France; Department Pediatric Oncology, Curie Institute, Paris, France; Department Surgery, Felix Guyon Hospital, Reunion Island, La Reunion, France; Department Anatomopathology, Necker Hospital Paris, Paris, France; Department Anatomopathology, Kremlin-Bicêtre Hospital, Le Kremlin Bicêtre, France; Department Genetics, Inserm U781 Paris, France; Department Genetics, Inserm U781, Paris, France.

Background and Aim: The occurrence of hepatoblastoma (HB) and hepatocellular carcinoma (HCC) in a
same family has not been reported before in the literature. Here, we report three related children with a liver cancer (hepatoblastoma, and hepatocellular carcinoma) associated with an underlying liver disease. Two of the children with hepatoblastoma and hepatocarcinoma were siblings, while the third hepatoblastoma patient was a second cousin. All patients originate from Reunion Island, a population prone to founder effects. The common genetic background as well as the existence of a primitive liver disease in these related cases, raises the possibility of a unique recessive trait predisposing to liver tumors in childhood.

Methods: To refine phenotypic characterization of liver disease, histopathology was performed on liver biopsies, either tumoral or not. Genome-wide linkage analysis was performed on the 8 related probands with the Affymetrix GeneChip Human Mapping 250K NspI array.

Results: Extended questioning of the two families revealed no known cases of adenomatous polyposis. All known causes for chronic liver disease were ruled out for each of the three patients. One child had a mixed epithelial form of HB with an early onset (antenatal) and a portal tract fibrosis. In the other branch of the family, one child had an embryonic form of HB with a later onset (5 years old) and precirrhosis stage. The other child had a typical form of HCC at 9 years old with underlying cirrhosis. Genotyping enabled to segregate a single region of 9 Mb with a number of candidate genes that could explain the association of chronic liver disease and liver tumour in child.

Conclusions: To the best of our knowledge, this is the first description of the occurrence of hepatoblastoma and hepatocarcinoma in a single extended family. Further analysis of the region may enable to identify a novel genetic predisposition factor involved in both liver disease and liver cancer.

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Poster Session 1

Nutrition

PNI-01

BIFIDOBACTERIUM TREATMENT IMPROVES INTESTINAL ADHERENS JUNCTION STRUCTURE IN EXPERIMENTAL NECROTIZING ENTEROCOLITIS

Presenter: B. Dvorak. University of Arizona, Tucson, USA.

Co-authors: K. Dvorak, L. Khailova, K. Arganbright, T. Kinouchi, B. Dvorak.

1University of Arizona, Tucson, USA; 2Meiji Dairies Corp, Odawara, Japan.

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PNI-02

LACTOBACILLUS GG IN THE PREVENTION OF GASTROINTESTINAL AND RESPIRATORY TRACT INFECTIONS IN CHILDREN ATTENDING DAY CARE CENTRES: A RANDOMISED, PLACEBO-CONTROLLED, DOUBLE-BLIND STUDY

Presenter: I. Hojsak. Children’s Hospital Zagreb, Zagreb, Croatia.

Co-authors: S. Abdović, S. Kolaček.

1Children’s Hospital Zagreb, Zagreb, Croatia.

Background and Aim: Children attending day care centres are at 2–3 times greater risk for getting respiratory infection and 2.2–3.5 times greater risk for gastrointestinal

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infections than children who stay at home. Our study’s objective was to investigate the role of Lactobacillus GG (LGG) in preventing gastrointestinal and respiratory tract infections in children attending day care centres.

**Methods:** We conducted a randomized, double-blind, placebo-controlled trial in 281 children attending day care centre. They were randomly allocated to receive during 3 months period LGG at a dose of 10^9 colony-forming units in 100 mL of a fermented milk product (LGG group, n = 139) or placebo that was the same postpasteurized fermented milk product without LGG (placebo group, n = 142).

**Results:** In the LGG group, compared to the placebo group, we found a significantly reduced risk of respiratory tract infections (43.2% vs 67.6%, P < 0.001), which was especially associated with younger age (P < 0.001). Patients receiving placebo had more gastrointestinal infections but difference was not statistically significant (22.5% vs 14.4%, P = 0.079). Groups did not differ in absence from day care centre (P = 0.069) (Table 7).

**Conclusions:** LGG administration can be recommended as a valid measure for decreasing the risk of respiratory infections in children attending day care centres.

**TABLE 7. Main outcome measurements and differences between study groups**

<table>
<thead>
<tr>
<th>Variable</th>
<th>LGG group, N = 139</th>
<th>Placebo group, N = 142</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory infections</td>
<td>60 (43%)</td>
<td>96 (67.6%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gastrointestinal infections</td>
<td>20 (14.4%)</td>
<td>32 (22.5%)</td>
<td>0.079</td>
</tr>
</tbody>
</table>

Difference analysed with χ² test.

**PN1-03**

FAVOURABLE EFFECT OF GROWING-UP MILK WITH SYNBIONTICS AND LCPUFA ON GROWTH AND GUT MICROBIOTA IN INDONESIAN TODDLERS

**Presenter:** A. Firmansyah. *Faculty of Medicine, University of Indonesia, Jakarta, Indonesia.*

**Co-authors:** P. Gayatri1, M. Kadim1, S. Alatas1, P. Steenhout2, N. Conus2, L. Lestarina2. 1Departement of Pediatrics, Faculty of Medicine, University of Indonesia, Jakarta; 2Nestle Nutrition, Nestec Ltd, Vevey.

**Aim:** To compare the growth of healthy toddlers given a growing-up milk (GUM) containing probiotics (*Bifidobacterium longum* [BL999] and *Lactobacillus rhamnosus* [LPR]), prebiotics (FOS) and LCPUFA with those given a standard GUM without probiotics, prebiotics and LCPUFA.

**Methods:** 393 healthy toddlers were enrolled with signed informed consent in a randomized, double-blinded, single-center study. At 12 months of age, they were randomly assigned in 2 groups: the symbiotic-containing GUM (BL999 6 x 10^8 CFU/day, LPR 1.2 x 10^7 CFU/day, FOS 2.2 g/day and DHA/ARA 0.3 g/day) and the standard GUM. After 4 months' supplementation, data on anthropology, antibody titer to measles and hepatitis A and stool pattern were collected. Stool microbiota was analyzed in a sub sample of 30 children per group. In addition, infant development was analyzed according to Bayley scales (BSID-III) at 12 and 24 months.

**Results:** 290 toddlers completed the study. There were no differences in demographic characteristics between the 2 groups, and the mean weight was similar at baseline. After 4 month supplementation, the children in the symbiotic group (n = 199) gained significantly more weight than those in the control group (7.57 vs 4.08 g/day; P = 0.0245) and their WHO z score was significantly better (mean z score −0.52 vs −0.62; P = 0.03). The symbiotic group had significantly higher lactobacilli count (7.54 vs 7.13 log10 in the control group; P = 0.02), with similarly higher % lactobacilli to total bacteria (0.77 vs 0.23, P = 0.05). The symbiotic group also tended to have lower enterobacteriace count (6.83 vs 7.2 in the control group; P = 0.40). Measles and hepatitis A antibody titers were not different between the 2 groups. Interestingly, the composite Bayley score change between 12 and 24 months of age was 5.16 points better in the symbiotic group compared to the control group (increase of 23.79 vs 18.63 points, respectively; P = 0.11).

**Conclusions:** Four months’ consumption of a GUM containing symbiotics and LCPUFA provides favorable effect on weight gain, nutritional status and gut microbiota in toddlers. The interesting observation of an improved Bayley score in infants supplemented with symbiotic GUM requires further investigation.

**PN1-04**

A MIXTURE OF PREBIOTIC OLIGOSACCHARIDES REDUCES IMMUNOGLOBULIN κ AND A FREE LIGHT-CHAIN LEVELS IN INFANTS AT HIGH RISK FOR ALLERGY

**Presenter:** B. Schouten. *Division of Pharmacology and Pathophysiology, UIPS, Utrecht University, Utrecht, The Netherlands.*

**Co-authors:** B. Van Esch1, G. Hofman1, G. Moro2, G. Boehm2, S. Arslanoglu2, L. Willemsen1, L. Knijpels4, J. Garssen1. 1Division of Pharmacology and Pathophysiology, UIPS, Utrecht University, Utrecht, The Netherlands; 2Center for Infant Nutrition, Macedonio
Melloni Maternity Hospital, Milan, Italy; ³Danone Research, Centre for Specialised Nutrition, Friedrichsdorf, Germany; ⁴Danone Research, Centre for Specialised Nutrition, Wageningen, The Netherlands.

Background and Aim: There is evidence that the intestinal microbiota play an important role in the development of the immune system of neonates. Prebiotic oligosaccharides influence the intestinal microbiota and can positively modulate the postnatal development of the immune system. In previous studies it has been shown that the prebiotic mixture of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides in a ratio 9:1 (Immunofortis) reduces the incidence of atopic dermatitis (Arch Dis Child 2006;91:814–9; Allergy 2008;May 27 [Epub ahead of print]). Recently it was shown that Ig free light chains (Ig-fLC) in plasma might be a biomarker for atopic dermatitis, here the involvement of κ and λ Ig-fLC in the modulation of infants fed a prebiotic diet was studied.

Methods: In a double-blind, randomized placebo-controlled trial; infants received a hypoallergenic whey formula with either 8 g/L of the prebiotics (n = 34) or maltodextrine (n = 40) as a placebo for 6 months. At the age of 6 months plasma samples were collected from the infants. Total plasma levels of κ and λ Ig-fLC were analyzed using ELISA.

Results: Total plasma levels of κ and λ Ig-fLC were lowered in infants on the prebiotic oligosaccharide mixture compared to the placebo (Ig-fLC κ 7.5 ± 0.4 vs 5.7 ± 0.4 μg/mL and Ig-fLC λ 8.8 ± 0.6 vs 6.3 ± 0.4 μg/mL; P = 0.0009 for both κ and λ Ig-fLC) (Fig. 1).

Conclusions: These data demonstrate that the studied prebiotic mixture is capable of lowering the κ and λ Ig-fLC plasma levels in infants at high risk for allergy, indicating their capacity to modulate the postnatal development of the immune system and their possible role in allergy prevention.

PN1-05

BIFIDOBACTERIAL COLONIZATION IN NONALLERGIC AND ALLERGIC INFANTS

Presenter: A. Waligora-Dupriet. Université Paris Descartes, Paris, France.

Co-authors: A. Waligora-Dupriet1, F. Campeotto2, G. Rouzaud1, O. Menard1, K. Da Silva1, P. Soulaines2, N. Kapel1, C. Dupont2, M. Butel1, ¹EA4065 Ecosystème Intestinal, Probiotiques, Antibiotiques, Université Paris Descartes, Paris, France; ²Neonatologie, APHP Saint Vincent de Paul, Paris, France; ³Coprologie Fonctionnelle, APHP Pitié-Salpêtrière, Paris, France.

Background and Aim: Some clinical studies have suggested a relation between allergic diseases and gut microbiota, especially for quantitative and/or qualitative Bifidobacterium colonization. However, there is no clear consensus concerning species involved. Our aim was to analyze bifidobacterial colonization in allergic and nonallergic French infants.

Methods: Ten allergic infants were included at their first clinical consultation, ie, before any food eviction and/or treatment. For each allergic patient 2 controls were included and matched for their age at sampling and 3 of the 4 following clinical parameters: per partum antibiotherapy, mode of delivery, type of feeding and antibiotherapy during the first weeks of life. The faecal microbiota was analyzed by culture methods. Ten to 20 colonies of Bifidobacterium per infant were picked up.

FIG. 1. Total plasma levels of both λ and κ Ig-fLC are reduced by immunofortis in children at high risk for allergy at 6 months of age. n = 40 for the placebo group and n = 34 for the prebiotic group; P = 0.0009 for both κ and λ Ig-fLC.
Strains were identified using rep-PCR fingerprinting with the BOXA1R primer and multiplex PCR. Faecal secretory IgAs were measured by ELISA (SIGA, ELISA Kit, Immunodiagnostik, Bensheim, Germany).

**Results:** No differences were observed between groups in the number of colonized infants or in the level of colonization by the main aerobic and anaerobic genera, i.e., staphylococci, enterococci, enterobacteria, Bacteroides, clostridia, and bifidobacteria. Infants were colonized by 1 to 5 Bifidobacterium species and 1 to 7 strains independently of allergic status or age at sampling. B breve, B bifidum and B pseudocatenulatum colonized 80%, 35% and 30% of the infants, respectively, with no link to allergic status. B longum tended to be associated with allergic status. Indeed this species was found in 78% of allergic infants but only in 36% of nonallergic ones. B adolescentis and B dentium were seldom isolated whereas B angulatum and B animalis were not detected.

No difference in secretory IgA levels was observed.

**Conclusions:** This study did not show any clear relationship between Bifidobacterium colonization and allergic diseases. Moreover, we did not observe any association between B adolescentis and the B catenulatum/pseudocatenulatum group and allergic status as reported by other authors. Thus, the link between Bifidobacterium colonization and allergic diseases, if it exists, is complex and can’t be restricted to a role for species.

**PN1-06**

**GUT MICROBIOTA AND WEIGHT GAIN IN OVERWEIGHT AND NORMAL WEIGHT PREGNANT WOMEN**

**Presenter:** Y. Sanz. Institute of Agrochemistry and Food Technology (IATA), Spanish National Research Council (CSIC), Burjassot-Valencia, Spain.

**Co-authors:** Y. Sanz1, M. Carmen Collado1, L. García-Valdés1, J. Martín-Lagos1, M. Segura2, M. Piqueras2, T. Anjos1, J. Martino2, R. López-Tarragona1, M. Martí2, J. Florido3, C. Campoy2, 1Institute of Agrochemistry and Food Technology (IATA), Spanish National Research Council (CSIC), Valencia, Spain; 2Department of Pediatrics, School of Medicine, University of Granada, Granada, Spain; 3Department of Obstetric and Gynecology, School of Medicine, University of Granada, Granada, Spain.

**Aim:** To analyse the gut microbiota composition in overweight and normal weight pregnant woman, and to establish possible relations between specific bacterial genera, body weight and weight gain over pregnancy.

**Methods:** Seventeen overweight women with a body mass index (BMI) >25 kg/m² (23.0 [20.8–24.3], median [interquartile range]) and 33 normal weight women with a BMI <25 kg/m² (28.7 [26.3–31.2]) and with uncomplicated pregnancies, were selected according to their pre-pregnancy BMI. After the recruitment at 10–12 weeks, the mothers were supervised at 24–25 weeks, 33–34 weeks and at delivery. At baseline and at each moment, weight, height and weight gain were determined. Faecal microbiota composition was analysed at 24–25 weeks of pregnancy by quantitative real-time PCR.

**Results:** BMI increased from 23.0 (20.8–24.3) to 26.6 (25.1–28.2) kg/m² in normal weight woman and from 28.7 (26.3–31.2) to 30.8 (28.9–35.5) kg/m² in overweight women at delivery. Weight gain was of 11.7 (8.8–14.3) kg and 10.0 (6.2–11.4) kg in normal and overweight pregnant woman, respectively. Weight at birth showed values of 3.20 (3.1–3.4) and 3.50 (3.2–4.0) kg in infants from normal and overweight women, respectively, and differed significantly. Bifidobacterium and Bacteroides counts were significantly higher in normal weight than in overweight women, whereas Enterobacteriaceae, E. coli and Staphylococcus counts were significantly lower in normal than in overweight women. Significant negative correlations between body weight at 24–25 weeks and bacterial counts of Bifidobacterium and Bacteroides were detected, while these correlations were positive for Staphylococcus, Enterobacteriaceae and E. coli. Relationships between gut microbiota composition and weight gain over pregnancy were also established. E coli counts were significantly higher in women with excessive weight gain than in women with normal weight gain over pregnancy. In contrast, Akkermansia muciniphila and Bifidobacterium counts were higher in women with normal weight gain than in those with excessive weight gain. Significant negative correlations were also detected between weight gain during pregnancy and Bifidobacterium, and A muciniphila counts, while positive correlations were found between Enterobacteriaceae and E coli counts and weight gain.

**Conclusions:** There are significant differences in the faecal microbiota of overweight and normal weight women, which are associated with weight gain. Thus, the study supports the hypothesis that the gut microbiota constitutes a novel factor that influences human obesity and may have further consequences in pregnant women and foetus health and later development.

**PN1-07**

**EVALUATING INFANTS IN DAY CARE FOLLOWING A PROBIOTIC SUPPLEMENTATION PERIOD: IS THERE LONG-TERM PROTECTION AGAINST COMMON INFECTIONS?**

**Presenter:** Z. Weizman. Soroka University Medical Center, Omer, Israel.
Co-authors: J. Abu-Abed^1, A. Nasasra^1, ^1Soroka University Medical Center, Beer-Sheva, Israel.

**Aim:** To evaluate whether day care infants, following a probiotic supplementation period, acquire a long-term protection against common infections.

**Methods:** In a previous prospective study, day care infants, aged 4–10 months, fed a formula supplemented with either *Lactobacillus reuteri* or *Bifidobacterium lactis* (BB-12), for 12 weeks, had significantly fewer and shorter episodes of infections, compared to controls. The present study is a retrospective analysis of these infants, during additional 12 weeks, following the supplementation period, looking at the rate of common infections compared to controls.

**Results:** Morbidity parameters of all 3 groups are presented in Table 8. There were no significant differences between groups. All data are means per infant per 12 weeks (95% confidence interval). Additional parameters such as visit to the local clinic, absence from day care, and prescription of antibiotics also did not reveal any significant differences between groups.

**Conclusions:** Both probiotic agents, *Lactobacillus reuteri* as well as *Bifidobacterium lactis* (BB-12), can provide protection against infection throughout the supplementation period only. There is no long-term protective effect following this period.

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**PN1-09**

**SYSTEMATIC REVIEW OF METHODS FOR ASSESSING ZINC STATUS IN CLINICAL TRIALS**

**Presenter:** K. Fekete. University of Pécs, Pécs, Hungary.

**Co-authors:** N. Lowe^2, T. Deccsi^1. ^1Department of Paediatrics, University of Pécs, Pécs, Hungary; ^2Centre for Applied Sport and Exercise Sciences, School of Psychology, University of Central Lancashire, Preston, UK.

**Aim:** To systematically review published data on the usefulness of different biomarkers to assess zinc status in clinical trials.
TABLE 9. Changes of plasma zinc concentration following supplementation at different doses

<table>
<thead>
<tr>
<th>Doses of zinc supplementation</th>
<th>No. studies and participants</th>
<th>Pooled effect size (μmol/L, mean, [95% CI])</th>
<th>Variability (I², %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15–25 mg/day</td>
<td>7 studies; n = 403</td>
<td>0.48 [0.20–0.45]</td>
<td>21.2</td>
</tr>
<tr>
<td>26–50 mg/day</td>
<td>17 studies; n = 648</td>
<td>2.83 [1.94–3.71]</td>
<td>80.7</td>
</tr>
<tr>
<td>51–100 mg/day</td>
<td>5 studies; n = 118</td>
<td>4.57 [3.33–5.70]</td>
<td>91.4</td>
</tr>
<tr>
<td>101–150 mg/day</td>
<td>11 studies; n = 206</td>
<td>4.94 [2.18–7.70]</td>
<td>91.4</td>
</tr>
<tr>
<td>All: 15–150 mg/day</td>
<td>40 studies; n = 1375</td>
<td>3.15 [2.40–3.89]</td>
<td>93.5</td>
</tr>
</tbody>
</table>

Methods: The MEDLINE, EMBASE and Cochrane Library CENTRAL databases were searched for intervention trials on zinc. The minimal duration of the interventions was set at 2 weeks. All types of supplements (including zinc sulphate, gluconate, methionate, and acetate) were considered. We used formal inclusion/exclusion criteria developed by the EURRECA consortium and applied standard operation procedures for data extraction, validity assessment and meta-analysis.

Results: In the 48 studies reviewed, a total of 16 biomarkers for zinc supplementation and 26 biomarkers for zinc depletion were identified. Only one study was carried out in adolescents and 3 studies included adolescents among adults. The primary analysis based on the greatest duration and greatest dose of supplementation indicated that 3 biomarkers effectively reflected changes in zinc intakes: plasma/serum zinc concentration (μmol/L, 50 studies with 1454 participants, pooled effect: 2.88 [95% CI: 2.24–3.51], I²: 93.6%) and hair zinc content (ppm, 3 studies with 93 participants, pooled effect: 13.24 [95% CI: 11.91–14.56], I²: 0) both for supplementation and depletion as well as urinary zinc excretion (μmol/mmol creatinine, 5 studies with 373 participants, pooled effect: 0.31 [0.20–0.43], I²: 0) for supplementation alone. The efficacy of another 29 biomarkers to reflect changes in zinc intakes remained unclear. In supplementation studies, pooled effect sizes of changes of plasma/serum zinc concentrations were related to dose of supplementation (Table 9).

Conclusions: In this systematic review, changes in plasma zinc concentration, urinary zinc excretion and hair zinc content effectively reflected enhanced zinc intakes. Until the availability of paediatric data, these biomarkers are suggested to be used in zinc supplementation studies on children.

Supported by the European Communities 6th Framework Programme (FP6-036196-2, EURRECA).

PN1-10

CRITERION VALIDITY AND INTRATER RELIABILITY OF THE PAEDIATRIC YORKHILL MALNUTRITION SCORE


Co-authors: K. Gerasimidis1, O. Keane1, I. Macleod1, E. Buchanan2, A. Maclean1, P. McGrogan1, G. Stewart1, M. McAuley1, D. Flynn1, C. Wright1, 1Human Nutrition Section, Developmental Medicine, University of Glasgow, Yorkhill Hospitals, Glasgow, UK; 2Department of Paediatric Gastroenterology Hepatology and Nutrition, Yorkhill Hospitals, Glasgow, UK; 3Women and Children Directorate, NHS Greater Glasgow and Clyde, Royal Alexandra Hospital, Glasgow, UK; 4PEACH Unit, Division of Developmental, University of Glasgow, Glasgow, UK.

Aim: To validate a novel nutritional paediatric screening tool in a tertiary children’s hospital and district general hospital.

Methods: A nutrition screening tool for children was developed using the European Society of Clinical Nutrition and Metabolism guidelines (Kondrup et al. Clin Nutr 2003;22:415–21), based on information easily collected by nursing staff. The Paediatric Yorkhill Malnutrition Score (PYMS) assesses 4 elements: body mass index (BMI; wt (kg)/height (m²)), history of weight loss, dietary intake, and predicted effect of the acute medical condition on nutritional status, with a score of 0–2 for each element. Patients with total score of 2 or more are referred for dietetic review. The performance of the tool has been presented elsewhere (Gerasimidis et al, personal communication, 2009). A 4-month pilot was launched in 3 medical and 1 surgical wards of a tertiary paediatric hospital and 1 general paediatric ward of a district general hospital. For a systematically selected sample of patients two dietitians blinded to the PYMS score recorded by nurses undertook a complete nutritional assessment using dietary history, anthropometry, and medical information to test the validity of the tool (criterion validity). An additional PYMS form was completed by the research dietitians and compared with that of the nurses in order to assess intratter agreement.

Results: 2192 patients were admitted and 1562 (71.3%) were screened for malnutrition by the hospital nursing staff (n~180); 247 of these children (144 boys, 8.1 years, range 1.1–16.4) were also assessed by the research dietitians. Of these children 10.9% were at actual risk of malnutrition, as identified by the research dietitians and would need dietetic intervention. Likewise 13.8% of the patients were screened at high risk of malnutrition
according to the PYMS completed by the nurses. Of those rated at high risk by full assessment, 59% were identified as at high risk by nurse-rated PYMS score. Likewise 92% of the patients who were judged not to be at high risk by full assessment were correctly identified by the PYMS. Of those identified at high risk by nurse completed PYMS, 47% were also judged at high risk after full assessment. Compared with the full assessment, PYMS presented moderate agreement with the nursing staff ($\kappa = 0.46$). 86% of the patients were screened at the same malnutrition risk by PYMS when completed by dietitians and nursing staff with a moderate interrater agreement ($\kappa = 0.53$).

Conclusions: The PYMS tool when completed by nurses on admission identifies a majority of true high risk cases with a low false positive rate in both a paediatric tertiary and district general hospital. Its relation to objective measures of body composition and other nutritional assessment/screening tools will be explored in future papers.

PNI-11

GROWTH AND BODY COMPOSITION CHANGES AFTER TERM IN SMALL-FOR-GESTATIONAL-AGE PRETERM INFANTS

Presenter: P. Roggero. Institute of Pediatrics and Neonatology, Fondazione IRCCS “Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena”, University of Milan, Italy, Milano, Italy.


Aim: To compare growth and body composition after term between preterm infants who were born small for gestational age (SGA) and those born adequate for gestational age (AGA).

Methods: An observational longitudinal study was conducted. Twenty-six preterm infants, born AGA, who did not show growth retardation at term (G1), 35 preterm infants, born AGA, who showed growth retardation at term (G2), 32 preterm SGA infants who remained growth retarded at term (G3) underwent assessment of growth and body composition by means of an air displacement plethysmography system (PEA POD Infant Body Composition System, Life Measurement, Inc, Concord, CA) at term, at 1 and 3 months of corrected age.

Results: Infants belonging to G3 showed significantly lower birth weight (BW), length (BL), head circumference (HC) than infants belonging to G1 and G2 (BW: 1076 ± 298 g vs 1326 ± 401 g and 1499 ± 341 g, $P < 0.0001$; BL: 35.5 ± 3.4 cm vs 40.8 ± 4.8 cm and 39.7 ± 2.5 cm, $P < 0.0001$; HC: 26.2 ± 2.4 cm vs 28.7 ± 2.2 cm and 29.2 ± 1.7 cm, $P < 0.0001$) whereas gestational age did not differ (30.9 ± 2.03 wk vs 31.3 ± 2.6 wk and 30.4 ± 2.6 wk, $P = 0.6$). At term, and at 1 and 3 months of corrected age infants belonging to G1 showed higher fat mass (Fig. 1), body length at 1 and 3 months of corrected age infants belonging to G2 showed lower body weight $P < 0.0001$ (Fig. 2), length $P < 0.0001$ and head circumference $P = 0.001$ as compared to infants belonging to G2. Daily fat mass increase (g) between term and 1 month of corrected age was higher in infants belonging to G2 than that of G3 (11.2 g/day vs 7.7 g/day,
P = 0.005); thereafter the G2 infants showed a trend towards the values of fat mass exhibited by the G1 infants (Fig. 1). The mean energy and protein intakes did not differ significantly between the three groups.

Conclusions: The different pattern of growth and body composition after term showed by the three groups of preterm infants may reflect the influence of the intrauterine growth rate.

PN1-12

THE EFFECT OF 2 DIFFERENT PROTEIN CONCENTRATIONS IN TERM INFANT FORMULA ON GROWTH

Presenter: J. Trabulsi. Wyeth, Collegeville, PA, USA.
Co-authors: M. Capeding, K. Ramanujam, P. Feng, S. McSweeney, B. Harris, P. Derusso. Wyeth Nutrition, Collegeville, PA, USA; JRF Health Center Complex, Muntinlupa City, Philippines; Wyeth Nutrition, Askeaton, Ireland.

Background and Aim: Lowering the protein concentration of infant formula closer to that of mature human milk (HM) may provide short and long-term benefits to the formula-fed (FF) infant. In the short-term, high protein intake in FF infants has been associated with concentrations of blood urea nitrogen (BUN), plasma amino acids, insulin, and insulin-like growth factor-1 (IGF-1) that are greater than HM-fed infants. In the long-term, high protein intake is proposed to be a factor in the greater weight gain velocity and weight-for-length z scores observed in FF infants, which in turn have been suggested as risk factors for obesity. The purpose of this study was to determine the effect of 2 different protein concentrations in term infant formula on metabolic and growth outcomes.

Methods: Healthy term infants, age 5 to 14 days old were enrolled in this randomized, controlled, double-blind study. FF infants (n = 224) were randomized to receive either experimental formula (EF: 12.8 g/L protein, 662 kcal/L) or standard formula (SF: 14.1 g/L protein, 662 kcal/L) or standard formula (SF: 14.1 g/L protein, 662 kcal/L) or standard formula (SF: 14.1 g/L protein, 662 kcal/L) for 4 months; a HM arm (n = 112) was included as a reference group. Blood samples were collected at baseline, day 60, and day 120; anthropometric measures were assessed at baseline and monthly thereafter.

Results: Three hundred twenty-one of the 336 infants enrolled completed the study. Age (mean ± SD) at enrollment was 9.6 ± 2.9 days. Mean concentrations of albumin, total protein, blood urea nitrogen (BUN), plasma essential amino acids, and IGF-1, were within normal ranges for all groups. Mean concentration of insulin did not differ among groups. Weight gain velocity (mean ± SE, g/day) from baseline to day 120 was SF: 28.1 ± 0.5; EF: 27.8 ± 0.5; HM: 26.6 ± 0.5. Mean weight gain velocity did not significantly differ between EF and SF nor between EF and HM; however weight gain velocity was significantly greater in the SF versus HM group (P = 0.04). Weight gain velocity was not associated with IGF-1 or insulin concentrations. Weight for length z scores did not differ among groups at baseline or study day 120.

Conclusions: A term infant formula containing 12.8 g/L protein (EF) supported age appropriate growth, normal protein status, and a growth pattern similar to HM-fed infants. This study did not find a relation between insulin or IGF-1 concentrations and weight gain velocity. The protein concentration of infant feeding does appear to play a role in growth since weight gain in lower protein EF formula was closer to HM.

PN1-13

THE EFFECT OF BREAKFAST CONSUMPTION ON BODY WEIGHT IN CHILDREN AND ADOLESCENTS IN EUROPE: A SYSTEMATIC REVIEW

Presenter: R. Marek. Medical University of Warsaw, Warsaw, Poland.
Co-authors: M. Ruszczyński, H. Szajewska. Medical University of Warsaw, Warsaw, Poland.

Background and Aim: It is estimated that approximately 8% of children and 20%–30% of adolescents skip breakfast. Previous studies have shown that breakfast consumption may have an effect on body weight. However, data are inconsistent, and there is still uncertainty regarding whether breakfast consumption can contribute to or protect against becoming overweight or obese. The aim of the study was to systematically review the evidence on the effects of breakfast consumption on body weight outcomes in children and adolescents in Europe.

Methods: The Cochrane Library, MEDLINE, EMBASE databases were searched in December 2008; additional references were obtained from reviewed articles. We included prospective cohort studies and cross-sectional studies that assessed the effect of breakfast consumption on body weight outcomes. Only studies conducted in Europe were included.

Results: We identified 16 studies. Thirteen trials (n = 56,880) consistently showed that eating breakfast has a protective effect against becoming overweight or obese. One of the included trials (n = 886) showed that this effect was significant only for boys. The effect of eating breakfast on the body mass index (BMI) was analyzed in 4 studies involving 2897 participants. All of these studies showed a significant increase in BMI in breakfast skippers. In 1 study (n = 1245), this effect was significant only for boys.

Conclusions: The results of this analysis suggest that eating breakfast is associated with a reduced risk of...
becoming overweight or obese and a reduction in the BMI. However, all of the data in this review were gathered from observational studies, thus, causality should not be assumed based on these findings. For future studies, a clear definition of breakfast and breakfast consumption, a reliable means of collecting breakfast consumption data, and a clear means of controlling for important confounders (eg, birth weight, parental obesity, dietary factors, physical activity, socioeconomic status, age, sex) are needed.

PNI-14

RELATION BETWEEN BMI AND FOLIC ACID PLASMA LEVELS, LIPID PROFILE, AND LYMPHOCYTE SUBSETS AT 24 WEEKS OF GESTATION

Co-authors: J. Romeo1, C. Campoy2, L. García-Valdés2, M. Marti3, J.A. Martín-Lagos3, M. Segura2, T. Anjos3, E. Martín-Bautista2, R. López-Tarragona1, J. Florido1, L.E. Díaz1, A. Marcos1. Immunonutrition Research Group, Department of Metabolism and Nutrition, Institute of Food Science, Technology and Nutrition (ICTAN), Instituto del Frio, Spanish National Research Council (CSIC) Madrid, Spain; 2Pediatrics Department, University of Granada, Granada, Spain; 3Gynecology and Obstetric Department, University of Granada, Granada, Spain.

Background and Aim: Obesity during pregnancy is related with several serious health complications; folic acid (FA) deficiency has been linked with an increased cardiovascular disease risk. Moreover, a link between obesity and cardiovascular disease and bacterial infection risks has been suggested. Since FA seems to be a key element for the proper functioning of the immune system, the aim of the current study was to assess the relationship between body max index (BMI) and folic acid plasma levels, lipid profile and lymphocyte subsets at 24 weeks of gestation.

Methods: 80 pregnant women were divided according to prepregnancy BMI into 2 groups: nonoverweight (<25 kg/m²; n = 58) and overweight/obesity (≥25 kg/m²; n = 22) and according to FA nutritional status (plasma) (*): low FA status (<12.5 nmol/L; n = 41), and high FA status (≥12.5 nmol/L; n = 39). Plasma lipid profile (LP), triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol fraction (HDL-c) and low density lipoprotein cholesterol fraction (LDL-c), leukocyte and lymphocyte subsets (CD3+, CD4+, CD8+ and CD16+56+) were measured at 24 weeks of gestation. Frequency and normality tests were performed. ANOVA and χ² test were done. SPSS version 15.0. Minimal level of significance: P < 0.05.

Results: The nonoverweight pregnant women group with high FA status showed HDL-c higher levels (P = 0.01) (77.74 ± 14.91 vs 89.41 ± 17.49; mg/dL) and lower TG levels (P = 0.04) (182.8 ± 68.52 vs 147.5 ± 56.52; mg/dL) than nonoverweight pregnant women group with low FA status. While total lymphocytes and its subpopulations were similar in both groups, neutrophils (10.88 ± 2.85 vs 9.74 ± 2.56; 10^3/L) were lower (P = 0.0014 and P = 0.0042, respectively) in the group with higher status of FA. Total leukocytes (10^3/L) were almost significantly lower in this group.

Conclusions: In the prepregnancy nonoverweight women group, adequate FA levels throughout the gestational period are associated with a favorable lipid profile at 24 weeks of gestation. The results suggest also a positive immunomodulator effect of FA. This outcome is important to take into account in order to prevent medical complications during gestation and in their offspring.

This is part of the PREOBE study, funded by Consejería de Innovación, Ciencia y Empresa de la Junta de Andalucía. (P06-CTS-02341), Spain.

PNI-15

BIOACTIVITY OF TRANSFORMING GROWTH FACTOR (TGF-β) IN HUMAN MILK AND INFANT FORMULA

Presenter: Z. Jouni. Mead Johnson Nutrition, Evansville, IN, USA.
Co-authors: Z. Jouni1, P. Ferguson1, E. Van Tol1, F. Rosales1, A. Morrow2, R. McMahan1. 1Mead Johnson Nutrition, Evansville, IN, USA; 2Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, USA.

Background and Aim: TGF-β is a functional protein present in human milk thought to be vital for gut and immune development as well as the induction of oral tolerance. The aim of this study was to assess the bioactivity of TGF-β present in human milk (HM) and some infant formulas (IF).

Methods: 260 human milk samples from clinically healthy mothers delivering term babies were obtained from three cohorts in the US and Mexico during the first 26 weeks postpartum. Commercially available infant formula (Enfamil LIPIL) was obtained from 5 countries (US, China, Mexico, Thailand, and the Philippines). TGF-β1 and TGF-β2 were determined using ELISA, and TGF-β bioactivity was assessed by dose dependent inhibition of IL-4 stimulated HT-2 cell growth.

Results: In HM, most of the TGF-β (≥90%) was in a latent form with the -β2 isoform 5- to 10-fold higher than TGF-β1. Total TGF-β (β1 + β2) concentrations
were positively skewed and exhibited high variability both within and between mothers, which was not explained by clinical status, protein concentration, lactation period or collection site. A geometric mean and variance was calculated for each collection time and location, yielding a grand geometric mean of 2700 pg/mL. TGF-β in IF was largely in the active form and, similar to HM, TGF-β was the predominant isoform. Dose-dependent bioactivity of TGF-β was observed in both HM and IF. TGF-β-related activity in HM did not substantially differ between US and Mexican mothers, and TGF-β-related activity in IF did not vary by marketed region. HM, at a concentration of TGF-β equivalent to that found in these infant formulas, exhibited approximately 2-fold higher total bioactivity, which may be explained in part by additional functional factors in HM that also inhibit HT-2 proliferation, differences in physiochemical properties of the two liquids, or potential matrix effects. Anti-TGF-β2 neutralizing antibody decreased activity in both IF and HM between 60–90%, suggesting that TGF-β2 contributes substantially to the total observed activity.

**Conclusions:** These results demonstrate that both human milk and some infant formulas contain TGF-β, and that in both TGF-β is active as demonstrated by a cell-based bioassay.

**PN1-16**

**TGF-β: A KEY FUNCTIONAL PROTEIN PRESENT IN HUMAN MILK AND INFANT FORMULA, SURVIVES DIGESTION AS REVEALED BY SIMULATED DYNAMIC STOMACH DIGESTION**

**Presenter:** E. Van Tol, Mead Johnson Nutrition, Nijmegen, The Netherlands.

**Co-authors:** Z. Jouni1, M. Wickham2, R. Faulks3, M. Denichilo1. 1Mead Johnson Nutrition, Evansville, IN, USA; 2Institute of Food Research, Norwich, UK; 3TGR BioSciences Ltd, Thebarton, Australia.

**Background and Aim:** TGF-β is a key functional protein present in breast milk that is vital for gut and immune development as well as tolerance induction in infants. Little is known about TGF-β levels and bioactivity in infant formula. Hence, we sought to determine whether infant formula can provide significant TGF-β levels and activity to the infant’s gut. The latter is difficult to predict since adequate in vitro models mimicking infant stomach digestion are lacking. We implemented a novel dynamic gastric digestion model to more accurately simulate gastric digestion and study survival of functional proteins from human milk and infant formula.

**Methods:** The dynamic stomach model was fed an acid solution (HCl, NaCl, CaCl2, and NaH2PO4) to simulate residual gastric condition after emptying. Reconstituted commercially available infant formula (Enfamil LIPIL) or human milk samples were introduced into the stomach model, and gastric-enzymatic secretions, NaCl, CaCl2, NaH2PO4, phosphatidylcholine, pepsin and gastric lipase were added to mimic real-time digestion at 37°C. Both pH and enzyme concentrations were adjusted over time to better simulate digestion physiology. Samples were collected over an hour to encompass stay-through passage time for liquid nutrition (30–45 min). TGF-β levels were measured using ELISA, and TGF-β bioactivity determined in a Mv1Lu (mink lung cells) growth inhibition assay. Specificity of TGF-β levels and activity were demonstrated using TGF-β antibodies. All samples were tested in dose-response experiments using triplicate determinations. Inhibition of cell growth was determined by measuring DNA content.

**Results:** TGF-β concentrations in infant formula and human milk were measured before and after dynamic stomach digestion. Substantial levels of TGF-β were detected even after 36 min of digestion, followed by a gradual decline over time. TGF-β bioactivity measurements followed a similar pattern, revealing almost complete retention within 36 min of digestion. The calculated EC50 value for TGF-β bioactivity in both human milk and formula modestly increased, indicating retention of activity up to 36 min of stomach digestion.

**Conclusions:** Substantial recovery of TGF-β immune reactivity and bioactivity was measured during infant formula and human milk digestion using an infant stomach digestion model. These data indicate that infant formula can provide bioactive TGF-β to the infant’s gastrointestinal tract similar to breast milk.

**PN1-17**

**TGF-β-2 CAN BE PRESENT IN INFANT FORMULA, RESIST DIGESTION IN VITRO, AND BE BIOLOGICALLY ACTIVE**

**Presenter:** B. Lonnerdal. University of California Davis, Davis, CA, USA.

**Co-authors:** X. Du1, K. Morris2, Z. Jouni2, G. Raj2, R. Waworuntu2. 1University of California, Davis; 2Mead Johnson Nutrition, Evansville, IN.

**Background and Aim:** TGFβ2 is present in human milk and is believed to be important for IgA production and oral tolerance induction during development. TGF-β signal through cell surface serine/threonine kinase receptors to intracellular signaling components known as Smads, which in turn translocate to the nucleus leading to assembly of the transcriptional apparatus of target genes. The concentration of TGF-β2 is much lower in bovine milk than in human milk, but by utilizing milk protein sources high in TGF-β2, formula content of...
Conclusions: TGF-β2 can be considerable. The purpose of this study was to investigate whether TGF-β2 in human milk and formula can resist proteolysis under conditions similar to those in the infant gut.

Methods: Human milk and infant formulas (reconstituted from powder) were exposed to pepsin at pH 5.0, 3.5 or 2.0 (reflecting increasing maturity of the infant gut) at 37°C for 30 min, neutralized to pH 7.0 by bicarbonate and incubated for 30 min with pancreatic enzymes. TGF-β2 in original samples, pepsin digests and pepsin+pancreatin digests was analyzed by enzyme-linked immunosorbent assay (ELISA). TGF-β activity was determined by a Smad2 redistribution assay which utilizes the MDA-MB-468 cell line and measures Smad2 translocation from the cytoplasm to the nucleus. The assay response is read using the Cellomics ArrayScan VTI HCS system.

Results: The level of TGF-β2 in infant formula was variable and in some cases exceeded that of human milk samples. Of the formulas tested, the highest levels were found for Enfamil LIPIL, whereas other cow milk formulas contained lower levels and partially hydrolyzed formula contained no detectable TGF-β2. Digestion with pepsin at pH 2.0 or 3.5, followed by digestion with pancreatic enzymes substantially increased the immunodetectable TGF-β2 in human milk and Enfamil by ~400-600%. Pepsin digestion at pH 5.0 resulted in lower increases in TGF-β2. This strongly suggests that acidification and/or proteolysis play important roles in liberation of immunodetectable TGF-β2. Additionally, the TGF-β2 in these digests (pepsin+pancreatin) was highly bioactive as measured by the Smad2 Redistribution assay.

Conclusions: TGF-β2 present in some infant formulas and human milk continues to be immunodetectable and retains activity after in vitro digestion, strongly suggesting that TGF-β2 can survive in the infant gut and exert its biological activities.

PN1-18

ANALYSIS OF CYTOKINES IN BREAST MILK RECEIVED BY INFANTS WITH GROSS BLOOD IN STOOLS

Presenter: M. Durilova. 2nd Faculty of Medicine, Charles University in Prague, Prague, Czech Republic.
Co-authors: K. Tesarova-Flajsmanova¹, K. Stechova¹, V. Stavikova¹, T. Ulmannova¹, J. Neoral¹. ¹Department of Paediatrics, 2nd Faculty of Medicine, Charles University in Prague, Prague, Czech Republic.

Aim: To analyze breast milk composition of cytokines received by infants with gross blood in stools and compare it to composition of breast milk received by healthy infants.

Methods: Breast milk samples were collected from mothers of infants with gross blood in stools (n = 20) at time of examination at the Department of Paediatrics (at the infant’s age of 16.8 weeks average; min-max 2–27 weeks) and from mothers of healthy infants (n = 20) with negative history of allergy at the infant’s age of 12 weeks. Commercial ELISA kits were used for detection of the following cytokines, chemokines and growth factors: interleukin (IL)-4, IL-6, IL-10, IL-17, IL-23, interferon-gamma (IFN-γ), transforming growth factor 1 (TGF-β1), epidermal growth factor (EGF) and eotaxin. Statistical analysis was performed with SPSS programme, differences between groups were analyzed by Mann-Whitney U test and probability level P < 0.05 was considered to be statistically significant.

Results: Significant difference was seen in concentration of IFN-γ (Th1 cytokine), which was higher (P < 0.001) in breast milk received by infants with gross blood in stools (range 0–8.45 pg/mL, average 2.1 pg/mL) in comparison with healthy infants (range 0–3.41 pg/mL, average 0.35 pg/mL). Regulatory cytokine IL-10 was not detectable in any of the tested samples in both groups. Concentration of TGF-β1 was lower in breast milk received by infants with gross blood in stools, but the difference was not significant and needs to be confirmed on a larger group.

Conclusions: Cytokines present in breast milk may influence the developing immune system of breast-fed infants. Interindividual differences in their composition are known, as well as the fact that it depends on many factors including mother’s atopy status. In the present study we found higher concentrations of IFN-γ (Th1 cytokine) in breast milk received by infants with gross blood in stools. In contrast, regulatory cytokine TGF-β1, which is considered to have a protective effect from development of allergic diseases, showed lower levels in this group. In the present study half of the breastfeeding mothers of infants with bloody stools had positive history of allergy. The results of this preliminary study show rather risk pattern of cytokine composition in breast milk of mothers whose infants present with gross blood in stools, but the results need to be confirmed in larger groups.

PN1-19

THE EFFECT OF STORAGE ON VITAMIN C CONCENTRATION IN EXPRESSED BREAST MILK

Presenter: C. Conlon. Massey University, Auckland, New Zealand.
Co-authors: Y. Cui¹, IFNHH, Massey University, Auckland, New Zealand.

Background and Aim: New Zealand guidelines for the storage of expressed breast milk recommend that breast milk can be stored at ambient temperature for up to 4 hours, at 4°C for up to 48 hours and at −20°C for up to 6 months. However it is likely that temperature sensitive nutrients
such as vitamin C will degrade under these storage conditions. Aim: To evaluate the changes in vitamin C concentrations in breast milk during storage according to NZ guidelines for storage of expressed breast milk.

**Methods:** 10 subjects were recruited and a sample of expressed breast milk was obtained from each subject before the first breast feed of the day from a single breast. Baseline levels of vitamin C were immediately measured after the first breast feed of the day from a single breast. Baseline concentrations in breast milk were determined after just 1 day of storage (4°C) or at −20°C. The concentration of vitamin C in the stored samples was determined at designated time points.

**Results:** Vitamin C concentration significantly dropped after storage for 2 hours at ambient temperature compared to baseline concentrations (37.9 ± 6.8 mg/L vs 61.4 ± 8.6 mg/L, P < 0.05). A significant decrease was observed after just 1 day of storage (48.2 ± 8.6 mg/L) at 4°C (P < 0.05) and at −20°C (56.7 ± 7.6 mg/L) (P < 0.05) compared to baseline levels (61.4 ± 8.6 mg/L). Vitamin C in samples stored at 4°C were significantly lower compared to storage at −20°C after 24 hours (48.2 ± 8.6 mg/L vs 56.7 ± 7.6 mg/L, P < 0.05) and 48 hours (41.1 ± 9.0 mg/L vs 54.6 ± 8.3 mg/L, P < 0.05).

**Conclusions:** Vitamin C decreases during storage at ambient temperature, at 4°C and at −20°C. To preserve the vitamin C concentration of expressed breast milk storage should be limited to as short as time as possible. Storage at −20°C appears to be more protective than storage at 4°C.

**PN1-20**

**LIPID PEROXIDATION AND ANTIOXIDANT STATUS OF VERY-LOW-BIRTH-WEIGHT NEONATES ENTERALLY FED BY BREAST MILK OR FORMULAS**

Presenter: N. Shilina. Institute of Nutrition of RAMS, Moscow, Russian Federation.

Co-authors: N. Shilina1, E. Kurbatova1, N. Beketova1, E. Baibarina1, I. Kon1. 1Institute of Nutrition of RAMS, Moscow, Russia.

**Background and Aim:** The prevention of oxidative stress in very-low-birth-weight babies (VLBW) with severe pulmonary disorders is an important component of these patients’ treatment. At the same time free radicals play an important role in regulation of some body functions (immunity, control of apoptosis, cell differentiation). So it is necessary to reach an optimal balance between prooxidant effects of free radicals and efficiency of antioxidant systems. One of the effective approaches for solving this problem may be optimal feeding. However adequate nutritional strategy for VLBWB has not been developed till now. So the aim of the study was to compare blood levels of malonic dialdehyde (MDA) as an index of lipid peroxidation (LPO), total antioxidant activity (AOA), antioxidant vitamins A and E in VLBWB enterally fed by either breast milk (BM) or two different infant formulas: extensively hydrolyzed protein formula (EHPF) or special formula for preterm babies (PF).

**Methods:** 45 VLBWB (birth weight less than 1500 g, gestational age 26–32 weeks) were enrolled in the study and divided into 3 groups fed by BM or randomly escribed to either EHPF or PF feeding groups. MDA level measured by thiobarbituric acid method, AOA (egg lipoprotein model), vitamins A and E (by HPLC) blood levels were studied at birth and 20 days later.

**Results:** MDA level. AOA, vitamin A and E blood levels in VLBWB at the beginning of the study (M ± SD: 0.37 ± 0.3 nmol/mL; 1.35 ± 0.16 rel.u., 10.1 ± 2.5 mg/dl, 0.3 ± 0.1 mg/dl, respectively) were significantly lower comparatively to the data in term infants received by us earlier (0.82 ± 0.6 nmol/mL, 0.16 ± 0.1 rel.u., 15 mg/dl, 0.8 mg/dl, respectively). 20 days enteral feeding by BM or EHPF but not PF restored MDA level up to the term infants level (0.61 ± 0.5 nmol/mL and 0.92 ± 1.2 nmol/mL for BM and EHPF respectively, P < 0.05). Feeding by BM but not by the formulas kept initial AOA level and provided normal vitamins A (19.8 ± 14 mg/dl) and E (1.53 ± 0.97 mg/dl) status of ELBWB. Feeding of neonates by EHPF increased the vitamin E level (1.57 ± 0.99 mg/dl) but did not influence the level of vitamin A (9.39 ± 1.38 mg/dl).

**Conclusions:** The data on blood LPO, AOA, vitamins A and E level confirm the opinion that enteral feeding by BM is the best way to reach an optimal balance of LPO/antioxidant status in VLBWB comparatively with EHPF and PF.

**PN1-21**

**QUANTIFICATION OF FAECAL β-DEFENSIN 2 LEVELS IN NEONATES: UPREGULATION DURING DIGESTIVE EVENTS?**

Presenter: F. Campeotto. Hôpital St Vincent de Paul, Paris, France.

Co-authors: M. Baldassare, N. Benahmed, V. Viallon, N. Kalach, M. Butel, C. Dupont, N. Kapel. 1Hôpital St Vincent de Paul, Paris, France; 2Universita di Bari, Bari, Italy; 3Hôpital Pitié Salpêtrière, Paris, France; 4Hôpital Cochin, Paris, France; 5Université Paris Descartes, Paris, France.

**Background and Aim:** Newborns display high intestinal permeability and a naïve adaptive immune system, but infections are rarely, indicating strong innate defence mechanisms. We measured faecal β-defensin 2 (HBD2), an inducible endogenous antibiotic peptide that...
produced by intestinal epithelial cells, in healthy full-term and preterm neonates and in preterm neonates with intestinal symptoms.

**Methods:** A first study enrolled 30 full terms (mean gestational age 39 weeks, range: 36–41) and 20 preterms (30 weeks, 25–33). Faecal samples were collected at day 3, 7, 12 and 30 for full term neonates and at days 15, 30 and 60 for preterms. A second study enrolled 10 neonates with intestinal distress (3 with NEC, Bell’s stage III, and 7 with rectal bleeding) and 20 healthy neonates as control matched for age, sex, and gestational age. Stool samples were collected and immediately stored at −80°C before ELISA measurement (β-Defensin 2 ELISA Kit, Immunodiagnostik, Bensheim, Germany). Statistical analyses were based on Wilkoxon or Sign rank tests and correlation were analysed using the non parametric Spearman test.

**Results:** HBD2 was reproducibly detectable in all stool samples. In full term newborns, faecal HBD2 decreased statistically from day 3 to day 7 (227 ng/g; 14–440 vs 117 ng/g; 30–470; \( P = 0.01 \)) then moderately decreased until day 30 (84 ng/g; 10–500). In contrast, healthy preterms exhibited stable levels between days 15 and 60 (82 ng/g; 30–154 and 85 ng/g; 26–390, respectively). No correlation with gestational age or mode of feeding was detected. No difference in HBD2 was observed between full term and preterm neonates at either days 12–15 or 30. Faecal HBD2 increased significantly \((P = 0.04)\) in preterm neonates with NEC. Indeed, HBD2 levels increased dramatically during Bell stage NEC III, median 412 ng/g (range 92–1271) vs 49 ng/g (range 2–1178) in patients with bleeding and 56 ng/g (range 31–164) in controls.

**Conclusions:** This pilot study shows for the first time the quantification of faecal HBD2 in neonates, providing normal reference values. Comparison with adult values (31 ng/g ± 15.4), suggests local inflammation in the neonatal period, likely due to bacterial and food antigens. Upregulation seems to occur during intestinal suffering.

**PN1-22**

**DIETARY SUPPLEMENTATION WITH LONG-CHAIN POLYUNSATURATED FATTY ACIDS IN THE PREVENTION OF ASTHMA AND OTHER ALLERGIC DISEASES IN CHILDREN—A SYSTEMATIC REVIEW OF RANDOMIZED CONTROLLED TRIALS**

**Presenter:** A. Horvath. *Medical University of Warsaw, Warsaw, Poland.*

**Co-authors:** A. Horvath¹, P. Dziechciarz¹, I. Kossak¹, H. Szajewska¹, R. Shamir², J. Garssen¹, A. Nauta², G. Boehm³, J. Garssen¹, L. Willemsen¹.

**Methods:** The Cochrane Library, MEDLINE, and EMBASE databases were searched in December 2008 for RCTs comparing the effects of PUFA supplementation with placebo or no supplementation.

**Results:** Six RCTs met the inclusion criteria. In 1 RCT \((n = 83)\), maternal supplementation with PUFA from 20 weeks of gestation until delivery did not show any protective effect on the occurrence of asthma (relative risk \(RR = 0.39, 95\% CI 0.08–1.67\)), cough without infectious symptoms \(RR = 0.37, 95\% CI 0.15–0.91\), or recurrent wheezing \(RR 0.9, 95\% CI 0.44–1.84\) in children 12 months of age. Another RCT \((n = 399)\) showed a significant protective effect of supplementing pregnant women with fish oil on the subsequent development of asthma in 16-year-old children \(RR = 0.38, 95\% CI 0.15–0.91\). Three RCTs evaluated the effects of postnatal PUFA supplementation in the same cohort of children \((n = 616)\), who were assessed at the ages of 18 months, 3 years, and 5 years. There was no effect of PUFA supplementation on the incidence of asthma in children at 3 years \((RR 1.13, 95\% CI 0.82–1.57)\) or at 5 years of age \((RR 1.03, 95\% CI 0.89–1.18)\). PUFA supplementation reduced the risk of wheezing symptoms in children at 18 months of age \((RR 0.82, 95\% CI 0.68–0.96)\) with no effect found in children at 3 years \((RR 1.03, 95\% CI 0.89–1.18)\), and at 5 years of age \((RR 1.13, 95\% CI 0.82–1.57)\). PUFA supplementation had a small but statistically significant protective effect on the incidence of cough without infectious symptoms only in children at 3 years of age \((RR 1.28, 95\% CI 1.06–1.56)\) Dietary supplementation with PUFA, either pre- or postnatally, did not show any protective effect on the occurrence of atopic dermatitis, allergic rhinitis, and gastrointestinal allergy.

**Conclusions:** Postnatal dietary supplementation with PUFA does not appear to provide substantial protective effect on the occurrence of asthma symptoms and any other allergic diseases in children. Evidence from 2 RCTs that assessed the effect of maternal PUFA supplementation on asthma symptoms in children shows conflicting results.

**PN1-23**

**NONSENSITIZING PARTIAL WHEY HYDROLYSATES INDUCE ORAL TOLERANCE TO WHEY IN A MOUSE MODEL OF COW’S MILK ALLERGY**

**Presenter:** B. Van Esch. *Utrecht University, Utrecht, The Netherlands.*

**Co-authors:** B. Van Esch¹, B. Schouten¹, G. Hofman¹, A. Nauta², G. Boehm³, J. Garssen¹, L. Willemsen¹.
Background and Aim: Hypoallergenic formulae are a good option for infants at risk for cow’s milk allergy (CMA). IgE mediated CMA may predispose the development of allergies later in life. Therefore, preventing CMA by inducing oral tolerance is preferred over avoidance of the allergenic protein. The capacity of a nonsensitizing partial whey hydrolysate (pWH) to induce oral tolerance in mice orally sensitized to whey was studied.

Methods: Mice were sensitized orally for 5 times with 20 mg whey using cholera toxin (CT) as an adjuvant. Mice were pretreated orally with 50 mg whey, pWH or extensive whey hydrolysate (day 7–day 2) prior to whey-CT sensitization. The acute skin response was measured after intra dermal whey challenge. Mouse mast cell protease-1 (mMCP-1), whey-specific splenocyte proliferation, and relative number of regulatory T-cells (CD4+/CD25+/Foxp3+) in mesenteric lymph nodes (MLN) and spleen were determined.

Results: Whey sensitized mice showed an acute allergic skin response after intra dermal. Whey challenge (10.9 ± 12.4 vs 35.4 ± 5.6 for CT controls). In contrast to the extensive whey hydrolysate, pretreatment with whey or pWH significantly reduced the acute skin response in mice (54.3 ± 6.1 μm and 83.6 ± 5.6 μm, respectively). In addition, the serum mMCP-1 concentration, reflecting mast cell degranulation after oral challenge, was reduced in mice pretreated with whey or pWH (24.7 ± 5.6 ng/mL and 35.09 ± 6.2 ng/mL, respectively vs 69.8 ± 13.4 ng/mL; for whey-CT sensitized mice) and similar tendencies were found for whey-specific T-cell proliferation. Pretreatment of mice with pWH before sensitization elevated regulatory T-cell numbers in MLN but not in spleen.

Conclusions: Both whey and pWH retained capacities to induce oral tolerance to whey in a mouse model of cow’s milk allergy using oral sensitization. This effect may be exerted by enhancing regulatory T-cell numbers in mesenteric lymph nodes. In previous studies it was shown that in contrast to whey, pWH had limited sensitizing capacities. Hence, the pWH might still be able to induce oral tolerance to whey without the risk of sensitization to whey proteins.

PN1-24

HEPTANOATE, AN ODD-Carbon MEDIUM-CHAIN FATTY ACID, ENHANCES RESISTANCE TO STRESS OF SPONTANEOUSLY HYPERTENSIVE RAT HEARTS

Presenter: F. Labarthe. CHU Tours, Tours, France.
Co-authors: F. Labarthe1, B. Bouchard1, M. Khairallah2, R. Gelinas2, H. Brunengraber3, C. Des Rosiers2. 1CHU Tours, Tours, France; 2University of Montreal, Montreal, Canada; 3Case Western Reserve University Cleveland, Ohio USA.

Background and Aims: Heptanoate, an energy and anaplerotic substrate, has been recently proposed in the treatment of inherited long-chain fatty acid (LCFA) oxidation defects, more specifically for patients with persistent cardiac or muscular symptoms. In a previous study, we demonstrated that spontaneously hypertensive rat (SHR) hearts, a genetic model of cardiomyopathy with a FA translocase/CD36 gene defect, have an impaired capacity to withstand an acute adrenergic stress, which can be improved by a supplementation with octanoate, an even-carbon medium-chain FA (MCFA). The aim of the present study was to document the functional and metabolic effects of heptanoate supplementation in SHR hearts submitted to a stress challenge.

Methods: Hearts were perfused ex vivo in a working mode with physiological concentrations of substrates and hormones, with either 0.49 mM oleate (OLE), or 0.2 mM heptanoate (HEP) and subjected to an adrenergic stimulation (epinephrine, 10 μM). 13C-labeled substrates were used to assess substrate selection for energy production.

Results: SHR hearts perfused with OLE demonstrated an impaired capacity to withstand the adrenergic challenge, with decreased contractility and developed pressure, decline in the aortic flow, and a progressive cardiac tissue damage reflected by an increased lactate dehydrogenase (LDH) release. Supplementation with HEP improved resistance to stress as evidenced by an increase in developed pressures and improved membrane integrity (reflected by a 27% lower LDH release, 5196 ± 615 vs 7123 ± 210 mU min−1 with OLE, P < 0.01). At the metabolic level, HEP increased the energetic contribution of exogenous FA (25 ± 2% vs OLE 16 ± 1%, P < 0.01), principally related to the contribution of the MCFA (+5%). HEP also induced a large increase in the tissue concentration of Krebs cycle intermediates (HEP 1124 ± 46 nmol mg−1, vs OLE 764 ± 39, P < 0.0001), according to an anaplerotic effect that was demonstrated using [13C]heptanoate differently labelled on carbon 1 or 5, 6 and 7.

Conclusions: Supplementation with 0.2 mM heptanoate enhances resistance to stress of ex vivo perfused SHR hearts, an effect that was associated with enhanced energy production and anaplerosis. Collectively, data from this and our previous study with octanoate emphasize the cardioprotective effects of MCFA, including HEP, in SHR hearts, a model with restricted exogenous LCFA utilization as it occurs in inherited LCFA oxidation defects. Additional benefits would also be expected in...
achieved. 13C-PA and 13C-OA in maternal plasma, were tracer enrichment in all maternal lipid fractions was 12h after tracer administration, a steady stable topen enrichments by GC-isotope ratio mass spectrometry. FA concentrations in individual lipid fractions were determined by gas chromatography (GC) and iso-turerized fermented milk product without LGG (placebo n = 366). They were randomly allocated to receive for their hos-pitalization duration LGG at a dose of 10^9 colony-form-ing units in 100 mL of a fermented milk product (LGG group, n = 376) or placebo that was the same postpas-teurized fermented milk product without LGG (placebo group, n = 366).

Results: In the LGG group, compared to the placebo group, we found a significantly reduced risk of gastro-intestinal infections (relative risk [RR] 0.4, 95% confidence interval [CI] 0.25–0.7, number needed to treat [NNT] 15, 95% CI 9–34), respiratory tract infections (RR 0.38, 95% CI 0.18–0.85, NNT 30, 95% CI 16–159), vomiting episodes (RR 0.5, 95% CI 0.3–0.9), diarrheal episodes (RR 0.24, 95% CI 0.1–0.5), episodes of gastro-intestinal infections lasting longer than 2 days (RR 0.4, 95% CI 0.25–0.7), and episodes of respiratory tract

When considering enrichment values, NEFA showed the highest incorporation of each 13C-FA in placenta. 13C-DHA showed significantly higher placenta/maternal plasma ratios than the other FA (13C-PA 7.45 ± 2.2b; 13C-OA 3.59 ± 1.1b; 13C-LA 6.96 ± 2.1b; 13C-DHA 38.82 ± 11.7a), and also significantly higher cord/ maternal plasma ratios (13C-PA 0.45 ± 0.1b; 13C-OA 0.39 ± 0.1b; 13C-LA 0.37 ± 0.1b; 13C-DHA 1.6 ± 0.5a).

Conclusions: This study demonstrates in vivo selective and preferential maternal-placental as well as maternal-fetal transfer of DHA in pregnant women at term, as compared to PA, OA and LA. 13C-DHA was predomin-antly esterified into PL and TG of maternal plasma, while PA and OA acid were found primarily in TG.

OP3-02

LACTOBACILLUS GG IN THE PREVENTION OF NOSOCOMIAL GASTROINTESTINAL AND RESPIRATORY TRACT INFECTIONS: A RANDOMISED, PLACEBO-CONTROLLED, DOUBLE-BLIND STUDY

Presenter: I. Hojsak. Children’s Hospital Zagreb, Zagreb, Croatia.

Co-authors: S. Abdovic1, H. Szajewska2, S. Kolaček1.
1Children’s Hospital Zagreb, Zagreb, Croatia; 2Medical University of Warsaw, Warsaw, Poland.

Background and Aim: The incidence of nosocomial infections, predominantly gastrointestinal and respira-tory, in children in developed countries is high, ranging from 5 to 44%. Presently, there is no effective strategy for preventing these infections. Our study’s objective was to investigate the role of Lactobacillus GG (LGG) in preventing nosocomial gastrointestinal and respiratory tract infections at a pediatric hospital.

Methods: We conducted a randomized, double-blind, placebo-controlled trial in 742 hospitalized children. They were randomly allocated to receive for their hos-pitalization duration LGG at a dose of 10^9 colony-form-ing units in 100 mL of a fermented milk product (LGG group, n = 376) or placebo that was the same postpas-teurized fermented milk product without LGG (placebo group, n = 366).

Results: In the LGG group, compared to the placebo group, we found a significantly reduced risk of gastro-intestinal infections (relative risk [RR] 0.4, 95% confidence interval [CI] 0.25–0.7, number needed to treat [NNT] 15, 95% CI 9–34), respiratory tract infections (RR 0.38, 95% CI 0.18–0.85, NNT 30, 95% CI 16–159), vomiting episodes (RR 0.5, 95% CI 0.3–0.9), diarrheal episodes (RR 0.24, 95% CI 0.1–0.5), episodes of gastro-intestinal infections lasting longer than 2 days (RR 0.4, 95% CI 0.25–0.7), and episodes of respiratory tract
infections lasting longer than 3 days (RR 0.4, 95% CI 0.2–0.9). Groups did not differ in hospitalization duration ($P=0.1$) (Tables 10 and 11).

**Conclusions:** LGG administration can be recommended as a valid measure for decreasing the risk of nosocomial gastrointestinal and respiratory infections in pediatric facilities.

**OP3-03**

**IRON SUPPLEMENTS REDUCE THE RISK OF IRON DEFICIENCY ANEMIA AT 6 MONTHS IN SWEDISH MARGINALLY LOW-BIRTH-WEIGHT INFANTS**

Presenter: M. Domellöf. *Umeå University, Umeå, Sweden.*

Co-authors: M. Domellöf¹, S. Berglund¹, B. Westrup². ¹*Umeå University, Umeå, Sweden;* ²*Karolinska Institute, Stockholm, Sweden.

**Background and Aim:** Due to increased risk for iron deficiency, iron supplements are often recommended for low birth weight infants. Most low birth weight infants have only marginally low birth weight (MLBW, 2000–2500 g). For this group, local recommendations for iron supplementation vary considerably and there is a lack of European guidelines. The objective was to study the effects of iron supplementation on iron status and risk of anemia in Swedish MLBW infants in a randomized, controlled, blinded trial.

**Methods:** We randomized 285 healthy MLBW infants to receive one of three doses of iron drops: 0, 1 or 2 mg/kg/day from 6 wk to 6 mo. Hemoglobin (Hb), serum ferritin, transferrin saturation and erythrocyte mean cell volume (MVC) were analyzed at 6 wk, 3 mo and 6 mo.

**Results:** Of the infants, 44% were preterm and 56% were term but small for gestational age. Infants with anemia at baseline (n=13) were excluded. At the start of the intervention (6 wk), mean (SD) Hb was 107.0 (11.6) g/L, ferritin was 120 (1.8) µg/L and MCV was 74.3 (4.5), 76.1 (3.3) and 76.7 (3.4) fl. (P < 0.001); geometric mean serum ferritin was 20.5 (2.0), 36.4 (2.1) and 47.7 (2.3) µg/L (P < 0.001) and mean transferrin saturation was 13.5 (6.9), 19.1 (8.4) and 22.4 (12.2) % (P < 0.001). At 3 months of age, 9 infants (5 from the placebo group, 2 each from the iron groups) were taken off the blinded intervention and given iron supplements due to anemia (Hb <95 g/L). The risk of developing iron deficiency anemia at any time during the intervention was 16% in the placebo group, 4% in the 1 mg group and 0% in the 2 mg group (P < 0.001). In the respective groups at 6 months, mean MCV was 74.3 (4.5), 76.1 (3.3) and 76.7 (3.4) fl. (P < 0.001); geometric mean serum ferritin was 20.5 (2.0), 36.4 (2.1) and 47.7 (2.3) µg/L (P < 0.001) and mean transferrin saturation was 13.5 (6.9), 19.1 (8.4) and 22.4 (12.2) % (P < 0.001). At 6 months of age, 9 infants (5 from the placebo group, 2 each from the iron groups) were taken off the blinded intervention and given iron supplements due to anemia (Hb <95 g/L). The risk of developing iron deficiency anemia at any time during the intervention was 16% in the placebo group, 4% in the 1 mg group and 0% in the 2 mg group (P < 0.001).

**Conclusions:** Healthy Swedish infants with marginally low birth weight have a high risk of developing iron deficiency anemia. Iron supplementation from 6 wk to 6 mo gives a dose dependent response in Hb, MCV, ferritin and transferrin saturation and a dose of 2 mg/kg/day effectively reduces the risk of iron deficiency anemia at 6 mo.

**OP3-04**

**METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) 677C>T POLYMORPHISM AND LEVELS OF HOMOCYSTEINE AND FOLATES IN EUROPEAN PREGNANT WOMEN AND IN THEIR OFFSPRING: THE NUHEAL STUDY**

Presenter: C. Campoy. *University of Granada, Granada, Spain.*

**TABLE 10. Main outcome measurements and differences between study groups**

<table>
<thead>
<tr>
<th>Variable</th>
<th>LGG group N = 376</th>
<th>Placebo group N = 366</th>
<th>P</th>
<th>Relative risk (95% CI)</th>
<th>NNT (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastrointestinal infections</td>
<td>19 (5.1%)</td>
<td>44 (12.0%)</td>
<td>&lt;0.001</td>
<td>0.4 (0.25–0.7)</td>
<td>15 (9–34)</td>
</tr>
<tr>
<td>Respiratory infections</td>
<td>8 (2.1%)</td>
<td>20 (5.5%)</td>
<td>0.017</td>
<td>0.38 (0.18–0.85)</td>
<td>30 (16–159)</td>
</tr>
</tbody>
</table>

**TABLE 11. Secondary outcome measurements and differences between study groups**

<table>
<thead>
<tr>
<th>Variable</th>
<th>LGG group N = 376</th>
<th>Placebo group N = 366</th>
<th>P</th>
<th>Relative risk (95% CI)</th>
<th>NNT (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vomiting episodes</td>
<td>17 (4.5%)</td>
<td>33 (9.0%)</td>
<td>0.015</td>
<td>0.5 (0.3–0.9)</td>
<td>23 (13–110)</td>
</tr>
<tr>
<td>Diarrheal episodes</td>
<td>7 (1.9%)</td>
<td>28 (7.7%)</td>
<td>&lt;0.001</td>
<td>0.24 (0.1–0.5)</td>
<td>18 (11–35)</td>
</tr>
<tr>
<td>Duration of gastrointestinal infection over 2 days</td>
<td>19 (5.1%)</td>
<td>45 (12.3%)</td>
<td>&lt;0.001</td>
<td>0.4 (0.25–0.7)</td>
<td>14 (9–31)</td>
</tr>
<tr>
<td>Duration of respiratory infection over 3 days</td>
<td>8 (2.1%)</td>
<td>19 (5.2%)</td>
<td>0.026</td>
<td>0.4 (0.2 to 0.9)</td>
<td>33 (17–257)</td>
</tr>
<tr>
<td>Duration of hospital intervention (days)*</td>
<td>5 (3–7)</td>
<td>4 (4–6)</td>
<td>0.109</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* Median (interquartile range). Difference analyzed with Mann-Whitney U test.

Co-authors: C. Campoy1, J.A. Martín-Lagos1, M. Escolano1, M. Parrilla1, A. Molloy2, E. Martín-Bautista1, H. Demmelmaier3, E. Szabó4, T. Décsí4, B. Koletzko5.
1Department of Paediatrics, School of Medicine, University of Granada, Granada, Spain; 2Department of Clinical Medicine, School of Biochemistry and Immunology, Trinity College, Dublin, Ireland; 3Department of Paediatrics, University of Pécs, Pécs, Hungary.

Background and Aim: Methylentetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-MTHF). A common genetic variant in MTHFR (MTHFR 677C>T) causes an increase of total homocysteine (tHcy) (hyperhomocystinemia: >15 μmol/L). The aim of the study was to analyze differences in MTHFR polymorphisms between three European countries and the impact of folate (F) and docosahexaenoic acid (DHA) supplementation during pregnancy on mothers’ and offspring F and tHcy plasma levels depending on their genetic polymorphisms. 311 healthy pregnant women recruited from Germany, Hungary, and Spain (Nuheal study) were randomized into a double-blind trial; they received daily either a preparation with 5-MTHF was lower in the MTHFR 677TT polymorphism group than in the other 2 (TT: 6.18 ± 2.7*; CT: 7.62 ± 3.23; CC: 8.60 ± 3.69).

Conclusions: F mother’s levels decreased during pregnancy. Mother’s and neonate’s F plasma concentrations were correlated. Mother’s with MTHFR 677TT genotype supplemented with 5-MTHF had lower tHcy concentrations than other genotypes.

This work is a part of the 5. EU Framework Program [NUHEAL], grant no. CLK1-CT-1999-00888.

OP3-05

BILE SALT–STIMULATED LIPASE AND PANCREATIC LIPASE RELATED PROTEIN 2 TOGETHER PROMOTE EFFICIENT LIPID DIGESTION AND PRODUCT ABSORPTION IN THE NEWBORN

Presenter: E. Andersson. Umeå University, Umeå, Sweden.
Co-authors: S. Lindquist1, L. Bläkberg2, O. Hernell1.
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Background and Aim: The aim of this study was to elucidate the combined effect of bile salt–stimulated lipase (BSSL) and pancreatic lipase related protein-2 (PLRP2) on fat digestion and absorption into cultured epithelial cells under conditions resembling the small intestine of a newborn infant. Background: Recently, we showed that BSSL together with PLRP2, and not pancreatic triglyceride lipase (PTL), are the dominating lipases expressed in exocrine pancreas at birth, and suggested them to be key enzymes in intestinal fat digestion as long as milk is the main food.

Methods: Cells of the human epithelial colorectal adenocarcinoma cell line Caco-2 were grown for 15 days post confluence on a 0.4-μm polycarbonate membrane in a transwell system. Bile salt concentrations and pH were chosen to resemble the milieu of small intestinal contents of newborn infants. Purified lipases (BSSL and PLRP2) and radioactively labelled lipids [triglycerides (TG), retinyl esters (RE) and cholesterol esters (CE)] were added to the apical compartment and incubated with the cells at 37°C. At different time points, lipid hydrolysis and cellular uptake was determined.

Results: Both BSSL and PLRP2 hydrolyzed each TG completely to 3 free fatty acids (FFAs) and one glycerol.
in the apical compartment, and the FFAs were readily
taken up by the cells and reesterified to TG. In contrast,
due to its stereospecificity PTL, the dominating digestive
lipase in adults, hydrolyzed each TG to 2 FFAs and 1 sn-2-monglyceride. When BSSL and PLRP2 operated
together, the effect was synergistic, and the uptake into
the cell was ~50% higher than the sum of the cellular
uptake with each lipase alone. Under conditions used the
combined effect was most evident at a bile salt concentra-
tion of 3–4 mM of a bile salt mixture physiologic to the
newborn. Both BSSL and PLRP2 hydrolyze RE, but
only BSSL was active against CE.

Conclusions: BSSL is considered to be of particular
importance for efficient fat digestion during the neonatal
period. We now demonstrate that PLRP2, also highly
expressed in neonates, is well adapted for function during
this period in life. Although the two enzymes share many
features, there are also differences, eg, in substrate
specificity. A striking new observation is that BSSL
and PLRP2 have a synergistic effect in fat digestion and absorption under in vitro conditions resembling
upper intestinal contents of newborn infants.

**OP3-06**

**CHARACTERIZATION OF PEPTIDES DERIVED FROM \( \alpha \)-LACTALBUMIN DIGESTION AND DEMONSTRATION OF THEIR PREBIOTIC EFFECT THROUGH STIMULATION OF BIFIDOBACTERIA IN A MODEL OF THE GASTROINTESTINAL TRACT**

**Presenter:** M. Kullen. Wyeth Nutrition, Collegeville, PA, USA.

**Co-authors:** T. Lambers2, K. Venema3, J. Bettler1, 1Wyeth Nutrition, Collegeville, PA, USA; 2NIZO Food Research B.V., Ede, The Netherlands; 3Wageningen Center for Food Sciences, Wageningen, The Netherlands.

**Background and Aim:** We have recently demonstrated that infants fed an \( \alpha \)-lactalbumin enriched formula had fecal bifidobacteria concentrations that were similar to human milk-fed infants. The objective of this study was to characterize the peptides that are derived from digestion of \( \alpha \)-lactalbumin and to evaluate the influence of these peptides on populations of bifidobacteria in a simulated in vitro model of the gastrointestinal tract.

**Methods:** \( \alpha \)-Lactalbumin (95% pure) was subjected to digestion in an in vitro model of the stomach and small intestine (TIM-1). \( \alpha \)-Lactalbumin digestion samples were collected from the gastric, duodenal, jejunal, and ileal compartments of the gut model and were subjected to reverse phase HPLC for general profiling, size exclusion HPLC for identification of large proteins, Edman degradation-based peptide sequencing to determine the peptide length distribution and Nano-LC LTQ FTMS to identify the peptide sequences in the digest samples. Additionally, ileal effluents of the digested alpha-lactalbumin were introduced into an in vitro model of the large intestine (TIM-2) to assess their impact on fecal bifidobacteria populations, which were assessed via a microbiota array chip composed of 16S rRNA-directed oligonucleotides targeted to select members of the microbiota.

**Results:** Digestion of \( \alpha \)-lactalbumin was progressively more extensive as the protein continued through the gastrointestinal tract model. Quantitative analysis of samples from the gastric compartment demonstrated that only a small amount of digestion had occurred. In the duodenal compartment, 77% of the protein was composed of peptides less than 17 amino acids; proteomics data revealed that 148 unique peptides were present. Quantitative data from jejunal compartment revealed that 82% of protein was composed of 79 unique peptides. Quantitative data on protein collected in the ileum revealed that 86% of the protein fraction was composed of peptides smaller than than 16 AA; a total of 61 unique peptides, of which 23 were specifically derived from \( \alpha \)-lactalbumin, were detected in the ileum. The majority of ileal peptides derived from \( \alpha \)-lactalbumin were related to the calcium-binding loop of the intact protein. Further, \( \alpha \)-lactalbumin-derived peptides from the ileal compartment induced a large increase in the signal for bifidobacteria while specifically stimulating \( B. \) adenescens, \( B. \) bifidum, \( B. \) breve, and \( B. \) catenulatum.

**Conclusions:** Work in this in vitro model demonstrates that a large number and significant diversity of peptides are generated from the digestion of \( \alpha \)-lactalbumin. Further, these peptides have a stimulatory effect on the growth of bifidobacteria in an in vitro model of the gastrointestinal tract. These findings provide mechanistic support for previous clinical observations where infants fed an \( \alpha \)-lactalbumin-enriched formula have fecal concentrations of bifidobacteria that are similar to human milk-fed infants.

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**Plenary Session 4:**

**Gastroenterology**

**OP4-01**

**INTESTINAL MICROBIOTA IN COELIAC DISEASE PATHOGENESIS**

**Presenter:** Y. Sanz. Institute of Agrochemistry and Food Technology (IATA), Spanish National Research Council (CSIC), Burjassot-Valencia, Spain.
Co-authors: Y. Sanz1, G. De Palma1, M. Medina1, I. Nadal1, E. Donat2, C. Ribes-Koninckx2, M. Calabuig3, 1Microbial Ecophysiology and Nutrition Group, Institute of Agrochemistry and Food Technology (IATA), Spanish National Research Council (CSIC), Valencia, Spain; 2Hospital Universitario La Fe, Valencia, Spain; 3Hospital General Universitario, Valencia, Spain.

Aim: To evaluate the possible associations between intestinal dysbiosis and the presentation of coeliac disease (CD), and the potential role of specific bacteria in the inflammatory process associated with this disorder.

Methods: Altogether 64 children were included in the study: 26 untreated CD patients (mean age 5.5 years, range 2.1–12.0 years) on a normal gluten-containing diet, 18 symptom-free coeliac disease (SFCD) patients (mean age 5.5 years, range 1.0–12.3 years) treated with a gluten-free diet for at least 2 years, and 20 healthy children (mean age 5.5 years, range 1.8–10.8 years) without known food intolerance. Microbiological analyses of faecal samples were carried out by using fluorescent in situ hybridization and flow cytometry techniques. Immunoglobulin-coated bacteria were detected using fluorescent-labelled antibodies by flow cytometry (FCM). The contribution of the faecal microbiota of CD patients to the pro-inflammatory milieu characteristic of CD and the possible benefits of Bifidobacterium strains were evaluated by analysing cytokine production and cell surface antigen expression in peripheral blood mononuclear cells (PBMCs) after stimulation with faecal samples and selected Bifidobacterium strains. Cytokines were analysed by ELISA and cell surface antigen expression by using fluorescent-conjugated antibodies and flow cytometry.

Results: IgA-coated faecal bacterial levels were significantly lower in both untreated and treated SFCD patients than in healthy controls. IgG and IgM-coated bacterial levels were also significantly lower in treated CD patients than in untreated CD patients and controls. Gram-positive to Gram-negative bacteria ratio was significantly reduced in untreated CD patients and in treated SFCD patients compared to controls. Bifidobacterium and Faecalibacterium prausnitzii groups were less abundant (P < 0.050) in untreated CD patients than in healthy controls. In contrast, Bacteroides-Prevotella group was more abundant (P < 0.050) in untreated CD patients than in controls. Faeces of both active CD and SFCD patients, representing an imbalanced microbiota, significantly increased TNF-α production and CD86 expression in PBMCs, while decreased IL-10 cytokine production and CD4 expression compared with control samples. Active CD-patient samples also induced significantly higher IFN-γ production compared with controls. However, Bifidobacterium strains suppressed the pro-inflammatory cytokine pattern induced by the large intestinal content of CD patients and increased IL-10 production.

Conclusions: CD is associated with intestinal dysbiosis and reductions in IgA-coated bacterial levels. Moreover, the intestinal microbiota of CD patients could contribute to the Th1 pro-inflammatory milieu characteristic of the disease, while B longum ES1 and B bifidum ES2 could reverse these deleterious effects. These findings provide new insights into the possible role of the intestinal microbiota in CD pathogenesis and hold future perspectives of interest in the disease therapy.

OP4-02

SMALL INTESTINE CONTRAST ULTRASONOGRAPHY (SICUS): AN ALTERNATIVE TO RADIOLOGY IN THE ASSESSMENT OF SMALL BOWEL IN PEDIATRIC PATIENTS WITH CROHN DISEASE

Presenter: F. Civitelli. Sapienza University, Rome, Italy.
Co-authors: N. Pallotta2, F. Viola1, E. Romeo1, G. Di Nardo1, O. Borrelli1, N. Abdulkadir Hassan2, G. Vincoli2, M. Carabotti2, B. Ciccantelli2, N. Cavallari1, M. Barbato1, E. Corazziari2, S. Cucchiara1, 1Pediatric Gastroenterology and Liver Unit, Sapienza University, Rome, Italy; 2Clinical Science Department, Sapienza University, Rome, Italy.

Aim: Radiological assessment, with either small bowel follow-through (SBFT) or enema, is the reference standard in Crohn disease (CD) for diagnosis of small bowel (SB) lesions. However radiation exposure limits the use of conventional radiology in the follow-up of CD patients. In adults SICUS, performed after distension of SB lumen with a macrogol solution is comparable to radiological examination in detecting presence, extension and site(s) of SB lesions (Lancet 1999;353(9157):985–6; Inflamm Bowel Dis. 2005;11:146–53). We evaluate the diagnostic accuracy of SICUS in pediatric patients with known or suspected CD.

Methods: Twenty-seven consecutive patients (F 12; age range 11–24 years) 21 with CD (6 previously submitted to surgery) and 6 with suspected CD were evaluated after the ingestion of 375 mL of macrogol solution. SICUS findings were compared with those of ileocolonscopy, SBFT, wireless capsule endoscopy (WCE), and surgery. SICUS was performed by a sonologist, unaware of radiological and endoscopic findings.

Results: SICUS was well tolerated by all patients. In undiagnosed patients CD lesions were detected at SICUS in 5/6 confirmed at endoscopy, at SBFT and/or WCE. In 1 patient with a final diagnosis of IBS no lesion was found at SICUS, endoscopy and WCE. According to Montreal classification CD patients showed 11 B1, 10 B2 and 5 B3 (2 B3p) behavior. 20 patients presented ileocolonic (L3) location, 7 also with upper involvement (L4), 5 patients ileo-terminal location (L1), 2 with L4 and 3 patients
Conclusions: Our data suggest that the noninvasive, radiation-free procedure SICUS is highly accurate for diagnosing CD lesions of the SB; it is comparable to endoscopic and radiological examination in detecting presence and site of SB lesions and furthermore enables to assess their extension. These findings support the use of SICUS as a first choice examination in the diagnostic workup and follow-up of pediatric patients with CD.

OP4-03

A-GLIADIN PEPTIDE P31-43 INTERFERES WITH HRS LOCALIZATION TO ENDOCYTIC VESICLES


Co-authors: M. Barone1, M. Nanayakkara1, M. Maglio1, D. Zanzi1, S. Santagata1, G. Lania1, R. Auricchio1, R. Troncone1, S. Auricchio1. 1University of Naples “Federico II,” Naples, Italy.

Background and Aim: We previously observed that gliadin peptide P31-43 induces effects similar to epidermal growth factor (EGF) in cultured cell lines and in enterocytes from celiac disease (CD) patients, and that these effects are mediated by delayed EGF degradation and prolonged EGF receptor (EGFR) activation in endocytic vesicles. The aim of this study was to investigate the molecular mechanisms underlying the effects of gliadin peptides on trafficking and maturation of vesicles responsible for EGFR endocytosis.

Methods: Western blot and immunofluorescence microscopy were used to determine Hrs localization to endocytic vesicles and cytosol. We used pulse and chase labeling in time-lapse experiments to monitor uptake and subcellular localization of gliadin peptides in CaCo-2 cells and enterocytes from CD patients and controls.

Results: A sequence similarity search revealed that P31-43 is strikingly similar to a region of Hrs, a key molecule involved in endocytic maturation. A-gliadin peptide P31-43 interfered with Hrs localization to early endosomes. Both P31-43 and the control P56-68 peptide entered CaCo-2 cells and interacted with the endocytic compartment, but P31-43 was localized to vesicles carrying early endocytic markers at time points when P56-68-carrying vesicles matured into late endosomes. P31-43-labelled vesicles were delayed, irrespective of the cargo they carry: dextran-containing vesicles, behaved similarly to EGFR-containing vesicles. Transferrin receptors and Lamp, markers of recycling pathway, were increased on the surface of P31-43-treated cells.

Conclusions: P31-43 delays vesicle trafficking by interfering with Hrs-mediated maturation to late endosomes and promotes the recycling pathway. Consequently, in P31-43-treated cells, EGFR activation is extended and more transferrin receptor finds its way to the surface. This may explain the role played by gliadin peptides in CD.

OP4-04

INTESTINAL T-CELL RESPONSE TO GLUTEN IN PATIENTS WITH TYPE 1 DIABETES ARE DETECTABLE ONLY IN THE PRESENCE OF CELIAC DISEASE–ASSOCIATED AUTOANTIBODIES

Presenter: C. Gianfrani. Institute of Food Sciences-CNR, Avellino, Italy.

Co-authors: C. Gianfrani1, A. Camarca1, M. Maglio2, R. Valentino1, R. Auricchio1, A. Franzese2, R. Troncone2, 1ISA-CNR, Avellino, Italy; 2Department of Pediatrics & ELFID, University of Naples “Federico II,” Naples, Italy; 3Institute of Experimental Endocrinology and Oncology-G. Salvatore-CNR, Naples, Italy.

Background and Aim: Type 1 diabetes (T1D) and celiac disease (CD) are two strongly associated immunological disorders. Different studies have suggested an implication of GALT and of dietary components, such as wheat gluten and cow’s milk proteins, in the pathogenesis of type 1 diabetes. We have previously shown that gliadin, the major gluten protein, is able to elicit an inflammatory response in the in vitro cultured jejunal mucosa of T1D patients. Herein we investigated the T-cell mediated reactivity to gliadin in the intestinal mucosa of T1D in the presence or absence of CD.

Methods: Sixteen T1D patients were enrolled (mean age 11 years, range 1–24); of these, 5 were negative for CD-associated autoantibodies (EMA/tTG-IgA), and had a normal mucosa histology; 6 had potential CD (ie, EMA/tTG-IgA positive serology and normal intestinal mucosa), and 5 were EMA/tTG-IgA positive and had villous atrophy. Furthermore, 6 adult CD patients with no T1D were also enrolled in this study (24 years, range 18–34). Mucosal explants were processed for generation of gliadin-specific T-cell lines (iTCLs), and gliadin-specificity was assessed by both IFN-γ-ELISPOT and cell
proliferation. We also investigated whether regulatory pathways might control the adaptive response to gliadin in T1D patients by using antibodies neutralizing IL-10 and TGF-β.

**Results:** No IFN-γ-secreting T-cells or cell proliferation to gliadin were observed in iTCLs from T1D without CD, neither in the presence of anti-IL-10R or anti-TGF-β MoAbs. By contrast, five of six iTCLs from T1D with potential CD were responsive to gliadin: (average of IFN-γ-secreting forming cells/10^6 cells (SFC): 1992 ± 1291); surprisingly, in the remaining patient the gliadin-specific T-cells were evident only in the presence of neutralizing MoAbs. Gliadin-reactive T-cell lines were obtained from all T1D patients with active CD, and the frequency of these cells (SFC: 3246 ± 4280) was not significantly different from that of T1D patients with potential CD (P > 0.5), or of CD patients without T1D (SFC: 3046 ± 1396, N = 6, P = 0.25).

**Conclusions:** Gliadin-reactive T-cells were detected only in the intestinal mucosa of T1D patients that developed an anti-tTG autoimmune response, either with active or potential CD. No IL-10 and/or TGFbeta-dependent regulation of T-cell response to gliadin were observed in T1D mucosa in the absence of CD. In conclusion, our data do not support the hypothesis of a role of adaptive immunity to dietary gluten in T1D pathogenesis; notwithstanding, an innate non-T-mediated immune response might be involved in the gliadin-induced inflammatory response observed in the small intestine of T1D patients without CD.

**OP4-05**

**IMPACT OF THE ROME II PEDIATRIC CRITERIA ON THE APPROPRIATESNESS OF UPPER AND LOWER GASTROINTESTINAL ENDOSCOPY IN CHILDREN**


Co-authors: E. Giannetti1, M. Martinelli1, G. Boccia1, L. Greco1, A. Staiano1. 1Department of Pediatrics, University of Naples “Federico II,” Naples, Italy.

**Background and Aim:** The Rome II pediatric criteria for functional gastrointestinal disorders (FGIDs) were created in 1999 as diagnostic tools and as a way to advance empirical research, providing clinicians with a positive approach to treating patient. The demand for pediatric gastrointestinal endoscopy has been increased in most developed countries, resulting in an important rise in overall costs for endoscopic procedures. The aim of our study was to assess the clinical impact of the Rome II pediatric criteria for FGIDs in selecting pediatric patients who underwent to upper and/or lower gastrointestinal endoscopy.

**Methods:** A total of 1624 consecutive children (732 boys and 892 girls; mean age: 7.4 years, range: 2 months – 18 years), referred to our pediatric unit for GI endoscopy from January 1998 to December 2006 were retrospectively evaluated. Indications and findings of GI endoscopic procedures performed before the institution of the Rome II pediatric criteria were compared with those procedures performed under the criteria. Upper and lower GI endoscopies not showing direct therapeutic or prognostic consequences were classified as “negative.”

**Results:** Upper GI endoscopy was performed in 1124 children, whereas 500 subjects underwent to colonoscopy. After the institution of the Rome II criteria, the mean rate of the upper GI endoscopy performed for celiac disease was significantly higher (42.9% vs 21.4%; 95% CI: −30.1 to −12.7; P = 0.004); no significant difference was observed in the mean rate of the procedures performed for ulcer peptic disease (21.7% vs 20.6%; 95% CI: −11.5 to 13.6; P = 0.84); whereas a significant decrease of the mean rate of negative upper GI endoscopy was found (78.5% vs 36.4%; 95% CI: 28.8 to 55.2; P = 0.001). A significant increase of the mean rate of lower GI endoscopy for inflammatory bowel disease was observed when performed under the Rome II criteria (22.9% vs 63.9%; 95% CI: −58.6 to −23.2; P = 0.001); furthermore, we found a significant decrease of the mean rate of lower endoscopy performed for GI polyps (18.5 vs 7.3%; 95% CI: 0.0 to 22.4; P = 0.05), as well as a significant decrease of the mean rate of negative colonoscopies (77.0% vs 28.7%; 95% CI: 31.7 to 64.7; P = 0.001).

**Conclusions:** This is the first pediatric, retrospective, observational study, evaluating the impact of the Rome II pediatric criteria on the appropriateness of GI endoscopy. This study highlights that the use of the criteria for FGIDs makes a significant positive impact in reducing the unnecessary GI endoscopic procedures, improving the diagnostic yield and the cost-effectiveness of the pediatric endoscopy.

**OP4-06**

**ENTEROPATHOGENIC ESCHERICHIA COLI MODULATION OF α- AND β-DEFENSIN ANTIMICROBIAL PEPTIDE EXPRESSION IN HUMAN INTESTINAL ORGAN CULTURE**


Co-authors: M. Lucas1, J. Kaper2. 1Royal Free Hospital, London, UK; 2University of Maryland School of Medicine, Baltimore, MD, USA.

**Aim:** To characterise the transcription of the Paneth cell specific α-defensins (AD5 and AD6) and the transcription and secretion of enterocyte specific β-defensins (BD1, BD2, BD3 and BD4) during ex vivo infection of duodenal biopsies with enteropathogenic Escherichia coli (EPEC).
**Methods:** Matched duodenal biopsies were inoculated with a) EPEC strain E69, b) a type three secretion system (T3SS) deficient mutant (E69ΔespB) and c) left uninfected. In vitro organ culture (IVOC) was performed for 6, 8, or 12 hours, and real-time PCR was used to measure gene expression. BD2 protein expression in IVOC supernatants was measured by ELISA following antibiotic treatment at 8 hours and a further 16-hour incubation. Semi-thin (1 μm) sections of resin embedded tissue were used to examine tissue preservation in 12 hour IVOC samples.

**Results:** There was no difference in mRNA expression of BD1, BD3 and BD4 in E69 infected biopsies relative to uninfected controls at any time point. In E69 and E69ΔespB infected biopsies BD2 mRNA expression versus uninfected controls was induced at 8 hours (mean 8.5 fold induction, n = 10, P < 0.05) and 12 hours (mean 10 fold induction, n = 14, P < 0.05). After 24 hours the BD2 peptide concentration in IVOC supernatants from E69 and E69ΔespB infected biopsies (mean 243 pg mL⁻¹ and 282 pg mL⁻¹, respectively, n = 6) was higher (P < 0.01) than from uninfected biopsies (54 pg mL⁻¹, n = 6). In contrast to BD2 mRNA expression at 12 hours, E69 infected biopsies showed a 3-fold decrease in expression for AD5 (P < 0.05, n = 14) and a 4-fold decrease in expression for AD6 (P < 0.05, n = 14) relative to both E69ΔespB infected biopsies and uninfected controls. Expression of the Paneth cell–specific trypsin gene was the same in all samples (n = 10); semithin resin embedded sections (n = 4) showed no differences in Paneth cell numbers and crypt morphology between infected and uninfected samples.

**Conclusions:** Ex vivo human intestinal infection with E69 produces a T3SS-independent secretion of BD2. Further work will be carried out to determine which bacterial ligand(s) induce BD2 secretion. In contrast, AD5 and AD6 expression was suppressed by E69 infection in a T3SS-dependent manner. Thus, EPEC appears able to actively down regulate Paneth cell α-defensin expression during infection of human tissue, which may contribute to the ability of this organism to colonise the small intestinal tract.

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**Poster Session 4**

**Gastroenterology: Coeliac Disease**

**PG4-01**

**THE INCIDENCE OF CHILDHOOD COELIAC DISEASE CONTINUES TO RISE IN SOUTHEAST SCOTLAND**

Poster: D. Basude. Bristol Hospital for Sick Children, Bristol, UK.

Co-authors: P. Gillett, C. Bradshaw, R. Russell, D. Wilson. ¹Royal Hospital for Sick Children, Edinburgh, UK; ²Royal Hospital for Sick Children, Glasgow, UK.

**Aim:** We aimed to identify the incidence of childhood coeliac disease in southeast Scotland from 1990 to 2006, including the incidence of classical disease in the under–2-year-old.

**Methods:** A retrospective case note review was undertaken of all the children under 16 years at the initial diagnosis of coeliac disease residing in the southeast of Scotland from 01/01/1990 to 31/12/2006 (17 years). All patients were identified using a combination of a departmental database, pathology records, coeliac serology database, hospital records and endoscopy logbooks. A diagnosis of coeliac disease was based on the revised ESPGHAN criteria. Population data were obtained from the Registrar General for Scotland.

**Results:** 173 patients met the diagnostic criteria for the study period. The population for Scotland and the south-east remained relatively stable over the period with only a net gain of 1% from migration. The average incidence rates for 1990–94, 1995–99, 2000–04 and 2005–06 were 1.81, 4.3, 6.27, and 6.09 per 100,000 childhood population, respectively (P < 0.05 comparing 1.81 with 6.09). The incidence of classic coeliac disease remained stable. The median age at diagnosis for the 4 periods in years were 2.0, 3.8, 5.9, and 7.6, respectively. The point prevalence for the condition in the childhood population was 49.9 per 100,000 at the end of 2004.

**Conclusions:** The incidence of coeliac disease has risen significantly in the childhood population of southeast Scotland from 1990 to 2006. There does not appear to be any decrease in the incidence of classic coeliac disease but a certain rise in the median age at diagnosis. This rise is probably due to the increased screening of at-risk populations and heightened awareness of the condition in the primary and secondary care services.

**PG4-02**

**CELL SHAPE AND ACTIN REARRANGEMENTS CAN DISCRIMINATE BETWEEN CELIAC PATIENTS AND CONTROLS DENDRITIC CELLS**


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**Aim:** Characterize the cell shape and actin rearrangements in the dendritic cells from patients with coeliac disease (CD) and controls.

**Methods:** Flow cytometry was used to assess the cell shape and then the actin rearrangements were measured by fluorescence recovery after photobleaching (FRAP).

**Results:** A significant higher percentage of coeliac patients' dendritic cells had a rounder (2.5 fold increase, n = 20, P < 0.05) and lower (2 fold decrease, n = 20, P < 0.05) percentage of dendritic cell density relative to healthy controls. The mean FRAP values for the coeliac patient group were 0.35 ± 0.04 (n = 6), while the healthy control group was 0.65 ± 0.04 (n = 6). The mean FRAP values for the patients with classical CD were 0.30 ± 0.05 (n = 4), while the healthy control group was 0.67 ± 0.05 (n = 4). The mean FRAP values for the patients with atypical CD were 0.35 ± 0.05 (n = 2), while the healthy control group was 0.70 ± 0.05 (n = 2).

**Conclusions:** Dendritic cells from patients with coeliac disease have a different cell shape and actin rearrangements compared to healthy controls. This difference may be useful in the diagnosis and monitoring of coeliac disease.
Aim: The cell shape in normal and celiac disease (CD) dendritic cells (DC) and their ability to interact with a substrate was investigated. In particular, the morphology of DC and actin rearrangement when in contact with fibronectin, a physiological substrate for DC, was analyzed.

Methods: DC were used from controls and both active CD and remission CD patients. Immature DC were generated by extracting monocytes from peripheral blood and stimulating them with IL-4 and GM-CSF for 6 days. Cell shape and adhesion was determined by crystal violet staining. The shape was analyzed by counting the cells that present, or not, more than 3 small dendrites and/or 1 long dendrite. DC from 6 random photos were counted from at least 5 independent experiments per group. Confocal microscopy of phalloidin staining was used to highlight the actin cytoskeleton.

Results: DC of CD patients have a different shape when interacting with the substrate compared to control cells. After 1 and 3 hours of incubation on fibronectin 68% and 73%, respectively, of the DC of patients present more than 3 small and/or 1 long dendrite, compared to 46% and 37%, respectively, in the controls. The DC are HLA-DR-low and CD1a positive, CD3 and CD14 negative suggesting that, only DC adhere to fibronectin (and that the DC of the celiac disease patients and controls are in the same stage of maturation). The alteration of the shape is observed in patients in the active as well in the remission fase of the disease.

Conclusions: These results suggest that in CD patients genetic background seems to influence DC shape and rearrangement of actin cytoskeleton more than inflammatory state.

PG4-03

ANDERSON DISEASE: INVESTIGATIONS IN 2 NEW FAMILIES AND 1 UNDESCRIBED MUTATION

Presenter: A. Georges. Children’s Hospital Brabois, Nancy, France.

Co-authors: M. Varret1, D. Rousselot3, J. Champagneull1, J. Guedenet1, M. Abifadel2, J. Rabeș2, E. Bruckert1, C. Boileau1, M. Bouma2, A. Morali1. 1CHU Brabois, Nancy, France; 2INSERM U781, Paris, France; 3CHU La Pitié Salpêtrière, Paris, France.

Aim: Anderson disease (or chylomicron retention disease) is a rare autosomic recessive disorder characterized by absence of post-prandial chylomicrons and apolipoprotein B48 in sera from affected patients. Molecular basis for this chylomicron secretory defect has been identified in 2003. Mutation in SARA2 gene encoding for Sar1b protein disturbs vesicular transport of lipoproteins from endoplasmic reticulum to Golgi apparatus. To own knowledge, 11 mutations have been described in patients with Anderson’s disease. Aim of this work is to report two new families with mutation in SARA2 gene. One of them carries an undescribed mutation.

Methods: “Zohra” is a Moroccan girl from unrelated parents. Celil and Pinar are brother and sister from Turkish consanguineous parents. In first months of life, they were suffering from diarrhea and failure to thrive. Blood analysis revealed very low cholesterol (<1 g/L) and apoA1 (0.7 g/L) levels whereas triglycerides and apoB levels were normal. Vitamin E (< 3 mg/L) but not vitamin A levels were very low. An abnormal steatorrhea was only seen in Pinar (5 g/day when 2 months old). Liposoluble vitamins supplementation was early begun and neuro-ophthalmologic complications were limited. Low-fat diet allowed normalization of digestive symptoms but Zohra’s growth curve remained under normal range and she presented fat “intolerance” with self-limitation. Despite no strict adherence to low fat diet, Celil and Pinar had normal growth. Based on SARA2 multigene expression, complementary studies were realized, showing a slight increase for CK (2*N), but an absence of hepatic steatosis and a normal US cardiac examination. To confirm genetic hypcholesterolemia, standardized lipid load, digestive endoscopy with ultrastructural analysis and SARA2 genetic studies were performed.

Results: The absence of postprandial chylomicrons in sera was confirmed by an oral fat load. In fasting state, video endoscopy showed a typical “white hoary frosting” aspect at duodenal level. Ultrastructural analysis of intestinal biopsies exhibited fat loaded enterocytes with both lipid droplets and lipoprotein-like particles. Using SARA2 gene sequencing, 2 homozygous mutations could be described in exon 3 leading to, respectively, a 83% and 68% truncation; one new deletion c.142delG-p.Asp48 ThrfsX64 was observed in Celil and Pinar.

Conclusions: Three new cases of Anderson disease are reported and one new mutation in SARA2 gene is described. This study is a good example for diagnosis difficulties. A large phenotypic heterogeneity could be observed, emphasizing interest for genotype-phenotype correlations.

PG4-04

CYTOKINES, MATRIX METALLOPROTEASES, AND FOXP-3 IN SITU EXPRESSION IN LATENT CELIAC DISEASE

Presenter: M. Ben Hariz. Mongi SLIM Hospital, Tunis, Tunisia.

Co-authors: M. Kallel-Sellami1, M. Ben Ahmed2, L. Laadhar1, A. Maherzi1, S. Makni1. 1La Rabta Hospital, Tunisia; 2CHU Ibn Sina, Tunis, Tunisia.
Tunis, Tunisia; 2 Pasteur Institute, Tunis, Tunisia; 3 Mongi
SLIM Hospital, Tunis, Tunisia.

Aim: Cytokine expression in intestinal biopsies of CD patients has been widely studied. Most studies concerned symptomatic CD patients with both positive serology and profound villous atrophy. However, few data are available about cytokine in situ expression in latent CD diagnosed in screening programs. The aim of the study is to assess the expression of IFN-γ, TNF-α, IL-10, matrix metalloproteases (MMP)-3, MMP-12 and FOXP-3 in intestinal biopsies from latent CD patients compared to active forms.

Methods: This study included 43 schoolchildren, aged 9 to 12 years old, who participated in a CD mass screening study: group 1: 25 children with symptomatic and active CD, group 2: 5 children with asymptomatic latent CD (positive IgA anti-endomysium and anti-transglutaminase antibodies) and a normal intestinal histology, and group 3: 23 children with positive IgA anti-transglutaminase antibodies, a negative anti-endomysium antibodies and a normal histology.

Results and Discussion: A significant increase in INF-γ, MMP-3 and MMP-12 expression was found in CD active children compared to the two other groups. These data confirm the central role of IFN-γ in CD pathogenesis by sustaining a Th-1 response in intestinal mucosa. In the latent group, no significant increase of these mediators was seen which is in line with the absence of intestinal damage. IL-10 and FOXP-3, involved in feedback-nega-
tive regulation was also not upregulated. These data do not agree with a role of IL-10 and regulatory T cells in maintaining homeostasis in latent CD patients. Further investigations focusing on a second immunosuppressive cytokine i.e. TGF-β are needed to elucidate the exact mechanisms accounting for the absence of an inflammatory response in spite of autoantibodies production.

PG4-06

CELIAC CHILDREN LESS THAN 2 YEARS OLD: SENSITIVITY AND SPECIFICITY OF ANTIBODIES TO DEAMIDATED GLIADIN PEPTIDES

Presenter: M. Barbato, Sapienza University of Rome, Rome, Italy.

Background and Aim: Recently, a new generation of antibodies has been developed to deamidated gliadin peptides (aDGP), which have shown an interesting performance with high sensitivity and specificity. Actually in literature study population for aDGP assays included predominantly adult patients with suspected celiac disease (CD). In these studies specificity and sensitivity were, respectively, about 83.6% and 90.3% for IgA and 84.4% and 98.5% for IgG. The aim of the study was to value the sensitivity and the specificity of aDGP assay for CD screening in children less than 2 years. We enrolled 143 children less than 2 years with normal values of serum IgA: 43 with CD, diagnosed according to ESP-GLAN criteria and 100 healthy children. In all children we tested IgA and IgG AGA, IgA anti-endomysial (EMA), IgA anti-tissue transglutaminase (anti-tTG), IgA, and IgG aDGP antibodies.

Methods: IgA and IgG AGA, anti-tTG were tested by an enzyme immunoassay, IgA and IgG EMA were tested by
indirect immunofluorescence assay (kit by Eurospital SpA, Trieste, Italy). IgA and IgG aDGP were tested by an enzyme-linked immunoabsorbent assay (Kit Inova Diagnostics). All patients underwent upper endoscopy and small intestinal biopsy; duodenal biopsy specimens were graded according to Marsh’s classification, modified from Oberhuber.

**Results:** 29/43 of celiac children had positive IgA AGA and 43/43 IgG AGA, 41/43 IgA EMA, 41/43 IgA anti-tTG, 31/43 IgA aDGP and 41/43 IgG aDGP (Table 12); 12/100 of healthy children had positive IgA AGA and 44/100 IgG AGA, all had IgA anti-tTG, IgA EMA and IgG aDGP negative, 3/100 IgA aDGP positive (Table 12). Sensitivity of IgA AGA 67.5% IgG AGA 100% IgA EMA 93% IgA anti-tTG 95% IgA aDGP 72% IgG aDGP 95% and specificity IgA AGA 88% IgG AGA 56% IgA EMA 100% IgA anti-tTG 100% IgA aDGP 96% IgG aDGP 100% (Table 12).

**Conclusions:** IgG aDGP assay seems to have the same high specificity and sensitivity than IgA anti-tTG, and a bit more sensitivity than IgA EMA for screening of CD in children less than 2 years (Table 12). However the last two tests, are reported sometimes negative in very young children as EMA in 2 of 38 our CD. In conclusion, aDGP test, especially of IgG class seems useful in very young children with suspected celiac disease.

**TABLE 12. Results**

<table>
<thead>
<tr>
<th></th>
<th>AGA</th>
<th>AGA</th>
<th>aDGP</th>
<th>aDGP</th>
<th>Anti-tTG</th>
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<tr>
<td></td>
<td>IgA</td>
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<tr>
<td>43 Celiac patients</td>
<td>29</td>
<td>43</td>
<td>31</td>
<td>41</td>
<td>41</td>
<td>40</td>
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<tr>
<td>100 Healthy patients</td>
<td>12</td>
<td>44</td>
<td>4</td>
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<td>0</td>
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<tr>
<td>Specificity, %</td>
<td>88</td>
<td>56</td>
<td>96</td>
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<td>Sensitivity, %</td>
<td>67.5</td>
<td>100</td>
<td>72</td>
<td>95</td>
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**PG4-07**

**PSYCHE AND COMPLIANCE WITH GLUTEN-FREE DIET: THE ROLE OF LOCUS OF CONTROL**

Presenter: C. Zanchi. *University of Trieste, Trieste, Italy*. Co-authors: A. Bellini1, S. Martelossi1, M. Montico1, G. Di Leo1, T. Not1, A. Ventura1. 1IRCCS Burlo Garofolo, Trieste, Italy.

**Background and Aim:** The psychological construct of locus of control (LoC) esteems the degree to which an individual perceives events that happen to him as result of his own behaviour (internal LoC) or of luck, chance, and powerful others (external LoC). In literature, chronic disease is associated to an external LoC, which leads to an unsatisfactory control of therapy. The aims of the study were to verify if celiacs show a more external LoC in comparison to healthy controls, and evaluate if an external LoC interferes with the compliance to gluten-free diet (GFD) and quality of life.

**Methods:** We evaluated 151 celiacs on GFD from at least 1 year (105 f, mean age 10.4 years, range 6–16 years) and 353 healthy controls (202 f, mean age 12 years, range 6–16 years). All subjects were administrated tests on the Nowicki-Strickland LoC scales to evaluate the degree of externality of LoC; celiacs fill in also the Kindl Test, to measure compliance to GFD and quality of life.

**Results:** There is no difference in LoC scores between younger celiac subjects, aged 6–8 years (Primary School Nowicki-Strickland LoC scales) or older celiac subjects, aged 9–16 years (Nowicki-Strickland LoC Scales for Children), and healthy controls (younger coeliac subjects 4.88±1.86 vs younger healthy subjects 4.64±1.7; older celiac subjects 13.18±4.32 vs older healthy subjects 13.45±4.25). Celiacs who consider to have a satisfactory quality of life, have a more internal LoC than celiacs who believe their lives are negatively affected (P = 0.011). Furthermore, celiacs with a good compliance with GDF show a more internal LoC than celiacs who are not compliant (P = 0.014).

**Conclusions:** Unlike children affected by other chronic diseases, celiacs do not have a more external LoC if compared to their healthy counterparts. Probably, this internality corresponds to high degree of personal responsibility for the treatment of the disease (GDF). Specific metacognitive trainings could improve internality, compliance to GFD and quality of life of celiacs.

**PG4-08**

**FECAL CALPROTECTIN CONCENTRATIONS IN CHILDREN WITH UNTREATED COELIAC DISEASE**

Presenter: N. Balamtekin. *Hacettepe University, Ankara, Turkey*. Co-authors: H. Demir1, N. Uslu1, G. Demir1, D. Orhan2, Z Akçören2, A. YUCE1. 1Hacettepe University, Faculty of Medicine, Department of Pediatrics, Section of Gastroenterology, Hepatology and Nutrition, Ankara, Turkey; 2Hacettepe University, Faculty of Medicine, Department of Pediatrics, Section of Pathology, Ankara, Turkey.

**Aim:** The aim of this study was to investigate fecal calprotectin concentrations (FCC) in children with newly diagnosed celiac disease (CD) and to determine the relation between FCC levels and clinical presentation, severity of histopathological injury, and infiltration of neutrophils in the small bowel mucosa.

**Methods:** The study included 26 children with newly diagnosed CD as a study group and 31 healthy children as...
a control group. The presenting signs and symptoms of celiac associated conditions in all the patients were reported. The patient group was divided into 2 categories based upon the clinical presentations as with gastrointestinal symptoms and with nongastrointestinal symptoms. Moreover, all the subjects in both study and control groups were evaluated for the diseases and conditions which effects of the levels of FCC such as inflammatory bowel diseases and the ones with these diseases and conditions were excluded. Serum IgA levels, tissue transglutaminase antibodies IgA and endomysial antibodies IgA were measured both study and control groups. The fecal samples were obtained before upper endoscopy in patients and were stored at −20°C and FCC levels were measured with ELISA method. Upper endoscopies were performed and at least four biopsy samples were obtained from proximal small bowel mucosa of the patients. Histopathological diagnosis was made according to modified Marsh criteria. CD was diagnosed based on the revised criteria of ESPGHAN.

**Results:** 57 children (26 with celiac and 31 healthy control) were included in the study. Female/male ratios were 17/9 for celiac and 16/15 for healthy control group. Female/male ratios were 1.8 years. Median FCC concentration was significantly higher in celiac disease patients [121.7 (8.7–306)] with respect to control group [7.5 (range 1–70)] (P < 0.001). Fecal calprotectin concentration was higher in patients with Marsh 3b and 3c with respect to Marsh 3a, 2 and 1 (142.1 ± 80.6 vs 109.0 ± 96.2). Patients presenting with gastrointestinal symptoms had higher FCC concentration compared to patients with nongastrointestinal symptoms (141.4 ± 95.6 vs 96.9 ± 74.1). Presence of neutrophilic infiltration in the biopsy samples did not affect the FCC concentration. FCC was not related to gender, age, weight or height.

**Conclusions:** Fecal calprotectin concentrations are higher in children with newly diagnosed CD. This test may be used as a noninvasive biomarker for screening of CD.

**PG4-09**

**IN DIAGNOSING CELIAC DISEASE IN CHILDREN, SMALL BOWEL BIOPSY BY ENDOSCOPY IS MORE RELIABLE THAN BY SUCTION CAPSULE: EXPERIENCE FROM A SCREENING STUDY**

**Presenter:** O. Sandström. **Umeå University, Umeå, Sweden.**

**Co-authors:** O. Sandström1, C. Webb2, A. Carlsson2, L. Danielsson3, B. Halvarsson4, A. Ivarsson3, E. Karlsson6, F. Norström5, L. Stenhammar7, L. Högberg2, 1Umeå University, Department of Clinical Science, Pediatrics, Umeå, Sweden; 2Lund University, Department of Pediatrics, Lund, Sweden; 3Norrålo Hospital, Pediatric Outpatient Clinic, Norrtälje, Sweden; 4University Hospital Malmö, Department of Clinical Pathology and Cytology, Malmö, Sweden; 5Umeå University, Department of Public Health and Clinical Medicine, Epidemiology, Umeå, Sweden; 6Växjö Hospital, Pediatric Clinic, Växjö, Sweden; 7Linköping University, Department of Clinical and Molecular Medicine, Pediatrics, Linköping, Sweden.

**Aim:** To study the distribution of the celiac disease (CD) enteropathy in proximal and distal duodenum, and to compare biopsy by suction capsule and endoscopy as diagnostic procedure.

**Methods:** A school-based CD screening study involving 5 Swedish centers, with 10,041 invited 12-year-olds, and 7567 consenting participation. Elevated serological markers were found in 192 children who were recommended to undergo small bowel biopsy. Biopsies were performed either with suction capsule or by endoscopy according to local clinical routine. Histopathological preparation and evaluation initially took place at the local pathology laboratory, and thereafter all mucosal specimens were reevaluated by an expert pathologist. When there was diagnostic divergence, specimens were further evaluated by a second expert pathologist and diagnostic consensus reached.

**Results:** Small bowel biopsies were performed in 183 children; 129 by endoscopy and 54 by suction capsule. Sixteen children with normal biopsies, 4 after endoscopy and 12 after suction capsule, were later re-biopsied by endoscopy. Out of the totally 145 endoscopic biopsies 2 were not diagnostically conclusive (1.4%), as to be compared with 4 out of the 54 biopsies performed by suction capsule (7.4%). Conclusive fractions from both proximal and distal duodenum were available from 111 of the endoscopic biopsies. Among these 90 cases had pathologic changes in both proximal and distal duodenum, and 10 cases had normal mucosa in both locations. Remaining 10 cases (9%) had an irregular distribution of the enteropathy; 7 with pathological changes only revealed in proximal duodenum and 3 with such changes only in distal duodenum.

**Conclusions:** We found an irregular, or so called patchy distribution of enteropathy, in almost a tenth of endoscopic performed biopsies. Several cases of CD would have been missed if relying only on duodenal biopsy by suction capsule. Another disadvantage of suction capsule is that the obtained specimens were more often diagnosisally inconclusive, which is unsatisfactory as re-biopsy then is needed.
TIME COURSE OF FAECAL CALPROTECTIN (FC) IN SICK AND HEALTHY PRETERM NEWBORNS (PN)

Presenter: J. Blasco. Hospital Materno-Infantil Málaga, Malaga, Spain.

Aim: FC has been proposed as a useful marker of inflammatory bowel disease in adults and children. Increased FC levels have been reported in healthy term and preterm newborns but there are sparse data regarding the normal range for FC changes in preterm infants over the first postnatal month, and its relation with intestinal illnesses. Our aim was to determine normal baseline levels of FC and to measure changes related to gestational age (GA), birth weight, sex, postnatal age, type of feeding (maternal human milk, preterm formula), type of delivery and clinical condition (sick vs nonsick).

Methods: FC (µg/g stool) levels of 87 very low birth weight (VLBW) infants (gestational age (GA) <32 weeks or birth weight ≤ 1500 g) were measured at day 4, 8, 15, 30 and 60. Infants were considered as “sick” if they underwent an evaluation for sepsis, had feeding intolerance, necrotizing enterocolitis, required treatment with antibiotics or vasopressor drugs, or needed respiratory support.

Results: Statistically significant differences between sick and nonsick PN (Tables 13 and 14) in all days were studied. When only taking into account healthy PN, there were statistically significant differences in FC between day 4 and days 8, 30, 60, but there were no significant differences between day 4 and 15. No differences can be proved neither according to predominance of human milk feeding nor GA, birth weight or type of delivery and stepwise multivariate regression analysis showed no interference between different factors analyzed. Mean FC was similar in patients suffering from necrotizing enterocolitis and clinical sepsis without intestinal symptoms. When performing ROC plots, the likelihood that a randomly selected sick PN has a higher FC than a healthy one is 95.2%. A cutoff point of 367 µg/g for FC has a sensitivity of 100% and 81.5% specificity.

Conclusions: FC was higher in PN than in children after first year of life and adults. FC was significantly higher in sick PN, with no differences between intestinal or systemic diseases. FC tended to decrease at the end of first week after birth (intestinal closure) with a slight increase at 15 days and similar levels afterwards for several weeks. There are no statistically significant differences in the FC levels of maternal human milk-fed infants versus preterm formula-fed ones. It may be useful to measure FC serially in VLBW in order to identify an early process of intestinal inflammation or clinical sepsis, probably related with different clinical events.

QUESTIONNAIRE-BASED CASE-FINDING OF LOW-SYMPTOMATIC COELIAC DISEASE (CD) IN AN UNSELECTED POPULATION OF DANISH CHILDREN

Presenter: P. Toftedal. Odense University Hospital, Odense C, Denmark.

Aim: Antibody screening and diagnosis of CD in children with type 1 diabetes suggested that a considerable
proportion of children with CD may in fact have preclinical (not doctor-diagnosed) symptoms (Diabetes Care. 2006;11:2452–6). We aimed in an unselected population of 8–9 year old children to test if a questionnaire may lead to significant case finding. To our knowledge such an approach has not been employed previously.

**Methods:** The population included all 8–9 year old children born in the island of Funen, a total of 9980, comprising a representative 9 % of the Danish population at that age. Prior to the study 13 children of the study population were known with CD. We developed a questionnaire based on five simple items suggestive of CD. The questionnaire was validated in a pilot study with 200 families:

1. Did your child suffer from abdominal pain more than twice during the last 3 months?
2. Did your child ever have diarrhoea lasting more than 2 weeks?
3. Does your child have tendency for firm and hard stools?
4. Does your child gain enough weight?
5. Does your child gain enough height?

**Results:** In total 7029 families returned the questionnaire (70%), 2835 children had 1 or more symptoms. These children were invited for a blood sample to determine serum IgA anti-tissue transglutaminase (tTG) antibody (Inova). Of 1720 tested for anti-tTG antibody 24 children were positive (20 to >150 units). Upper endoscopy was performed in 17. Histological signs of CD as Marsh III lesions were found in 11 children, 2 had Marsh I, and 1 had Marsh II lesions. All 14 children fulfilled diagnostic criteria for CD. The minimal prevalence proportion of preclinical CD was 0.14% [95% CI 0.07–0.22] (14/9967). The total minimal prevalence proportion of children with CD was 0.27% [95% CI 0.18–0.39] (27/9980). The maximal prevalence proportion of preclinical CD was 1.2% [95% CI 0.75–1.9] (21/1720) including those with positive anti-tTG, but without a final diagnosis of CD.

**Conclusions:** A number of preclinical/low-symptomatic CD patients may be found by a mailed questionnaire. However, the population is probably not enriched considerably as the prevalence proportion is close to those obtained in other populations (approx. 1%).

**PG4-12**

**SALIVARY CD SCREENING IN 6- TO 8-YEAR-OLD ITALIAN SCHOOLCHILDREN: PRELIMINARY FOLLOW-UP RESULTS**

Presenter: R. Nenna. *Sapienza University of Rome, Rome, Italy.*

Co-authors: M. Montuori1, R. Luparia1, M. Mennini1, L. Montuori1, L. Petrarca1, D. Masotti2, F. Lucantoni2, C. Tiberti2, M. Bonamico1. 1Department of Paediatrics, *Sapienza University of Rome, Rome, Italy;* 2Department of Clinical Sciences, *Sapienza University of Rome, Rome, Italy.*

**Aim:** The usefulness of coeliac disease (CD) screening is highly controversial mainly because of the low compliance with the therapy in asymptomatic subjects. The high prevalence and the complications of an undiagnosed CD prompted us to perform a CD screening using salivary anti-transglutaminase antibodies (tTGAb) detection. 34 asymptomatic out of 4048 screened children showed positive tTGAb values. The overall prevalence in the population investigated (including 22 CD known cases) was 1.38%. The aim of this study was to evaluate the compliance to the gluten-free diet (GFD) during the follow-up in screening-detected children.

**Methods:** 32 children (11 males, 21 female) (29 biopsy proved CD) started a GFD. During the follow-up, compliance to the diet, weight/stature increase, Ab reduction and well-being were evaluated.

**Results:** During the follow-up on a GFD, all CD children showed a strict adherence to the GFD, a weight and stature increase (Table 15), an Ab reduction and an improvement in well-being.

**Conclusions:** We demonstrated that salivary tTGAb detection is a powerful, noninvasive, simple, well-accepted, reproducible and sensitive screening method (*J Pediatr.* 2004;144:632–6; *Aliment Pharmacol Ther.* 2008;28:364–70). The compliance with the GFD in screened CD children (aged from 6 to 8 years) has been optimal with a significant increase of the anthropometric parameters. A longer follow-up might show if an early diagnosis in asymptomatic children and a strict GFD will permit proper growth and prevent complications in CD children.

**TABLE 15.**

<table>
<thead>
<tr>
<th>Follow-up, no. mo</th>
<th>No. patients</th>
<th>Weight increase (Mean ± SD)</th>
<th>Stature increase (Mean ± SD)</th>
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<td>1.8 ± 1.2</td>
<td>3.4 ± 1.5</td>
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<td>6</td>
<td>11</td>
<td>2.9 ± 1.4</td>
<td>4.4 ± 1.4</td>
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<td>5</td>
<td>2.5 ± 1.8</td>
<td>6.5 ± 2</td>
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<td>12</td>
<td>6</td>
<td>4.7 ± 2</td>
<td>6.4 ± 0.5</td>
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</table>

**PG4-13**

**REFRACTORY COELIAC DISEASE AND FOOD ALLERGY—A SINGLE CENTRE EXPERIENCE**

Presenter: F. Kiparissi. *Great Ormond Street Hospital, London, UK.*

**Co-authors:** M. Montuori1, R. Luparia1, M. Mennini1, L. Montuori1, L. Petrarca1, D. Masotti2, F. Lucantoni2, C. Tiberti2, M. Bonamico1. 1Department of Paediatrics, *Sapienza University of Rome, Rome, Italy;* 2Department of Clinical Sciences, *Sapienza University of Rome, Rome, Italy.*

**Aim:** The usefulness of coeliac disease (CD) screening is highly controversial mainly because of the low compliance with the therapy in asymptomatic subjects. The high prevalence and the complications of an undiagnosed CD prompted us to perform a CD screening using salivary anti-transglutaminase antibodies (tTGAb) detection. 34 asymptomatic out of 4048 screened children showed positive tTGAb values. The overall prevalence in the population investigated (including 22 CD known cases) was 1.38%. The aim of this study was to evaluate the compliance to the gluten-free diet (GFD) during the follow-up in screening-detected children.

**Methods:** 32 children (11 males, 21 female) (29 biopsy proved CD) started a GFD. During the follow-up, compliance to the diet, weight/stature increase, Ab reduction and well-being were evaluated.

**Results:** During the follow-up on a GFD, all CD children showed a strict adherence to the GFD, a weight and stature increase (Table 15), an Ab reduction and an improvement in well-being.

**Conclusions:** We demonstrated that salivary tTGAb detection is a powerful, noninvasive, simple, well-accepted, reproducible and sensitive screening method (*J Pediatr.* 2004;144:632–6; *Aliment Pharmacol Ther.* 2008;28:364–70). The compliance with the GFD in screened CD children (aged from 6 to 8 years) has been optimal with a significant increase of the anthropometric parameters. A longer follow-up might show if an early diagnosis in asymptomatic children and a strict GFD will permit proper growth and prevent complications in CD children.

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<td>3</td>
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<td>11</td>
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<td>4.7 ± 2</td>
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</table>
Co-authors: L. Michaelis1, M. Elawad1. 1Great Ormond Street Hospital for Sick Children, London, UK.

Aim: This study evaluated the incidence of IgE and non-IgE mediated food allergy in patients with typical serological/histological features of coeliac disease (CD) who responded poorly to a gluten-free diet.

Methods: A retrospective analysis of clinical, serological and histological data on N = 189 patients with histologically confirmed CD over an 8-year period (age range 2 years 1 month to 22 years 9 months, median 9 years). Female n = 115. Refractory CD patients with raised tIgE levels ± positive specific IgE (sIgE) to cow’s milk and/or egg, wheat or soya were identified.

Results: 71/189 (37.5%) had persistent symptoms/histology despite a strict gluten free diet and seroconversion. 65/189 patients (34.4%) had raised tIgE levels, and 27/65 (41.5%) had positive sIgEs. 20/27 (74%) had positive sIgE to cow’s milk, 7/27 (26%) had sIgE to egg, wheat or soya (CMPA). 2/189 patients (1%) had a normal tIgE level (CMPI). All responded well to a cow’s milk free diet (CMFD). 4/189 (2.1%) patients who had normal tIgE went on to immunosuppressive therapy. 37/189 (19.5%) had IgA deficiency, of whom 11 (29.7%) had selective IgA deficiency (<0.06 g/L). Of the 11 patients with selective IgA deficiency, 8 (72.7%) had raised IgE levels.

Conclusions: Our study suggests that IgE-mediated CMPA appears to be more common than non-IgE mediated CMPI in children with CD. A total IgE and sIgE should be part of the screening process for children with CD. CMFD should be considered in patients with refractory CD. The higher incidence of IgA deficiency may suggest an association between CD and CMPA.

PG4-15

PRE- AND POSTMARKETING SAFETY PROFILES OF RACECADOTRIL SACHETS, A “NEW” ANTI DIARRHOEAL DRUG


Co-authors: Y. Joulin1. 1Bioprojet, Paris, France.

Aim: Among antidiarrhoeal drugs, racecadotril is known as effective to reduce stool output and duration of diarrhoea in children (Aliment Pharmacol Ther. 2007;26:807–13), but published safety data is considered as insufficient. Our aim was to report the overall experience with racecadotril tolerance from pre- and postmarketing data.

Methods: The individual case safety reports (ICSRs) and the adverse events (AEs) from all the clinical trials (CT) were collected. Periodic safety update reports were used for postmarketing analysis.

Results: During CT, the number of AEs is given in Table 10. The relative risk (RR) of AEs racecadotril/placebo or
ORS is RR = 0.765, 95% CI [0.611–0.962]. The most frequent AEs (frequency greater than 1%) were (a) vomiting, 5.1% in racecadotril groups, 5.8% in groups treated only by oral rehydration solution or by placebo and 12% in loperamide group and (b) fever, 2.3%, 4.6% and 4.0%, respectively. The frequency of withdrawals due to AE were the same in racecadotril groups (15/1129 = 1.3%) and in the control groups (15/1118 = 1.3%). Allergic AE were reported in 1.3% and 1.4%, respectively. From launch in November 2000 to March 2008, worldwide safety management (25 countries) reported 43 ICSRs records (62 AEs) among 14.54 million patients, that was about one ICSR for 338 000 patients (95% upper limit of confidence = 0.414 x 10^-5). The annual frequency of ICSRs was between 0.0009% and 0.0012% during the first 2 years, then below 0.0003%. There were 13 serious and 30 nonserious ICSRs. Half of these AEs belonged to “skin and subcutaneous tissue disorders,” mainly rash and urticaria. Nature and frequency of AEs did not significantly differ between racecadotril 10 mg for infants and children less than 30 months old and racecadotril 30 mg for older children.

Conclusions: Racecadotril cumulative tolerance data are consistent with a safe profile, not different from placebo and most AE are due to the gastroenteritis syndrome.

**TABLE 15. Number of adverse events (AEs) during clinical trials in acute diarrhoea in children**

<table>
<thead>
<tr>
<th>Loperamide</th>
<th>Placebo or ORS</th>
<th>Racacetroltril</th>
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<tbody>
<tr>
<td>No. treated patients</td>
<td>50</td>
<td>704</td>
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<tr>
<td>No. AEs</td>
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<td>No. patients with AE</td>
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<td>114</td>
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<td>Frequency of AEs, %</td>
<td>22.0</td>
<td>16.2</td>
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**PG4-16**

HOW PATCHY IS PATCHY VILLOUS ATROPHY? DISTRIBUTION PATTERN OF HISTOLOGICAL LESIONS IN THE DUODENUM OF CHILDREN WITH CELIAC DISEASE

Presenter: C. Monfredini. *Children’s Hospital, Brescia, Italy.*

Co-authors: F. Gottardi, S. Bolognini, S. Martinazzi, V. Villanacci, A. Ravelli. *Children’s Hospital, Brescia, Italy.*

**Background and Aim:** In celiac disease (CD) villous atrophy (VA) may have a patchy distribution and VA confined to the duodenal bulb has also been reported, possibly leading to underdiagnosis due to sampling error. However in a recent study of > 100 celiac children no biopsies with entirely normal mucosa were found from the bulb to the duodeno-jejunal flexure. The aim of the study was to prospectively verify the degree and distribution of histological lesions among duodenal biopsies as well as within each biopsy in CD.

**Methods:** Over the last 4 years, in each patient with suspected CD (positive anti-transglutaminase antibodies) 4–5 endoscopic biopsies were taken from the duodeno-jejunal flexure/distal duodenum (D3), intermediate duodenum (D2), proximal duodenum (D1) and duodenal bulb (B). Biopsies were subjected to H&E staining and immunostaining with anti-CD3 monoclonal antibodies for intraepithelial lymphocyte count. Duodenal lesions were classified according to Marsh-Oberhuber and CD was diagnosed according to the ESPGHAN criteria.

**Results:** 354 children did have CD. VA was found in 340/354 (96%) and total VA was present in 281/354 patients (79%). 160 of 354 (45.2%) had different lesions at different sites, as follows: grade 3B+3C (109/160), 3A+3B+3C (41/160), 3A+3B (9/160) and 2+3C (1/160), and none of these patients had entirely normal biopsies. Sixty of 354 (16.9%) had variable lesions within the same biopsy, as follows: grade 2+3A (26/60), 2+3A+3B (16/60), 2+3A+3B+3C (13/60), 3A+3B (2/60), 2+3B+3C (1/60), 2+3C (1/60) and 3B+3C (1/60). The majority of these 60 patients also had histologically normal areas within the same biopsy, i.e. true patchy villous atrophy. In these 60, the most frequent lesions per site were: grade 2 in B (90%); 3A in D1 (52%); D2 (48%) and D3 (48%); 3B in D2 (48%), with significantly increasing severity in an aborad fashion (P < 0.01).

**Conclusions:** Our study shows for the first time that in newly diagnosed CD patients variability of histological lesions may exist even within the same duodenal biopsy, where areas of entirely normal mucosa often exist. Our study also confirms that (a) in a significant proportion of CD patients duodenal lesions can be variable amongst different biopsies, (b) lesion severity has a proximal-to-distal gradient so that total VA is most likely found close to the duodeno-jejunal flexure, and (c) no patient with newly diagnosed CD has entirely normal duodenal biopsies. Consequently, even a single endoscopic or capsule biopsy from the distal duodenum can be reliable for the diagnosis of CD.

**PG4-17**

LONG-TERM FOLLOW-UP OF INFANTILE GASTROINTESTINAL JUVENILE POLYPOSIS: EVIDENCE OF HIGH-GRADE DYSPLASIA ADENOMAS


Co-authors: F. Ruemmele, C. Delnatte, D. Sanlaville, P. Vannerom, D. Canioni, F. Jaubert, D. Stoppa-Lyonnet.

In celiac disease (CD) villous atrophy (VA) may have a patchy distribution and VA confined to the duodenal bulb has also been reported, possibly leading to underdiagnosis due to sampling error. However in a recent study of > 100 celiac children no biopsies with entirely normal mucosa were found from the bulb to the duodeno-jejunal flexure. The aim of the study was to prospectively verify the degree and distribution of histological lesions among duodenal biopsies as well as within each biopsy in CD.
Methods: Infantile gastrointestinal juvenile polyposis (IJP), defined by the presence of multiple juvenile polyps developed from the stomach to the rectum, is characterized by its very early and severe manifestations within the first 2 years of life. We report 2 patients (1 boy [patient 1] and 1 girl [patient 2]) with a long follow-up of 17 and 5 years, respectively.

Results: Disease onset was respectively at 6 and 3 months of life. Clinical symptoms were rectal bleeding with marked anaemia, and severe protein-losing enteropathy requiring regular transfusions or IV iron substitution and albumin perfusion; macrocephaly (>+2DS) present since birth, facial dysmorphism and in patient 1 speckled penis at adolescence. Multiple juvenile polyps were identified in the stomach, duodenum, jejunum, ileon and colon, with the highest density of polyps in the colon. The course was complicated by bouts of abdominal pain due to repeated intestinal intussusceptions which resolved spontaneously. Given persistent rectal bleeding, and increased albumin requirement in spite of multiple endoscopic polypectomies, a total colectomy with ileorectal anastomosis was performed at the age of 10 and 2 years, respectively. In patient 1, rectal bleeding stopped for over 5 years and no more albumin perfusion was required. In patient 2, macroscopic bleeding, and abdominal pain resolved; the need for albumin substitution persisted, but at lesser extent. Endoscopic follow-up revealed adenomas in the stomach, duodenum and proximal jejunum with low-grade dysplasia at 4 and 2 years, respectively. Patient 1, now 16 years old, showed the first signs of high-grade dysplasia duodenal adenomas at the age of 12. In both cases, genetic analysis demonstrated, for the first time, de novo contiguous germine deletions of 10q encompassing PTEN and BMPRIA, 2 tumor suppressor genes.

Conclusions: We report the longest follow up of IJP in 2 children who have undergone early colectomy. The demonstration of hamartomatous-adenomatous sequence in upper GI polyps shows that the risk of cancer is major in this syndrome. We hypothesize that the severity of this particular form of IJP reflects a cooperation between the two tumor-suppressor genes deleted in our patients.

PG4-18

PAEDIATRIC GASTROINTESTINAL ENDOSCOPY IN THE UK

Presenter: R. Muhammed. Birmingham Children’s Hospital, Birmingham, UK.

Co-authors: H. Jenkins1. 1 University Hospital of Wales, Cardiff, Wales.

Background and Aim: Endoscopy is an essential tool in delivering a paediatric gastroenterology service. In order to plan endoscopy services for children, it is important to determine the pattern of paediatric gastroenterological endoscopy services in the U.K. and identifying the number of endoscopies performed in each unit, the number of operators performing these endoscopies and whether the endoscopies were performed under sedation or general anaesthesia. In addition, information was sought regarding out of hours emergency provision.

Methods: A questionnaire was sent to each paediatric gastroenterology unit in the U.K requesting the number of endoscopies (upper and lower) performed each year and by whom, the location of endoscopy services for children, the method of sedation/general anaesthesia, the provision of percutaneous endoscopic gastrostomy placement and the provision of emergency endoscopy services.

Results: The questionnaire was sent to 31 units in the U.K. performing paediatric endoscopy and responses were received from 25 (81% response rate). The median number of total endoscopies (upper and lower) performed each year was 332 (range 64–2040), with the median number of oesophago-gastro-duodenoscopy (OGD) being 225 (range 54–1176) and the median number of colonoscopy being 107 (range 10–864). Endoscopies were often performed by several consultants and trainees within each unit, with the median number of OGD per year per consultant in each unit being 101 (range 20–288) and the median number of colonoscopies performed per consultant 49 (range 10–215). 19 units had trainees in paediatric gastroenterology and the median number of OGD and colonoscopies per year per trainee was 175 (range 25–375) and 96 (range 19–275), respectively. Paediatric endoscopies were performed in designated paediatric endoscopy units in 2/25, operating theatres/endoscopy units (shared with adult colleagues) in 23/25 units. 18/25 units performed all endoscopies under general anaesthesia with 7 centres using sedation as well as general anaesthesia. Percutaneous endoscopic gastrostomy (PEG) insertion was performed in 24 out of 25 units with the service undertaken solely by paediatric surgeons in 11 centres, by paediatric gastroenterologists in 4 centres and jointly by both in 9 centres. Only 11/25 units provided formal out of hours endoscopy services with median number of endoscopies performed per unit out of hours was 10 (range 2–100) per year.

Conclusions: We have shown that there is a wide variation in the provision of paediatric endoscopy services across the UK. To the best of our knowledge, there is no similar study comparing paediatric endoscopy provision.
and these data from our survey will be invaluable in helping to plan for training and service provision in the UK.

**Poster Session 5**

**Gastroenterology: Food Allergy, Immunology and Gut Infection**

PG5-01

**COW’S MILK EPICUTANEOUS IMMUNOTHERAPY IN CHILDREN: A DOUBLE-BLIND PILOT TRIAL OF SAFETY AND EFFICACY**


**Background and Aim:** Cow’s milk allergy (CMA) decreases with age, but long lasting conditions, most of the time related to an IgE-mediated mechanism, need a treatment. Even promising, oral immunotherapy may induce a wide range of side effects (SEs). In order to minimize those, we developed a method based on repeated allergen application on the skin with a new device (Viaskin, DBV Technology; Paris), used for the first time in humans, after successful animal testing (J Allergy Clin Immunol. 2008;121:793). A bicentre double blind placebo-controlled study investigated the safety and efficacy of epicutaneous immunotherapy (EPIT) in children with severe IgE-mediated CMA.

**Methods:** A Viaskin coated with 1 mg of cow milk proteins (active) or glucose (placebo) was applied every other day, during 3 months. An open oral food challenge (OFC) was performed at days 0 (D0) and 90 (D90). The cumulated tolerated milk dose (CTD) was measured during the OFC. A CTD index was calculated ([CTD at D90 – CTD at D0]/CTD at D0). Potential SEs, clinical symptoms, milk skin prick tests (SPT), and milk sIgE and sIgG subclasses were assessed every month. All children, both in active and placebo groups, were offered to continue an open phase of active EPIT for 3 additional months.

**Results:** 19 children (median 3 y, 8 m–6 y) satisfied the enrolment criteria, active (n = 11, 2 dropouts) and placebo (n = 8, 1 dropout). Tolerance and safety were good, SEs being minor and similar in both groups. At D90 of EPIT, in the active group (n = 13), the CTD increased more than 10 fold in 5 children, between 3- and 10-fold in 3, less than 3-fold in 1, remained unchanged in 3 and decreased in 1. With placebo (n = 7), the CTD increased less than 3-fold in 4 and was unchanged in 3. Taking into account all active treatments (n = 20), the median CTD index improved significantly in the active group, 5.61, vs placebo, 0.17, P = 0.02. As a whole, milk SPT, sIgE and sIgG subclasses did not vary significantly.

**Conclusions:** This pilot study, although performed on a limited number of patients, suggests for the first time in children that EPIT is safe and could constitute a therapeutic option in children with severe IgE-mediated CMA.

PG5-02

**PROTECTIVE ROLL OF CPG-ODN1826 ON INTESTINAL BARRIER FUNCTION AND TH1/TH2 BALANCE IN A RAT FOOD ALLERGY MODEL**

Presenter: J. Huang. Xinhua Hospital affiliated with Shanghai Jiao Tong University, Shanghai, China.

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**Aim:** Recent in vitro studies suggest that sequences comprise unmethylated CpG motifs, which are abundant in probiotic bacterial DNA has multiple and complex effects on the immune system. They might offer an alternative way to prevent and potentially reverse Th2-biased immune deregulation that leads to allergy. This study gives us a deep prospection into the role of CpG-ODN1826 on intestinal barrier function and Th1/Th2 balance in a rat food allergy model compared with probiotics and probiotic bacterial DNA.

**Methods:** Sixty 3-week-old female Brown Norway (BN) rats were randomly divided into six groups: negative control (PBS gavage group), positive control (ovalbumin (OVA) sensitization group), probiotics treatment group (OVA sensitization + probiotic treatment for 8 weeks), bacterial DNA treatment group (OVA sensitization + intraperitoneal bacterial DNA immunization), CpG ODN treatment group (OVA sensitization + intraperitoneal CpG
ODN 1826 immunization) and non-CpG ODN control group (OVA sensitization + intraperitoneal CpG ODN 1982 immunization). The severity of food allergy was evaluated by serum OVA-IgE level. Intestinal barrier function and Th1/Th2 immune balance were analyzed.

**Results:** OVA-IgE level was significantly higher in the positive control than those in the negative control group ($P < 0.05$). Probiotics treatment led to significantly decreased OVA-IgE levels and improved intestinal permeability compared with positive control group ($P < 0.05$). Both CpG ODN and probiotic DNA immunization can achieve comparable effects on decreasing OVA-IgE levels, and they also resulted in significantly lower eosinophil and mast cell infiltration in small intestinal mucosa ($P < 0.05$). In CpG ODN and probiotic DNA immunization groups, a cytokine profile towards that of Th1 (increased IFN-$\gamma$/IL-4 ratio) was observed, but no difference in small intestinal crypt depth and villous height compared with the positive control group. While probiotic treatment group can improve morphology, inflammatory cell infiltration of intestinal tissue and the Th1/Th2 balance when compared with the positive control group.

**Conclusions:** Although CpG ODN 1826 can only copy the immune modulation effect of probiotics but not the intestine trophy function, it can still achieve significant treatment effect on food allergy objects. This also implies that the immune modulation effect exerted by probiotic bacteria is induced by the abundant unmethylated CpG motif contained in them.

**PG5-03**

**IMMUNOGLOBULIN-FREE LIGHT CHAINS PLAY A PIVOTAL ROLE IN CASEIN ALLERGY IN MICE AND MAY BE A BIOMARKER FOR ATOPIC DERMATITIS IN INFANTS**

**Fig. 1.** Effect of F991 (Ig-fLC inhibitor) on the induction of an acute allergen-specific skin reaction for (A) casein- and (B) whey-sensitized mice. F991 decreased casein-induced ear swelling significantly but whey-induced ear swelling was unaffected. Ear swelling ($\mu$m) is calculated as the increase in ear thickness induced by the corresponding antigen at 1 hour after challenge. $**P < 0.01$, $***P < 0.001$ and $n = 6$. 

**Background and Aim:** Cow’s milk allergy (CMA) is affecting 2.5% of young infants. Up to 40% of the CMA patients have no detectable cow’s milk specific serum immunoglobulin (Ig) E. In previous studies differences in IgE responsiveness between casein- and whey-sensitized mice were observed, therefore the involvement of Ig free light chains (fLC) in allergic sensitization was studied. To confirm these findings the involvement of Ig-fLC in infants with and without atopic dermatitis (AD) was studied.

**Methods:** Mice were orally sensitized with casein or whey using cholera toxin as an adjuvant. Serum Igs, Ig-fLCs and the acute allergic skin reaction were determined with and without the presence of a specific Ig-fLC inhibitor (F991) to reveal systemic hypersensitivity and the contribution of Ig-fLC. Furthermore, passive immunization studies were carried out with supernatants of spleen cells from casein or whey allergic mice. Sera from infants at high risk for allergy with and without mild AD (SCORAD 5.2 – 24.7) were analyzed for total $\lambda$ and $\kappa$ Ig-fLC.

**Results:** Serum levels of casein-specific IgE were not increased when compared to control mice (4.7 ± 1.6 vs 6.0 ± 3.5 AU, respectively). Casein-specific IgG1 levels

**Presenter:** B. Schouten. Division of Pharmacology and Pathophysiology, UIPS, Utrecht University, Utrecht, The Netherlands.


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were enhanced, although this was not significant due to 2 nonresponders. For the whey-sensitized mice both specific IgE (2.5 ± 1.1 vs 239.4 ± 123.6 AU; *P < 0.05) and IgG1 (*P < 0.001) were augmented compared to sham-sensitized mice. Ig-fLC levels were only enhanced in casein-sensitized mice (sham: 66.6 ± 5.5, whey 64.2 ± 8.7 and casein 92.3 ± 9.5 OD mm⁻²; *P < 0.05). Ig-fLC blocker F991 diminished ear swelling in casein (from 147.4 ± 16.0 µm to 83.3 ± 8.3 µm, *P < 0.05) recipients of both actively and passively sensitized mice (Fig. 1). No effects were found in whey mice. Total levels of κ Ig-fLC in infants with atopic dermatitis were enhanced (8.8 ± 0.85 vs 7.1 ± 0.40 µg/mL, *P = 0.0075), λ Ig-fLC showed similar tendency (7.7 ± 0.42 vs 6.2 ± 0.33 µg/mL, *P = 0.14). In these infants cow’s milk-specific IgE, IgG1 and total levels of IgE, IgG1, and IgG2 were not found to be enhanced.

Conclusions: This study demonstrate that Ig-fLC drives the acute allergic skin reaction in mice orally sensitized with casein and that they might be used as a biomarker for atopic dermatitis.

PG5-04

EPICUTANEOUS IMMUNOTHERAPY: FEASIBILITY AND EFFICACY OF A NEW TREATMENT FOR PEANUT ALLERGY IN SENSITIZED MOUSE


Co-authors: L. Mondoulet², V. Dioszeghi³, J. Vanoorbeek³, B. Nemery³, C. Dupont¹. ¹Hôpital Saint Vincent de Paul, Paris, France; ²DBV Technologies, Paris, France; ³Katholieke University, Leuven, Belgium.

Background and Aim: Peanut allergy, a life-threatening disease, needs a cure. Subcutaneous immunotherapy is considered the most efficacious, but cannot be used in clinical practice due to the risk of systemic reactions. The aim was to evaluate in a controlled study with a large cohort of sensitized mice, the efficacy of epicutaneous immunotherapy (EPIT) as a new alternative pathway with the skin.

Methods: 60 BALB/c mice were sensitized to whole peanut protein extract (PPE) by means of gavages with an adjuvant (cholera toxin). Sensitization was manifested by a significant increase of serum specific IgE (sIgE) levels as compared non sensitized animals (group NS, n = 20). 20 sensitized mice remained untreated (group NT). Conventional immunotherapy (SC injection of PPE, 1/week) was carried out during 8 weeks (group SC, n = 20). A skin device, coated with 100 µg of PPE (VIASKIN, DBV Technologies, Paris), was applied on shaved skin every week during 48 hours (group EP; n = 20). NS and NT had a sham treatment with empty VIASKIN. sIgE, sIgG1 and sIgG2a were monitored during the treatment. At the end of the experiment mice were challenged with aerosol of peanut powder and injections of methacholine. Airway hyperreactivity (AHR) was evaluated by plethysmography and resistance/compliance using a FlexiVent system.

Results: After treatment, sIgE decreased significantly in EP and SC (respectively from 250 ng.mL⁻¹ to 90 ng.mL⁻¹ and from 210 ng.mL⁻¹ to 50 ng.mL⁻¹) as compared to NT, *P < 0.001. IgG1/IgG2a ratio significantly decreased in EP and SC, respectively from 58 to 4 and from 28 to 2, as compared to NT, *P < 0.01. With the highest dose of methacholine, Penh values decreased significantly in EP and SC (respectively 7.7 and 6.7) as compared to NT (11, *P < 0.01) and were not different from ND values (5.1). Dynamic resistance studied at different doses of methacholine was also reversed by EPIT. The resistance and compliance values were not different in EP, SC and ND and lower than NT (*P < 0.001).

Conclusions: In this model of sensitized mouse, analysing both biological and physiological responses, epicutaneous immunotherapy is as efficient as the conventional subcutaneous treatment, the reference method in immunotherapy.

PG5-05

DIFFERENT TIMING OF USING PROBIOTICS ON RAT FOOD ALLERGY MODEL: PREVENTION OR TREATMENT

Presenter: Y. Zhong. School of Medicine, Shanghai Jiao Tong University, Shanghai, China.

Co-authors: Y. Zhong³, W. Cai², J. Huang², H. Zhang², W. Tang¹, B. Cheng¹. ¹Department of Nutrition, School of Medicine, Shanghai Jiao Tong University, Shanghai, China; ²Clinical Nutrition Center, Xinhua Hospital, Shanghai Jiao Tong University, Shanghai, China.

Background and Aim: Early colonization of the bowel with probiotic bacteria such as lactobacilli and bifidobacteria can effectively stimulate the immune system and maturation of gut function, thus promoting tolerance to nonbacterial antigens. The aim of the present study was to investigate the effect of different timing of probiotic bacteria administration on alleviating food allergy in a rat food allergy model.

Methods: Fifty 3-week-old female Brown Norway (BN) rats were randomized into 5 groups: negative control (PBS gavage group), positive control (ovalbumin (OVA) sensitization by oral gavage), prevention (probiotics administration for 2 weeks before OVA sensitization), treatment (probiotics administration for 2 weeks after
OVA sensitization period) and whole-course intervention (probiotics administration to 2 weeks before OVA until sensitization to end of the experiment). The probiotics mix used was *Lactobacillus rhamnosus* GG and *Bifidobacterium longum* BB536. The severity of food allergy was evaluated by serum OVA-IgE level. Intestinal Intestinal permeability was assessed by enteral administration of FITC-conjugated dextran 4000. Intestinal sIgA, inflammatory infiltration, morphologic change and the balance of Th1/Th2 cytokines were analyzed.

**Results:** OVA-IgE level was significantly higher in the positive control group than that in the negative control group ($P < 0.05$). All three probiotics administration regimens led to significantly decreased OVA-IgE levels and improved intestinal permeability compared with positive control group ($P < 0.05$). Probiotics administration prior to sensitization (both prevention and whole-course intervention) resulted in significantly lower eosinophil and mast cell infiltration in small intestinal mucosa ($P < 0.05$), and a cytokine profile towards that of Th1 (increased IFN-γ/IL-4 ratio), but no difference in small intestinal villus height and crypt depth was observed. In contrast, probiotics treatment could significantly improve both morphology and inflammatory cell infiltration in intestinal tissue, but did not alter the Th1/Th2 cytokine balance when compared with the positive control group.

**Conclusions:** Both probiotics prophylaxis and treatment could attenuate food allergy by decreasing OVA-IgA level, but the underlying mechanisms of their effects might be different.

**PG5-06**

THE DIET IN CHILDREN WITH FOOD ALLERGY: COMMON ERRORS AND EFFECT OF A NUTRITIONAL COUNSELLING ON GROWTH AND NUTRIENT INTAKE

**Presenter:** S. Ruotolo. University of Naples “Federico II,” Naples, Italy.

**Co-authors:** A. Coruzzo1, G. Terrin1, M. Tardi 1, L. Cosenza1, A. Braucci1, R. Troncone1, E. Riva2, C. Agostoni2, R. Berni Canani1. 1Department of Pediatrics, University of Naples “Federico II,” Naples, Italy; 2Department of Pediatrics, University of Milan, Milan, Italy.

**Background and Aim:** The only available therapy for children with food allergy (FA) is elimination diet, which could be responsible for inadequate dietary intake. We investigated dietary intake and body growth in pediatric outpatients on elimination diet because FA and the effect of a nutritional counselling on body growth and dietary intake.

**Methods:** Prospective multicenter controlled study including children with FA (3–36 months of age) on elimination diet for ≥30 days, consecutively observed in 2 tertiary centers. For comparison healthy children were also evaluated. At the enrollment and after 2, 4, and 6 months, anthropometric indices (z score for weight and length) were collected and dietary intake was assessed by a 3-day diary. The nutrient intake was analysed using a specific software (Nutriclick) based on the Italian food composition tables. A professional dietary intervention was then provided by specialized pediatric dieticians.

**Results:** The study included 151 children. Of these, 86 (54 M, median age 19.8, 95% CI 17.0–20.6 months) had FA, and 65 were healthy controls (41 M, median age 20.5, 95% CI 18.0–23.0 months). Clinical symptoms of FA were gastrointestinal (80.2%, vomiting, chronic diarrhea, and hematochezia), atopic dermatitis (54.7%), acute urticaria and angioedema (4.7%), asthma (7.9%) and anaphylaxis (1.2%). 76 patients presented allergy to cow’s milk (CMA) (88.4%), almost (1.2%) or in combination with hen’s egg (45.2%), wheat (5.8%), tomato (5.8%), fish (8.1%), soybean (6.1%), rice (3.5%); the 10 patients without CMA were allergic to hen’s egg (5), soybean (2), fish (2) and rice (1). The mean length of the elimination diet was 10.8 months (8.5–13.1, 95% CI). At the enrolment, FA-affected patients presented a median z score for weight (−0.48 vs 0.45, $P < 0.001$) and for length (−0.82 vs 0.65, $P < 0.005$) lower than healthy controls. Six out of 86 (7.0%) FA affected children, and none in the control group, presented a dietary reference intake (DRI) below <67% ($P < 0.005$) and 39 (45.3%) children with FA presented a DRI between 67 and 90%, compared to 7 (10.6%) in the control group ($P < 0.001$). After 2 months of controlled dietary intervention, the DRI became normal in all subjects (average 101% of DRI, 97.0–105.4, 95% CI), and these data were confirmed during the follow-up.

**Conclusions:** An unbalanced diet with deficient caloric intake is a frequent feature of FA affected children, potentially determining a significant impact on body growth. This condition could be prevented and resolved by a nutritional counselling. These data should encourage the application of a pediatric medical nutrition protocol for FA affected children.

**PG5-07**

HEMATOCHEZIA: ALLERGIC BOWEL INFLAMMATION IN INFANTS?

**Presenter:** A. Cseh. Semmelweis University, Budapest, Hungary.

**Co-authors:** B. Szebeni1, B. Szalay1, B. Vsrhelyi1, A. Ara2, G. Veres2. 1Research Group for Pediatrics and Nephrology, Semmelweis University, and Hungarian

**Background and Aim:** The only available therapy for FA affected children.
Academy of Sciences, Budapest, Hungary; 1Department of Pediatrics, Semmelweis University, Budapest, Hungary.

Background and Aim: Hematochezia (HC, fresh blood in the stool) is an alarming symptom of otherwise healthy infants that can be cured in most cases with maternal elimination diet. While several data suggest an allergic disorder, its pathomechanism is still unclear. The objective of our study was to describe the alteration of adaptive immune system in HC infants.

Methods: 10 infants with histologically verified eosinophilic or allergic colitis were recruited. None of them had evidence of a bleeding diathesis, bacterial enteritis or fissures. Peripheral blood Th1 and Th2 cytokines were measured with a cytokine chip before and after the introduction of a 1 to 3 months long maternal eliminating diet. In addition the prevalence of lymphocyte subgroups (i.e., activated CD4 and CD8 lymphocytes expressing CD45, CD25, CD69, CD62L or HLADR markers) along with the iNKT/NKT/NK cells (CD3, 6B11, CD161 markers) and Treg cells (FoxP3 positive) were investigated with flow cytometry. 10 healthy infants without HC served as controls.

Results: The levels of total Th2 cytokines were higher in HC before the diet ($P = 0.02$) compared to the controls. The CD4CD45R0/CD4CD45RA was lower in HC patients before the diet than in controls ($P = 0.03$). The iNKT and NKT cell prevalence were increased ($P = 0.008$, $P = 0.03$, respectively) before the diet than in controls. The prevalence of Treg cells was lower before the diet than in controls ($P = 0.03$) and compared to the prevalence after the diet there was an increase in the number of Treg cells ($P = 0.02$).

Conclusions: These results support the notion that the immune system of HC infants is skewed toward Th2 directions, indicating that an allergic reaction may be responsible for the development of HC. Decreased Treg cells and increased iNKT and NKT cells observed in patients with HC may have a potential role in the pathomechanism of eosinophilic/allergic colitis.

Background and Aim: We have recently demonstrated that the atopy patch test (APT) is an useful diagnostic tool in children with gastrointestinal symptoms and suspected food allergy (FA) (Allergy, 2007;62:738). The interpretation of the APT to foods is not yet completely standardized, it is still subjective and prone to intra- and inter-observer variations. We aimed to test the diagnostic accuracy of APT in the diagnosis of FA-related gastrointestinal diseases and to validate the reading of the APT in terms of the diagnostic accuracy of individual skin signs.

Methods: Children referred for suspected FA-related gastrointestinal symptoms were subjected to APT using fresh food (cow’s milk, hen’s egg, wheat). 12-mm Finn chambers were applied for 48 hours, and results were read after 48 and 72 hours. Skin changes were graded for erythema, induration and papule formation. Food allergy was assessed by double blind, placebo-controlled food challenges (DBPCFC). Sensitivity, specificity and predictive values were calculated for each skin signs in relation to challenge outcome.

Results: 117 DBPCFC were performed in 99 patients (65 boys, media age 26.6 months, ±19.25 DS, range 3–48 months): 39 tested positive for cow’s milk (CM), 19 for hen’s egg (HE), and 2 for wheat. The results of APT with fresh food were CM: sensitivity: 66.7%, specificity 84.1%, positive predictive value (PPV) 78.8% and negative predictive value (NPV) 74.0%; and HE: sensitivity 84.2%, specificity 100%, PPV100% and NPV 75.0%.

The combination of skin induration plus presence of papules, with or without moderate erythema, had a PPV and specificity for the challenge outcome of 95.4% (CI 95%, 77.1–99.9) and 97.8% (CI 95%, 88.2–9.9), respectively, in CM allergy.

Conclusions: The diagnostic properties of APT improved significantly if skin induration and presence of papules were used in combination. These skin signs were highly predictive of challenge outcome. Other skin signs (ie, erythema) were found to be less accurate, particularly if used in isolation. Careful and standardized documentation of the severity of single APT skin signs may improve the reproducibility of APT interpretation and reduce intra- and interobserver error.

PROPOSAL FOR A STANDARDIZED INTERPRETATION OF THE ATOPY PATCH TEST IN CHILDREN WITH GASTROINTESTINAL SYMPTOMS AND SUSPECTED FOOD ALLERGY


Co-authors: S. Ruotolo1, L. Cosenza1, A. Passariello1, G. Terrin1, C. Puzone1, M. Passaro1, A. Braucci1, R. Troncone1, R. Berni Canani1. 1Department of Pediatrics, University of Naples Federico II, Naples, Italy.

INTESTINAL INNATE IMMUNITY AND APOPTOSIS IN RESPONSE TO PATHOGENIC ROTAVIRUS AND VACCINES PREPARATIONS


Co-authors: G. De Maco1, V. Bacciagrossi1, C. Armellino1, A. Guarino1. 1University of Naples “Federico II,” Naples, Italy.
Background and Aim: Adaptive immune response does not consistently reflect protection following RV natural infection or vaccination. The role of innate immunity in RV infection is also unknown. Cathelicidins are important components of intestinal innate immunity. LL-37, the only known human cathelicidin, shows a broad range of antimicrobial and inflammatory effects and modulates cell proliferation and apoptosis. We investigated LL-37 expression and its correlation with apoptosis in enterocytes infected with a pathogenic RV or with the live strains contained in the 2 available vaccines.

Methods: Caco-2 cells were infected with RV SA-11, or with monovalent attenuated human rotavirus (Rotarix) or with the pentavalent attenuated human-bovine rotavirus (Rotateq) vaccines at increasing concentrations (1, 5, 25 PFU/cell). LL-37 was evaluated by the number of cells expressing LL-37 by indirect immunofluorescence with a monoclonal antibody at different time postinfection (1, 3, 6, 24, 48 hours). Apoptosis was determined by Hoechst DNA staining.

Results: RV SA-11 infection significantly increased the number of cells expressing LL-37. LL-37 expression was directly dependent on viral load and showed a time-dependent pattern, reaching a plateau at 48 hours. RV infection was associated with enterocyte apoptosis. Apoptotic cells showed stronger LL-37 expression than nonapoptotic cells. Both viral preparations Rotarix and Rotateq induced LL-37 expression and apoptosis (Table 16). However, an inverse correlation pattern between apoptosis and LL-37 expression was elicited by the 2 vaccine preparations: Rotarix induced a significantly higher apoptosis and lower LL-37 expression, compared to Rotateq ($P<0.001$).

Conclusions: Innate immunity is involved in the response to RV and correlates with apoptosis. The viral preparations contained in the 2 vaccines stimulate LL-37 expression and induce apoptosis with a different pattern.

### PG5-10

**CLINICAL AND GENETIC HETEROGENEITY IN IMMUNE DYSREGULATION, POLYENDOCRINOPATHY, ENTEROPATHY X-LINKED (IPEX) SYNDROME—A 5-YEAR FOLLOW-UP STUDY**

**TABLE 16. LL-37 expression and apoptosis in Caco-2 cells at 48 hours postinfection**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>RV SA-11</th>
<th>Rotarix</th>
<th>Rotateq</th>
</tr>
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<tr>
<td>LL-37, % of cells</td>
<td>4 ± 2</td>
<td>42 ± 7</td>
<td>33 ± 6</td>
<td>55 ± 9</td>
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<td>Apoptosis, % of cells</td>
<td>3 ± 1</td>
<td>32 ± 8</td>
<td>36 ± 7</td>
<td>22 ± 6</td>
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</tbody>
</table>


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Background and Aim: Immune dysregulation, polyendocrinopathy autoimmune enteropathy X-linked (IPEX) syndrome is a most often lethal systemic autoimmune disease caused by mutations in the FOXP3 gene. In the present study we analyzed the variability of clinical presentation and evolution of IPEX and underlying molecular events.

Methods: Clinical data of a single center series of 13 boys with a molecularly proven diagnosis of IPEX-syndrome were collected and analyzed. All patients had a complete immunological work-up and FOXP3 genotyping. In a subgroup of seven patients, mRNA and FOXP3 protein expression were analyzed using RT-PCR, FACS, and confocal immunofluorescence. Functional assays of regulatory (reg) and effector T cells were performed using coculture systems.

Results: Two different clinical phenotypes could be identified: patients with insulin-dependent diabetes mellitus (IDDM) and severe autoimmune enteropathy (IPEX type 1), and patients without IDDM, but severe allergic reactions and autoimmune enteropathy (IPEX type 2). Outcome was markedly better in patients with IPEX type 2. Missense mutations of FOXP3 were detected in 5 patients, whereas eight had a deletion. Consequently, variable FOXP3 protein expression levels and cellular location were detected ranging from absent to subnormal or aberrant location. All patients had markedly altered function of CD4+CD25high T cells.

Conclusions: The clinical picture of IPEX-syndrome is more heterogeneous than previously described, with the occurrence of IDDM associated to enteropathy as pejorative symptom. No correlation between the type of FOXP3 mutation and T reg. function or IPEX disease type or severity could be established.

### PG5-11

**IS TNFA A POTENTIAL TARGET FOR THE TREATMENT OF COLITIS IN CHRONIC GRANULOMATOUS DISEASE?**


Co-authors: C. Delfert1, A. Rougemont1, D. Belli1, K. Krause1, 1Dept Pediatrics, University Hospital Geneva, Geneva, Switzerland; 2Dept Padiatrics, University Hospital Geneva, Geneva, Switzerland; 3Dept Pathology, University Hospital Geneva, Geneva, Switzerland; 4Dept Pathology.
Background and Aim: Chronic granulomatous disease (CGD) is an immunodeficiency and hyperinflammation disorder. Sterile hyperinflammation in the colon, CGD colitis, contributes significantly to the morbidity of patients. Presently available treatment options for CGD colitis include corticosteroids and interferon-α. Over-shooting TNF-α production in CGD phagocytes has been proposed as a mechanism contributing to hyperinflammation. In this study we have investigated whether a deletion or suppression of TNF-α resulted in a diminution of the hyperinflammation in a murine CGD model.

Methods: For genetic deletion experiments, wild-type (N = 6), CGD (= NOX2-deficient, N = 6), and CGD/TNF-α-deficient mice (N = 6) were injected intradermally with b-glucan to induce hyperinflammation. For pharmacological experiments, CGD mice were treated with the TNF-α scavenger sTNFR2-Fc (Ethanercept) before b-glucan injection. Seven days later, the severity of inflammation was assessed on H&E stained slides of ear sections by a histological score (from 0–4, with 4 being the most severe inflammation).

Results: The b-glucan generated a mild inflammation score in the wild-type mice (mean score 1.5 ± 0.5), while the CGD mice showed severe inflammation (mean 3.5 ± 0.2). Genetic deletion or pharmacological inhibition of TNF-α did not decrease the severity of the inflammation in CGD mice: histological scores for CGD/TNF-α-deficient mice and for sTNFR2-Fc treated CGD mice were 3.7 ± 0.3 and 3.7 ± 0.4, respectively. In both TNF-α-suppressed mice, a hyperinflammatory syndrome associated with necrotic lesions was observed.

Conclusions: Neither genetic deletion of TNF-α nor treatment with TNF-α scavenger prevented skin hyperinflammation in CGD mice. Thus unlike previously suggested, TNF-α probably does not represent a potential target for the treatment of CGD hyperinflammation. However, our results were obtained in skin hyperinflammation and future research will have to investigate the similarities and differences between hyperinflammation in the skin and in the colon.

PG5-13

RACECADOTRIL DIRECTLY INDUCES ION ABSORPTION UNDER BASAL CONDITION AND INHIBITS ROTAVIRUS CHLORIDE SECRETION IN HUMAN ENTEROCYTES


Background and Aim: Advances in the understanding of bacterial pathogenesis have greatly benefited from the use of human epithelial cell lines which require aerobic conditions for optimal survival. However, in the target site of infection, the human intestine, conditions are generally anaerobic. The aim of the study was to investigate the influence of anaerobic apical conditions on bacterial colonisation of human intestinal epithelial cells.

Methods: Polared T84 human colon carcinoma cells were placed in a vertical Ussing chamber as the interface between an anaerobic (5% H₂, 90% N₂, 5% CO₂) or aerobic (95% oxygen, 5% CO₂) apical chamber and an aerobic basal chamber. The cells were infected with enterohaemorrhagic E.coli (EHEC) as a model organism and studied by immunofluorescence microscopy, scanning EM, and Western blotting for bacterial type 3 secretion system (T3SS) effector proteins.

Results: Immunofluorescence staining and scanning electron microscopy showed intimate bacterial adherence, EspA filament formation and actin polymerisation beneath adherent EHEC after 6-hours of infection. The adherence phenotype was similar under aerobic and anaerobic conditions. Determination of adherent bacteria by plating out cell lysates showed an increase in bacterial colonisation under anaerobic compared to aerobic conditions. Secretion of the T3SS effectors Tir, EspA and EspB was investigated by SDS-PAGE and Western blot analysis and was found to be considerably enhanced under apical anaerobic conditions, and to be dependent on the presence of host cells. The effect of nitrate and TMAO as terminal electron acceptors for anaerobic respiration was studied and no increase in bacterial colonisation or secretion of T3SS effector proteins was observed compared to anaerobic conditions without electron acceptor.

Conclusions: The vertical Ussing chamber system provides a model that can reproduce luminal anaerobic conditions yet maintain host cell survival. In this system EHEC colonisation of polarised T84 cells is increased under anaerobic apical conditions which more closely resemble the natural environment in the human gut. The translocation of bacterial effector proteins via the T3SS is also enhanced, indicating that bacterial virulence may be underestimated in aerobic cell culture conditions.
**Background and Aim:** Racecadotril (RAC) is an inhibitor of enkephalinase, an enzyme that inhibits the enkephalins, neurotransmitters of the enteric nervous system (ENS) with antisecretory activity. RAC is effective in acute diarrhoea in in vivo studies. Rotavirus (RV) is the major cause of childhood gastroenteritis. A putative mechanism of RV diarrhoea is the activation of ENS. However we have previously reported that RV induces early chloride secretion in human enterocytes. We tested the hypothesis that RAC stimulates ion absorption in basal conditions and reduces chloride secretion induced by RV and does this through a direct interaction with the enterocyte. Since MAP Kinases ERK1/2 and p38 are involved in the regulation of transepithelial ion transport, we also tested the hypothesis that these may be involved in the effects induced by RAC on intestinal ion transport.

**Methods:** Short-circuit current (Isc), an electrical parameter of chloride secretion, was measured in Caco-2 cell monolayers infected with RV strain SA-11 (5 PFU/cell) or control (not infected) using Ussing chambers and exposed to increasing concentration of RAC in different phases of RV infection (pre- and postinfection). MAPK involvement was evaluated by preincubation with the specific inhibitors of ERK1/2 and p38, PD908059 and SB0203580, respectively. In addition, expression of ERK1/2 and p38 active forms was evaluated by western blot.

**Results:** RAC addition to the mucosal side of enterocytes induced a decrease in Isc, indicating ion absorption. The effect was time and dose dependent. Isc peaks 1 hour after 10-μM RAC exposure. MAPK inhibitors totally inhibited the effects of RAC, indicating a direct involvement of MAPK ERK1/2 and p38. This was confirmed by Western blot analysis. Rotavirus secretion was inhibited when RAC was added in postinfection phase. The addition of RAC before and during RV infection had no effect on secretion.

**Conclusions:** RAC has a potent direct proabsorptive effect on intestinal ion transport in basal condition. This effect involves both ERK1/2 and p38 MAPK. In addition RAC is able to abolish chloride secretion induced by RV acting directly on intestinal epithelial cells. These results depict an entirely novel mechanism of RAC that explain why RAC is highly effective in RV infection.

**PGS-14**

**ZINC ACTS AS INTESTINAL FUEL PROMOTING ENTEROCYTE PROLIFERATION WITH A MAPK- AND GSH-DEPENDENT MECHANISM**


**Background and Aim:** Infectious diarrhea may be osmotic or secretory. Zinc directly promotes chloride absorption either in basal conditions and in conditions of active electrolyte secretion induced by enteric pathogens (*J Infect Dis.* 2005;191:1072–7). However, zinc deficiency in malnourished children is associated with severe diarrhea and intestinal atrophy. Zinc supplementation to oral rehydration solution (ORS) has been proposed in order to reduce the risk, severity and duration of diarrhea in children particularly in developing countries. We tested the hypothesis that zinc acts as intestinal fuel promoting enterocyte proliferation and differentiation. We also evaluated zinc effect on MAPK kinase ERK1/2, the major target signaling in intestinal cell proliferation, and glutathione (GSH), the major compound of intracellular antioxidant defence.

**Methods:** Intestinal cell proliferation was monitored by 3H-thymidine incorporation in Caco-2 cells exposed to increasing ZnCl2 concentrations. The role of MAPK ERK1/2 was investigated evaluating the activated form by Western blot and using its specific inhibitor PD908059. The activation of GSH was investigated through the modifications of its intracellular levels by a colorimetric assay and by inhibition experiments with buthionine sulfoximine (BSO), its specific inhibitor. Intestinal differentiation was evaluated by lactase and sucrase enzymatic activities with Dalqvist method.

**Results:** ZnCl2 induced a significant increase in 3H-thymidine incorporation in Caco-2 cells exposed to increasing ZnCl2 concentrations. The role of MAPK ERK1/2 was investigated evaluating the activated form by Western blot and using its specific inhibitor PD908059. The activation of GSH was investigated through the modifications of its intracellular levels by a colorimetric assay and by inhibition experiments with buthionine sulfoximine (BSO), its specific inhibitor. Intestinal differentiation was evaluated by lactase and sucrase enzymatic activities with Dalqvist method.

**Conclusions:** ZnCl2 induced an increase in the activated-ERK1/2 form and inhibition of GSH production abolished the trophic effect of ZnCl2.

**PGS-15**

**GASTROINTESTINAL INFECTION BY CYTOMEGALOVIRUS INFECTION IN IMMUNOCOMPETENT CHILDREN**

Presenter: M. Barbato. *Sapienza University of Rome, Rome, Italy.

Background and Aim: Cytomegalovirus (CMV) is a double-stranded DNA herpes virus causing infections or diseases mainly in immunodeficient humans by a vertical or horizontal transmission. There is experimental evidence that it has a peculiar tropism to enteric nervous system and intestinal immunological cells. There have been isolated reports of CMV infections in children with gastrointestinal (GI) motility abnormalities as well as in children with acute attacks of inflammatory bowel disease (IBD). Furthermore, the role of CMV in these disorders has not been defined in depth. We aimed at reporting the presence of CMV infection in immunocompetent children with GI dysmotility and with IBD.

Methods: CMV infection was investigated in 22 children (age range 3–24 months) with GI dysmotility and a diagnosis of severe gastroesophageal reflux (GER) disease, resistant to prokinetic and antisecretory drugs as well as to elemental diets. Additional 10 patients with active IBD or IBD-like disorders, resistant to conventional drugs, were investigated for CMV infection. Either congenital or acquired immunodeficiency was excluded in all. CMV infection was defined as the detection of CMV DNA in urine, blood and gastric fluid using RTPCR; CMV was also detected by histopathologic testing and immunohistochemistry in biopsy taken from intestinal biopsies. These procedures were performed after antiviral IV treatment with ganciclovir (GCV), at a dose of 5 mg/kg/every 12 hours for 14 days, followed by oral valganciclovir, until negativity of the tests. The therapy was conducted for at least 3 months.

Results: CMV infection was documented in 11 patients with refractory GI dysmotility (all ≤ 1 year-old), characterized by delayed gastric emptying (documented by ultrasonography) in 10, endoscopic esophagitis in 7 and abnormal GER (detected by 24-hour pH test) in 9; in 1 patient a manometric diagnosis of esophageal achalasia was done. Surgical anti-reflux fundoplication (with pyloroplasty in some cases) had been planned in most of them. CMV infection was detected in one 10-month old patient with bleeding diarrhea and an endoscopic picture of ulcerative colitis-like lesions. Four IBD patients with an acute attack, resistant to corticosteroids and immunomodulators showed a CMV infection. Protracted anti-CMV therapy determined a clinical improvement in all infected children.

Conclusions: In immunocompetent children CMV infection can play a role in GI motility disorders refractory to pharmacologic therapy and diet restriction and in episodes of reactivation of IBD. It is advisable testing CMV in children with such entities before other more invasive options (surgery for GI dysmotility, biological therapy for IBD) are considered.
successful bacterial clearance and generation of protective immunity in a healthy host versus skewing towards a more inflammatory, autoimmune response leading to disease. Both bacterial virulence and host susceptibility are likely to contribute to the latter.

PG5-17

A RANDOMIZED CONTROLLED TRIAL ON THE EFFECT OF A COMBINATION OF LACTOBACILLUS ACIDOPHILUS, LACTOBACILLUS DELBRUECKI SUBSP. BULGARICUS AND BIFIDOBACTERIUM BIFIDUM IN THE PROPHYLAXIS OF VOMITING AND DIARRHOEA OF HOSPITALISED CHILDREN 1 TO 7 YEARS OF AGE

Presenter: R. Pancheva. St Marina University Hospital, Varna, Bulgaria.

Co-authors: R. Pancheva1, K. Stoeva2, M. Georgieva1, D. Bliznakova3, M. Gylbova1, L. Ivanova2, D. Petrov1, V. Tseaneva1. 1Department of Pediatrics and Medical Genetics, St Marina University Hospital, Varna, Bulgaria; 2Department of Social Health, Medical University, Varna, Bulgaria; 3Laboratory of Virusology, St Marina University Hospital, Varna, Bulgaria.

Background and Aim: Gastrointestinal disorders occur frequently in patients who are hospitalised. Most disturbing are vomiting and diarrhoea. A risk group is that aged 1 to 7 years. Some of the reasons are frequent antibiotic prescription, hospital stay, changes in dietary pattern. The aim of the study was to determine whether a combination of probiotic bacteria (Lactobacillus acidophilus, Lactobacillus delbruecki subsp. bulgaricus and Bifidobacterium bifidum) can prevent gastrointestinal disturbances such as vomiting and diarrhoea (defined as ≥3 loose or watery stools/24 hours) of hospitalised children aged 1–7 years.

Methods: A total of 156 patients (age 1–7 years) with acute respiratory or urinary tract infection were enrolled in a double-blind randomised placebo controlled study. They were assigned at admission to receive standard intravenous antibiotic treatment plus either Lactobacillus acidophilus, Lactobacillus delbruecki subsp. bulgaricus and Bifidobacterium bifidum (n=79) at a dose of 10^10 three times daily, or placebo (n=77) for the course of their hospital stay. Stool samples were collected on the first day and fifth day after admission and tested for the presence of rotavirus Ag and Clostridium difficile toxin A and B.

Results: The probiotic combination reduced the risk of vomiting and diarrhoea in comparison with placebo (69.9% vs 30.1%; relative risk: 0.55 [95% CI: 0.38–0.80]). Patients receiving the probiotic combination had a lower rate of infection by rotavirus [4 of 78 (5%) vs 10 of 78 (13%)] and Clostridium difficile [6 of 78 (8%) vs 17 of 78 (22%)] compared with placebo. There was no statistical significance in the prevalence of rotavirus (16% vs 23%; relative risk: 1.36 [95% CI: 0.55–3.32]) and Clostridium difficile (26% vs 34%; relative risk: 1.33 [95% CI: 0.61–2.92]) between probiotic treated and placebo group.

Conclusions: Probiotics are a good tool for preserving the functional and biological integrity of the intestines during hospital stays of patients at highest risk for gastrointestinal disturbances such as children aged 1 to 7 years.

PG5-18

CALPROTECTIN AND IL-8: MARKERS OF INTESTINAL INFLAMMATION IN (PRE)TERM INFANTS?

Presenter: E. Westerbeek. VU University Medical Center, Amsterdam, The Netherlands.

Co-authors: E. Moerch1, R. Knol1, W. Fetter1, H. Lafeber1, R. Van Elburg1. 1Vu University Medical Center, Amsterdam, The Netherlands.

Background and Aim: In newborns, a systemic inflammatory response occurs within 15 minutes after birth. The gastrointestinal tract may also participate in this postnatal inflammatory response. Fecal calprotectin (f-calprotectin) and IL-8 (f-IL8) may reflect this inflammatory response. F-calprotectin and f-IL8, occurs after birth and that this response is more pronounced in preterm than in term infants. In addition, we hypothesize that perinatal factors influence f-calprotectin and f-IL8. The aim of the study was to compare f-calprotectin and f-IL8 in preterm and term infants, and to determine the influence of perinatal factors on f-calprotectin and f-IL8 in preterm infants.

Methods: In preterm infants (gestational age <32 weeks and/or birth weight <1500 g) and term infants (gestational age >37 weeks), fecal samples were collected within 48 hours after birth. F-calprotectin was measured by ELISA (Buhlmann, Basel, Switzerland) and f-IL8 by random-access chemiluminescence immunoassay (Siemens, Breda, The Netherlands). Perinatal factors of preterm infants were recorded. Data are expressed as median and range. Data were analysed by Mann-Whitney U test, Spearman correlation and linear...
background and Aim: Study of the pharmacokinetics (PK) of the extended release formulation of tacrolimus (ADVAGRAF) once daily, compared to tacrolimus (PROGRAF) twice per day.

Methods: The study was approved by the institutional review board. 20 adolescents (9 males; age: 12–17 years) fulfilled selection criteria: normal graft function, no rejection in preceding months, and informed consent. Median time from OLT was 11.7 years (range: 1.4–15.5). Present immunosuppression consisted of prednisolone (19 patients, median: 4 mg every other day), plus tacrolimus. Four patients received mycophenolate 11.5–25 mg/kg/day. After 7 days of taking their current doses of tacrolimus bid they were admitted for PK (samples at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 11.5, 12, 12.5, 13, 13.5, 14, 15, 16, 18, 20, 24 hours). On day 8 they were converted to ADVAGRAF on a 1:1 (mg/mg) basis for their total daily dose, given once daily. On day 14 a PK study of ADVAGRAF was done (samples at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 15, 24 h). Pharmacokinetics analysis was performed using a noncompartmental approach (WinNonlin 2.0). The relative bioavailability was assessed by the ratio: area under the concentration–time curve (AUC) of ADVAGRAF/AUC of PROGRAF and its corresponding 90% confidence interval (CI).

Results: (tacrolimus levels in ng/mL) Day 7: PK of PROGRAF: C_{max} of the afternoon dose was lower than C_{max} of the morning dose. Mean values were C0: 7.09, C12: 7.08 C_{max}: 19.5. AUC: 234.8 ng/mL·h. Day 14: PK of ADVAGRAF values were C0: 7.09 C_{max}: 18.9. AUC: 238.5 ng/mL·h. The mean ratio between formulations (AUC_Advagraf/AUC_Prograf) was 101% (90% CI: 91.3–111.85%). The correlation of trough level with AUC was r^2: 0.72 (PROGRAF) and r^2: 0.74 (ADVAGRAF). Patients were assessed 1 month after conversion. Dose was modified after day 14 in 5/20 patients. Trough levels at 1 month were 6.3 ± 2.2. Renal and graft function did not show changes compared to baseline. Patients were satisfied with the once daily administration of the drug.

Conclusions: In adolescents with normal graft function, tacrolimus (PROGRAF) bid and extended-release tacrolimus (ADVAGRAF) once daily are bioequivalent.

PH2-02

FACTORS IMPACTING ADOLESCENT QUALITY OF LIFE AFTER LIVER TRANSPLANTATION


Co-authors: L. Franck, F. Gibson, A. Dhawan. 1UCL Institute of Child Health, London, UK; 2King’s College London, London, UK.

Aim: To identify factors affecting quality of life (QoL) in young people after liver transplantation.

Methods: 55 young people, 21 (38%) male, aged 12–18 years participated. QoL was measured using a validated, self-report questionnaire (CHQ-CF87). Impacting factors included transplant, psychological and family-related variables measured through standardised questionnaires, including: impact of immunosuppression (MTSOSDS); self-esteem (PH2); emotional health (CDI); behaviour (SDQ), family function (FES) and parental impact (CHQ-PF50). Clinical information was also obtained.

Poster Session 2

Hepatology

PH2-01

CONVERSION FROM PROGRAF TO ADVAGRAF IN ADOLESCENTS WITH NORMAL GRAFT FUNCTION AFTER LIVER TRANSPLANTATION: BIOEQUIVALENCE ON 1:1 DAILY DOSE

Presenter: L. Hierro. Children’s University Hospital La Paz, Madrid, Spain.


Background and Aim: Study of the pharmacokinetics (PK) of the extended release formulation of tacrolimus (ADVAGRAF) once daily, compared to tacrolimus (PROGRAF) twice per day.
from the medical notes. The domains of the CHQ-CF87 were reduced using factor analysis to provide physical, psychological and social domains and factors impacting on QoL were identified through stepwise, multiple regression analysis.

Results: Young people had lower QoL compared to the general population in every domain (median 10.8 (range 3–22.7) points lower, \( P < 0.05 \)) but similar to that of young people with other chronic illnesses. A secondary chronic illness developed as a result of immunosuppression was noted in 40 (75%) young people and they reported the occurrence of 18 (4–31/33) symptoms related to immunosuppression. Twelve (22%) had received psychiatric or psychological treatment 3.5 (1–14) years from the time of transplant. Self-esteem was noted in 40 (75%) young people and they received psychiatric or psychological treatment 3.5 (1–14) years from the time of transplant. Self-esteem and emotional health were similar to the general population but emotional impact was significantly worst (19.6 vs 9.2, respectively, \( P > 0.05 \)). However, young people had significantly worst behaviour (19.6 vs 10.3, respectively, \( P < 0.001 \)). Forty (73%) were living in a 2-parent family. The organisation and control aspects of family function were worst than the general population (4.7 vs 2, \( P = 0.02 \)). Impact on parental time was similar to the general population but emotional impact was significantly worst: 65.6 vs 80.3, \( P < 0.001 \). Follow-up was available in 74/77 patients. All biopsies were reviewed by a single pathologist. Histological hepatitis was graded according to the Metavir scoring system.

Results: Four major histological categories were found in the biopsies: (1) normal findings (n = 23; 31%), (2) reactive changes (n = 7; 10%), (3) portal fibrosis (n = 25; 34%), (4) hepatitis (n = 17; 23%). Miscellaneous findings were found in 2 biopsies. Compared with our earlier study using a CsA-based immunosuppressive regimen, the incidence of fibrosis was similar (CsA 31%; FK 34%; NS). The patients with portal fibrosis had significantly higher liver enzymes (AST 49 ± 28 IU/L, ALT 59 ± 40 IU/L) compared with patients with normal histology (AST 36 ± 11 IU/L, ALT 30 ± 13 IU/L). Histological hepatitis was more frequently observed in FK-treated patients (23%) compared with the CsA group (CsA 1%; \( P < 0.05 \)). This hepatitis was predominantly mild (Metavir score “mild” in 13/17, 76%; “moderate” in 4/17, 24%). Histological hepatitis was not associated with elevated liver enzymes. The etiology of the hepatitis could not be identified: Serology for hepatitis A, B and C was negative. In situ hybridisation for EBV (EBER) was negative in all hepatitis cases. Serum IgG concentrations were similar in the two groups (hepatitis 11.9 ± 4.5, other 10.2 ± 3.3, NS).

Conclusions: The incidence of graft fibrosis at 1 year after pediatric LTx is similar after a CsA- or an FK-based immunosuppressive regimen. The FK-based regimen is specifically associated with a high incidence of histological mild hepatitis of unknown origin.

**PH2-03**

HIGH INCIDENCE OF HEPATITIS AND PORTAL FIBROSIS 1 YEAR AFTER PEDIATRIC LIVER TRANSPLANTATION


Background and Aim: Recent studies show a high incidence of abnormal histological features during follow-up after pediatric liver transplantation (LTx). Previously we described portal fibrosis in 30% of first-year biopsies after LTx in a group of pediatric patients treated with an immunosuppressive scheme based on cyclosporine (CsA; *Transplantation* 2000; 70: 1581–7). It has remained unclear whether the development of fibrosis differs using a tacrolimus- (FK) based immunosuppressive regimen, for example, due to differences in induction of hepatic TGF-β1 expression and hence stimulation of fibrosis.

The aim of this study was to assess development of fibrosis in liver grafts at 1 year after pediatric LTx using a FK-based immunosuppressive scheme.

Methods: We included patients transplanted between 1999 and 2006, who had a 1-year post-LTx survival and were treated with a FK-based immunosuppressive scheme (n = 77). A 1-year post-LTx protocol biopsy was available in 74/77 patients. All biopsies were reviewed by a single pathologist. Histological hepatitis was graded according to the Metavir scoring system.

Results: Four major histological categories were found in the biopsies: (1) normal findings (n = 23; 31%), (2) reactive changes (n = 7; 10%), (3) portal fibrosis (n = 25; 34%), (4) hepatitis (n = 17; 23%). Miscellaneous findings were found in 2 biopsies. Compared with our earlier study using a CsA-based immunosuppressive regimen, the incidence of fibrosis was similar (CsA 31%; FK 34%; NS). The patients with portal fibrosis had significantly higher liver enzymes (AST 49 ± 28 IU/L, ALT 59 ± 40 IU/L) compared with patients with normal histology (AST 36 ± 11 IU/L, ALT 30 ± 13 IU/L). Histological hepatitis was more frequently observed in FK-treated patients (23%) compared with the CsA group (CsA 1%; \( P < 0.05 \)). This hepatitis was predominantly mild (Metavir score “mild” in 13/17, 76%; “moderate” in 4/17, 24%). Histological hepatitis was not associated with elevated liver enzymes. The etiology of the hepatitis could not be identified: Serology for hepatitis A, B and C was negative. In situ hybridisation for EBV (EBER) was negative in all hepatitis cases. Serum IgG concentrations were similar in the two groups (hepatitis 11.9 ± 4.5, other 10.2 ± 3.3, NS).

Conclusions: The incidence of graft fibrosis at 1 year after pediatric LTx is similar after a CsA- or an FK-based immunosuppressive regimen (~30%). The FK-based regimen is specifically associated with a high incidence of histological mild hepatitis of unknown origin.

**PH2-04**

EFFECTS OF HYponATREMIA ON PRETRANSPLANT AND POSTTRANSPLANT OUTCOME IN PEDIATRIC LIVER RECIPIENTS

Presenter: C. Arikan. Ege University School of Medicine Organ Transplantation and Research Center, Izmir, Turkey.
**Background and aims:** Serum sodium (Na) concentrations have been suggested as a useful predictor of mortality in adult patients with end-stage liver disease awaiting liver transplantation. We aimed to assess the effects of hyponatremia (Na < 130 mEq/L) on pretransplant mortality rate and survival, as well as posttransplantation outcome in pediatric patients.

**Methods:** One hundred-fifty-nine consecutive patients with cirrhosis listed for liver transplantation during December 2003–December 2006 were included in the study. The effect of Na status, PELD scores, portal hypertension, variceal bleeding, ascites and renal resistive index on both pre–posttransplant outcome searched by Cox regression and multivariate logistic regression analysis.

**Results:** The median age, PELD score and serum Na were 4.5 yrs (0.3–17), 20 (3–54), 130 mEq/L (119–147), respectively. Biliary atresia was the primary diagnosis in 42%, and the pretransplant mortality rate on the waiting list was 23.7%. Hyponatremia was detected in 51.6% (n=82). The risk for death on the waiting list was 23.7% (P<0.0001, 95% CI 1.97–3.6) compared to patients without hyponatremia. Additionally, patients who subsequently died on the waiting list had higher PELD scores (P<0.0001) and ascites (P<0.0001, OR = 2.49, 95% CI 1.57–3.5) than patients survived without transplantation.

**Conclusions:** The presence of hyponatremia was associated with a high rate of mortality, and reduced 3-month transplant survival. So, it should be considered not only a risk factor of death before transplantation but also a risk factor for early posttransplantation outcome.

**PH2-05**

**ERYTHROCYTOSIS: ALSO A POSTTRANSPLANT COMPLICATION IN LIVER RECIPIENTS?**

Presenter: P. Vajro. Università di Napoli, Napoli, Italy.

**Background and Aim:** Erythrocytosis may complicate renal transplantation with a prevalence of the order of 10–15% varying from as low as 3.2% in the pediatric age to as high as 20% in adults and has not been reported in other types of organ transplantation. It is suggested that factors like erythropoietin (EPO), renin-angiotensin system, male gender, and renal cysts may play a role. We report 3 cases of erythrocytosis developing after liver transplantation (OLT).

**Methods:** 90 Italian OLT children (F=42; age: 12.8±6.7 years) followed up at our institution were retrospectively evaluated. Mean age at OLT was 3.5±3.6 years (range 0.2–12.5 years); biliary atresia was the main OLT indication (70%). Cyclosporin A and tacrolimus were the primary immunosuppressors in 26 and in 64 patients, respectively. Family and clinical history, complete physical examination, blood cells count, hemoglobin levels, hematocrit (Hct), renal and hepatic function tests including ultrasonographic parameters were recorded. Hemoglobin electrophoresis, arterial blood gas analysis, spirometry, EPO, and abdominal CT scan or MMR were investigated in 94 patients. An increase in Hct (P<0.0001) and ascites (P=0.003, 95% CI 0.9–0.97) were independently associated with pretransplant death. Significant predictors of survival within 3 months at listing were also hyponatremia (P=0.002, 95% CI 0.14–0.64) and PELD score (P=0.031, 95% CI 1.01–1.06). Among risk factors hyponatremia (P=0.05, 95% CI 0.05–0.92) and presence of ascites (P=0.04, 95% CI 1–28.2) were independent predictive factors of early posttransplantation survival.

**Conclusions:** These are the first 3 cases of erythrocytosis developing after OLT. In the symptomatic patient erythrocytosis may be due to high levels of EPO: it is unclear whether renal cysts may increase EPO production directly or by local ischemia of adjacent renal tissue. Our preliminary results suggest the need of searching for erythrocytosis also in OLT recipients’ follow-up.

**PH2-06**

**MYCOPHENOLATE MOFETIL–ASSOCIATED REVERSAL OF GLOMERULAR FILTRATION RATE LOSS IN LIVER-TRANSPLANTED CHILDREN**

Presenter: S. Rauschenfels. Medical School Hannover, Hannover, Germany.
Background and Aim: Calcineurin inhibitors (CNI) provide effective immunosuppression after orthotopic liver transplantation (OLT) in children but are associated with chronic renal failure. Combined immunosuppressive therapy of mycophenolate mofetil (MMF) and reduced CNI is known to be a safe and nephroprotective therapy. The long term outcome in children has not been fully evaluated. The aim of this study was to proof the efficiency of a combined immunosuppressive therapy of MMF and cyclosporin A (CSA) in stable liver transplanted children. The development of renal function under MMF and CSA was compared to renal function under CNI.

Methods: In this prospective study development of renal function of 25 children (14 male) in median 11.1 (range 2.0–16.5) year old, in median 4.7 (range 1.1–15.6) years after OLT under a combined immunosuppressive therapy of MMF and CSA was compared with development of renal function in a historic control group of 51 children (28 male) in median 10.6 (range 1.5–15.9) year old in median 5.1 (range 1.0–10.7) years after OLT under immunosuppressive therapy with CNI (36 CSA, 15 Tacrolimus). In the study group target CSA-C\textsubscript{0} trough-level were 80–120 ng/mL or level were 35–65 ng/mL. The historical control group aimed CSA-C\textsubscript{0} through-levels of 80–120 ng/mL or Tacrolimus-C\textsubscript{0} of 3–6 ng/mL. The target MMF AUC (area under the plasma concentration time-curve 0–12 h) was 30–65 \mu g*h/mL. In a yearly routine renal function was evaluated by Cr-EDTA-clearance (Sapirstein) and plasma-cystatin-C-values.

Results: In the study group there was a decrease of 28% in plasma Cystatin-C values, from 1.56 ± 0.45 mg/L to 1.12 ± 0.39 mg/L at 24 months. The GFR increased from 60 mL/min to 77 mL/min at 24 months. In the control group plasma cystatin-C values increased of 7% from 0.44 ± 0.35 mg/L to 1.55 ± 0.44 mg/L. The GFR showed a decrease of 11% from 67 ± 23 mL/min to 59 ± 22 mL/min at 24 months. In the study group MMF-AUC were in median 61.4 ± 26.4 mg*h/L. MMF dosage had to be reduced due to gastrointestinal side effects in 2 patients. In 1 patient of the study group acute rejection occurred after refusal of medication, whereas in the control group no rejection was reported. Liver function tests and full blood cell counts in both groups showed no significant changes.

Conclusions: Introduction of MMF combined with reduced CSA dosage leads to improvement of renal function of liver-transplanted recipients. The combined immunosuppressive therapy is potent and safe. Side effects under a close MMF monitoring were rare.

PH2-07

EVOLUTION OF POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDER MANAGEMENT IN PEDIATRIC LIVER TRANSPLANT RECIPIENTS: A SINGLE-CENTRE EXPERIENCE

Presenter: A. Verma. Pediatric Liver Centre, King’s College Hospital, London, UK

Co-authors: N. Shanmugam\textsuperscript{1} Z. Ahmadinejad\textsuperscript{1}, D. Hadzic\textsuperscript{1}, A. Baker\textsuperscript{1}, G. Mieli-Vergani\textsuperscript{1}, N. Heaton\textsuperscript{1}, M. Rela\textsuperscript{1}, M. Zuckerman\textsuperscript{2}, S. Height\textsuperscript{1}, A. Dhawan\textsuperscript{1}.

\textsuperscript{1}Pediatric Liver Centre, King’s College Hospital, London, UK; \textsuperscript{2}Institute of Liver Studies, King’s College Hospital, London, UK.

Aim: The aims of this study were to review the incidence, clinical presentation, histological patterns, different therapeutic modalities, and outcome of post transplant lymphoproliferative disorder (PTLD) in the pediatric liver transplant recipients (PLTR) in our institution.

Methods: A retrospective analysis was done for 24 cases of PTLD diagnosed on histology between June 1990 and December 2007 in 658 PLTR.

Results: The incidence of PTLD was 3.7%. The median age at diagnosis of PTLD was 57 months (range 16–152) with median interval for PTLD presentation from liver transplant was 112.5 weeks (range 13–404). Clinical manifestations were gastrointestinal in 16 patients, neurological in 2, and neck pain and lump in 2. Lymph node enlargement was observed in 20 patients, splenomegaly in 17, fever in 15, and hepatomegaly in 13. Primary immunosuppression was cyclosporine in 13 patients and tacrolimus in 9. Histopathologic findings were high-grade lymphoma for 9 patients and polymorphic PTLD for 6. In situ hybridization for EBV was positive in 18 of 21 cases. Pretransplant EBV serology was negative in 14 patients. Initial therapy for 21 patients was reduction of immunosuppression (RDI). Nine patients received rituximab, but only 3 responded. Fourteen children underwent chemotherapy of whom 12 responded. Four patients had surgical removal of the lesions. Twelve patients received an acyclovir antiviral agent. One patient died before any intervention. Two patients had improvement with RDI along with surgery. Six (27.3%) patients died during the follow-up period with PTLD being the predominant cause in 4.

Conclusions: The treatment of PTLD continues to evolve because of the availability of newer agents. The
efficacy of rituximab as sole agent in the treatment of PTLD remains to be proved.

**PH2-08**

**INFECTIOUS COMPLICATIONS IN CHILDREN AFTER LIVER TRANSPLANTATION**

Presenter: M. Cakir. Ege University School of Medicine, Izmir, Turkey.

Co-authors: M. Cakir1, C. Arikan1, H. Yuksekaya1, M. Baran2, G. Tumgor1, M. Kilic1, S. Aydogdu1. 1Ege University School of Medicine, Organ Transplantation and Research Center, Izmir, Turkey.

**Aim:** Infectious complications still represent a major cause of morbidity and mortality in children after liver transplantation (LT). The objective of this prospective study is to analyze the occurrence, timing, determinants and outcome of infectious complications after pediatric LT.

**Methods:** The study included 44 consecutive pediatric patients (3.9 ± 4.3 years, 45% male) who underwent LT between January 2006 and October 2007. Biliary diseases were leading cause (57.5%) for LT. Peripheral blood, urine and other cultures were obtained in all children during febrile episodes. Laboratory findings, site and type of infection and culture results were prospectively recorded in all patients.

**Results:** There were 24 documented infections in 19 children (0.54 infection episode per patients and 1.26 infection episodes per infected patients) including; 18 bacterial (75%), 3 viral (12.5%) and 3 fungal (12.5%) infections. Sites of infections were blood stream infection in 11 (45.8%), urinary tract in 6 (25%), and intraabdominal in 2 (8.3%). The median follow-up was 413 days. Overall, cumulative infection incidence and infection incidence density were 54.4 and 1.72 per 1000 patient days, respectively. Most of the infections (83.3%) occurred within first 6 months. Infection related death was seen in only 3 patients. Positive predictive value of leucocytosis, thrombocytopenia, elevated CRP and ESR and procalcitonin for the proven infection episodes were 41.6%, 25%, 70.8%, 50% and 87.5%. After antibiotherapy; both CRP and procalcitonin levels decreased to normal values in noncomplicated patients. None of the factors such as gender, age, donor type, primary diagnosis, duration of ICU after LT, type of immunosuppression were found to be associated with infectious complications except presence of malnutrition and weight at the time of LT in multivariate analysis.

**Conclusions:** Infectious complications are still a common problem in liver transplanted patients; mainly bacterial origin and presents in the early period. Malnutrition and low body weight are the major risk factors. It is important to differentiate the infections from other complications such as rejection. Procalcitonin may be used for to predict and response to antibiotherapy for the infectious complications with its high predictive value and clinical correlation with the treatment response.

**PH2-09**

**DERMATOLOGIC COMPLICATIONS AFTER LIVER TRANSPLANTATION IN PEDIATRIC PATIENTS**

Presenter: M. Baran. Ege University School of Medicine, Izmir, Turkey.

Co-authors: M. Baran1, H. Yuksekaya1, M. Cakir1, M. Kilic1, S. Aydogdu1, C. Arikan1. 1Ege University School of Medicine, Organ Transplantation and Research Center, Izmir, Turkey.

**Aim:** To determine frequency, characteristics, and outcome of dermatological complications after pediatric liver transplantation.

**Methods:** Between March 1997 and December 2008, 158 pediatric liver transplantation procedures were performed (62 DDLT and 96 LDLT, 10 retransplantation) in 148 patients (median 4.6; range 6 mo–17 years old). Medical records of patients evaluated for posttransplant dermatologic problems.

**Results:** 50 of 148 patients (33.8%) experienced dermatologic problem during posttransplant follow up. The most common complication are pressure-induced alopecia areata (n = 10) and hypomagnesemia induced hair loss (n = 4) were recovery within posttransplant 6 months period. Cyclosporine-induced gingival hyperplasia (n = 5) and hypertrichosis (n = 6) treated with conversion to tacrolimus. Fissured tongue and buccal mucositis developed in 4 patients who received tacrolimus. These patients also had high total serum IgE level and dramatically respond to ketotifen and tapering immunosuppression. Eight patients who presented recurrent urticaria and maculopapular rash with itching diagnosed as food allergy. Infection-related problems zona zoster (n = 1), Varicella zoster (n = 4), herpetic stomatitis (n = 4) and candidal mucositis were self-limited. Perianal ulceration (n = 1) and brown-blue polyloid and plate lesions on the hard palate (n = 1) due to Kaposi sarcoma were treated conversion to sirolimus-based immunosuppression. Graft-versus-host-disease (GVHD) diagnosed in 2 patients and despite aggressive treatment and support they died.

**Conclusions:** Dermatologic problems are common in pediatric liver transplant recipients. They are mostly related to immunosuppression. Despite most of them being self-limited, some dermatologic signs may be
omnious and should alert physicians to early diagnosis and appropriate treatment.

**PH2-10**

**SURVIVAL OF PAEDIATRIC TRANSPLANT RECIPIENTS MORE THAN 15 YEARS AFTER ORTHOTOPIC LIVER TRANSPLANTATION IN CHILDREN**

**Presenter:** U. Baumann. Hannover Medical School, Hannover, Germany.

**Co-authors:** L. Schwadtke¹, L. Kousoulas¹, E. Pfister¹, I. Goldschmidt¹, K. Bindeballe¹, K. Schubert¹, M. Bohn¹, S. Rauschenfels¹, F. Lehnert¹, T. Becker¹, U. Baumann¹. ¹Hannover Medical School, Hannover, Germany.

**Background and Aim:** Paediatric liver transplantation has become an established treatment option with excellent short and medium term outcome. Limited data exist as to long-term prognosis and late graft loss in children who were transplanted more than 15 years ago. We wish to report our single-centre experience on survival rates and graft loss in children transplanted at our institution.

**Methods:** We performed a retrospective analysis of patient records of children aged 0–17 years who underwent liver transplantation at our institution between November 1972 and December 1993. Of 896 transplant procedures performed in total, 186 (20.8%) were children (96 boys and 90 girls). These 186 transplants were performed on 152 children. 2 children died following road traffic accidents and were excluded from further analysis. Data collected included gender, diagnosis at OLT, number of retransplants, type of transplantation, type of graft, complications and cause of death.

**Results:** 31 of 186 (16.7%) transplant procedures were retransplants, 5 (1.6%) were third transplants. 150 children aged 01/12–1710/12 years were transplanted either for acute hepatic failure (n = 20, 13.3%) or cirrhosis (biliary atresia (n = 50, 33.3%), metabolic liver disease (n = 43, 28.7%), and other (n = 37, 24.7%). None of the children underwent combined liver–kidney transplantation. 37 of 150 (24.7%) children required retransplantation 1 day–107/12 years after their first graft, 5 (3.3%) children received a 3 liver transplant 2 days–9 months after their second graft. Reasons for graft loss were vascular complications (n = 5, 11.9%), biliary complications (n = 6, 14.3%), rejection (n = 19, 45.2%) and other (n = 12, 28.6%). Altogether 63 of 150 (42%) children died between 1 day and 13 11/12 years after their last graft. 87 children (48%) are alive 15–30 years after their first liver transplant procedure. Primary diagnoses of the 60 deceased patients were acute hepatic failure (n = 11, 17.5%); biliary atresia (n = 24, 38%) and metabolic diseases (n = 11, 17.5%). All transplanted organs were of cadaveric origin. In 186 transplant procedures 126 (67.7%) full-size grafts were used, 60 (32.3%) were split grafts. In total, causes of death were infections (n = 20, 31.8%), liver related complications (n = 11, 17.5%), cerebrovascular complications (n = 7, 11.1%), pulmonary (n = 5, 7.9%), gastrointestinal (n = 3, 4.7%), cardiovascular complications (n = 3, 4.7%) and others (n = 14, 22.3%). Overall survival of children more than 15 years following OLT is fairly good. Primary diagnosis of liver disease seems to influence clinical outcome. Infections after OLT are the most common cause of death.

**Conclusions:** Long-term outcome of liver transplantation in childhood is good and likely to improve further with modern surgical and medical management.

**PH2-11**

**ANGIOPLASTY OF SYSTEMIC VEINS AFTER PAEDIATRIC LIVER TRANSPLANTATION**

**Presenter:** H. Bertram. Hannover Medical School, Hannover, Germany.

**Co-authors:** E. Pfister¹, U. Baumann¹, T. Becker², S. Schoof³. ¹Paediatric Gastroenterology and Hepatology, Hannover Medical School, Hannover, Germany; ²Transplantation Surgery, Hannover Medical School, Hannover, Germany; ³Paediatric Cardiology, Hannover Medical School, Hannover, Germany.

**Background and Aim:** Vascular complications account for a substantial perioperative or late morbidity after pediatric liver transplantation, especially in small patients after use of split grafts. Modern catheter technology enables endovascular interventions in the vast majority of patients. We present our preliminary experience in treating vascular complications in systemic veins in all paediatric age groups.

**Methods:** Retrospective evaluation of all endovascular interventions in systemic veins (inferior caval vein [IVC] or liver vein anastomosis [LV A]) during the last 5 years. Results: Between 2003 and 2008, 170 visceral angiographies were performed in 69 patients [infants (n = 28), children (n = 124), adolescents (n = 18)] after pediatric liver transplantation for suspected stenotic lesions in IVC and/or LV A or follow-up investigations. 57 patients were treated with interventional recanalization and/or balloon angioplasty for stenotic lesions, tortuous veins, or complete vessel occlusion during their first procedure. Despite successful balloon angioplasty, restenosis after endovascular therapy was frequent. In intention to avoid primary stenting in small children whenever possible, repeated angioplasty was performed in 60% of patients. 33 patients received 2–4 interventions, 5 patients >5
angioplasties with decreasing severity of residual stenosis during follow-up. Of 13 complete occlusions, all but 2 could be recanalized. Stent angioplasty (n = 12) was used in severe LVA obstruction in the early postoperative period with large amounts of ascites forcing the patients on ICU, or after hemodynamically relevant restenosis despite repeated balloon angioplasties, respectively, with complete relief of vessel occlusion in all patients. Accept from small wire perforations during revascularization procedures without significant bleeding we did not notice any complications.

Conclusions: Endovascular treatment of stenotic lesions in systemic veins after pediatric liver transplantation is effective and safe. Balloon angioplasty alone will result in frequent restenosis, especially if the primary lesion is caused by vessel torsion instead of scar formation. Stent angioplasty is more effective to relieve the obstruction, but may cause problems in the growing patient during follow-up. An individual strategy is recommended.

PH2-12

DIAGNOSTIC AND THERAPEUTIC DIRECT SELECTIVE PORTAL VEIN ANGIOGRAPHY
Presenter: H. Bertram. Hannover Medical School, Hannover, Germany.
Co-authors: E. Pfister, B. Ulrich, B. Thomas, S. Stephan. 1Paediatric Gastroenterology and Hepatology, Hannover Medical School, Hannover, Germany; 2Transplantation Surgery, Hannover Medical School, Hannover, Germany; 3Paediatric Cardiology, Hannover Medical School, Hannover, Germany.

Background and Aim: Selective angiography is the gold standard for detailed evaluation of vascular structures. Modern catheter technology enables endovascular interventions during the same procedure. We present our preliminary experience in pre- and postoperative diagnostic as well as therapeutic catheterizations of the portal vein circulation.

Methods: Direct portal vein catheterizations were performed percutaneously in general anaesthesia using the transhepatic or transsplenic approach, respectively, with ultrasound as well as fluoroscopic guidance. The track in the spleen left by the sheath was closed with coils in all but one patient, we only used coils after transhepatic catheterization once.

Results: Between 2006 and 2008, 17 direct portal vein angiographies were performed in 11 patients [age: 2–16 years, body weight: 12–68 kg]. Transsplenic direct selective angiography (n = 8) of the extraportal portal vein was predominantly used to display splenic and mesenteric veins as well as the extent and the distribution of collateral vessels in patients with portal vein thrombosis to define surgical [shunt procedure vs liver transplantation] or endovascular [recanalization and embolization of collateral vessels, respectively] options. Transsplenic stent angioplasty of portal vein stenosis and transsplenic recanalization of complete portal vein thrombosis was successfully performed in one patient each. Transhepatic catheterization of the portal vein was chosen for balloon (n = 2) or stent angioplasty (n = 5) of portal vein stenosis after liver transplantation. In general, these are time-consuming procedures requiring sophisticated equipment and experienced investigators. Most patients needed multiple punctures, before a wire could be advanced into the portal vein. In 3 patients we were not able to enter the portal vein percutaneously by the transhepatic (n = 2) or the transsplenic route, respectively. All 3 were catheterized successfully during a second procedure using transsplenic access. We faced an oozing bleeding into the abdominal cavity along the sheath requiring blood transfusion in one patient. A thrombus without compromise of blood flow was noticed after stent angioplasty of portal vein stenosis which resolved during anticoagulation therapy. Transitory mild fever and moderate abdominal pain within 48 h after intervention occurred in 60% of patients. Fever with positive blood culture was noticed in one patient after transhepatic stent angioplasty of portal vein stenosis.

Conclusions: Direct selective portal vein angiography is a technically demanding procedure. It uniquely displays the extraportal portal venous system including collateral vessels and gives hemodynamic data that may help to take therapeutic decisions. Endovascular catheter interventions may successfully be performed using the transhepatic or transsplenic approach.

PH2-13

OUTCOME OF LIVER TRANSPLANTATION IN CHILDREN WITH CYSTIC FIBROSIS
Presenter: S. Loganathan. Birmingham Children’s Hospital, Nottingham, UK.
Co-authors: I. Van Mourik, J. Clarke, C. Lloyd, D. Kelly. 1Birmingham Children’s Hospital, Nottingham, UK.

Aim: To assess the long term outcome of liver transplantation (LTx) in children with cystic fibrosis—associated liver disease (CFLD) in our unit.

Methods: Retrospective review of all children with CFLD transplanted between 1989 and 2007. Data reviewed are demographic details, indications for LTx, diabetic status, survival, renal function (calculated glomerular filtration rate (cGFR) mL/min/1.73 m²), lung function (FEV1 and FVC % scores) and nutritional status.
Results: 20 children (12M, 8F) underwent LTx. Median age at transplant was 11.7 years (2.1–16.5). Indications were progressive liver failure with deteriorating pulmonary function (n = 19) and acute liver failure (n = 1). 13/20 are alive. Median follow up is 8.4 years (0.07–15.5). 3/20 had retransplant (2 primary graft failure, 1 chronic rejection after 9 years). 6 were diabetic pre-LTx and 6 developed IDDM post-LTx, in 2/6 this resolved between 6 and 9 months post-LTx (Table 17).

Conclusions: 1- and 5-year survival was 90% and 80%, respectively. Nutritional status post-LTx for CFLD does not improve which is likely multifactorial. Pulmonary function remained stable in first two years and renal function deteriorated in first 6 months before stabilising. Provided lung function is reasonably preserved, LTx is an effective therapeutic option for end-stage CFLD and has comparable long-term survival to LTx for other indications.

### PH2-14

**CAN PAEDIATRIC PROFESSIONALS RELIABLY IDENTIFY PALE STOOL?**

Presenter: B. Vadamalayan. *Kings College Hospital NHS Trust, London, UK.*

Co-authors: O. Akindolie1, A. Sutcliffe2, A. Baker1. 1*King’s College Hospital, London, UK; 2University College Hospital London, London, UK.*

**Background and Aim:** Biliary atresia (BA) is an important surgically remediable cause of neonatal cholestasis. Early recognition by identification of abnormal (pale) stool colours allows earlier surgery and better results. Unfortunately there are no objective means of identifying abnormal stools. Before designing a measurement device, we wished to know how reliably trained professionals recognise pale stool.

**Methods:** Following ethical approval, stools were collected from normal and cholestatic infants and photographed against a white background with a colour calibration card. The colours were standardised by computers to allow for ambient light. Photographs of 5 normal, 3 indeterminate and 4 acholic stools were chosen and shown to professionals with a questionnaire asking them to classify each stool as “healthy” or “suspect.”

**Results:** A total of 81 questionnaires were completed by 36 paediatric doctors and 45 paediatric nurses in 3 London teaching hospitals, 1 of them is a National Referral Centre for paediatric liver disease. Doctors and nurses correctly identified suspect stools at 62.7% and 62.9%, respectively:

**Conclusions:** There were no significant differences between the 3 institutions or between doctors and nurses in identifying pale stool but doctors were better than nurses at identifying normal stool (71.5% vs 58.5%). Professionals can not recognise suspect stools to the level of reliability required for identification of biliary atresia. There is a need for objective methods of identifying stool colour.

### PH2-15

**CHOLANGITIS AND OUTCOMES AFTER KASAI PORTOENTEROSTOMY FOR BILIARY ATRESIA**

Presenter: P. Rao. *St James University Hospital, Durham, UK.*

Co-authors: S. Davison1, V. Karthik1, S. Rajwal1, N. Alizai1, M. Stringer1, P. Mcclean1. *St James University Hospital, Leeds, UK.*

**TABLE 17.**

<table>
<thead>
<tr>
<th>Time</th>
<th>Pre-LTx (n = 20)</th>
<th>Post-LTx, 12 mo (n = 18)</th>
<th>Post-LTx, 24 mo (n = 18)</th>
<th>Post-LTx, 60 mo (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight SDS</td>
<td>-0.74 (–4.25 to 1.22)</td>
<td>-1.27 (–3.36 to 0.46)</td>
<td>-0.99 (–4.5 to 0.62)</td>
<td>-1.7 (–5.2 to 0.08)</td>
</tr>
<tr>
<td>Height SDS</td>
<td>-1.25 (–3.0 to 1.080)</td>
<td>-1.68 (–3.84 to 0.96)</td>
<td>-1.75 (–4.23 to 0.66)</td>
<td>-1.4 (–4.3 to 0.8)</td>
</tr>
<tr>
<td>cGFR</td>
<td>120.9 (77 – 165)</td>
<td>71 (30–133)</td>
<td>77 (53–149)</td>
<td>85.5 (38.0–105)</td>
</tr>
<tr>
<td>FEV1 SDS</td>
<td>-1.51 (–3.7 to 0.79)</td>
<td>-1.00 (–3.79 to 1.05)</td>
<td>-1.21 (–3.78 to 1.19)</td>
<td></td>
</tr>
<tr>
<td>FVC SDS</td>
<td>-0.76 (–3.66 to 1.28)</td>
<td>-0.63 (–3.24 to 1.49)</td>
<td>-0.3 (–2.96 to 1.03)</td>
<td></td>
</tr>
</tbody>
</table>

1. Hospital “A” (Referral Centre)

<table>
<thead>
<tr>
<th>Stool</th>
<th>Normal</th>
<th>Suspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correct</td>
<td>69/100 (69%)</td>
<td>93/139 (66.9%)</td>
</tr>
<tr>
<td>Incorrect</td>
<td>31/100 (31%)</td>
<td>46/139 (33.1%)</td>
</tr>
</tbody>
</table>

2. Hospital “B”

<table>
<thead>
<tr>
<th>Stool</th>
<th>Normal</th>
<th>Suspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correct</td>
<td>121/205 (59.0%)</td>
<td>186/287 (64.8%)</td>
</tr>
<tr>
<td>Incorrect</td>
<td>84/205 (41%)</td>
<td>101/287 (35.2%)</td>
</tr>
</tbody>
</table>

3. Hospital “C”

<table>
<thead>
<tr>
<th>Stool</th>
<th>Normal</th>
<th>Suspect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correct</td>
<td>70/95 (73.7%)</td>
<td>72/133 (54.1%)</td>
</tr>
<tr>
<td>Incorrect</td>
<td>25/95 (26.3%)</td>
<td>61/133 (33.1%)</td>
</tr>
</tbody>
</table>

**Background and Aim:** The diagnosis of cholangitis following Kasai portoenterostomy (KP) for biliary atresia is difficult to prove and antibiotic treatment is often commenced on the basis of a high index of suspicion. This study aims to examine the clinical features of children treated as cholangitis, their response to antibiotics and need for subsequent orthotopic liver transplant (OLT).

**Methods:** A retrospective case note review of 72 consecutive children with biliary atresia who underwent KP at a single centre between June 1994 and April 2008. Cholangitis was defined as “definite” in the presence of unexplained fever, deterioration in liver function tests (LFT’s) with positive blood cultures or “probable” if unexplained fever accompanied either deranged LFT’s and/or pale stools but without positive blood cultures. Children without fever but with deteriorating LFT’s and/or pale stools were defined as having “possible” cholangitis. Response to antibiotics was defined as improvement in symptoms of cholangitis by end of treatment.

**Results:** Fifty-two patients with cholestasis underwent MR cholangiography and duodenal fluid examination. Diagnosis of extrahepatic biliary atresia was based on nonvisualization of either common bile duct or common hepatic duct, duodenal fluid examination showed colorless and surgical exploration. Diagnosis of infantile hepatitis syndrome was confirmed with visualization of common bile and common hepatic ducts, duodenal fluid color was yellow and clinical follow-up until jaundice resolved.

**Results:** 34 of 52 infantile patients were diagnosed as IHS, 18 was diagnosed as EHBA. In 30 of the 34 patients with HIS, MR cholangiography clearly depicted common hepatic and common hepatic duct were not depicted. In 31 of the 34 patients with IHS, duodenal fluid examinations were bile acid positive. In 18 patients with EHBA, the common bile ducts and common hepatic ducts were not depicted by MR cholangiography in 13, and duodenal fluid examinations were bile negative in all 18 cases.

**Conclusions:** Sensitivity and specificity of duodenal fluid examination in diagnosis of EHBA is 100% and 91.1%. Sensitivity and specificity of MR cholangiography in diagnosis of EHBA is 94.4% and 88.24%. Sensitivity and specificity of MR cholangiography combined with duodenal fluid examination in diagnosis of EHBA is 94.4% and 97.06%. MR cholangiography and dynamic examination of duodenal fluid are useful in the differential diagnosis between IHS and EHBA.

**PH2-17**

HEPATIC OSTEODYSTROPHY IN CHILDREN WITH PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS (PFIC)

**Presenter:** Z. Huang, Tongji Medical College, Huazhong Science and Technology University, Wu Han, China.

**Co-authors:** Z. Huang, Y. Hu, L. Xia, Tongji Hospital, Tongji Medical College, Huazhong Science and Technology University, Wu Han, China.

**Background and Aim:** PFIC encompasses a group of autosomal recessive disorders due to defective transport of bile salts from the hepatocyte into the canaliculus. Some children have severe bone disease resulting in
fractures while others escape this complication. The aim of this small pilot study was to examine possible factors associated with severe bone disease.

**Methods:** The case notes of all children with PFIC attending a supraregional paediatric liver unit were reviewed. **Results:** 23 children diagnosed with PFIC were included in the study. Median age at presentation to hospital was 7 weeks (range 2–68). Their mean birth weight was 3.1 kg (95% CI ± 0.2) and mean gestation at birth 38.5 weeks (95% CI ± 0.67). 74% were of Asian origin. 14 children (61%) presented with neonatal jaundice. Other modes of presentation included failure to thrive (17%), abnormal liver function without jaundice (22%), hypocalcaemia (13%), late-onset jaundice (8.7%) and pruritus (4.3%). 9 (39%) children had fractures. In 8 the lower limb was involved and in 5 there were multiple fractures. There was no difference in the sex ratio, ethnicity, mean birth weight, median age at presentation, and number of children needing a liver transplant between the two groups. Of the 9 children with fractures, 7 had x-rays available for radiological examination and all (100%) had features of rickets. All 9 children with fractures had episodes of hypocalcaemia but 6 (67%) required treatment with intramuscular vitamin D and/or prolonged courses of intravenous calcium. Of the 14 children without fractures, x-rays were available in 11 children, 6 (55%) of whom had evidence of rickets. Five (36%) patients in this group required intramuscular vitamin D and/or intravenous calcium therapy. Weight z score at 6 months of age was significantly worse in the fracture group ($P=0.005$). Thereafter, up to 6 years follow-up, there was no significant difference in the weight z score between the groups. Genetic mutations and immunohistochemistry findings were only available for 17 children and did not seem to distinguish between the two groups. For comparison, in the same study period, only 2 out of 67 (3%) children with biliary atresia and 2 of 19 (10.5%) children with Alagille syndrome had fractures.

**Conclusions:** A subgroup of children with PFIC seem to develop severe bone disease. Severe failure to thrive in the first 6 months of life may be associated with this complication. More data from a larger cohort of children is needed.

**PH2-18**

**LEUCOCYTE VACUOLATION IN NEONATAL ICHTHYOSIS–SCLEROSING CHOLANGITIS SYNDROME (NISCH) ASSOCIATED WITH CLDN1 DISEASE/CLAUDIN-1 DEFICIENCY: ABSENCE AFTER LIVER TRANSPLANTATION**

**Presenter:** A. Knisely. King’s College Hospital, London, UK.

**Abstract:**

**Background and Aim:** Mutation in CLDN1, encoding the tight-junction protein claudin-1, underlies neonatal ichthyosis–sclerosing cholangitis syndrome (NISCH). Several patients with NISCH are reported, with two CLDN1 mutations. An early description of NISCH as “ichthyosis, leucocyte vacuolation (LV), alopecia, and sclerosing cholangitis” highlighted nonlipidic LV in these patients. We present a patient with NISCH and CLDN1 disease/claudin-1 deficiency in whom, after paternal-donor liver transplantation (LTX), LV was not observed. We conclude that LV is not an inherent, non-variant, or obligatory feature of NISCH and speculate that it may be a secondary phenomenon.

**Methods and Results:** Patient: One of different-sex consanguineous twins developed neonatal icterus. Extrahepatic biliary atresia was diagnosed. Hepatic portoenterostomy was performed, without clinical improvement. As alopecia, icterus, and ichthyosis were prominent, NISCH was considered. LTX at 12 months found micronodular cirrhosis. Genetic and Cell Biology Studies: DNA was extracted from proband, co-twin, and parental leucocytes. Keratinocytes were cultured from control, proband, and paternal skin punch biopsies. RNA and proteins were extracted from keratinocytes and cDNA was prepared from RNA. CLDN1 and CLDN4, encoding the tight-junction protein claudin-4, were sequenced. Claudin-1 and -4 synthesis was assayed by Western blot. The proband was homozygous for the novel CLDN1 mutation IVS1+5insG. Each parent was heterozygous for the same mutation; the co-twin was wild-type. No CLDN4 mutation was found. Claudin-1 cDNA and protein were demonstrable in proband materials and claudin-1 cDNA and protein were not. Morphologic Studies of Leucocytes: At 40 months, when the proband was well,uffy-coat preparations were fixed in situ and embedded in resin. Buffy-coat sections and simultaneous peripheral-blood films were unremarkable on light microscopy; buffy-coat sections were unremarkable ultrastructurally. LV was not observed.

**Conclusions:** CLDN1 is not expressed by leucocytes, which do not form tight junctions. We thus wondered how claudin-1 deficiency might lead to LV. LV may reflect a storage disorder, as with Jordan anomaly, in which nonmembrane-bound neutral lipid accumulates within leucocyte cytoplasm. It also may be a nonspecific finding, associated with metabolic stress. Although we do not know if our patient had LV before LTX, LV was not observed.

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find after LTX (after resolution of primary hepatobiliary disease). We conclude that LV is not an absolute feature of NISCH associated with claudin-1 deficiency, and that absence of LV may not exclude NISCH.

Conclusions: The majority of treated obese children remained overweight or obese in young adulthood. Early-started treatment, duration of follow-up and efficacy of weight reduction during childhood were not associated with normal BMI in young adulthood. The only factors of long-term prognosis significance were independent of any therapeutic interventions during childhood.

PN2-01

PREDICTIVE FACTORS OF LONG-TERM WEIGHT EVOLUTION IN TREATED OBESE CHILDREN

Presenter: P. Tounian, Armand-Trousseau Hospital, Paris, France.
Co-authors: L. Ficheux¹, O. Achtari¹, L. Santos¹, P. Tounian¹, ¹Pediatric Gastroenterology and Nutrition, Armand-Trousseau Hospital, Paris, France.

Aim: To track treated obese children from childhood to young adulthood in order to identify predictive factors of long-term evolution.

Methods: 307 obese children seen as outpatients were asked by mail and/or telephone 11.1 ± 2.7 y. after their first consultation to indicate their current weight and height. Based on current BMI (weight/height²), the patients were classified into 3 groups: normal (N), BMI <25 kg/m²; Overweight (Ow), BMI between 25 and 30 kg/m²; Obese (Ob), BMI >30 kg/m². Data collected during the outpatient visits were compared between children who become N and those who remained Ow or Ob. Results were expressed as odds ratios (OR) and [95% CI].

Results: 173 patients could be contacted and were distributed as follows: 60 N (n F, x ± y years), 62 Ow (n F, x ± y years), 51 Ob (n F, x ± y years). OR for remaining Ow or Ob in young adulthood were not significant for sex (1.4 [0.7–2.8]), age at the first visit (0.9 [0.8–1.0]), time from obesity onset to the first visit (0.9 [0.8–1.1]), duration of outpatient follow-up (1.1 [1.0–1.4]), and evolution of the BMI z score during outpatient follow-up (1.3 [0.9–2.3]). The only significant OR for remaining Ow or Ob in young adulthood were BMI z score at the first visit (2.3 [1.5–3.7], P < 0.0001), maternal overweight or obesity (2.8 [1.6–6.7], P = 0.015), and non-European origin (2.7 [1.0–8.3], P = 0.06). Conversely, high socioeconomic status of the parents (0.3 [0.1–0.7], P = 0.02) and high study level reached by the patients (0.3 [0.1–0.8], P = 0.02) were associated with a good prognosis.

PN2-02

RARE MUTATIONS IN MC3R GENE CAUSING FUNCTIONAL IMPAIRMENT OF THE RECEPTOR ARE ASSOCIATED WITH CHILDCHOOD OBESITY

Presenter: B. Dubern, Armand-Trousseau Hospital, APHP, Paris, France.
Co-authors: M. Mencarelli², L. Benajiba¹, R. Allili¹, S. Maestrenchi³, C. Simon³, P. Galan³, B. Costes³, A. Diblasio², K. Clément¹, P. Tounian¹, ¹INSERM Nutriomique U872(Eq7), Paris 6 University, Paris, France; ²Istituto Auxologico Italiano, Molecular Biology Laboratory, Verbania, Italy; ³Strasbourg University, EA 1801, Strasbourg, France; ¹UMR U557 INSERM, U 11125 INRA, Cnam; P13 University, CRNH, Idf Bobigny, France; ²Biochemistry and Genetics, Henri Mondor Hospital, APHP, INSERM U841, Créteil, France.

Background and Aim: The melanocortin 3 receptor (MC3R) plays a key role in the control of energy homeostasis. Few studies evaluated the frequency of MC3R mutations in large populations of obese and control subjects and their impact on obese phenotype. The aim of our study was to determine the role of MC3R mutations in early-onset obesity.

Methods: DHPLC and subsequent direct sequencing of the coding region of MC3R gene was performed in 444 obese children (age 13.4 ± 3.3 y, BMI z score 4.3 ± 1.1SD), 337 control children (age 11.5 ± 0.6 y, BMI z score 0.1 ± 1.0 SD) and 416 control adults (age 49.0 ± 6.6 y, BMI 22.1 ± 1.8 kg/m²). Phenotype in obese children included obesity history, body composition, resting metabolic rate, fasting lipids, insulinemia and glucose. In vitro functional analyses were performed through alpha-MSH stimulation and direct cAMP detection for all mutations.

Results: 6 heterozygous mutations (2.03%) were detected in 9 unrelated obese subjects (S17T, V177I, T280S, I335S) including 1 silent mutation (I226I) and one mutation in noncoding region (T-4C). In all cases, the variations were identified in a heterozygosity state. The frequency of MC3R mutations in controls was not significantly different (1.13% in control children and 1.68% in adults). Phenotype of obese children with MC3R mutation was not significantly different when compared
to children without mutation except for height, which was higher in mutation carriers. Segregation analysis in families revealed an autosomal transmission with variable expression. The in vitro functional analysis demonstrated that the mutations that impaired receptor functionality were characteristic only of the obese population, whereas the mutations without functional consequences were present both in control and obese subjects.

**Conclusions:** MC3R mutations are frequent in obese children but are also found in control populations; no specific phenotype was detected in MC3R mutation carriers except for height; and although rare variants in MC3R gene are present in general population, those mutations with significant functional consequences on receptor activity are always associated with an obese phenotype.

**PN2-03**

**RELATION BETWEEN MATERNAL AND NEONATAL PLASMA ADIPONECTIN CONCENTRATIONS AND ANTHROPOMETRICAL INDICES AT BIRTH AND AT THE AGE OF 4 YEARS**

**Presenter:** E. Szabo, University of Pecs, Pecs, Hungary.

**Co-authors:** E. Szabo¹, H. Demmelmaier², C. Campoy³, T. Decsi³, B. Koletzko². ¹University of Pecs, Department of Paediatrics, Pecs, Hungary; ²Dr von Hauner Children’s Hospital, Division of Metabolism and Nutrition, Munich, Germany; ³University of Granada, Department of Pediatrics, Granada, Spain.

**Aim:** To investigate the relationship between plasma adiponectin concentrations in maternal and cord blood to anthropometrical indices measured at birth and at 4 years of age.

**Methods:** We supplemented mothers with either fish oil, or 5-methyl-tetrahydro-folic acid, or both, or placebo from the 20th week of gestation. Plasma adiponectin concentrations were measured in a subgroup of expecting mothers and their newborns (n = 81) by ELISA at the 20th week of gestation, 30th week of gestation, and at delivery.

**Results:** We found no significant differences in adiponectin values between the 4 supplementation groups. Plasma adiponectin concentrations decreased significantly during pregnancy (20th week: 13.19 [6.24]; 30th week: 10.83 [5.18]; delivery: 10.15 [4.56]; n = 81; µg/mL, mean [SD], P < 0.05) and were about 3-fold higher in venous cord blood (35.33 [13.89]) than in maternal blood. There were significant positive correlations between adiponectin concentrations in cord blood and birth weight, ponderal index, triceps and subscapular skinfold thicknesses of the newborns; however, there were no correlations with maternal adiponectin concentrations (Table 18). In contrast, we found significant positive correlations between maternal adiponectin concentrations and weight and chest and waist circumferences of their children at 4 years of age (Table 18).

**Conclusions:** Neither fish oil nor folate supplementation influenced plasma adiponectin concentrations in expecting mothers or in their newborns. As maternal adiponectin does not cross the placenta, the correlation of maternal plasma adiponectin concentrations during pregnancy and anthropometrical data measured in their 4-year-old children might indicate that both are influenced, respectively programmed, by a not-yet-identified maternal factor or the programming effect of maternal adiponectin is mediated via a not-yet-identified mechanism.

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**PN2-04**

**ADIPONECTIN IN BREAST-FED INFANTS IN THE FIRST 6 MONTHS OF LIFE, IN THEIR MOTHERS, AND IN BREAST MILK**

**Presenter:** F. Savino, Ospedale Infantile Regina Margherita, Turin, Italy.

**Co-authors:** S. Liguori¹, E. Petrucci¹, M. Lupica¹, F. Fissore¹, G. Nanni¹, R. Oggero¹. ¹Ospedale Infantile Regina Margherita, Department of Pediatrics, Turin, Italy.

**Background and Aim:** Adiponectin, a hormone secreted by adipocytes, has been identified recently in breast milk. Although its biological role has not been firmly established, clinical and experimental researches indicate that it regulates lipid and glucose metabolism by increasing insulin sensitivity, affects foetal development, and exerts

**TABLE 18. Correlations between plasma adiponectin concentrations during pregnancy and anthropometrical indices at birth and at 4 y old**

<table>
<thead>
<tr>
<th>Anthropometric index</th>
<th>20th week</th>
<th>30th week</th>
<th>Delivery</th>
<th>Cord blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight</td>
<td>0.123</td>
<td>0.236</td>
<td>-0.008</td>
<td>0.271</td>
</tr>
<tr>
<td>Triceps skinfold</td>
<td>0.026</td>
<td>0.172</td>
<td>-0.107</td>
<td>0.378</td>
</tr>
<tr>
<td>Subscapular skinfold</td>
<td>-0.047</td>
<td>0.146</td>
<td>-0.081</td>
<td>0.410</td>
</tr>
<tr>
<td>Weight (4 y old)</td>
<td>0.334</td>
<td>0.393</td>
<td>0.350</td>
<td>0.213</td>
</tr>
<tr>
<td>Chest circumference</td>
<td>0.311</td>
<td>0.378</td>
<td>0.317</td>
<td>0.086</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>0.370</td>
<td>0.388</td>
<td>0.424</td>
<td>0.253</td>
</tr>
</tbody>
</table>

n = 81. ¹P < 0.05. ²P < 0.01.
anti-inflammatory and antiatherogenic effects. Scanty data are on serum adiponectin levels in infancy. Evaluation of this hormone in breast-fed (BF) infants could be of interest in order to clarify the role of breast-feeding in the prevention of obesity. The aim of the study was to evaluate the relation between serum adiponectin values in BF infants in the first 6 months of life, in their mothers, and in breast milk.

**Methods:** We studied 37 AGA healthy infants exclusively BF, aged 0–6 months, without chronic or acute gastrointestinal disease. Serum adiponectin concentrations have been determined by RIA test (Adiponectin human RIA-3765, DRG Diagnostics) at least 3 hours after feeding. Breast milk adiponectin concentrations have been determined by ELISA test (Adiponectin ELISA D-72770 Medigagnost, Reutlingen, Germany). The study protocol was approved by ethical committee and parents gave written consent. Statistical analysis: Spearman’s correlation; statistical significance was set at $P < 0.05$.

**Results:** Median (min-max) serum adiponectin concentration in infants was 136.8 (38.5; 189.7) ng/mL and in their mothers ($n = 18$) was 54.5 (23.2; 97.3) ng/mL. Median (min-max) adiponectin concentration in breast milk ($n = 25$) was 10.08 (1.92; 18.5) ng/mL. We observed a positive correlation between serum adiponectin concentration in mothers and hormone levels in breast milk, even though we didn’t define statistical significance ($r = 0.5; P = 0.06$). The correlation between serum hormone concentration in infants and in their mothers was not statistically significant.

**Conclusions:** In the present study we observed higher adiponectin levels in infants than in their mothers, with no correlation between infants’ and mothers’ hormone concentration. This observation confirms the results of previous studies in which adiponectin levels were higher in neonates than in adults and decreased between the first and the second year of life. Nevertheless our data don’t let us to understand the reason; we might hypothesize that hyperadiponectinemia in infants could be due to higher percentage of adipose tissue and to the immaturity of negative feedback regulation of this hormone. Further in our research we confirmed the presence of adiponectin in human milk with lower concentration than those found in infants and mothers serum. Others investigations are needed to clarify the role of adiponectin in infant nutritional status and metabolic development.

**PN2-05**

**BREAST MILK LEVELS OF PROTEIN, FAT, IGF-1, GRELIN, LEPTIN, ADIPONECTIN AND GROWTH OF BREAST-FED INFANTS DURING FIRST 3 MONTHS OF LIFE**

Presenter: N. Shilina. Institute of Nutrition of RAMS, Moscow, Russian Federation.

Co-authors: N. Shilina1, I. Kon3, M. Gmoshinskaya1.

1Institute of Nutrition of RAMS, Moscow, Russian Federation.

**Background and Aim:** It is proposed that protein excess intake, inducing increase of blood IGF-1 level, is an important risk factor of high growth rate (GR) and obesity of bottle-fed infants. However it is not clear whether the same factors may be the reason of high GR in breast-fed infants. So our aim was to study possible relation between breast-fed infant GR and breast milk protein, fat, IGF-1, ghrelin, leptin, adiponectin levels.

**Methods:** Body weight gain and length during each of first three months of life, daily intake of breast milk and its protein, fat, IGF-1, ghrelin, leptin and adiponectin content were studied in 71 breast-fed healthy infants. All observed infants were divided into three groups varying in their monthly weight gain: with low (less than 500 g increase of body weight per month), normal (from 500 g to 1000 g increase) and high weight gain (more than 1000 g increase). Daily milk intake was measured by weighing of infants before and after feeding, breast milk fat content by standard method, total protein by Kjeldal method and individual proteins were measured by ELISA.

**Results:** The protein and fat breast milk levels were not differed between all three groups, but the daily milk intake was significantly higher in infants with high GR than in infants with low and normal GR (at 1 month of age $555 \pm 32$ g, $820 \pm 49$ g, and $770 \pm 51$ g for low, high and normal GR, respectively, $P_{low-normal} = 0.006$; $P_{low-high} = 0.008$; at 2 months of age $888 \pm 73$ g, $740 \pm 49$ g for high and normal GR, respectively, $P = 0.021$; at 3 months of age $752.5 \pm 50$ g, $951 \pm 62$ g, $843 \pm 49$ g, for low, high and normal GR, respectively, $P_{low-high} = 0.03$. So the total daily intake of protein was higher in infants with high than with low GR. The IGF-1 breast milk levels was also higher in infants with high than in infants with low and normal GR, the difference was significant in 3-month-old infants (mean $\pm$ SD: $12.2 \pm 5.4$ vs. $3.15 \pm 2.4$ ng/mL, $P = 0.032$). There was a tendency to increase of ghrelin breast milk content in infants with high GR comparatively to infants with low GR. There were no difference in leptin and adiponectin breast milk levels between all 3 groups.

**Conclusions:** High growth rate of breast-fed infants was coupled with higher breast milk consumption, higher level of IGF-1 and a tendency to higher ghrelin content in breast milk. The increase of IGF-1 and possibly ghrelin content in breast-milk may be one of the reasons of high GR in infancy and overweight in older age in breast-fed infants.
TABLE 19.

<table>
<thead>
<tr>
<th>NAFL</th>
<th>Body mass index</th>
<th>Arterial pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient’s</td>
<td>Mother’s</td>
</tr>
<tr>
<td>+ n = 37</td>
<td>31.9 ± 8.9</td>
<td>31.6 ± 7.3</td>
</tr>
<tr>
<td>− n = 32</td>
<td>28.3 ± 3.2</td>
<td>27.9 ± 5.8</td>
</tr>
<tr>
<td>P</td>
<td>0.0002</td>
<td>0.004</td>
</tr>
</tbody>
</table>

TABLE 20.

<table>
<thead>
<tr>
<th>NAFL</th>
<th>γGt</th>
<th>SGOT</th>
<th>SGPT</th>
<th>Fibrinogen</th>
<th>HOMA</th>
<th>TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ n = 37</td>
<td>21.5 ± 9.3</td>
<td>27.1 ± 7.1</td>
<td>28.7 ± 13.5</td>
<td>446.2 ± 94.2</td>
<td>6.3 ± 5.3</td>
<td>3.65 ± 1.4</td>
</tr>
<tr>
<td>− n = 37</td>
<td>15.1 ± 3.6</td>
<td>22.1 ± 4.1</td>
<td>17.7 ± 5.7</td>
<td>380.6 ± 121.7</td>
<td>4.03 ± 2.1</td>
<td>2.8 ± 1.2</td>
</tr>
<tr>
<td>P</td>
<td>0.0003</td>
<td>0.0006</td>
<td>0.000004</td>
<td>0.01</td>
<td>0.04</td>
<td>0.01</td>
</tr>
</tbody>
</table>

PN2-06

COMPARISON OF SOMATOMETRIC INDICES, LABORATORY FINDINGS, AND FOOD INTAKE DATA BETWEEN OBESE CHILDREN WITH AND WITHOUT FATTY LIVER

Presenter: T. Lampoudi. Technological Education Institute, Thessaloniki, Greece.
Co-authors: K. Kitsios1, M. Papadopoulou1, A. Kolovou2, K. Koutroumanis2, A. Savvidou2.

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Aim: To suggest the main predisposing factors for the development of fatty liver in obese children by identifying characteristics specific to obese children with NAFL, compared to obese children without fatty liver.

Methods: Sixty-nine obese children were studied (boys 39, girls 30, median age 11.1 y). They were classified as obese based on their BMI values, according to IOTF criteria. According to the presence of NAFL in liver ultrasound they were divided in 2 groups: 37 with NAFL (boys 23, girls 14, median age 11.4, range 6–16 y), 32 without NAFL (boys 16, girls 16, median age 10.7, range 6–16 y). The following somatometric, laboratory and dietetic parameters were studied: a) body weight (BW), body height (BH), waist circumference (WC), hip circumference (HC), arterial pressure (AP, syst, diast), resting metabolic rate (RMR) assessed by indirect calorimetry; b) biochemistry profile: SGOT, SGPT, γGt, CRP, homocysteine, fibrinogen, insulin resistance (HOMA), TSH and lipid profile; c) weekly food intake records and analysis by specific software (FoodProcessor, ESHA, USA). The BMI of both parents was also assessed.

Results: The findings with statistically significant differences between the 2 groups are displayed in Tables 19 and 20. No statistically significant difference was found in RMR and food intake data, in terms of total energy intake or specific macronutrient intake. No difference was found in lipid profile parameters.

Conclusions: The obese children with fatty liver demonstrated by liver ultrasound represent a distinct subpopulation who displays clear cut clinical and laboratory characteristics suggesting a familial predisposition to obesity, central fat disposition, more severe obesity, and more pronounced metabolic and inflammatory changes. Since no differences were found in RMR values, dietetic intake or lipid profile it is suggested that factors other than personal dietetic preferences or energy and lipid metabolism, have to be studied in order to clarify whether the above characteristics are associated with primary predisposing factors or they represent just secondary findings.

PN2-07

FASTING LEPTINEMIA IN EUTROPHIC CHILDREN AND ADOLESCENTS

Presenter: C. Nogueira De Almeida. CESNI-UNAERP, Ribeirão Preto, São Paulo, Brazil.
Co-authors: A.P. Pinho Ramos1, R. Garcia Ricco2, M. Pepato3, I. Lourenças Brunetti3.

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Aim: To determine, in a group of eutrophic children and adolescents, the values of fasting leptinemia and its correlation with age and body mass index.

Methods: A cross-sectional study conducted in 2 public schools in Ribeirão Preto, São Paulo, Brazil. Anthropometric measurements and venous blood sample were obtained for determination of fasting leptinemia of 448
Background and Aim: The recent discovery of leptin and ghrelin has added new perspectives to the understanding of the control of food intake, metabolism and energy homeostasis, and growth. Regulation of these hormones in the perinatal period, when many metabolic, immune and hormonal pathways are being developed, is of great interest. We therefore investigated in mother–newborn pairs the relations between insulin and the IGF system (the 2 main established regulators of fetal growth), and ghrelin and leptin, in an effort to define possible associations between the maternal and offspring hormone concentrations.

Methods: Design—Prospective, descriptive study. Subjects—100 mothers and their healthy term, appropriate for gestational age babies. Measurements—Serum ghrelin, leptin, IGF-1, IGFBP-3 and insulin concentrations were measured in delivering mothers and their babies at birth. For babies GH and glucose were also determined.

Results: Cord ghrelin was significantly higher than maternal ghrelin concentration (P < 0.0001), while cord leptin, IGF-1, and IGFBP-3 were significantly lower (P < 0.0001). There was no significant difference between mothers’ and babies’ insulin levels. After correction for gestational age, baby’s gender, and perinatal and antenatal variables (Clin Chem. 2008;54:550–8), cord ghrelin was significantly positively associated with maternal ghrelin and leptin, but negatively with IGF-1. Moreover, maternal ghrelin was negatively associated with cord insulin. Cord leptin was significantly positively associated only with maternal leptin. None of the maternal hormones was significantly associated with baby’s GH.

Conclusions: New findings of this study are increases in insulin levels in maternal compartment were associated with decreases in ghrelin concentrations in the fetal compartment, suggesting that a low fetal ghrelin might represent a counterregulatory attempt to limit in utero the anabolic effect of maternal insulin; increases in maternal leptin was associated with increases in fetal ghrelin. Leptin production is regulated by a variety of factors, including inflammation. Considering that ghrelin may inhibit circulating proinflammatory cytokines, it possible that the observed positive association might represent an adaptive immune response to the inflammatory insults in the maternal environment; increases in maternal IGF-1 was associated with decreased ghrelin levels, suggesting that ghrelin may be required for the fetal adaptive response in defense against the maternal catabolic response during parturition. There is a complex interplay between maternal and fetal hormones and growth factors in the perinatal period. The results of this study may provide clues to the mechanisms which control fetal growth.
reduced in obesity. Ghrelin circulates in acylated (A-Ghr) and desacylated (D-Ghr) forms, but their potential differential associations with insulin resistance and whether they are differentially altered in childhood obesity remain undefined. Thus, we investigated the associations of ghrelin forms with insulin resistance in obese and non-obese children and the impact of metabolic syndrome (MS) on their plasma concentrations.

**Methods:** Design—Prospective cross-sectional study. Patients—A total of 103 obese children [age, 9.6 ± 2.8 y; body mass index-standard deviation score (SDS), 2.13 ± 0.57], and 97 lean subjects (age, 8.9 ± 3.7 y) with and without components of MS were included in the study. Measurements—Fasting blood glucose, insulin, lipid profile, and acylated and total ghrelin were examined. Insulin resistance was determined by a homeostasis model assessment of insulin resistance (HOMA-IR). Definitions—We defined MS in presence of 3 of the following criteria: obesity, hypertension, low HDL cholesterol, elevated triglycerides, and impaired fasting glucose and/or insulin resistance.

**Results:** Compared to lean subjects, obese children had highly significantly lower T-Ghr and D-Ghr levels (P < 0.0001), but only just significantly lower A-Ghr (P = 0.04), and higher A/D-Ghr ratio. In the entire population, after adjustment for age, gender, and Tanner stage, insulin levels and HOMA-IR were negatively associated with T-Ghr and D-Ghr, and positively associated with A/D-Ghr ratio. These associations remained significant even after correction for BMI-SDS. No correlation was observed for A-Ghr. T-Ghr as well as D-Ghr levels were significantly lower in the obese patients with MS compared to those without it, while A/D-Ghr ratio was higher, and they decreased or increased, respectively, with the number of the components of MS (P < 0.01 for trend). No differences were observed with respect to A-Ghr. Compared with lean healthy children, lean subjects with 1 or more components of MS had higher A-Ghr concentrations as well as A/D-Ghr ratio (P < 0.05), along with higher levels of insulin and Homa-IR. No differences were observed in T-Ghr and D-Ghr values.

**Conclusions:** Our study suggests that elevated A-Ghr in terms of a fraction of total circulating hormone could negatively modulate insulin action in obese as well as nonobese children, and contribute to the association of insulin resistance and metabolic syndrome.

**PN2-11**

**ARE TREATMENT TARGETS FOR HYPERCHOLESTEROLEMIA EVIDENCE BASED? SYSTEMATIC REVIEW AND META-ANALYSIS OF RANDOMIZED CONTROLLED TRIALS**

**Presenter:** P. Dziechciarz. **Medical University of Warsaw, Warsaw, Poland.**

Co-authors: P. Dziechciarz1, Y. Lebenthal2, A. Horvath1, H. Szajewska1, R. Shamir2. 1Medical University of Warsaw, Warsaw, Poland; 2Institute of Gastroenterology, Nutrition and Liver Diseases, Schneider Children’s Medical Center, Petach-Tikva, Israel.

**Background and Aim:** Recent pediatric guidelines recommend early initiation of statins in children with...
hypercholesterolemia. The aim of the study was to systematically review the literature for evidence in support of target age and levels for intervention as well as target values for low-density lipoprotein (LDL) cholesterol levels in treated children with familial hypercholesterolemia.

Methods: A literature search from 1966 to December 2007 was performed using PubMed; retrieved articles were screened for double-blind randomized controlled trials (RCT) evaluating statin therapy in children and adolescents younger than 18 years. Trials were included if they met predetermined criteria.

Results: Six trials involving 834 patients aged 8 to 18 years were included. Of the 6 RCTs, only one study described attainment of LDL cholesterol treatment target: 84 (60%) of the subjects in the treatment group and no subjects in the placebo group reached their target LDL cholesterol level (N = 87; RR 57.5; 95% CI 3.64–9.10). Only 1 RCT assessed the effect of a statin on the carotid intima-media thickness (N = 211; WMD 4.1; 95% CI 0.01–8.2); it demonstrated no clinically significant alterations in vital signs (systolic blood pressure and pulse rate) in the treatment group but a decreased systolic blood pressure in the placebo group, leading to a significant between-treatment group difference (P < 0.05). Only 1 RCT explored the effect on the carotid intima-media thickness (N = 211; WMD −0.01; 95% CI −0.03 to −0.00), with no significant difference found between the treatment and placebo groups. Another RCT showed that the mean absolute change in flow-mediated dilation over a short period of time in male C57BL/6 mice.

Conclusions: Recent recommendations that favor statins as the first-line drug treatment for hypercholesterolemia are evidence based. However, there is no firm evidence regarding when to start this treatment or what target LDL cholesterol level should be attained. Studying high risk groups (obese or diabetic patients) and incorporating composite endpoints (eg, measurement of endothelial dysfunction) may help define treatment guidelines.

PN2-12
A DIET RICH IN OMEGA-6 POLYUNSATURATED FATTY ACIDS AND SUCROSE REPRODUCES KEY FEATURES OF METABOLIC SYNDROME IN C57BL/6 MICE
Presenter: R. Himes. Baylor College of Medicine, Houston, TX, USA.
Co-authors: S. Cope-Yokoyama, C. Smith. Baylor College of Medicine, Houston, TX, USA. A. Hofman, E. Van Der Beek, E. Steegers, V. Jaddoe. Erasmus Medical Center, Rotterdam, The Netherlands; Danone Research-Centre for Specialised Nutrition, Wageningen, The Netherlands.

Aim: To determine whether a diet enriched in o-6 fatty acids and sucrose will reproduce features of metabolic syndrome in C57BL/6 mice.

Methods: 4- to 7-week-old male C57BL/6 mice were randomized to chow (13% kcal fat, lard and corn oil) or high fat/high sucrose (HF/HS) diet (48% kcal fat, corn oil) for a period of 4–6 weeks. Food consumption was monitored and weekly weights were obtained. Fasting blood samples were obtained at sacrifice for serum insulin, glucose, cholesterol and alanine aminotransferase (ALT). Portions of the liver were allocated for routine histology, oil-red-o staining and gene expression analysis by quantitative real-time PCR.

Results: Diet intake was isocaloric between groups. HF/HS mice had significantly greater weight gain than controls (6.99 vs 4.43 g, P = 0.0431). HF/HS mice had significantly greater fasting serum insulin (1.21 vs 0.32 ng/mL, P = 0.0401), HOMA-IR scores (7.38 vs 2.7, P = 0.008), cholesterol (130.4 vs 84.25 mg/dL, P = 0.0001) and serum ALT (52.7 vs 31 U/L, P = 0.018). HF/HS livers displayed microvesicular steatosis at 4 weeks with a tendency towards macrovesicular steatosis at 6 weeks. Twenty percent of HF/HS livers contained necroinflammatory foci consistent with nonalcoholic steatohepatitis by 6 weeks vs 0% in controls. Unexpectedly, hepatic expression of TNF-α was not different between the 2 groups.

Conclusions: The HF/HS diet is an efficient means of inducing obesity and features of metabolic syndrome over a short period of time in male C57BL/6 mice. Hepatic transcriptional regulation of TNF-α does not appear to play a role.

PN2-13
GROWTH IN FETAL LIFE AND INFANCY IS ASSOCIATED WITH ABDOMINAL ADIPOSITY AT THE AGE OF 2 YEARS: THE GENERATION R STUDY

Aim: To examine the associations of fetal and postnatal growth characteristics with abdominal fat mass at the age of 2 years.

Methods: This study was performed in 481 children participating in a prospective cohort study from early fetal life onward. Fetal and postnatal growth characteristics in
Conclusions: Our results suggest that rapid growth rates in fetal life and infancy are associated with increased abdominal subcutaneous and preperitoneal fat mass in healthy children. Further studies need to explore whether these associations persist in later life and are related to metabolic syndrome outcomes.

PN2-14

ABDOMINAL FAT IN CHILDREN MEASURED BY ULTRASOUND AND COMPUTED TOMOGRAPHY

Presenter: B. Durmuş, Generation R-Erasmus Medical Center, Rotterdam, The Netherlands

Co-authors: D. Mook-Kanamori1, S. Holzhauer1, L. Hollesteijn1, B. Durmuş1, R. Manniesing1, M. Koek1, G. Boehm2, E. Van Der Beeck2, A. Hofman1, J. Witteman1, M. Lequin1, V. Jaddoe1, 1Erasmus Medical Center, Rotterdam, The Netherlands; 2Danone Research-Centre for Specialised Nutrition, Wageningen, The Netherlands.

Aim: To examine whether measures of abdominal visceral fat can be measured by ultrasound in children and adolescents. First, we examined whether preperitoneal fat is a good approximation for visceral fat in children using computed tomography (CT) scans. Second, we compared measures of subcutaneous and preperitoneal fat in children using abdominal ultrasound and CT methodology.

Methods: First, we retrospectively examined 47 CT scans of nonobese children (median age 7.9 (95% range: 1.2–16.2) years). In these scans we compared visceral fat area with preperitoneal fat thickness and area at the umbilical level. Second, we examined 34 nonobese children (median age 9.5 (95% range: 0.3–17.0) by CT and ultrasound. We compared the maximum preperitoneal fat thickness, the subcutaneous fat thickness and areas of both fat layers measured by CT and ultrasound. CT measurements were used as reference.

Results: Within CT correlation coefficients between visceral and preperitoneal fat thickness and area were 0.58 (P < 0.001) and 0.76 (P < 0.001), respectively. Stronger correlations were found for within CT measured subcutaneous fat measurements. Ultrasound measurements of preperitoneal and subcutaneous fat were well correlated with CT measurements. Correlation coefficients ranged from 0.75 to 0.97 (all P < 0.001). Small systematic differences were observed when analyzing the results described by the method of Bland and Altman.

Conclusions: Our findings suggest that preperitoneal fat can be used as an approximation for visceral fat in children. Measuring abdominal fat with ultrasound in children is a valid method for epidemiological and clinical studies. The exact agreement between the ultrasound and CT scan was limited, which indicates that ultrasound should be used carefully for obtaining fat distribution measurements in individual children.

PN2-15

LONG-CHAIN ACYLCARNITINES REGULATE hERG WHILE MEDIUM-CHAIN ACYLCARNITINES DO NOT

Presenter: F. Labarthe, CHU Tours, Tours, France.

Co-authors: F. Ferro1, T. Tran1, A. Ouille1, D. Babuty1, J. Le Guennec1, F. Labarthe1, 1INSERM U921, Tours, France.

Background and Aim: Fatty acids (FA) are known to impact cardiac contractility and functions, but much remains to be learned about the implicated cellular mechanisms. In patients with inherited long-chain FA oxidation defects, altered levels of FA-derived metabolites, namely acylcarnitines (acyl-CAR), have been suggested to contribute to the development of cardiac symptoms, such as cardiomyopathies and conduction defects. The aim of this study was to document the potential effects of various acyl-CAR at different concentrations on the hERG channel, a cardiac K+ channel that has been implicated in lethal arrhythmias.

Methods: HEK293 cells stably expressing hERG channel were studied in the ruptured whole cell patch clamp configuration. Acyl-CAR derivatives from medium- (C8 and C10) and long-chain (C16 and C18:1) FA were applied intra- and extracellularly at 2 different concentrations: 3 μM (physiological) and 30 μM (pathological) and compared to the current in absence of acyl-CAR into and outside the cells.

Results: C8-CAR and C10-CAR had no effect at 3 μM and 30 μM whether they were applied intra- or extracellularly. C16-CAR and C18:1-CAR had no effect on the current when applied thought the patch pipette. When applied extracellularly, 3 μM C16-CAR or C18-CAR...
induced an increase of the current amplitude. This increase was associated with different effects on the activation and availability properties. At this concentration, the long-chain acyl-CAR induced also a speeding of the kinetic of deactivation of the I_{HERG}. At 30 µM, C18-CAR, and not C8-CAR, induced an initial increase of the current amplitude associated with the acceleration of deactivation kinetic. After less than one minute of 30 µM C18-CAR perfusion, a huge increase of the leak was observed and the cells died.

Conclusions: At physiological extracellular concentrations of long-chain acyl-CAR (around 3 µM), a regulation of I_{HERG} occurred, suggesting that at lower or higher pathological concentrations, there must be an impact on the action potential that can explain some of the cardiac symptoms reported in patients with inherited long-chain FA oxidation defects. Thus, results from this study highlight that, besides a toxic effect at high concentrations, long-chain acyl-CAR also exert a regulatory role on HERG channel, a mechanism that make a link between FA metabolism and cardiac functions. This effect appeared to be specifically related to long-chain acyl-CAR applied extracellularly.

RN2-16

RBC-FATTY ACIDS IN ABETALIPOPROTEINEMIA: CAN CUTANEOUS APPLICATION OF OIL AFFECT FATTY ACID COMPOSITION?

Presenter: E. Granot. Kaplan Medical Center, Rehovot, Israel.

Co-authors: E. Jakobovich¹, E. Berry². Kaplan Medical Center, Rehovot, Israel; ²Braun School of Public Health & Community Medicine, Faculty of Medicine, Hebrew University Hadassah, Jerusalem, Israel.

Background and Aim: Abetalipoproteinemia (ABL) is a rare autosomal recessive disorder characterized by defective assembly and secretion of plasma apoB-containing lipoproteins. Patients with ABL suffer from fat malabsorption and resultant deficiency of fat-soluble vitamins. Plasma and adipose tissue of ABL patients have been shown to be deficient in linoleic acid (C 18:2 w6). The aim of this study was to characterize fatty acid composition in RBC membranes (as a surrogate marker of other cell membranes) in ABL patients and determine whether cutaneous application of oils can affect cell membrane FA composition.

Methods: In 10 ABL patients and 25 age-matched controls blood drawn in EDTA and RBC-FA analyzed. 6 ABL patients underwent daily topical application (“massage”) of oilive oil (oleic acid C18:1 w9), safflower oil (linoleic acid C18: 2 w6) and flaxseed oil (alpha-linolenic acid C18:3 w3). Each oil administered for 6 weeks at a dose of 5 mL/m² on same body surface area, with a 6-week interval between each of the oils. Blood obtained before and following each 3-week course of oil application. Plasma RB-FA analysis: RBC separated and hemolyzed. Lipids extracted using chloroform-isopropanol (J Biol Chem, 1957, as modified by Rose and Oklander, J Lipid Res. 1965). Fatty acid methyl esters were prepared by transesterification with methanolic trimethylammonium hydroxide (Meth Prep II) in presence of butylated hydroxytoluene as antioxidant. Fatty acid methyl esters quantified by gas liquid chromatography (Migal Lab, Kiryat Shmona) and results expressed as % weight/weight composition.

Results: RBC-FA analysis (total of 37 ABL samples analyzed vs 25 control samples). Oleic acid levels significantly higher in ABL as compared to controls; 14.15 ± 1.96 (mean ± SD) vs 7.18 ± 1.7 P < 0.0001. Linoleic acid significantly lower in ABL; 3.502 ± 0.53 vs 5.12 ± 1.96 in controls, P < 0.0001. Mean % weight composition of arachidonic acid (C 20:4 w6), EPA (C 20:5 w3) and DHA (C22:6w3) did not differ between ABL and controls. Cutaneous application of oils did not affect RBC-FA composition. No change observed in oleic acid levels after application of olive oil nor in that of linoleic acid after safflower oil. Alpha-linoleic acid levels were nonmeasurable in all samples before and after flaxseed oil application.

Conclusions: Cell membranes of ABL patients are relatively deficient in linoleic acid (an essential w6 FA) although levels are within the lower range of the norm. Cutaneous application of C18 oils does not affect RBC-FA composition and does not result in an increase in membrane linoleic acid levels (C18:2 w6). In ABL patients, despite malabsorption of long-chain fatty acids, cell membrane levels of PUFA (≥C 20, w3 and w6) are within the normal range. In ABL, in absence of chylomicrons, long chain fatty acid absorption likely occurs via alternative/compensatory pathways which remain to be delineated.

PN2-17

HIGH-THROUGHPUT METHOD FOR THE ANALYSIS OF FATTY ACID COMPOSITION IN PLASMA

Presenter: C. Glaser. Dr von Hauner Children’s Hospital, Ludwig-Maximilians-University of Munich, Munich, Germany.

Co-authors: H. Demmelmaier¹, B. Koletzko¹. ¹Division of Metabolic Diseases and Nutritional Medicine, Dr von Hauner Children’s Hospital, Ludwig-Maximilians-University of Munich, Munich, Germany.

Background and Aim: Plasma fatty acid (FA) composition, a marker of FA status and dietary intake, is
associated with health outcomes on a short and long term basis. Detailed investigation of the relationships between plasma FA composition and health requires the analysis of high numbers of samples. Although the determination of FA composition of total plasma lipids provides valuable information, the sensitivity in the detection of effects can be increased by the analysis of individual lipid fractions. Most often phospholipids (PL) are analyzed. However manual sample preparation is cumbersome and time-consuming. Thus, we developed a high-throughput method for the analysis of FA in plasma phosphoglycerides (PG), which additionally increases sensitivity.

**Methods:**
Gas chromatographic (GC) FA analysis of individual lipid classes with established methods requires liquid-liquid extraction of plasma lipids, chromatographic separation of lipid classes, conversion of FA into methyl esters and their extraction into hexane before GC analysis. For plasma PG FA analysis according to the newly developed method proteins of 100-μL plasma are precipitated with methanol. In the methanolic supernatant the PG FA are selectively converted at room temperature into fatty acid methyl esters (FAME) by base catalyzed transesterification. After extraction into hexane FAME are analyzed by GC.

**Results:** Coefficients of variation for FA contributing more than 1% to total fatty acids, were found to be below 4% (N=8). Comparison of the results with results obtained with the established method, showed highly significant correlations (R > 0.90 for all FA except C22:0, C24:0, C24:1n-9). The new method leads to enormous savings in analysis time and solvent consumption.

**Conclusions:** The new method significantly reduces working time and costs and saves the environment. It provides equivalent results compared to the analysis of phospholipid FA by the established method and is especially suitable for the determination of long-chain unsaturated fatty acids, which are mainly bound in PG. The easy handling allows reproducible and robust analyses. Small sample volumes enable the analysis of plasma from infants.

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**PN2-18**

**SYSTEMATIC REVIEW OF METHODS FOR ASSESSING N-3 LONG-CHAIN POLYUNSATURATED FATTY ACID STATUS IN CLINICAL TRIALS**

**Presenter:** T. Decsi. *University of Pécs, Pécs, Hungary. Co-authors: T. Marosvölgy1, V. Jakobik1, K. Fekete1. 1Department of Paediatrics, University of Pécs, Pécs, Hungary.

**Aim:** To systematically review published data on the usefulness of different biomarkers to assess n-3 long-chain polyunsaturated fatty acid (LCPUFA) status in clinical trials.

**Methods:** The MEDLINE, EMBASE and Cochrane Library CENTRAL databases were searched for intervention trials on n-3 LCPUFAs. The minimal duration of the interventions was set at 2 weeks. All types of supplements (seafood and its derivates, single cell oils, eggs enriched with n-3 LCPUFA) were considered. We used formal inclusion/exclusion criteria developed by the EURRECA consortium and applied standard operation procedures for data extraction, validity assessment and meta-analysis.

**Results:** Here we focus on DHA status. We found 45 relevant studies (36 randomised, controlled trials and 9 before/after studies) reporting on 17 potential biomarkers. The vast majority of the studies (n=42) were carried out in adults. Based on the primary analysis of the greatest duration and the greatest supplementation dose, 8 biomarkers were found to effectively reflect changes in DHA intakes (Table 21). The efficacy of another 9 biomarkers to reflect DHA status remained unclear. Pooled effect sizes of changes of plasma phospholipid DHA were related to dose of supplementation up to 2500 mg/day (0 to <300 mg/day: 0.85 [0.54–1.71], 300 to <1500 mg/day: 1.99 [1.40–2.58], 1500 to <2500 mg/day: 3.83 [2.78–4.87], ≥2500 mg/day: 2.74 [2.03–3.44], % wt/wt, mean [95% CI]).

**Conclusions:** In this systematic review, 8 biomarkers were found to effectively reflect changes in n-3 LCPUFA intakes in clinical trials carried out mainly in adults. Until

**TABLE 21. Biomarkers found to be effective to assess docosahexaenoic acid (DHA) status in clinical trials**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>No. studies and participants</th>
<th>Pooled effect size (% wt/wt, mean, [95% CI])</th>
<th>Variability (I², %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma total DHA</td>
<td>6 studies; n = 262</td>
<td>1.13 [0.54–1.71]</td>
<td>88.7</td>
</tr>
<tr>
<td>Plasma phospholipid DHA</td>
<td>21 studies; n = 923</td>
<td>2.45 [1.87–3.02]</td>
<td>94.0</td>
</tr>
<tr>
<td>Plasma triacylglycerol DHA</td>
<td>5 studies; n = 116</td>
<td>0.86 [0.08–1.65]</td>
<td>92.1</td>
</tr>
<tr>
<td>Plasma cholesterol ester DHA</td>
<td>5 studies; n = 110</td>
<td>0.42 [0.13–0.71]</td>
<td>92.2</td>
</tr>
<tr>
<td>Plasma nonesterified DHA</td>
<td>3 studies; n = 72</td>
<td>1.35 [0.11–2.59]</td>
<td>95.0</td>
</tr>
<tr>
<td>Erythrocyte total DHA</td>
<td>6 studies; n = 277</td>
<td>2.33 [0.86–3.81]</td>
<td>94.0</td>
</tr>
<tr>
<td>Erythrocyte phospholipid DHA</td>
<td>6 studies, n = 229</td>
<td>0.97 [0.50–1.43]</td>
<td>72.3</td>
</tr>
<tr>
<td>Platelet DHA</td>
<td>8 studies, n = 235</td>
<td>1.25 [0.87–1.64]</td>
<td>79.9</td>
</tr>
</tbody>
</table>

the availability of paediatric data, these biomarkers are suggested to be used in supplementation studies on children.
Supported by the European Communities 6th Framework Programme (FP6-036196-2, EURRECA).

**Poster Session 3**

**Hepatology/Nutrition**

**PHN3-01**

**DETERMINANTS OF INSULIN RESISTANCE AND NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN CHILDREN**

Presenter: L. Pacifico. La Sapienza University of Rome, Rome, Italy.
Co-authors: L. Pacifico¹, C. Anania², E. Poggiogalle³, F. Martino⁴, G. Olivero⁵, F. Ferraro⁶, C. Chiesa⁷, ¹La Sapienza University, Rome, Italy; ²ASL C, Rome, Italy; ³National Research Council, Rome, Italy.

**Background and Aim:** The contribution of metabolic syndrome (MS) to chronic diseases is complex with insulin resistance as the underlying mechanism. In addition, insulin resistance and NAFLD appear to be correlated since childhood. Therefore, we compared the metabolic and cardiovascular risk factors among children and adolescents with and without obesity and/or metabolic abnormality, and investigated determinants of insulin resistance and NAFLD in this age group.

**Methods:** Design—Prospective cross-sectional study. Patients—A total of 118 obese children [age, 11.1 ± 3.2 y; BMI-standard deviation score (SDS), 2.18 ± 0.4] and 63 lean subjects (age, 10.7 ± 3.8 y) with and without components of MS were studied. Measurements—Fasting blood glucose, insulin, lipid profile, apolipoproteins (Apo) A and B, uric acid, and alanine aminotransferase (ALT) were examined. Sonographic findings of liver and carotid intima-media thickness (IMT) were determined. Measurements—Fasting blood glucose, insulin, lipid profile, apolipoproteins (Apo) A and B, uric acid, and alanine aminotransferase (ALT) were examined. Sonographic findings of liver and carotid intima-media thickness (IMT) were determined. Insulin resistance was determined by a homeostasis model assessment of insulin resistance (HOMA-IR). Definintions-HOMA-IR changes during childhood depending on the age, gender and pubertal stage. We have, therefore, considered HOMA-IR values above the 95th percentile for age and sex an indicator of insulin resistance. We defined MS in presence of 3 of the following criteria: obesity, hypertension, low HDL cholesterol, elevated triglycerides, and impaired fasting glu-
cose and/or insulin resistance, and NAFLD as the co-
existence of elevated serum ALT and increased liver echogenicity in the sonographic evaluation.

**Results:** The mean value of serum lipids, ApoB/ApoA ratio, insulin, HOMA-IR, ALT, and uric acid, as well as carotid IMT was higher in the obese group with MS followed by the groups of obese children without MS, lean subjects with one or more components of MS, and healthy lean subjects. The mean HDL cholesterol was lower in obese patients with MS than in the other groups. BMI and waist circumference, systolic blood pressure, triglycerides, HDL cholesterol, uric acid, ApoB/ApoA ratio, and carotid IMT had significant associations with HOMA IR and ALT, after controlling for age, gender and pubertal stage. Logistic regression analysis revealed that waist circumference, triglycerides, uric acid, and ApoB/ApoA ratio, increased the risk of insulin resistance and NAFLD among study children. Carotid IMT was significantly associated with insulin resistance and NAFLD. Conclusions: We found significant association between carotid IMT with insulin resistance and NAFLD with similar risk factors for these 2 interrelated disorders. The association of carotid IMT with insulin resistance and NAFLD might suggest that the liver and the vessels share common mediators.

**PHN3-02**

**VISCERAL FAT MEASURED BY MRI MAY PLAY THE CRUCIAL ROLE IN THE DEVELOPMENT OF NAFLD IN CHILDREN AND ADOLESCENTS**

Presenter: J. Neuhoff-Murawska. Children’s Memorial Health Institute, Warsaw, Poland.
Co-authors: E. Jurkiewicz¹, A. Wierzbicka², W. Janczyk², M. Litwin³, M. Golkowska³, P. Socha³. ¹Department of Radiology, Warsaw, Poland; ²Department of Biochemistry and Experimental Medicine, Warsaw, Poland; ³Department of Gastroenterology, Hepatology and Immunology, Warsaw, Poland; ³Department of Radiology, Kidney Transplantation and Hypertension, Warsaw, Poland; ³Department of Neonatal Pathology, Warsaw, Poland.

**Background and Aim:** Obesity is one of the major risk factors of nonalcoholic liver disease (NAFLD) and fat distribution seems to play the role in adults with NAFLD. The aim of the study was to test hypothesis that it is fat tissue distribution and relative excess of visceral fat content what is related to NAFLD in children.

**Methods:** 22 patients with NAFLD aged 13.5 (11.7–16.0) y with BMI 27.5 (24.6–28.9) and 22 healthy overweight/obese aged 13.9 (11.3–16.3) y with BMI 26.9 (23.9–30.1) [median(Q1–Q3)], matched for absolute BMI (±10%), age (±0.5 y) and gender were
compared. Amount of visceral:intrapertioneal visceral, extrapertioneal visceral and subcutaneous fat was measured by magnetic resonance imaging (MRI). Anthropometric parameters, blood lipids, fasted glucose, insulin, proinsulin were evaluated and HOMA-IR was calculated.

Results: There were significant differences between NAFLD and healthy children in extrapertioneal visceral fat content: 17.72 (12.41–20.16) cm² vs 11.91 (7.16–15.68) cm², respectively (P < 0.05), and total visceral fat content 33.46 (24.61–39.84) cm² vs 22,05 (15.21–34.00) cm², respectively (P < 0.05) [median (Q1–Q3)]. There were no differences in intrapertioneal visceral fat and subcutaneous fat content among NAFLD and healthy children. Visceral fat content was related to waist circumference (Spearman R = 0.61, P < 0.05). The ratio fat/subcutaneous content was significantly related to HOMA-IR as an indicator of insulin resistance (Spearman R = 0.91, P < 0.05).

Conclusions: The excess of total visceral and extrapertioneal visceral fat, but not BMI distinguishes NAFLD and healthy subjects and altered fat tissue distribution correlates with insulin resistance in NAFLD.

PHN3-03

BODY COMPOSITION, FAT DISTRIBUTION, AND BONE MASS IN OVERWEIGHT/OBESE CHILDREN SUFFERING FROM NONALCOHOLIC FATTY LIVER DISEASE (NAFLD)

Presenter: P. Pludowski. Children’s Memorial Health Institute, Warsaw, Poland.
Co-authors: J. Maciej, P. Socha, L. Mieczysław, N. Joanna, J. Wojciech, A. Wierzbicka. Children’s Memorial Health Institute, Warsaw, Poland.

Background and Aim: The association of NAFLD with body composition abnormalities leading to disturbed relations between bone, muscle (lean body mass, LBM) and fat (FM) and its distribution remains unclear in children. The study was aimed to evaluate body composition and FM distribution in NAFLD children compared to healthy controls matched for absolute BMI (±10%), age (±0.5 y) and gender.

Methods: Total body scans using DXA (Prodigy Advance, GE Healthcare, USA) were performed in 11 NAFLD children aged 14.0±2.4 y with a BMI of 26.9±2.3 kg/m² and 11 controls aged 14.0±2.4 y with a BMI of 26.7±3.0 kg/m² (ns), The magnitude of possible differences between NAFLD cases and BMI-matched controls was investigated utilizing z scores (age- and gender-matched) calculated for DXA assessed bone mineral content (TBBMC), LBM, FM and for FM and LBM fractions of body weight (FM/BW; LBM/BW), using previously established reference values. Relative bone strength index was calculated as TBBMC/LBM ratio. Fat mass distribution was evaluated using DXA assessed Android and Gynoid regions of interest.

Results: NAFLD cases had significantly increased z scores for FM/BW (3.33±0.96; P < 0.01) and FM/LBM (3.42±1.16; P < 0.05) compared to healthy BMI-matched controls (2.02±1.54 and 2.21±1.82, respectively). When body height was controlled for, NAFLD cases had significantly higher SD-scores for FM (3.01±1.31), FM/BW (2.52±0.77), FM/LBM (2.61±1.32) compared to respective values observed in controls (1.95±1.71; 1.43±1.25; 1.57±1.49; all P < 0.05). In both NAFLD and controls BMI appeared to be slightly higher for their age and height showing z scores of 0.84±1.21, 1.36±1.37 and SD scores of 0.78±1.16, 1.34±0.98, but LBM/BW in both groups was reduced (z scores of −1.68±0.66, −1.47±0.93 and SD scores of −1.27±0.74, −1.06±0.80, respectively). Both z scores and SD scores for TBBMC and TBBMC/LBM did not differ between NAFLD and BMI-matched controls and were generally close to that expected in physiology. Finally, despite the same BMI, NAFLD cases had significantly increased %FM in both Android (50.5±6.8) and Gynoid (45.1±6.6) regions compared to controls (42.2±12.7 and 38.7±9.9; all P < 0.05, respectively).

Conclusions: Age- or height-adjusted bone mass and bone strength appeared to be not affected by NAFLD. Children with NAFLD had increased FM and relative decrease of LBM compared with BMI-matched controls what suggests significant generalized alterations in body composition accompanying NAFLD that is not explained solely by BMI.

PHN3-04

SERUM CONCENTRATIONS OF ADIPOKINES IN CHILDREN WITH NONALCOHOLIC FATTY LIVER DISEASE

Presenter: D. Lebensztejn. Medical University of Białystok, Białystok, Poland.
Co-authors: D. Lebensztejn, A. Romanowska, E. Skiba, E. Tarasiów, I. Werpachowska, M. Kaczmarski. Department of Pediatrics, Gastroenterology and Allergology, Medical University, Białystok, Poland; Department of Radiology, Medical University, Białystok, Poland.

Background and Aim: Noninvasive markers that predict both fatty liver in obese children and the degree of liver steatosis and in result could replace liver biopsy are lacking. Research on adipokines involved in pathogenesis
of nonalcoholic fatty liver disease (NAFLD) seems to be promising. Therefore, the aim of the study was to evaluate serum adipokines levels in obese children with NAFLD.

**Methods:** 42 obese (BMI >97 pc) children, age range 7–17, mean 12 years, were admitted to our department with suspected liver disease (hepatomegaly, and/or ultrasonographic liver brightness and/or increased ALT activity). Viral hepatitis (HBV, HCV), autoimmune and metabolic liver diseases (Wilson’s disease, alpha 1 antitrypsin deficiency, CF) were excluded. Fasting serum levels of TNF-α, sTNFR1, sTNFR2, IL-6, sILR, adiponectin, RBP-4 and visfatin were determined (ELISA) in all patients and in 20 nonobese controls. The degree of liver steatosis in ultrasound (USG) was graded according to Saverymuttu et al. Advanced liver steatosis was defined as a score >1. 1HMR spectroscopy was performed with 1.5-T scanner and with PRESS sequence; total lipids concentration was assessed in relative units in comparison to unsuppressed water signal. Area under curve ROC analysis was used to calculate the power of the assays to detect fatty liver or advanced steatosis (AccuROC, Canada).

**Results:** Fatty liver was confirmed in 30 children both in USG and 1HMR spectroscopy (16 had also an increased ALT activity). Adiponectin level was lower (P = 0.02) and TNF-α higher (P = 0.04) in children with NAFLD compared to obese patients without liver abnormalities/total lipids concentration was assessed in relative units in comparison to unsuppressed water signal. ROC analysis was used to calculate the power of the assays to detect fatty liver or advanced steatosis (AccuROC, Canada). The ability of serum adiponectin (cutoff 8.0 μg/mL, Se = 90%, Sp = 50%) and TNF-α (cutoff 1.89 pg/mL, Se = 80%, Sp = 58%) to differentiate obese children with fatty liver from those without steatosis was significant (AUC = 0.7292, P = 0.022; AUC = 0.7028, P = 0.042, respectively). Adiponectin >6.5 μg/mL had a sensitivity of 100% and a specificity of 53%; AUC = 0.7919, P = 0.0086 in predicting advanced liver steatosis. All other markers did not allow a useful prediction. Significant negative correlation was found between the total liver lipid concentration in 1HMR and adiponectin (r = 0.32, P = 0.038), whereas positive correlation was observed in relation to RBP-4 (r = 0.42, P = 0.006).

**Conclusions:** Adiponectin seems to be the most suitable noninvasive biomarker in predicting both advanced liver steatosis in children with NAFLD and fatty liver in obese children.

**PHN3-06**

**UDP-GLUCORONYL TRANSFERASE (UGT1A1) TATA BOX POLYMORPHISM IN PEDIATRIC POPULATION AND GILBERT SYNDROME**

Presenter: O. Zaja Frantulovic. Sestre Milosrdnice University Hospital, Zagreb, Croatia.

**Background and Aim:** Polymorphism of the promoter region of the UGT1A1 gene was shown to be associated with Gilbert syndrome (GS) in Caucasians. Insertion of additional TA nucleotide in TATA gene sequence, normally composed of 6 repeating TA dinucleotide sequences, causes a reduced gene expression and the reduction of UGT1A1 enzyme activity to 30% of normal values. The aim of the study was to determine the
prevalence of (TA)n polymorphism of UGT1A1 gene and the genotype basis of Gilbert syndrome in Croatia, as well as to investigate the correlation between given genotypes and total serum bilirubin levels, in respect to the age and gender group.

**Methods:** Blood samples were taken under the standardized conditions from 157 children (male/female: 75/82) aged 6 to 18 years (control group), and from 174 children clinically diagnosed with Gilbert syndrome (male/female: 98/76) aged 9 to 18 years. Total bilirubin were determined automatically by Olympus AU 600. TATA sequence of the UGT1A1 promotor was amplified by PCR, and the identification of the three possible genotypes, TA6/6, TA6/7 and TA7/7 was performed by electrophoresis on polycracilamid gel using modified Sampietro’s method.

**Results:** The correlation between total serum bilirubin levels (TB) and age was found in boys (r=0.243; \(P=0.035\)), but not in girls (r=0.087; \(P=0.442\)). In the group aged 6–12 years there was no gender difference in serum TB, while in the group aged 13–18 years TB was significantly higher in boys (15.98 ± 12.65 \(\mu\)mol/L) than in girls (11.44 ± 7.15 \(\mu\)mol/L). TA6 allele frequency in control group is 0.649, and TA7 allele 0.351, with equal occurrence in both genders (\(P=0.990\)). In control group, TA6/6 genotype was present in 44.0%, TA6/7 in 42.0%, and TA7/7 in 14.0% of children, without deviation from the theoretical frequencies (\(P=0.792\)) and without any gender difference in distribution (\(P=0.880\)). Trimodal distribution of serum bilirubin concentration was found which depended on the genotype, with highest concentration in TA7/7 group. There was a statistically significant association of genotypes with serum TB (\(P<0.001\)) in all children as well as in gender groups. In GS patients TA7 allele frequency is 94.83% with symmetric distribution in both genders (\(P=0.913\)). TA7/7 genotype was found in 90.80% of GS patients, TA6/7 in 8.05%, and TA6/6 in 1.15%, distributed equally in both genders in accordance with Hardy-Weinberg equilibrium (\(P=0.409\)).

**Conclusions:** Insertion of additional TA dinucleotide in the promotor region of UGT1A1 gene is the main cause of GS phenotype in the Croatian pediatric population.

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**PHN3-07**

**INCREASED PREVALENCE OF CONGENITAL ANOMALIES OF THE UMBILICAL AND PORTAL VENOUS SYSTEM IN DOWN SYNDROME**

**Presenter:** N. Prince. King’s College London School of Medicine at King’s College Hospital, NHS Foundation Trust, London, UK.

**Co-authors:** T. Grammatikopoulos1, C. Dryden3, D. Flynn3, P. Kane2, N. Hadzic1. 1Paediatric Liver Centre, King’s College, London School of Medicine at King’s College Hospital, NHS Foundation Trust, London, UK; 2Department of Radiology, King’s College Hospital, NHS Foundation Trust, London, UK; 3Department of Paediatric Gastroenterology, Royal Hospital for Sick Children, Glasgow, UK.

**Background and Aim:** Trisomy 21 is often associated with congenital cardiac malformations and prolonged cholestasis. We report a series of children who presented to our centre with abdominal vascular malformations and minimal biochemical abnormalities.

**Methods:** Our database was reviewed for patients with trisomy 21 and congenital umbilico-portal venous system abnormalities, who presented to our unit between 1985 and 2008. Clinical, biochemical, and radiological findings were analysed.

**Results:** Out of 40 patients with Down syndrome, 5 (2 male) were identified with a median age at presentation of 5 weeks (range, antenatally – 5.5 months). Three patients were born prematurely at 32, 35 and 36 weeks gestation. One patient was diagnosed antenatally at 20-week ultrasound scan. Presenting features included conjugated (3) or unconjugated (2) hyperbilirubinaemia, signs of heart failure (1), poor feeding (2), hepatosplenomegaly (2), thrombocytopenia (2), coagulopathy (3), and hypothyroidism (1). Radiological findings on ultrasonography and CT scan along with cardiac findings on echocardiography are listed in the table below. Mean values of biochemical parameters at presentation were total bilirubin 96 \(\mu\)mol/L (range, 53–180), conjugated bilirubin 27 \(\mu\)mol/L (range, 10–51), AST 51 IU/L (range, 32–72), ALP 744 IU/L (range, 302–1757).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Abdominal vascular malformations</th>
<th>Cardiac malformations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Agenesis of ductus venous with shunting from portal vein to inferior vena cava</td>
<td>Patent foramen ovale</td>
</tr>
<tr>
<td>2</td>
<td>Intrahepatic portocaval shunt: left portal vein to left hepatic vein</td>
<td>Atroventricular septal defect</td>
</tr>
<tr>
<td>3</td>
<td>13-mm left portal vein aneurysm with arterioportal shunting and reversal of right portal vein flow</td>
<td>Atrial septal defect, ventricular septal defect, patent ductus arteriosus</td>
</tr>
<tr>
<td>4</td>
<td>Patent ductus venous with reversed flow in the portal system</td>
<td>Patent foramen ovale, patent ductus arteriosus, pericardial effusion</td>
</tr>
<tr>
<td>5</td>
<td>Fistulous connections between hepatic artery and right portal vein and between right portal vein and intrahepatic inferior vena cava with patent ductus venous communicating with left portal vein</td>
<td>Atrial septal defect, ventricular septal defect, patent ductus arteriosus</td>
</tr>
</tbody>
</table>
Results:

All children have been with normal appearance and requires ongoing respiratory support. One eighth of all children with Down syndrome referred to our unit had congenital umbilico-portal venous system abnormalities, associated with cardiac defects in all. The pathogenic mechanism of vascular and cardiac anomalies, albeit unknown, may be shared. Increased awareness could facilitate early diagnosis.

PHN3-08

MOLECULAR GENETIC ANALYSIS IN PATIENTS WITH NIEMANN-PICK DISEASE TYPE B IN NORTHEASTERN BULGARIA


Co-authors: M. Georgieva1, V. Sinigerska2. 1University Hospital St Marina, Varna, Bulgaria; 2National Genetic Laboratory, Sofia, Bulgaria.

Background and Aim: Niemann-Pick disease (NPD) is an autosomal recessive lipid storage disorder that results from the deficiency of a lysosomal enzyme, acid sphingomyelinase. Traditional classifications subdivide NPD phenotypes into type A (OMIM 257200), a lethal neurodegenerative condition with early onset and death in the first 2–3 years of life, and chronic visceral type B (OMIM 607616) with variable severity and compatible with survival into adulthood. The aim of this study is identifying mutational characteristics in patients with NPD type B in northeastern Bulgaria.

Methods:

Clinical evaluation, pedigree data, and mutational analysis were performed in patients, presenting with spleen and liver injury. For 6 years (2002–2008) the diagnosis NPD was proved in 6 children (4 boys and 2 girls of Gypsy ancestry; unrelated) by clinical and biochemical analysis–all patients were found homozygotes for a specific mutation (W391G) in SMPD1, typical for patients of Gypsy origin on Balkan peninsula.

Conclusions:

Mutational analysis becomes very important for the conformation of the disease NP, which in the near future will be crucial, as ERT becomes available.

PHN3-09

PARAMETERS OF LIPID METABOLISM AND ANTIOXIDANTS IN CHILDREN WITH A1-ATD

Presenter: P. Socha. Children’s Memorial Health Institute, Warsaw, Poland.

Co-authors: A. Bakula1, P. Socha1, J. Pawlowska1, A. Wierzbicka2, J. Socha1. 1Children’s Memorial Health Institute, Warsaw, Poland.

Background and Aim: The common finding on histological examination in children with alpha-1-antitrypsin deficiency is liver steatosis. However, the mechanisms of liver damage are not clearly understood and variable course of the disease is not explained. The aim of the study was to evaluate parameters of lipid metabolism and free radical defense in children with alpha1-ATD.

Methods:

We studied 45 children aged 10.5 (6.4–13.9) years [median (Q1 –Q3)], admitted to our hospital with chronic hepatitis, who were homozygous (PiZZ) for alpha1-ATD and 46 healthy age matched controls. We analyzed laboratory parameters of lipid metabolism and selected antioxidants (GSH, GPx).

Results:

Serum concentration of apolipoprotein E was significantly increased in alpha1-ATD when compared to controls: 18 (17–20) vs. 9.5 (6–13) mg/dL, respectively [median (Q1 –Q3) (P < 0.01)]. Patients with alpha1-ATD presented with decreased serum LCAT activity compared to controls: 126.8 (117.4–143.8) vs 161 (119.0–193.5) nmol/mL/hour (P = 0.005). GPx activity decreased significantly in alpha1-ATD: 31.8 (31.4–32.8) vs. 34.7 (30.8–39.0), respectively (P < 0.05). No differences in total cholesterol, LDL and HDL cholesterol, triglyceride, apo AI, apo B, Lp(a) and GSH concentration were observed between the groups. Lipid peroxidation was measured in a subgroup of 22 children with alpha1-ATD and 22 healthy age matched controls and there were no significant differences between the groups: 0.31 (0.22–0.59) vs. 0.46 (0.34–0.73) nmol/mL.

Conclusions:

Antioxidant defense seems to be slightly decreased in patients with alpha1-ATD as expressed by decreased GPx activity but it does not seem to contribute to free radical injury. There are no major disturbances in lipid metabolism except for decreased LCAT and cholesterol (6/6). Cherry-red spot found in 2/5. Myelogram with foamy cells–5/6. Specific laboratory test—decreased sphingomyelinase activity 6/6. Mutational analysis—all patients were found homozygotes for a specific mutation (W391G) in SMPD1, typical for patients of Gypsy origin on Balkan peninsula.
increased apo E concentration which do not seem to be related to liver damage.

**PHN3-10**

THE HEALTH ECONOMICS OF GASTROSTOMY INSERTION AND FUNDOPPLICATION IN CHILDREN WITH NEUROLOGICAL IMPAIRMENT

Presenter: A. Vernon-Roberts. Oxford University, Oxford, UK.

Co-authors: J. Leal 2, A. Gray 2, P. Sullivan 1, 1Oxford University, Department of Paediatrics, Oxford, UK; 2Oxford University, Health Economics Research Centre, Oxford, UK.

**Aim:** The study aim was to accurately assess all costs associated with gastrostomy (GT) insertion with or without fundoplication in children with neurological impairment (NI). Information was retrieved from the point of initial surgical referral. The primary outcome measure was the total cost to the health service of performing GT insertion with and without fundoplication.

**Methods:** Only children with NI were included in the study (exclusion criteria: genetic, metabolic or neurodegenerative disease). Study patients were identified from the operating and endoscopy diaries dating from January 2000 until December 2005. This allowed a minimum follow up period of 1 year. Of the 167 GT inserted in that time 76 children were eligible to take part and 52 gave consent. Case notes were retrieved and cost areas investigated were outpatient attendance, ward admissions, surgery/endoscopy, radiology, allied health professionals, and pathology. Costs were examined over a minimum period of 1 year per patient to capture maintenance and replacement costs and subsequent admissions. Unit costs were assigned using Trust Financial Returns, NHS Reference Costs, Personal Social Services Research Unit data, manufacturers and the British National Formulary, and each data point was inflated to 2006 rates using the Pay and Prices Index.

**Results:** A total of 84 gastrostomy insertions (including revision to button) were performed in the time period. Of these procedures 34/52 had concurrent fundoplication. The average cost of GT insertion was £408.42. The average cost of insertion of GT plus fundoplication was £1581.52. Significant differences (95% CI) in cost were associated with performing anti-reflux surgery (P = 0.0008) including the cost of outpatient follow up (P ≤ 0.0001) and allied health professional support (P = 0.04). The cost of ward admissions for the anti-reflux surgery itself was not significantly different from those associated with insertion of the GT (P = 0.059) but costs for subsequent admissions for related complications were significantly different (P = 0.018) in those who had a fundoplication.

**Conclusions:** A recent Cochrane review highlighted the uncertainty regarding the optimal treatment of fundoplication surgery versus antireflux therapy in the NI child undergoing GT insertion. These data contribute to this by showing the significant additional costs associated with fundoplication in these patients.

**PHN3-11**

CLINICAL AND BIOCHEMICAL FACTORS CORRELATED WITH ANTIOXIDANT CAPACITY IN 43 CYSTIC FIBROSIS CHILDREN

Presenter: A. Fabre. APHM, Marseille, France.

Co-authors: J. Garcia 1, J. Gaudart 2, S. Caspar-Bauguil 3, J.L. Rittie 4, F. Bremond 4, E. Mas 4. 1Hopital de Purpan, Toulouse, France; 2APHM, Marseille, France; 3Hopital de Rangueil, Toulouse, France; 4Hopital des enfants, Toulouse, France.

**Background and Aim:** An imbalance between oxidants and antioxidant defences is suspected to play a role in the pathogenesis of cystic fibrosis (CF). We used a method assessing the global antioxidant capacity to look for clinical or biological factors associated with an oxidative stress.

**Methods:** Global antioxidant capacity was measured on erythrocytes and whole blood using the KRL kit of Kirial in 43 cystic fibrosis children followed in the one CF unit. Several data were recorded the same day: anthropometric measures, pulmonary function, severity scores (Schwachman and Brasfield); blood samples: nutritional and inflammatory proteins, selenium and zinc, fat vitamins and erythrocyte fatty acids. Univariate analysis was performed with Spearman’s rank correlation and Wilcoxon-Mann-Whitney test, and multivariate analysis with a generalized estimating equation.

**Results:** Sex ratio was 1.26 and the mean age is 12.3 ± 3.1 years. The 13 factors correlated with a diminution of the antioxidant capacity evaluated on erythrocytes are for the univariate analysis: female sex (P = 0.013), sporanox therapy (P = 0.031), exacerbation (P = 0.026), bronchial congestion (P = 0.002), C-reactive protein >10 mg/l (P = 0.023), orosomucoid plasma level (−0.587, P < 0.001), ceruloplasmin plasma level (−0.587, P < 0.001), Brasfield score (−0.464, P = 0.005), blood leucocytes level (−0.440, P = 0.01), w6/w3 (−0.369, P = 0.029), cupper plasma level (-0.354, P = 0.04), FEV1 (0.395, P = 0.019) and antioxidant capacity on whole blood (0.51, P = 0.002). The multivariate analysis revealed 4 significant parameters; 3 of them are negatively correlated: ceruloplasmin plasma level (P = 0.009), Brasfield score (P = 0.003), w6/w3 (P = 0.007), and 1 is positively

correlated: the link between ceruloplasmin and Brasfield score ($P = 0.019$). The antioxidant capacity on whole blood was associated with 6 parameters on univariate analysis: female sex ($P = 0.004$), intake of vitamin E ($P = 0.05$), blood leucocytes level ($-0.395, P = 0.011$), Brasfield score ($-0.316, P = 0.039$), selenium plasma level ($0.368, P = 0.017$), and antioxidant capacity on erythrocytes ($0.510, P = 0.002$). The factors correlated with a diminution of the antioxidant capacity in CF are mostly related to pulmonary inflammation or infection. However, nutritional factors are poorly correlated with the antioxidant capacity; likewise blood vitamin A and E levels or the intake of vitamin are only related to the antioxidant capacity on whole blood, maybe because of a better bioavaibility. Ceruloplasmin result is most intriguing: it is an inflammatory protein but the importance of the correlation suggests a putative role of the protein by itself.

**Conclusions:** Antioxidant capacity is associated with inflammatory parameters rather than with nutritional factors. The precise role of ceruloplasmin needs to be further explored.

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**PHN3-12**

**CAREGIVERS’ PERCEPTIONS FOLLOWING PERCUTANEOUS ENDOSCOPIC GASTROSTOMY IN CHILDREN WITH CONGENITAL HEART DISEASE**

**Presenter:** R. Srinivasan. **Department of Paediatric Gastroenterology, Liverpool, UK.**

**Co-authors:** W. Blumenow$^1$, W. Blumenow$^1$, A. Dalzell$^1$.

$^1$Alderhey NHS Foundation Trust, Liverpool, UK; $^2$Medical School, University of Liverpool, Liverpool, UK.

**Background and Aim:** While the usefulness of percutaneous endoscopic gastrostomy (PEG) is clearly established in the nutritional support of children with neurodisability, the role in substituting for prolonged nasogastric feeding in children with congenital heart disease (CHD) is a relatively recent development. We aimed to study parent/carer perceptions following the insertion of PEG in children with CHD. There are no previously published experiences about the same.

**Methods:** Descriptive qualitative survey of parental perceptions using a semistructured questionnaire.

**Results:** Thirty-eight carers of children with CHD completed a 27-point semistructured questionnaire (Tables 22 and 23). Time taken to feed their children reduced significantly after PEG insertion; 30–60 min (mode) prior to PEG insertion compared to 15 min subsequently ($P = 0.0006$). The frequency of feeding reduced from 6 times/day to 4–5 times/day; $P = 0.0004$ (Wilcoxon matched pairs signed ranks test). Carers’ perceived significant reductions in pre procedure symptoms, ease of administering medications and an enhanced level of happiness in their children (binomial test). 97% of carers were highly satisfied with the procedure with 15 parents wishing that the operation was done earlier (40%), while 22 felt it was done at the appropriate time (57%).

**Conclusions:** Carers of children with CHD perceive significant improvements in their child’s symptoms, wellbeing and ease of administering medication after PEG insertion. 97% of parents regarded PEG as the correct decision in assisting nutritional support.

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**TABLE 22. Effect of PEG insertion of preoperative symptoms**

<table>
<thead>
<tr>
<th>Preoperative problems</th>
<th>No. subjects (n)</th>
<th>Perception of improvement</th>
<th>Perception of worsening</th>
<th>Perception of no change</th>
<th>$P$ sign (binomial) test, 1-tailed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fighting, difficulty at feeding</td>
<td>12</td>
<td>11</td>
<td>1</td>
<td>0</td>
<td>0.003</td>
</tr>
<tr>
<td>Coughing, choking</td>
<td>21</td>
<td>20</td>
<td>1</td>
<td>0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vomiting</td>
<td>29</td>
<td>26</td>
<td>0</td>
<td>3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fatigue while feeding</td>
<td>15</td>
<td>13</td>
<td>1</td>
<td>1</td>
<td>0.003</td>
</tr>
</tbody>
</table>

**TABLE 23. Impact of PEG on the child and carer**

<table>
<thead>
<tr>
<th>Criterion</th>
<th>No. respondents (n)</th>
<th>Perception of improvement</th>
<th>Perception of worsening</th>
<th>Perception of no change</th>
<th>$P$ sign (binomial) test, 1-tailed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child’s happiness</td>
<td>38</td>
<td>34</td>
<td>0</td>
<td>4</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Ease of giving medications</td>
<td>38</td>
<td>33</td>
<td>1</td>
<td>4</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Time to devote to other children (siblings)</td>
<td>31</td>
<td>19</td>
<td>1</td>
<td>11 (6 had no other children)</td>
<td>0.140</td>
</tr>
<tr>
<td>Carer’s quality of life</td>
<td>38</td>
<td>29</td>
<td>9</td>
<td>24</td>
<td>0.07</td>
</tr>
<tr>
<td>Time to devote to self (carer)</td>
<td>38</td>
<td>14</td>
<td>23 (not in work)</td>
<td>0.1279</td>
<td></td>
</tr>
<tr>
<td>Carer’s working life</td>
<td>38</td>
<td>15</td>
<td>23 (not in work)</td>
<td>0.1279</td>
<td></td>
</tr>
</tbody>
</table>

PHN3-13

RENAL DISEASE IN CHILDREN ON LONG-TERM PARENTERAL NUTRITION

Presenter: V. Colomb, Necker-Enfants Malades Hospital, Paris cerex 15, France.
Co-authors: C. Noto1, N. Patey2, M. Charbit3, F. Lacaille1, O. Goulet1, V. Colomb.

LONG-TERM PARENTERAL NUTRITION
RENAL DISEASE IN CHILDREN ON PHN3-13

Paris cedex 15, France.

Background and Aim: Renal failure has been reported in patients on long-term parenteral nutrition (LTPN). Its mechanism is unclear. Osmotic renal charge, amino acids, aminoside treatment may impair renal function. The aim of this study was to compare retrospectively renal biopsies (RB) in children on LTPN to search specific lesions suggesting a PN toxicity.

Methods: Eleven children on LTPN for more than 1 year were studied. Median age at RB was 8 years (1–24). Median PN duration was 6.5 years (1–14). Indication for PN was congenital diarrhoea (5 cases), short bowel syndrome (4), Hirschsprung disease (2). Indications for RB were: measured glomerular filtration rate <80 mL/min/1.73m² and tubular impairment (7 cases), proteinuria (2), evaluation before intestinal transplantation (ITx) (2).

Results: Histological anomalies were observed in 6 patients (1 RB before ITx and 5 for renal impairment). One patient presented with acute tubular necrosis after an episode of dehydration and one with congenital diarrhoea presented a membranous glomerulonephritis. In the last 4 patients ischemic lesions were observed (arteriolar myocyte vacuolization); 3 of these showed also calcifications and osmotic lesions (tubular microvacuolisation), with interstitial fibrosis in 2. Patients with histological lesions had a greater number of CVC and nephrotoxic treatments, dehydratation or shock or acute renal failure events.

Conclusions: Histological lesions observed in children on LTPN are nonspecific and probably of multifactorial origin. They can occur without biological disorders. Tubular microvacuolisation observed in 3 patients has been identified as a sign of toxicity of osmotic drugs. Then, hyperosmolar solutions, like PN, could cause such lesion. However, this lesion is not observed in all patients. Patients on LTPN are also exposed to several other risk factors, as chronic hyperfiltration or association between lysine and aminosides. Smaller kidney are described in adults on LTPN and observed in 30% of our patients showed smaller kidney with other anomalies. ITx is an alternative treatment of intestinal failure, which shows a high risk of renal side effects. A systematic renal histological evaluation in children on LTPN is mandatory, especially at the time of evaluation before ITx, for a correct interpretation of possible lesions after ITx.

PHN3-14

TOLERABILITY AND SAFETY OF SHORT-TERM OLIVE OIL–BASED INTRAVENOUS LIPID EMULSION IN PEDIATRIC PATIENTS UNDERGOING BONE MARROW TRANSPLANTATION

Presenter: C. Hartman, Schneider Children’s Medical Center of Israel, Petah Tikva, Israel.
Co-authors: C. Hartman1, D. Berkowitz2, R. Elhasid2, M. Ben Harush2, S. Hadad2, R. Shami1. Schneider Children’s Medical Center of Israel, Petah-Tikva; 2Rambam Health Care Campus, Haifa, Israel.

Background and Aim: Parenteral nutrition is an important element in the supportive care of patients undergoing bone marrow transplantation (BMT). The objectives of this prospective randomized study were to assess short term safety and metabolic effects of an olive oil–based (OO) lipid emulsion compared with a MCT/LCT (M/L) emulsion in the clinical setting of pediatric BMT.

Methods: Twenty-eight pediatric BMT patients (age 1–18 years) expected to need parenteral nutrition (PN) support for at least 2 weeks, were randomly assigned to receive either OO (n = 15) or M/L (n = 13) lipid emulsions within PN. The other PN constituents were similar in both groups. Clinical and routine laboratory parameters as well as plasma fatty acids profile, vitamin E and peroxidation status were recorded at baseline and after 14 days of PN.

Results: Patients’ age, sex, body weight and primary diagnosis were not significantly different between the 2 PN protocols. No significant differences were found for hematological parameters, liver enzymes, vitamins concentrations and plasma peroxidation status as well as the percentage and time to engraftment. OO group showed higher oleic acid (58.4 ± 6.9 vs. 91.2 ± 6.0, P = 0.012) enrichment and higher linoleic (15.8 ± 3.5 vs. 33.0 ± 3.0, P = 0.012) and arachidonic acid (17.8 ± 5.8 vs. 48.3 ± 5.1, P = 0.002) but similar eicosapentanoic and docosahexanoic acids compared to M/L group at day 14.

Cholesterol levels decrease significantly in the OO group after 14 days on PN ($P=0.017$).

**Conclusions:** OO lipid emulsion was well tolerated, maintained essential fatty acids and peroxidation status and generated a favorable plasma lipid profile. This study suggests that short-term use of OO as intravenous lipid emulsions is safe and can be used as an alternative in children in need of PN support during BMT.

**PHN3-15**

**A SUDDEN AND MARKED REDUCTION IN PN CHOLESTASIS ON CHANGING FROM A CONVENTIONAL INTRAVENOUS LIPID SOURCE TO SMOF LIPID**

**Presenter:** R. Muhammed. *Birmingham Children’s Hospital, Birmingham, UK.*

**Co-authors:** R. Bremner¹, P. Davies¹, S. Protheroe¹, C. Holden¹, T. Johnson¹, S. Murphy¹. ¹Birmingham Children’s Hospital, Birmingham, UK.

**Background and Aim:** Lipids used in PN are suspected of having detrimental effects on immunity leading to a risk of infection. Recently novel lipid formulations have been introduced aimed at positive modulation of immune function. Conventional lipid emulsions such as Intralipid (Fresenius-Kabi) are prepared from soybean oil. SMOF lipid (Fresenius-Kabi) is a complex mixed-type emulsion including soybean oil (30%), medium chain triglyceride (30%), olive oil (25%) and fish oil (15%). We describe our experience with a series of infants and young children with PN cholestasis who were changed from Intralipid to SMOF. A comparison is made with a historical pre-SMOF cohort.

**Methods:** We reviewed the records of all those changed to SMOF because of cholestasis over the year following its introduction in our unit, and compared them with cholestatic children receiving Intralipid during the previous year. Only those receiving PN for at least 6 months were included.

**Results:** In total 8 were changed to SMOF (short bowel syndrome = 4, phenotypic = 2, autoimmune enteropathy = 1, idiopathic protracted diarrhoea = 1). The comparison group consisted of 9 children with PN cholestasis. In both the SMOF and comparison groups although there was a fall in the amount of PN energy and lipids being received after 6 months of follow-up, both groups were still receiving large and comparable amounts of PN. In the SMOF group one died of liver disease and another is awaiting liver transplantation. However in remaining 6 there was a sudden, often dramatic and sustained fall in bilirubin about 1–3 months after commencing SMOF. There is a statistically significant fall in bilirubin during the period of follow-up in the SMOF group ($P=0.02$) without any significant difference between the 2 groups with regards to their enteral and parenteral nutrition (Tables 24 and 25). The comparison group (n=9) by contrast had a steady and significant increase in bilirubin during a 6 month follow-up period.

**Conclusions:** The sudden fall in bilirubin observed with SMOF was striking and unexpected. Lipids have potent biological effects, and these could be beneficial in preventing or treating cholestasis. An RCT should be undertaken to evaluate the efficacy of SMOF in preventing PN-related liver disease in young children.

**PHN3-16**

**MICRONUTRIENT STATUS IN CHILDREN ON LONG-TERM ENTERAL NUTRITION**

**Presenter:** F. Gottrand. *CHRU Lille, Hopital Jeanne de Flandre, Lille, France.*

**TABLE 24. Characteristics at onset**

<table>
<thead>
<tr>
<th></th>
<th>SMOF group, n=8</th>
<th>Control group, n=9</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median bilirubin mmol/L</td>
<td>143 (71–275)</td>
<td>91 (78–176)</td>
<td>0.193</td>
</tr>
<tr>
<td>Median PN energy, cal/kg/day</td>
<td>95 (64–108)</td>
<td>96 (65–114)</td>
<td></td>
</tr>
<tr>
<td>Median enteral energy, cal/kg/day</td>
<td>21 (0–50)</td>
<td>8 (0–58)</td>
<td>0.431</td>
</tr>
</tbody>
</table>

**TABLE 25. Characteristics at 6 months**

<table>
<thead>
<tr>
<th></th>
<th>SMOF group, n=8</th>
<th>Comparison group, n=9</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median bilirubin mmol/L</td>
<td>19 (6–213)</td>
<td>185 (11–269)</td>
<td>0.06</td>
</tr>
<tr>
<td>Median bilirubin change over 6 mo</td>
<td>$-110 (-269 to 94)$</td>
<td>$93 (-165 to 161)$</td>
<td>0.023⁷</td>
</tr>
<tr>
<td>Median PN energy, cal/kg/day</td>
<td>74.5 (48–90)</td>
<td>75 (25–95)</td>
<td></td>
</tr>
<tr>
<td>Median PN energy difference over 6 mo</td>
<td>$-27 (0 to –40)$</td>
<td>$49 (30–111)$</td>
<td>0.177</td>
</tr>
<tr>
<td>Median enteral energy, cal/kg/day</td>
<td>32.5 (0–83)</td>
<td>49 (30–111)</td>
<td>0.22</td>
</tr>
<tr>
<td>Median enteral energy difference over 6 mo</td>
<td>$17.5 (-18 to 48)$</td>
<td>$36 (5–111)$</td>
<td>0.09</td>
</tr>
</tbody>
</table>

⁷ Statistically significant.
Background and Aim: Although enteral nutrition (EN) is a well-established technique in children, few data on the micronutrient status are available in this population. The aims of the study were to evaluate the micronutrient status in a pediatric population receiving long-term EN, and to look for factors associated with possible deficiencies.

Methods: All children receiving for more than 6 months home EN covering more than 50% of the daily energy requirement were included in this cross-sectional single centre study. Patients affected by malabsorption or supplemented with micronutrients were excluded. Sixty-four patients (36 boys and 28 girls) aged less than 18 years (range: 2–18 years). Median age was 7 years (range: 2–18 years). Median z score weight/height was –1.2 (range: –5.5 to 3.2). Median duration of EN was 43 months (range: 6–140 months). For every child were noted the underlying disease, quantity and type of EN formula, and drug intake. During a routine visit in the outpatient clinic, calcium, phosphorus, iron, zinc, copper, selenium, as well as vitamins C, D, and E were measured in the plasma.

Results: EN represented 90.4% of the total energy intake (range: 50–100%), and 60% of the daily recommended caloric intake. EN covered from 9.4 to 625% of the recommended micronutrient intake. Iron deficiency was found in 15.6% of patients (with anemia in 3.1%), whereas zinc and copper deficiency were found in 21.9% and 10.9%, respectively. Iron deficiency was significantly less frequent in children with neuromuscular disease compared to the rest of the group (8.8% vs 31.6%; P = 0.022). There was no correlation between iron status and energy intake. There was no case of selenium, vitamin C, D, or E deficiency. We did not find any other factor (underlying disease, type and quantity of macronutrient, use of EN formula containing fibers, duration of EN) associated with micronutrient deficiency.

Conclusions: Deficiencies in micronutrients are rare in children receiving prolonged EN, and micronutrient status must be perhaps evaluated each year. However, longitudinal studies are required to assess the evolution of micronutrient status over time.

PHN3-17

NUTRITIONAL IMPACT ANALYSIS OF THE FOOD AID DISTRIBUTED IN THE SAHARAWI REFUGEES CAMPS: POSSIBLE IMPLICATION FOR CELIAC DISEASE

Presenter: S. Martinazzi. Children’s Hospital, Brescia, Italy.
Co-authors: C. Monfredini1, L. Menchini2, A. Ravelli1.
1Children’s Hospital, Brescia, Italy; 2International Committee for Peoples Development (CISP), Rome, Italy.

Background and Aim: The Saharawi are a formerly nomadic people exiled since 1975, whose population is estimated to be around 1 million. 250,000 live in refugee camps in the south of the Algerian desert, where the prevalence of celiac disease (CD) is 7–8%, the highest reported in the world so far, and malnutrition is also common. The Saharawi almost entirely depend on food supply provided by international solidarity. The aim of the study was to establish the nutritional impact of the food aid distributed to the Saharawi population in view of possible clinical correlations with CD.

Methods: We evaluated the nutritional composition of food supplies provided to the population of Saharawi refugee camps for 1 year (June 2006–July 2007) in comparison with the officially recommended amounts, i.e., RDA and LARN. An identical supply was assumed for all individuals.

Results: With a daily energy content ranging from 15–50% of RDA/LARN, there is a calorie deficiency of 300–1500 kcal daily, depending upon age, sex and physical activity. The calorie supply of food aids was distributed as follows: carbohydrate 68.5%, fat 19%, and protein 12.5%, i.e., fat and protein deficiency and carbohydrates excess. Wheat flour was the major source of carbohydrates and since cereals are often given early to Saharawi infants, this is one likely reason for precipitating or worsening CD. The Ca content of food supply was <20% of the minimum recommended amount and this, together with the known high phosphate content of water, could result in significant impairment of bone mineralization and growth. The iron content of food supply was less than 50% of RDA/LARN and the marked reduction of vitamin C (12% of recommended intake), together with the high consumption of tea would impair iron absorption. Vitamin B12 was also deficient in these food supplies (about 20% of RDA/LARN). Therefore anemia should be expected, especially in pregnant/lactating women and children, and a poor iron intake could significantly worsen the anemia related to CD. Indeed iron-deficiency anemia is a common finding in the Saharawi of all ages.

Conclusions: An insufficient and unbalanced dietary intake of several nutrients appears to be a major cause of malnutrition in the Saharawi. Some changes in the food aids such as an increase in meat supply (camel, mutton, lamb, chicken), dairy products, vegetables and fruit, and a reduction of wheat flour are advisable in order to raise the intake of animal protein, calcium and iron, and reduce the nutritional impact of CD.
FRUCTOSE INTOLERANCE IN FUNCTIONAL GASTROINTESTINAL DISEASES

Presenter: V. Bedi. Semmelweis University, Budapest, Hungary.
Co-authors: A. Nemes-Nagy1, N. Csosznszki1, K. Farkas1, E. Tomsits1. 1Semmelweis University, 2nd Department Pediatrics, Budapest, Hungary.

Background and Aim: The number of patients suffering from functional gastrointestinal diseases (FGIDs) has been growing in the last decade. So far, fructose intolerance, a contributor to abdominal pain and a significant factor in FGIDs has gained less focus with regards to pediatric patients than to adults. Dietetic guidelines aiming at the avoidance of fructose malabsorption could improve the quality of life of FGIDs patients. In our examination we would like to know in which Rome III category contains the most patients with fructose malabsorption and if we diagnose it, wheter low fructose diet can contribute to improve the quality of life of FGIDs patients.

Methods: Between October 30, 2006 and October 30, 2008 our gastroenterological unit treated 2866 patients, out of whom 137 were diagnosed with FGIDs. That means 0.05%. 65 patients have had unchained symptoms, neither had comorbid diseases, nor had undertaken chronic medical treatment, nor had taken antibiotics within 3 weeks qualified to become subjects of our study. It is these 65 patients who were administered a fructose breath test for 180 minutes after consuming 20 g fructose during normal gastroenterological control, int he framework of the study. The patients who have had fructose positive breath test were given guideline to restricted fructose consumption in their nutrition by dietician.

Results: From the 65 patients are 18 toddlers. We examined the toddlers group from 1 to 3 years of age, because the consuming of the fructose in this age is relevant. 65 patients belong to children/adolescents in Rome III category. Eight of 18 toddlers and 30 of 47 (63.8%) children/adolescents had a positive breath test result. The majority of toddlers (14 patients) who had positive fructose test results suffered from functional infant colic and belongs to Rome III/G4. Children/adolescents (35 patients) fell in the category of childhood functional abdominal pain in Rome III/H2d. One month later, 39 of 48 patients who restricted their fructose consumption reported that the presence or/severity of their symptoms reduced significantly.

Conclusions: In spite of the fructose intolerance seems to be an important role of the symptoms of FGIDs was relevant only some type of Rome III categories and only in the childhood/adolescents group it may recommended to conduct fructose breath test on patients with these special FGIDs categories. If the patients have fructose intolerance dietetic advice could help to improve the quality of life in these patients.

SYMPOSIUM 4

GI: Stem Cell Therapies for Gastrointestinal Diseases

IDENTIFICATION OF NOVEL LEFT-RIGHT AND ANTERIOR-POSTERIOR CANDIDATE GENES IN THE VISCERAL MESODERM

Presenter: V. McIn. Baylor College of Medicine, Houston, Texas, USA.
Co-authors: N. Desai1, S. Chad2, M. Jamrich2. 1Baylor College of Medicine, Dept of Pediatrics, Houston, TX; 2Department of Molecular and Human Genetics, Houston, TX.

Background and Aim: Left-right (LR) decisions and anterior-posterior (AP) decisions are important cell fate decisions in the developing vertebrate gastrointestinal tract and may be implicated in human syndromes, but to date very few genes participating in these developmental decisions have been characterized. It is generally accepted that the visceral mesoderm plays an important role in these developmental decisions, but its study to date has been impeded by its relative inaccessibility in mouse embryos, and lack of appropriate tools in other models. We aimed to identify novel genes in the early visceral mesoderm by using the experimental advantages of Xenopus laevis, namely the ability to isolate tissue in very early embryos. The rationale for using the X.laevis model is that developmental paradigms are highly conserved across species, and that understanding early cell fate decisions in vitro should ultimately aid researchers aiming to direct cell fate in vitro for the purposes of cell-based therapies.

Methods: Using a novel technique to isolate visceral mesoderm in neurula stage Xenopus laevis embryos, we have performed gene expression analysis of right vs left and anterior vs posterior visceral mesoderm using the Affymetrix Xenopus laevis genome 2.0 array. Gene expression profiling was performed in triplicate. Significance was considered for a fold change $>2$ or $<-2$ or $P$ value $<.05$. Candidate genes were selected for their differential expression. Their expression was validated by RT-PCR and whole mount in situ hybridization.

Results: We identified $>200$ differentially expressed genes between left and right visceral mesoderm, while
approximately 150 genes were differentially regulated between anterior and posterior. Examples of known genes not previously implicated in LR and AP decisions are in Table 26.

**Conclusions:** We have developed a robust method to isolate genes involved in early LR and AP decisions in the visceral mesoderm. There appears to be a molecular signature for both the LR and AP axes in the early visceral mesoderm. Characterization of candidate genes will offer insight into novel pathways regulating import-ant decisions in the development of the GI tract and may contribute to understanding pathways involved in human syndromes associated with laterality defects or gastrointestinal malformations. In addition, understanding developmental paradigms is relevant to the study of gastrointestinal diseases since they may be reactivated in regeneration in cancer.

**TABLE 26.** Genes not previously implicated in LR and AP decisions

<table>
<thead>
<tr>
<th>Left</th>
<th>Right</th>
<th>Anterior</th>
<th>Posterior</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-fos A</td>
<td>sFRP2</td>
<td>myc</td>
<td>Tbx6</td>
</tr>
<tr>
<td>Xoct-91</td>
<td>T-cell diff protein</td>
<td>sFRP2</td>
<td>Hox36</td>
</tr>
<tr>
<td>BMP-7</td>
<td>mt-A</td>
<td>twist</td>
<td>bHLH-WRPV</td>
</tr>
</tbody>
</table>

**SY4-02**

**UNRAVELLING THE GENETIC ORIGIN OF MICROVILLIOUS INCLUSION DISEASE: DISEASE-CAUSING MUTATIONS IN MYOSIN VB GENE**

Co-authors: T. Müller², O. Goulet¹, S. Lechner², M. Hess², N. Schiebermeier², P. Heinz-Erian², F. Lacaille¹, F. Sauvat¹, Y. Revillon¹, J. Schmitz¹, V. Colomb¹, D. Canioni¹, L. Huber², A. Janecke². ¹Hôpital Necker Enfants Malades, Paris, France; ²Children’s Hospital, Innsbruck, Austria.

**Background and Aim:** Microvillus inclusion disease (MVID) is a rare autosomal recessive diarrheal disorder presenting with severe intractable diarrhea within the first few weeks of life. MVID is characterized by a lack of microvilli on the surface of mature enterocytes and occurrence of intracellular vacuolar structures containing microvilli. Recently, we suggested mutations in MYO5B encoding the unconventional type Vb myosin motor protein, as causal in MVID patients from consanguineous Turkish families. The aim of this study was to extend mutation analyses in MYO5B to different cohorts of patients from different ancestry of consanguineous and nonconsanguineous origin and to validate its significance.

**Methods:** Direct sequencing of the MYO5B exons and all splice sites was performed in 14 unrelated MVID patients of a single center. Anti-CD10 immunohistochemical staining was performed to detect neutral endopeptidase expression at the apical brush-border membrane in MVID enterocytes.

**Results:** Clinically, all patients presented with an early onset form of MVID characterized by massive and life-threatening watery diarrhea within the first days of life. Mutations in MYO5B gene were identified in all patients, both in consanguineous and not consanguineous families: 15 distinct nonsense and missense mutations were identified in the MYO5B gene. These mutations result in a predicted truncated or completely abolished MYOB5B protein expression. No difference in the clinical or histological phenotype of MVID was observed between patients of consanguineous and nonconsanguineous origin. In all patients with MVID the apical membrane marker neutral endopeptidase (CD10) was mislocalized to the subapical cytoplasm.

**Conclusions:** In the present work, we confirm that mutations in MYO5B characterize early onset MVID. The type of mutation indicates a loss of function of MYO5B and therefore we demonstrate that these genetic inborn errors are causal in the development of MVID. This discovery will help to develop prenatal diagnostic tools and it will help to unravel the pathophysiology of this congenital diarrheal disorder.
Results: 193 OGDs were performed in our department during the study period of whom 132 children had oesophageal biopsies taken with an age range from 6 months to 17 years, 50% of who were male. All had distal oesophageal biopsies taken unless the oesophageal biopsy was suggested clinically, when proximal oesophageal biopsies were also then taken. 3/132 children met the histological criteria for EoE on distal oesophageal biopsies and correlated with those children in whom EoE was considered on clinical grounds. There was no variation in numbers of eosinophils between distal and proximal oesophageal biopsies. The rest of the children who had oesophageal biopsies for other clinical indications did not meet the histological criteria for EoE, although only distal oesophageal biopsies were taken.

Conclusions: Diagnosis of EoE depends on both clinical and histological criteria. Despite EoE being reported as a new condition with evidence of increasing prevalence similar to inflammatory bowel disease (IBD), we identified only 3 patients (approximate prevalence of 0.2/100,000 children) with this condition compared to 28 new cases of children with IBD within the same time period (approximate prevalence 30/100,000 children). Children whose oesophageal biopsies met the histological criteria for EoE, had clinical symptoms suggestive of EoE, and distal oesophageal biopsies alone were diagnostic. None of the children without clinical suspicion of EoE had oesophageal biopsies suggestive of EoE on histology.

SY6-02

EOSINOPHILIC ESOPHAGITIS IN CHILDREN: STUDY OF 13 CASES; ENDOSCOPIC, ALLERGOLOGIC, AND MANOMETRIC INVESTIGATIONS

Presenter: P. Dumond. CHU Nancy-Brabois, Vandoeuvre les Nancy, France.
Co-authors: J. Champigneulle, P. Athias, D. Moneret-Vautrin, A. Morali. CHU Nancy Brabois Vandoeuvre les Nancy, France; CHU Dijon, Dijon; CHU Nancy Hopital Central, Nancy, France.

Background: Eosinophilic esophagitis has become increasingly recognized in children over the 15 years. Diagnosis implies a precise eosinophils count per high-power field (hpf) in biopsies.

Methods: Thirteen cases (boys = 85%) could be diagnosed after esophageal biopsies in our unit during the last 3 years; median age was 12.5 years (1.9–15.5). Investigation schedule included in all cases endoscopy with hpf examination, extended allergic evaluation, parasitological analysis.

Results: Clinical symptoms were mainly dysphagia and failure to thrive (54%), bolus impaction (38%), odynophagia and pyrosis (31%), vomiting and recurrent abdominal pain (15%). Clinical atopy was frequent (62%): asthma (38%), rhino-conjunctivitis (31%), and atopic dermatitis (23%). An increase was observed for several blood parameters: eosinophilia in 85% (median value: 1100/mm³), ECP in 91% (61.5 μg/L; 10.5–210); IgE = 5.7 * n value for age (0.1–25). Endoscopic aspects included esophageal longitudinal furrowing (53%), whitish exudates (35%) or mucosal rings (12%) in all cases. Eosinophils count (EC) was increased (>15/hpf) in upper (100%) and lower (85%) esophagus. Coloscopy could be realized in 7 cases: nodular aspect (2), high EC (6). GER was observed in 4/7 esophageal contrast studies (ringlike indentation 1/7). Eosphageal manometric evaluation showed an abnormal profile: LOS hypertension and body dyskinesias (4/5). Food allergy (2/9, fish), food sensitization (3/9, egg, leguminous, hazelnut, umbelliferous) and respiratory allergy (5/9) were identified. Outcome data were available for 8 patients. Clinical improvement was observed (4/8) after a proton pomp inhibitor therapy (1), a dietary restriction but without endoscopic improvement (1), a moisture evicition (2) substituted by “viscous budesonide” (2). There were 4 treatment failures: wheat evicition (2) substituted by “viscous budesonide” (2) and then oral prednisolone (1), cow’s milk evicition (twins).

Conclusions: Clinical signs of eosinophilic esophagitis have to be kept in mind; contribution of eosinophilic count in hpf should be emphasized; rectocolic infiltration is probably underestimated; manometric investigations need to be precised in children; efficiency of dietary evicition is not constant and “topical” or oral corticosteroids seem to be often useful (Spergel 2008, Bohm 2008).
AHP-01

EVALUATION OF THE EFFECTS OF HIGH-FAT DIET ON LIPIDS METABOLISM IN CHILDREN WITH DRUG-RESISTANT EPILEPSY TREATED WITH KETOGENIC DIET

Presenter: A. Stolarczyk. Children’s Memorial Health Institute, Warsaw, Poland.
Co-authors: M. Bachanski1, A. Wierzbicka2, E. Krawiecka3, P. Socha4

Background and Aim: The ketogenic diet with strictly defined ratio of fat to protein and carbohydrates was developed in the 1920s to treat patients with difficult-to-control seizures. High-fat diet is generally recognised as negatively affecting lipids metabolism. Our objective was to determine the effect of high-fat diet on plasma levels of the major indicators of lipid metabolism (apo-B, apo-A, LDL, VLDL, HDL, chol and TG) in children treated with ketogenic diet.

Methods: Fifteen children aged 3.5 to 12 years, treated with ketogenic diet for mean 2.5 years (1.5–5 years) were observed. All of them presented with seizures despite adequate use of a few medications. Ketogenic diet was introduced and continued for at least 1.5 years. For most patients (73%) diet was initiated at ambulatory conditions. All patients were under regular (every 3 months or on demand) control of therapeutic team (physician and nutritionist) and adhere to the diet. Lipid metabolism parameters were assessed every 3 months at first year of observation and every 6 months in following years. Fifty percent of children received standard diet at 4:1 ratio, others needed diet ratio modification for better control of seizures, ketosis, and growth parameters.

Results: All enrolled children remained on the diet and presented with at least 50–60% reduction in seizure frequency and severity. Two patients presented with seizure decrease above 90%. For all but 1 child, plasma levels of lipids, lipoproteins and apolipoproteins remained within the normal ranges. One boy with increased TG >600 mg/dL and Chol >400 mg/dL after initiation of the diet reduced his parameters to normal after adjusting the ratio to 1.5:1. After 3 months of treatment HDL cholesterol decreased from 46.9 ± 10.9 mg/dL to 39.4 ± 9.4 mg/dL and ApoA1 decreased from 1.26 ± 0.35 g/L to 1.08 ± 0.35 g/L.

Conclusions: High-fat, low-carbohydrates and low-protein ketogenic diet seems to be helpfull in therapy of children with difficult-to-control seizures and it does not produce significant adverse effects on plasma lipid levels, except for decreasing HDL-cholesterol, if adequate fat profile is used in the diet.

AHP-02

CHARACTERISATION OF AND CHANGES IN THE ANTHROPOMETRIC AND NUTRITIONAL PARAMETERS OF A LARGE COHORT OF PAEDIATRIC COELIAC PATIENTS AT DIAGNOSIS AND FOLLOW-UP

Presenter: T. Cardigan. Royal Hospital for Sick Children, Glasgow, UK.
Co-authors: T. Cardigan1, S. Vasileiad2, E. Buchanan1, P. Mcgrogan2, R. Russell 2. 1Department of Nutrition and Dietetics, Royal Hospital for Sick Children, Glasgow, UK; 2Department of Paediatric Gastroenterology, Hepatology and Nutrition, Royal Hospital for Sick Children, Glasgow, UK.

Aim: To review the characterisation of and changes in the anthropometric and nutritional parameters of a large cohort of paediatric coeliac patients at diagnosis and follow-up.

Methods: All children diagnosed with coeliac disease (CD) at Royal Hospital for Sick Children, Glasgow from 1995–2008 based on the ESPGHAN criteria were retrospectively studied. Data were collected at the time of diagnosis from both dietetic and medical records and then 6, 12, and 24 months after diagnosis. Data collected included weight, height, BMI, haematological and biochemical data. Minitab version 14 was used for statistical analysis. A t test was used with a paired t test used for paired data and a 2-sample t test for unpaired data.

Results: 112 children (42 male and 70 female) were diagnosed with CD in the study period. Growth parameters are shown in Table 27. Haemoglobin, haematocrit, and mean cell volume were abnormal at diagnosis in 31%, 54.5% and 45.5% of patients, respectively (n = 99). At 12 months 24/100 of patients had bloods rechecked and 37.5% still had abnormal levels. By 24 months abnormal results were found in 10% of patients who had bloods checked (n = 28). Abnormalities in other parameters including B12, albumin, calcium, prothrombin time and vitamin D were abnormal at diagnosis for 4%, 22.9%, 26.5%, 33.9%, and 21.7% of children, respectively. PTH at diagnosis was abnormal in 42.1% of children (n = 19).
Conclusions: Following initiation of a gluten-free diet (GFD) weight gain had improved significantly by 6 months with further improvement at 12 months. Height improved significantly in the first 6 months and continued to improve up to 24 months. Whilst many biochemical and haematological parameters were abnormal at diagnosis they remained abnormal following initiation of a GFD. It is clinically perceived that these abnormalities will correct themselves once a GFD is commenced but we have shown that this is not always the case. This review shows the importance of a clinical pathway in CD to monitor, correct, and follow up this group of patients.

AHP-03

FOOD REINTRODUCTION FOLLOWING EXCLUSIVE ENTERAL FEEDING IN CROHN DISEASE


Co-authors: T. Johnson1, E. Buchanan2, J. Smith3, K. Maxwell4, J. Caines5.1Birmingham Children’s Hospital, Birmingham, UK; 2Royal Hospital for Sick Children, Glasgow, UK; 3Addenbrookes Hospital, Cambridge, UK; 4Kings College Hospital, London, UK; 5Northwick Park Hospital, London, UK.

Background and Aim: Elemental feeds were initially used for managing exacerbations of Crohn disease with gradual food reintroduction (Aliment Pharmacol Ther. 1997;11:17–31). Recent studies suggest that the efficacy of liquid diet therapy (LDT) is unrelated to specific food allergens (Gut. 1993;34:783–7). Gradual food reintroduction remains common practice throughout the UK despite the majority of centres using a cow’s milk–based feed for exclusive enteral nutrition (EEN). The aim of this audit was to establish current food reintroduction practices amongst dietitians and clinicians in the UK following EEN.

Method: All full BSPGHAN members were sent a questionnaire asking about type of feed used, duration of EEN, other foods allowed, and food reintroduction practices and reasons for them. A literature search was also undertaken.

Results: 84 questionnaires (42%) were completed and analysed. The results follow:

<table>
<thead>
<tr>
<th>Type of feed</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elemental</td>
<td>14 (17)</td>
</tr>
<tr>
<td>Polymeric</td>
<td>56 (66)</td>
</tr>
<tr>
<td>Both</td>
<td>14 (17)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration, wk</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4</td>
<td>0</td>
</tr>
<tr>
<td>4–6</td>
<td>2 (2)</td>
</tr>
<tr>
<td>6</td>
<td>56 (67)</td>
</tr>
<tr>
<td>6–8</td>
<td>3 (4)</td>
</tr>
<tr>
<td>8</td>
<td>23 (27)</td>
</tr>
</tbody>
</table>

38% of respondents allowed no additional food or drink during EEN. The remaining respondents allowed a combination of the following drinks and food: water, squash, fizzy drinks, black tea, herbal tea, Bovril, boiled sweets, chewing gum, jelly sweets and ice lollies. 89% of respondents did not think that children with Crohn disease had an increased incidence of food allergy. Food reintroduction practices varied widely. 18% of respondents introduced normal diet from day 1 and 50% by day 7. Single food reintroduction was favoured by 28% of respondents, the remainder practising empirical exclusion diets. 68% of respondents reported the reason for their practice was historical rather than evidence based. In a study of 102 children undergoing food reintroduction following EEN only 2 remained persistently intolerant (J Pediatr Gastroenterol Nutr. 2007;44(Suppl 1):G2–03).

Conclusions: Whilst the majority of centres use a polymeric feed for management of exacerbations of Crohn disease, single food reintroduction and exclusions remain common practice. There is no evidence to exclude single or multiple foods whilst reintroducing diet after LDT and delaying the introduction of a normal diet prolongs a difficult treatment, may compromise nutritional intake and status, and may make further courses of treatment

<table>
<thead>
<tr>
<th>TABLE 27. Growth parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>At diagnosis</td>
</tr>
<tr>
<td>Weight (n = 107) Mean z score</td>
</tr>
<tr>
<td>Height (n = 103) Mean z score</td>
</tr>
<tr>
<td>BMI (n = 103) Mean z score</td>
</tr>
</tbody>
</table>

* P < 0.05 compared with diagnosis.
** P < 0.05 compared with 6 months.
*** P < 0.05 compared with diagnosis.
AHP-04

DIETARY HABITS OF SLOVENIAN ADOLESCENTS

Presenter: N. Fidler Mis. University Children’s Hospital, Ljubljana, Slovenia.

Co-authors: H. Kobe1, M. Timeč1, C. Krinik1. 1University Children’s Hospital, Ljubljana, Slovenia.

Background and Aim: This is the first national representative study on dietary habits of Slovenian adolescents. We aimed to investigate their dietary habits and to compare them with the recommendations.

Methods: A representative sample of 2,813 adolescents, 14–17 years old (10% of population entering high school) was recruited from Feb. 2003 – Apr. 2005. The participation rate was 95% (BMI for boys and girls (mean (SD)): 21.4 (3.8) and 21.2 (3.5) kg/m^2). Dietary habits during the past one year were assessed by the semi-quantitative food frequency questionnaire (FFQ). The present nutrition was assessed by 3-day weighted dietary protocols (3 DPs) in a subgroup of 197 adolescents. Energy and nutrient intakes were evaluated by computerized programme Prodi 5.2 expert plus (Stuttgart, Germany) and compared with the D-A-CH reference values for nutrient intake and the joint WHO/FAO recommendations.

Results: Adolescents reported energy intake slightly below the D-A-CH recommendations for moderate physical activity (ie, PAL = 1.75) (boys and girls: 98% and 92% of D-A-CH; 12.7 and 9.7 MJ/day). The proportion between macro nutrients was adequate (carbohydrates, fats and proteins: 57, 28 and 15% for boys and 57, 29 and 14% for girls). The composition of ingested fats (13% saturated, 10% monounsaturated and 5% polyunsaturated fats) and carbohydrates (16% energy from free sugars) were unsuitable. The absolute intakes of cholesterol, water and dietary fibres were adequate (boys and girls: 300 and 218 mg/day; 2972 and 2633 mL/day; 33 and 27 g/day). The evaluation of 12 vitamins and 12 minerals showed low intakes of folic acid (72% of D-A-CH), vitamin D (88%) and fluoride (32%), whereas excessive sodium intake (127% above the WHO/FAO upper limit) in boys. Girls reported low intakes of folic acid (60% of D-A-CH), pantothenic acid (80%), vitamin D (64%), calcium (85%), iron (94%) and fluoride (30%), whereas excessive sodium intake (82% above the WHO/FAO upper limit).

Conclusions: Slovenian adolescents reported a rather low energy intake. The proportion between macronutrients is adequate. The composition of ingested fats (too high saturated, too low polyunsaturated fats intake) and carbohydrates (too high free sugars intake) is unsuitable. There is a low intake of folate, vitamin D and fluoride in both genders and also of: pantothenic acid, calcium, iron and dietary fibre/MJ in girls. The sodium intake is markedly too high in both genders. The dietary habits of Slovenian adolescents, especially of girls, are not healthy and need to be improved.

AHP-05

CHANGES IN THE INCIDENCE, PRESENTING SYMPTOMS, AND AGE AT DIAGNOSIS IN PAEDIATRIC PATIENTS WITH COELIAC DISEASE IN A REGIONAL CENTRE OVER A 14-YEAR PERIOD

Presenter: E. Buchanan. Royal Hospital for Sick Children, Glasgow, UK.

Co-authors: E. Buchanan1, S. Vasileiadi2, T. Cardigan1, P. Mcgrogan2, R. Russell2. 1Department of Nutrition and Dietetics, Royal Hospital for Sick Children, Glasgow, UK; 2Department of Paediatric Gastroenterology, Yorkhill Hospital, Glasgow, UK.

Aim: To review changes in the incidence, presenting symptoms and age at diagnosis in paediatric patients with coeliac disease referred to a regional referral centre over a 14-year period.

Methods: All children diagnosed with coeliac disease (CD; based on the ESPGHAN criteria) at Royal Hospital for Sick Children, Glasgow, from 1995–2008 were studied retrospectively. Data were collected at the time of diagnosis from both medical and dietetic records and also at 6, 12, and 24 months post diagnosis. This included demographics, source of referral, presenting symptoms and any comorbidity. Annual incidence was calculated. Minitab version 14 was used for statistical analysis. Comparison of medians was performed using a Mann-Whitney U test.

Results: 112 children were diagnosed with CD during the study period. The study period was divided into period 1 (1995–2001) and period 2 (2002–2008). The median age of diagnosis overall was 5.25 years (IQR 2.98–9.23). In period 1 the median age of diagnosis was significantly younger at 2.42 years (IQR 1.62–4.6) compared to 7.25 years (IQR 3.58–10.56, P = 0.00001) in period 2. The median number of children diagnosed in period 1 was 4 (IQR 1–6) compared with 11 (IQR 5–21, P = 0.02) in period 2. Of 24 diabetic children all were diagnosed in the second time period. There were significant changes in presenting symptoms between the 2 time periods. The number of children presenting with diarrhoea decreased in the second time period (69.2% vs 46.5%, P = 0.04). The number of children presenting with reported poor
weight gain also decreased in the second time period (42.3% vs 17.4%, \(P = 0.008\)). In contrast no asymptomatic patients were reported in period 1 compared to 14 in period 2, \(P = \infty\). 25 had a positive family history of CD, 24 had type 1 diabetes, 4 Down syndrome, 2 hypothyroidism, 1 hypoparathyroidism and 1 dermatitis hepatoformis. 93/95 children had positive anti-endomysial antibodies (EMA) at diagnosis. 58/60 also had hepatic antibodies (tTG) measured at diagnosis. 11 out of the 112 (9.8%) were IgA deficient.

**Conclusions:** The number of patients diagnosed with CD has risen significantly between the 2 time periods. The patient population has changed with a reduction in those presenting with diarrhoea and reported poor weight gain but an increase in those who are asymptomatic. There is also an increase in age at diagnosis and a notable increase in the number of diabetics diagnosed with CD.

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**Allied Health Professionals**

**Poster Presentations**

**AHP-06**

**PROVIDING NUTRITION TO BABIES BORN WITH GASTROSCHISIS**

Presenter: T. Dunne. *Children’s University Hospital, Dublin, Ireland.*

Co-authors: V. Kelly\(^1\). *Children’s University Hospital, Dublin, Ireland.*

**Aim:** The aim of this study was to analyse the provision of nutrition to infants born with gastroschisis and evaluate the need for clinical feeding guidelines.

**Methods:** The medical and dietetic records of 42 patients with gastroschisis managed at a single institution between 2004 and 2008 were retrospectively reviewed. For the purpose of interpreting the data patients with atresia, necrotising enterocolitis (NEC), severe dysmotility or who required additional gut surgery were classified as complex cases.

**Results:** 24 (57%) patients were born before 37 weeks’ gestation. Their mean gestational age was 34.9 weeks and mean birthweight was 2.08 kg. The mean birthweight of the 18 term infants was 2.61 kg. All patients required parenteral nutrition which was commenced between days 1–5 of life with a mean starting age of 2.5 days. 23 patients (54.8%) received PN via central line, 7 patients (16.7%) via peripherally inserted central catheter (PICC) and 12 patients (28.6%) via peripheral line. The average duration of PN for all patients was 34 days (simple cases: 23 days, complex cases: 54 days). Mean calorie intake per kilogram body weight per day for the first and second weeks of life was 43 and 83, respectively. The average time taken to initiate enteral nutrition was 16 days (simple cases: 14 days, complex cases: 19 days). Time taken to reach full feeds (150 mL/kg/day) was 39.5 days. Complex cases took 62 days and simple cases 28 days. 33 (79%) patients received mother’s own expressed breast milk and of these, 11 (26%) were still receiving breast milk at discharge. For those that received breast milk but were discharged on formula, they received breast milk for an average of 14 days duration. Of the 31 patients who received formula all were initially given a hydrolysed cow’s milk protein formula. 5 patients (12%) developed NEC between 28 and 73 days of age. 4 of these 5 patients received EBM for a mean of 11 days but all were receiving infant formula at onset of NEC.

**Conclusions:** Patients with gastroschisis require parenteral nutrition for at least 3 weeks. They are also at increased risk of NEC and in this study NEC developed between 3 weeks and 3 months of age. Clinical feeding guidelines for this group are indicated and should include recommendations on central access for PN and the promotion and continuation of breast milk as the feed of choice.

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**AHP-07**

**LACTOBACILLUS REUTERI EFFECT ON ATOPIC ECZEMA IN CHILDHOOD**


Co-authors: L. Axelsson\(^1\). *Lund University, Lund, Sweden.*

**Aim:** To determine whether simple dietary supplementation with *L. reuteri* can affect atopic eczema in young children currently under standard treatment.

**Methods:** In this prospective double-blind randomized study, patients (3 months to 4 years of age) were included after a diagnosis with moderate atopic dermatitis. The patients were randomised in 2 groups, each containing 25 subjects. One group (A) received *L. reuteri* supplementation daily (1 \(\times\) \(10^8\) CFU/day suspended in 5 drops of food oil) whilst the other group (B) was given an identical placebo. To determine whether oral supplementation with *L. reuteri* can affect the extent and severity of atopic eczema in children given standard treatment we examined responses to prick test to egg, milk, fish, cat, and peanut allergens after 12 months treatment with *L. reuteri*. Steroid treatment was measured. The extent and severity of eczema were assessed according to a validated scoring system (SCORAD). Analysis of total blood IgE was made at the beginning and at the end of the study.

**Results:** The extension of the eczema were significantly decreased over time in the group given *L. reuteri* compared to the group given placebo (\(P = 0.024\)). Subjective
symptoms such as itching and loss of sleep were significantly lower in the group given lactobacillus ($P = 0.024$).

From the study start to the last visit (one year) there were significantly increased IgE-levels in the group given placebo ($A P = 0.133$ vs $B, P = 0.022$). The skin prick test for peanut was significantly reduced in the group given active substance ($P = 0.023$). There were no recorded differences in the steroid consumptions between the groups.

**Conclusions:** These results indicate that *L reuteri* may have an effect on the immune system and reduce allergic symptoms in children. However more similar studies are needed to confirm the results in this study.

### AHP-08

**IMPLEMENTING A NOVEL PAEDIATRIC NUTRITIONAL SCREENING TOOL (PAEDIATRIC YORKHILL MALNUTRITION SCORE): CHALLENGES AND IMPACT IN PAEDIATRIC NURSING PRACTICE**

Presenter: I. Macleod. NHS Greater Glasgow and Clyde, Yorkhill Hospital, Glasgow, UK.

Co-authors: I. Macleod¹, K. Gerasimidis¹, O. Purcell¹, T. Mohammed², I. Swinbank¹, W. Charlotte², D. Flynn¹, M. McAuley¹. ¹NHS Greater Glasgow and Clyde, Yorkhill Hospital, Glasgow, UK; ²Acute Services, NHS Greater Glasgow and Clyde, Yorkhill Hospital, Glasgow, UK; ³PEACH Unit, Division of Developmental Medicine, University of Glasgow, Yorkhill Hospitals, Glasgow, UK.

**Aim:** To evaluate the effect of the implementation of a novel paediatric nutritional screening tool on clinical nursing practice, within current staffing resources, in medical and surgical wards in a tertiary paediatric and district general hospital.

**Methods:** A paediatric malnutrition screening tool (Paediatric Yorkhill Malnutrition Score-PYMS) was developed locally for children above 1 year using Body Mass Index (BMI) and information collected on admission (Gerasimidis et al. BSPGHAN 2009 winter meeting abstract. Accepted for oral presentation). The availability of measuring and weighing equipment was reviewed in the pilot areas and their accuracy assessed. Impact of the tool on the number of children weighed and measured, including plotting of growth data was assessed through case review (n = 177) before and after implementation in a random number of admissions. The impact on nursing practice was evaluated using an anonymous questionnaire at the end of a 4-month pilot period. This project did not require ethics committee approval but was registered with the hospital audit office.

**Results:** Weighing equipment and standing stadiometer provision were available in all areas but, 50% of the clinical areas lacked supine length equipment. The accuracy of the majority of the weighing equipment (96%) was within the specifications detailed in current guidelines (Guidance Notes Relating to the Legal Prescription of Medical Weighing Scales. Northampton: UK Weighing Federation; 2002), but 40% of the height equipment was out of calibration ($>$1 mm) Servicing and calibration records were unavailable in all clinical areas. A significant increase in height recording was observed before and following the introduction of PYMS (2.3% vs 68.2%, $P < 0.001$) although completion of growth charts did not improve (7% in both periods). No improvement in length recording was seen in children who were not eligible for a PYMS (ie, those <1 year). Although only one third of respondents had previous knowledge of malnutrition screening, following the implementation of PYMS, 86% of them believed that malnutrition screening can directly improve patient care. The majority of the nurses who responded to the questionnaire (n = 80) found PYMS easy to use (96%), quick (85%), and easy to integrate into admission (82%), although 83% of the respondents agreed that workload increased.

**Conclusions:** Malnutrition screening with PYMS is achievable within current nursing practice. Implementation may be hindered by deficits in equipment availability and accuracy. In addition, awareness of nutritional screening needs to be raised amongst other staffing groups to ensure best practice in nutritional care.