Mycoplasma pneumoniae and Respiratory Virus Infections in Children With Persistent Cough in England

A Retrospective Analysis

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Background: Persistent cough following an acute respiratory tract infection is common in children, but clinicians may find it difficult to give accurate prognostic information on likely duration of cough without a microbiologic diagnosis. This study estimates the prevalence of *Mycoplasma pneumoniae* (Mp) and assesses the prognostic value of detecting Mp and respiratory viruses in children with persistent cough.

Methods: We retrospectively analyzed blood samples, nasopharyngeal aspirates (NPAs), and cough duration data from 179 children with persistent cough lasting 14 days or longer. Of these children, 37% had serologically confirmed *Bordetella pertussis* (pertussis). We detected Mp by polymerase chain reaction of NPAs and IgM serology, and respiratory viruses (human rhinoviruses, influenza viruses, respiratory syncytial viruses, and human metapneumovirus) by polymerase chain reaction of NPAs. We used Kaplan–Meier analyses to calculate median cough durations with 95% confidence intervals (CIs).

Results: We detected Mp in 22 of 170 children with sufficient blood and/or NPAs (12.9%, 95% CI: 8.7–18.8). Cough duration in children with positive Mp serology (median: 39 days, 95% CI: 24–54) was significantly shorter than in children with positive pertussis serology (median: 118 days, 95% CI: 82–154, $P < 0.001$). The presence of respiratory viruses did not significantly lengthen cough duration in children with pertussis (median: 154 days, 95% CI: 74–234, $P = 0.810$). Only 3 children had both Mp and respiratory virus infections.

Conclusions: Mp is an important infection in children with persistent cough and is associated with a significantly shorter duration of cough than pertussis. However, detecting respiratory viruses does not add prognostic value in children with pertussis.

Key Words: *Mycoplasma pneumoniae*, viruses, infection, cough, child

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Persistent cough is a common symptom among school-age children, which can lead to considerable parental stress and recurrent medical consultations.[1] Parents of children with persistent cough tend to consult when the child appears distressed or unwell and are therefore likely to find precise diagnostic and prognostic information helpful.[2]

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Most coughs in children are caused by acute respiratory tract infections (RTIs), which usually settle within 2 weeks.[3] However, persistent cough following a RTI is common in children. A multicenter European parent questionnaire survey reported that 12.7% of children 7 to 11 years of age had experienced a persistent cough lasting 3 months or longer during the previous autumn/winter season, 7.6% had a persistent productive cough even when they did not have a cold, and 11.5% had a persistent dry cough at night.[4]

Children with persistent cough are sometimes diagnosed with asthma. However, asthma is less likely in children with persistent cough that is not accompanied by wheeze or other respiratory symptoms.[5] Persistent or secondary RTIs are likely to account for a substantial proportion of persistent cough in children. In a prospective cohort study of 108 children referred to a tertiary center with persistent cough of at least 3 weeks' duration, almost 40% were diagnosed with protracted bacterial bronchitis involving a range of respiratory pathogens including *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Streptococcus pneumoniae*.[6]

In community studies, various bacterial and viral infections have been implicated in childhood persistent cough. These include *Bordetella pertussis* (pertussis),[7–9] *Mycoplasma pneumoniae* (Mp),[8–10] respiratory syncytial virus, and influenza viruses.[9,10] Most acute RTIs are caused by respiratory viruses.[11] However, Mp is one of the most common bacterial causes of RTIs in children, and its highest incidence is found in the 5- to 9-year age group (4 per 1000 children per year).[12]

Between October 2001 and March 2005, we prospectively recruited a cohort of 179 children who presented in UK primary care with persistent cough lasting 2 weeks or longer. We found that 37% had evidence of recent pertussis infection based on serology, and that duration of cough in children with pertussis was significantly longer than that in children without pertussis.[7] We also sought consent to obtain nasopharyngeal aspirates (NPAs) from these children, but have not previously analyzed or reported these.

In this study, we retrospectively analyzed blood samples, NPAs, and cough duration data from this cohort. Our objectives were to estimate the prevalence of Mp, compare duration of cough between children with Mp and pertussis, and to determine whether the presence of respiratory viruses further prolongs the duration of cough in children with these infections.

MATERIALS AND METHODS

We performed a retrospective analysis of a previously recruited cohort of children 5 to 16 years of age who presented to their general practitioner with a cough lasting 14 days or longer, which was either unexplained or triggered by an acute RTI. Children were prospectively recruited from 18 general practices in Oxfordshire. We sought consent to obtain blood and NPA samples from each child. For children who had been coughing for between 14 and 28 days at the time of study entry, we sought consent to obtain a second blood sample 4 to 6 weeks after the initial sample.[7]

We excluded children in whom the date of onset of cough could not be established. We also excluded children with chronic underlying medical conditions which might have been the cause of their persistent cough, including cystic fibrosis, bronchiectasis, and cardiac failure. We excluded children with asthma if clinicians felt that their persistent cough was asthma-related, but not if they felt that the cough was unexplained or triggered by an acute RTI.

We took detailed medical and cough histories on study entry and performed a standardized clinical examination including temperature, peak expiratory flow rate, and height. We also asked the parent(s) of each child to complete a cough diary for the duration of the child's cough with input from the child if it was felt that he or she had sufficient understanding of the task to do this reliably. For the first 14 days after study entry, we asked parents to record in the diary whether or not the child's cough was present on a daily basis. At the end of each subsequent week, we asked them to record whether or not the cough was still present until there had been an absence of cough for 2 consecutive weeks. We estimated the total duration of cough in each child based on the sum of the duration of cough reported at the time of study entry, the number of days that cough was recorded as being present during the first 14 days after study entry, and the number of subsequent weeks for which cough was recorded as being present in the cough diary.

Laboratory Methods

Sample Storage

Both NPAs and serum samples were initially collected between October 2001 and March 2005. Serum samples were stored at −20°C and NPAs at −80°C until testing in 2010.

Mp Serology

IgG was adsorbed from sera before IgM testing with the RF-SorboTech Kit (Virotech, Genzyme Diagnostics) according to the manufacturer's instructions. The presence and quantity of IgM in the serum samples was analyzed by enzyme-linked immunosorbent assay using the Mp enzyme-linked immunosorbent assay (recombinant) IgG/IgM Testkit (Virotech, Genzyme Diagnostics) according to the manufacturer's instructions. Samples were deemed positive if the Virotech units were above 11.0, borderline if Virotech units were 9.0 to 11.0, and negative if Virotech units were below 9.0.

Nucleic Acid Extraction

Total nucleic acid was extracted from the samples using the QIAsymphony robot using QIAsymphony Virus/Bacteria Mini Kit (Qiagen) according to the manufacturer's instructions and examined for the presence of Mp and respiratory viruses by real-time polymerase chain reaction (PCR). Total nucleic acid extracts were stored at −80°C before testing.

Mp PCR

Real-time PCR that amplifies the P1 gene encoding the major surface adhesion P1 protein[13] was used to determine the presence of Mp DNA in 5-μL sample of extracted NPAs. Assays were carried out using a LightCycler (Roche, United Kingdom) to amplify both Mp and an internal processing control that detects inhibitory samples, with detection of 10 copies or more of the Mp target sequence in 5 μL sample DNA.[13] Uracil-DNA glycosylase (Roche) was added to each reaction to eliminate carry over contamination.[14] Data were analyzed using Roche LightCycler software version 3.5 and an estimation of detectable Mp DNA was made in direct comparison to a positive standard curve.

Respiratory Virus PCR

Extracted nucleic acid from NPAs was also subjected to real-time PCR screening for the following respiratory viruses: influenza A (H1 and H3), influenza B, respiratory syncytial virus A and B, and human metapneumovirus, as described previously.[15–17] Samples were also screened for human rhinovirus in a real-time 1-step reverse transcription-PCR reaction which targeted the conserved 5′ untranslated region (primer and probe sequences available on request). All real-time PCR/reverse transcription-PCR assays were conducted on either ABI TaqMan or Qiagen RotorGene platforms.

Data Analysis

We summarized the characteristics of children whose blood and/or NPA samples were still sufficient for analysis using percentages for categorical variables and medians and interquartile ranges for continuous variables. We also summarized data on infections detected by serology and PCR analysis of NPAs. We compared proportions using Fisher exact test.

We used Kaplan–Meier analyses to calculate median total cough duration with 95% confidence intervals (CI) in children with different infections. We compared total cough duration from the time the cough started between children with different infections using the log rank statistic. We only included children whose blood and NPA samples were both sufficient for laboratory testing and whose Mp serology results were not borderline in our cough duration analyses. Analyses were performed using PASW Statistics version 18.0.

RESULTS

Table 1 summarizes the baseline characteristics of children whose blood and/or NPA samples were sufficient for analysis (n = 170). Of these, 62 had previously been found to have serologically confirmed pertussis infections; 16 of 62 children had been prescribed β-lactams, and 8 of 62 had been prescribed macrolides because they had already started coughing. Forty-one children (24.1%) had asthma; of these, 5 also had eczema and 1 also had hay fever. One child had cerebral palsy, the main manifestations of which were related to motor function and coordination; the condition was therefore not considered to be related to the child's persistent cough. All but 4 children (166/170, 97.6%) had a temperature of less than 37.5°C and peak flow expiratory rate was

TABLE 1. Patient Characteristics (Blood and/or Nasopharyngeal Aspirate Analyzed), n = 170

Characteristic	Median (Interquartile Range) or Number (%)
Age (y)	8.7 (6.5–12.2)
Cough duration on study entry (d)	33 (24.8–49.5)
Sex (male)	95 (55.9)
Ethnicity (white)	162 (95.3)
Medical conditions	
Asthma	41 (24.1)
Eczema	10 (5.9)
Hay fever	3 (1.8)
Diabetes mellitus	2 (1.2)
Cerebral palsy	1 (0.6)
Household smoker	63* (39.1)
Antibiotics prescribed since cough started	53 (31.2)
Positive pertussis serology	62 (36.5)
Samples suitable for analysis	
Blood and NPA	136 (80)
Blood only	15 (8.8)
NPA only	19 (11.2)

*n = 161.
NPA indicates nasopharyngeal aspirate.

at least 80% of predicted in 149/166 children in whom peak flow expiratory rate was measured (89.8%).

Table 2 compares detection of Mp based on IgM serology versus PCR. In total, we detected Mp in 22/170 children (12.9%, 95% CI: 8.7–18.8). Of these, 4 children had been prescribed β-lactams and 2 had been prescribed macrolides since the start of their cough. Mp was detected by both serology and PCR in 8 children, by serology only in 11 children, and by PCR only in 3 children. Paired serum samples were still sufficient for analysis in 27 children. Of these, both samples were positive for Mp IgM in 4 children and the second sample only in 2 children. The NPAs of 3 children in whom we detected Mp by serology were not sufficient for analysis. Among children whose blood and NPA samples were both sufficient for analysis (n = 136), the annual prevalence of Mp varied between 0% (0/25) in 2004 and 25.5% (14/55) in 2002. The prevalence of Mp was not significantly higher in children with asthma (6/41, 14.6%) than without asthma (16/129, 12.4%; $P = 0.790$).

Table 3 summarizes the detection of respiratory viruses in NPAs. The most commonly detected viruses were human rhinoviruses (24/155, 15.5%), which accounted for 75% of respiratory virus infections (24/32). We detected rhinoviruses in the NPAs of similar proportions of children with and without asthma (asthma: 6/38, 15.8%; no asthma: 18/117, 15.4%; $P = 1.00$). Seventeen children with respiratory viruses also had evidence of infection with pertussis or Mp (53.1%). Excluding the 4 children in whom we detected both pertussis and Mp, we found respiratory viruses in a higher proportion of children with pertussis (14/46, 30.4%) than with Mp (3/15, 20%). However, the difference between these 2 proportions was not statistically significant ($P = 0.524$).

Total durations of cough (from time cough started) were almost identical between children with single Mp infections detected by serology (median: 39 days, 95% CI: 24–53 days) and PCR (median: 39 days, 95% CI: 22–56 days). Based on serology, children with single Mp infections had a significantly shorter duration of cough than children with pertussis (median: 118 days, 95% CI: 82–154 days, $P < 0.001$) or children in whom neither infection was detected (median: 70 days, 95% CI: 40–101 days, $P = 0.001$) (Fig. 1). Duration of cough was not significantly longer in children with positive pertussis serology and respiratory virus infections than in children with positive pertussis serology alone (median: 154 days, 95% CI: 74–234 days, $P = 0.810$). Only 3 children had evidence of both Mp and respiratory virus infections (human rhinoviruses). In children with human rhinoviruses who did not have evidence of infection with either Mp or pertussis, median duration of cough was 60 days (range, 42–72 days).

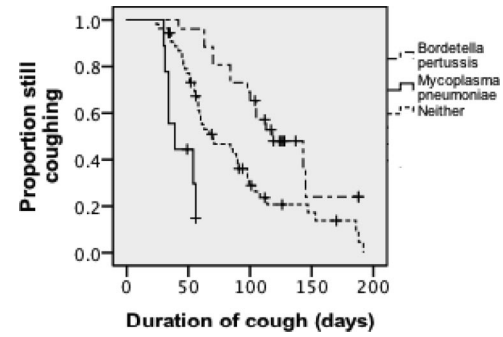

FIGURE 1. Duration of cough in children with *Bordetella pertussis*, *Mycoplasma pneumoniae*, or neither infection detected on serology. Children with *Mycoplasma pneumoniae* had a significantly shorter duration of cough (median: 39 days, 95% CI: 24–54 days) than children with pertussis (median: 118 days, 95% CI: 82–154 days, $P < 0.001$) or children in whom neither infection was detected (median: 70 days, 95% CI: 40–101 days, $P = 0.001$). Vertical lines denote censored data. Children whose cough diaries did not indicate that their cough had stopped for at least 2 weeks were censored at the time of the latest diary entry.

TABLE 2. Detection of *Mycoplasma pneumoniae* (n = 136)

	Serology Positive*	Serology Negative	Serology Borderline
PCR positive	8†	2	1
PCR negative	8‡	114§	3‡

*Three children were serology positive but NPA samples were insufficient for analysis.
†Pertussis serology positive, n = 1; rhinovirus, n = 1.
‡Pertussis serology positive, n = 3; rhinovirus, n = 2.
§Pertussis serology positive, n = 43.
PCR indicates polymerase chain reaction.

TABLE 3. Detection of Respiratory Viruses in Nasopharyngeal Aspirates (n = 155)

Virus	No. Children	% (95% Confidence Interval)
Human rhinoviruses	24*	15.5 (10.6–22.0)
Influenza	4	2.6 (1.0–6.5)
AH1	1	
AH3	2	
B	1	
Respiratory syncytial virus (RSV)	3	1.9 (0.7–5.5)
RSV A	1†	
RSV B	2†	
Human metapneumovirus	1	0.7 (0.1–3.7)

*Pertussis serology positive = 12, *Mycoplasma pneumoniae* serology positive = 2, *Mycoplasma pneumoniae* serology, and PCR positive = 1.
†Pertussis serology positive = 1.

DISCUSSION

Summary of Main Findings

We found evidence of Mp infection in one-eighth of children with persistent cough. However, duration of cough in children with Mp was only one-third of that in children with pertussis. The additional presence of respiratory virus infections did not significantly prolong cough in children with pertussis.

Comparison With Existing Literature

Our estimate of the prevalence of Mp in children with persistent cough based on PCR of NPAs (11/155, 7.1%) is considerably higher than estimates reported in children with acute cough and fever (2.6%)[18] and asymptomatic household contacts of Mp cases (0.25%).[19] Our estimate was also higher than estimates of the prevalence of Mp reported in a 2-year prospective study of children with a 1- to 6-week history of cough, which found Mp in only 4.4% of 136 cough episodes based on PCR of nose and throat swabs[9] and in a prospective cohort study of children referred to a tertiary hospital with more than 3 weeks of cough, which found Mp in only 1.9% of patients based on rising total antibody titers and PCR of bronchoalveolar lavage fluid.[6]

Detection rates of Mp in children with persistent cough are likely to vary with patient age and timing of recruitment. Versteegh et al[9] and Marchant et al[6] may have detected Mp in a lower

proportion of coughing episodes because their samples consisted mainly of preschool and young school-age children. However, the highest incidence of Mp is found in school-age children, especially those 5 to 9 years of age.[12] In addition, Mp occurs in cyclical epidemics which happen at approximately 4 yearly intervals.[19] The increased prevalence of Mp during 2002 which we observed in our cohort was also observed nationally.[20] However, the recruitment periods of shorter studies may not coincide with Mp epidemics. Mp also occurs with increased frequency during spring and winter in England and Wales (Djomo PN. unpublished data, 2009); seasonality of recruitment may therefore also influence estimates of Mp prevalence.

Mp and respiratory viruses, particularly parainfluenza virus and respiratory syncytial virus, have previously been implicated in asthma pathogenesis and exacerbations.[21–23] However, in our cohort, the proportions of children in whom we detected Mp and rhinovirus were similar in children with and without asthma. Because most coughs in children start as acute RTIs, it is likely that many children in our cohort had persistent coughs caused by "postinfectious" coughs (ie, nonspecific persistent coughs following an acute RTI). These have previously been reported to account for almost half of persistent coughs lasting 3 to 8 weeks.[24] Children with persistent cough following RTIs may have evidence of bronchial hyper-reactivity, but this is associated with increased neutrophil, rather than eosinophil, levels in bronchoalveolar lavage and induced sputum samples.[6,25,26] We observed that children with Mp had the shortest duration of cough; Mp may be cleared more efficiently by the immune system than other infections because the organism replicates slowly and has limited capacity for protein biosynthesis.[27]

Strengths and Limitations

We were still able to analyze blood and/or NPA samples from 95% of our originally recruited cohort. However, the quality of samples may have deteriorated in storage over time, which may have reduced our detection rate of the infections studied.

For pragmatic reasons, we asked parents, with input from their children if appropriate, to record the absence or presence of cough on a weekly rather than daily basis if the child was still coughing more than 14 days after study entry. This resulted in a high rate of diary completion (119/136, 87.5%) which we may not have achieved with daily diaries, because these would have had to be completed for 2 months or longer from the time of study entry for 37% of children (44/119 completed diaries). However, we accept that this is a limitation of our study and that daily cough diaries for the entire duration of the cough would have improved the accuracy of our total cough duration estimates. Asking parents to record whether or not the child's cough was present at the end of each week may have resulted in us underestimating total duration of cough in some children. However, since we excluded children in whom the date of onset of cough could not be established, the maximum magnitude of any underestimation would only have been 6 days; this is unlikely to have changed the conclusions of our study.

To maximize our detection of Mp, we used both PCR and IgM serology methods. PCR is superior to IgG or IgM serology in detecting Mp in patients with acute respiratory symptoms. However, Mp detection rates using PCR are likely to be lower in patients with persistent cough because bacterial load declines in relation to the time interval from onset of illness to sampling.[28] Although we detected evidence of Mp infection in 11 children on IgM serology but not on PCR, we felt that positive IgM serology alone was still a reliable indication of recent Mp infection. A prospective study involving adult patients with community-acquired pneumonia, which used the same Virotech kit that we used in our study on single serum samples, reported that IgM antibodies to Mp were only very rarely detected in healthy subjects (2/602 blood donors and orthopedic patients) and that patients who tested positive for Mp on PCR of respiratory samples (sputum or other respiratory secretions) had very similar demographic and clinical characteristics compared with patients with IgM antibodies to Mp only.[29] We also demonstrated similar durations of cough in children with single Mp infections based on PCR and serology. This strongly suggests that our methods of detecting Mp in this cohort had good specificity. However, the role of IgM serology in detecting respiratory viruses is limited as IgM levels are often low due to repeated exposure to circulating virus.[30]

We were unable to determine whether the presence of respiratory virus infections lengthens duration of cough in children with Mp because we only detected both types of infection in 3 children. The high proportion of mixed infections we observed among children with respiratory virus infections suggests that these viruses may predispose children to secondary infections. However, in this study, we were unable to confirm the exact timing of different infections in the same child, which, with hindsight, could have been addressed by serial sampling.

The numbers of children with Mp or pertussis who had been prescribed macrolides since their cough started were too small for us to be able to determine the effect of macrolides on duration of cough in children with these infections. We are unable to comment on the etiology of persistent cough in children in whom we did not detect any infections. This highlights an important area for further research.

CONCLUSIONS

Although Mp is usually associated with acute RTIs, it may also be found in school-aged children with persistent cough, particularly during periods of high Mp activity. Early detection of Mp in pertussis-negative children with persistent cough can help reassure children, parents, and clinicians that the cough is likely to settle after approximately 6 weeks. However, establishing the presence of respiratory viruses in children with persistent cough is currently of limited prognostic value. Our findings highlight the need for more rapid and accessible techniques for detecting Mp in children with persistent cough in primary care.

REFERENCES

1. Marchant JM, Newcombe PA, Juniper EF, et al. What is the burden of chronic cough for families? *Chest*. 2008;134:303–309.
2. Wyke S, Hewison J, Russell IT. Respiratory illness in children: what makes parents decide to consult? *Br J Gen Pract*. 1990;40:226–229.
3. Shields MD, Bush A, Everard ML, et al. Recommendations for the assessment and management of cough in children. *Thorax*. 2008;63:1–15.
4. Leonardi GS, Houthuijs D, Nikiforov B, et al. Respiratory symptoms, bronchitis and asthma in children of Central and Eastern Europe. *Eur Respir J*. 2002;20:890–898.
5. Faniran AO, Peat JK, Woolcock AJ. Persistent cough: is it asthma? *Arch Dis Child*. 1998;79:411–414.
6. Marchant JM, Masters BM, Taylor SM, et al. Evaluation and outcome of young children with chronic cough. *Chest*. 2006;129:1132–1141.
7. Harnden A, Grant C, Harrison T, et al. Whooping cough in school age children with persistent cough: a prospective cohort study in primary care. *BMJ*. 2006;33:174–177.
8. Hallander HO, Gnarpe J, Gnarpe H, et al. *Bordetella pertussis*, *Bordetella parapertussis*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and persistent cough in children. *Scand J Infect Dis*. 1999;31:281–286.
9. Versteegh FG, Weverling GJ, Peeters MF, et al. Community-acquired pathogens associated with prolonged coughing in children: a prospective cohort study. *Clin Microbiol Infect*. 2005;11:801–807.
10. von Konig CH, Rott H, Bogaerts H, et al. A serologic study of organisms possibly associated with pertussis-like coughing. *Pediatr Infect Dis J*. 1998;17:645–649.

11. van Gageldonk-Lafeber AB, Heijnen ML, Bartelds AI, et al. A case-control study of acute respiratory tract infection in general practice patients in The Netherlands. *Clin Infect Dis.* 2005;41:490–497.
12. Hammerschlag MR. *Mycoplasma pneumoniae* infections. *Curr Opin Infect Dis*. 2001;14:181–186.
13. Pitcher D, Chalker VJ, Sheppard C, et al. Real-time detection of *M. pneumoniae* in respiratory samples with an internal processing control. *J Med Microbiol*. 2006;55:149–155.
14. Longo MC, Berninger MS, Hartley JL. Use of uracil DNA glycosylase to control carry-over in polymerase chain reaction. *Gene*. 1990;93:125–128.
15. Fleming DM, Andrews NJ, Ellis JS, et al. Estimating influenza vaccine effectiveness using routinely collected laboratory data. *J Epidemiol Community Health*. 2010;64:1062–1067.
16. Elliot AJ, Powers C, Thornton A, et al. Monitoring the emergence of community transmission of influenza A/H1N1 2009 in England: a cross sectional opportunistic survey of self sampled telephone callers to NHS Direct. *BMJ*. 2009;339:b3403.
17. Ellis J, Iturriza M, Allen R, et al. Evaluation of four real-time PCR assays for detection of influenza A(H1N1)v viruses. *Euro Surveill*. 2009;14: pii.19230.
18. Harnden A, Perera R, Brueggemann AB, et al. Respiratory infections for which general practitioners consider prescribing an antibiotic: a prospective study. *Arch Dis Child*. 2007;92:594–597.
19. Chalker VJ, Stocki T, Mentasti M, et al. *Mycoplasma pneumoniae* infection in primary care investigated by real-time PCR in England and Wales. *Eur J Clin Microbiol Infect*. Dis 2011;30:915–921.
20. Laboratory reports to CfI of infections due to *Mycoplasma pneumoniae* England and Wales by date of report 1990–2011 (4 weekly), 2011. Available at: http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1194947359371.
21. Jackson DJ, Lemanske RF Jr. The role of respiratory virus infections in childhood asthma inception. *Immunol Allergy Clin N Am*. 2010;30:513–522.
22. Tang LF, Shi YC, Xu YC, et al. The change of asthma-associated immunological parameters in children with *Mycoplasma pneumoniae* infection. *J Asthma*. 2009;46:265–269.
23. Maffey AF, Barrero PR, Venialgo C, et al. Viruses and atypical bacteria associated with asthma exacerbations in hospitalized children. *Pediatr Pulmonol*. 2010;45:619–625.
24. Kwon NH, Oh MJ, Min TH, et al. Causes and clinical features of subacute cough. *Chest*. 2006;129:1142–1147.
25. Fitch PS, Brown V, Schock BC, et al. Chronic cough in children: bronchoalveolar lavage findings. *Eur Respir J*. 2000;16:1109–1114.
26. Zimmerman B, Silverman FS, Tarlo SM, et al. Induced sputum: comparison of postinfectious cough with allergic asthma in children. *J Allergy Clin Immunol*. 2000;105:495–499.
27. Yus E, Maier T, Michalodimitrakis K, et al. Impact of genome reduction on bacterial metabolism and its regulation. *Science*. 2009;326:1263–1268.
28. Nilsson AC, Bjorkman P, Persson K. Polymerase chain reaction is superior to serology for the diagnosis of acute *Mycoplasma pneumoniae* infection and reveals a high rate of persistent infection. *BMC Microbiol*. 2008;8:93.
29. von Baum H, Welte T, Marre R, et al. *Mycoplasma pneumoniae* pneumonia revisited within the German Competence Network for Community-acquired pneumonia (CAPNETZ). *BMC Infect Dis*. 2009;9:62.
30. Loffelholz M, Chonmaitree T. Advances in diagnosis of respiratory virus infections. *Int J Microbiol*. 2010;126049.

Current Abstracts

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Vaccine Discovery and Translation of New Vaccine Technology

Rappuoli et al. *Lancet*. 2011;378:360–368.

In this second in a series of 5 articles on the New Decade of Vaccines, examples of new technologies for the development and preclinical and clinical assessment of vaccines are reviewed. New methods of discovery, such as reverse vaccinology, structural biology, and systems biology, promise new vaccines for different diseases and efficient development pathways for these vaccines.

In any vaccine, the selection of antigens is a crucial step. In the past, although a rational approach was used, vaccine antigens were identified largely with empirical approaches. However, empirical methods are limited by the fact that some pathogens did not have easily identifiable immunogenic or protective vaccine antigens, and in some cases, identified target antigens seemed to be unsafe or poorly immunogenic. Knowledge of the genome of an organism can now be used to develop vaccines, for example by application of reverse vaccinology, which is the use of genomic information of an organism to identify potential antigenic targets, which cannot be identified with classic techniques. Reverse vaccinology can also be combined with new adjuvants that allow the type of immune response required for protection to be targeted.

Additionally, the application of structural biology to vaccinology, or structural vaccinology, could boost the development of vaccines against diseases in which other approaches have not been successful. Structural biologic studies allow the atomic resolution of antigen structure, enabling rational design of specific target epitopes for use as vaccine candidates. Structural studies have led to improved understanding of the various mechanisms by which different paramyxoviruses use their attachment glycoproteins to hijack specific protein and glycan cell-surface receptors for viral entry.

Although many vaccine antigens are still identified through empirical techniques, use of genomic and structural biologic approaches will probably increase. Although the ability to identify possible antigens increases the likelihood of developing a successful vaccine, identification of many possible antigens poses a challenge because new approaches then need to be used to select which of the possible antigens to take forward for vaccine development.

The selection process is complicated, and development of models that predict protection through systems biology analyses could help with this process. Systems biology is an integrative method combining knowledge of cytokine and immunologic response patterns to identify markers that predict the safety and effectiveness of vaccines. Ideally such markers would allow early identification of unsafe vaccine candidates and guide selection of the most effective vaccine combinations.

Comment: Vaccine targets should expand beyond diseases of childhood to include healthy adults, pregnant women, and elderly people, and new indications, such as autoimmune disease and cancer. Development of improved preclinical assessment of vaccine safety and effectiveness to speed development of new, safer vaccines through translational medicine and systems biology approaches remains a challenge for the next decade.