

Aetiology of community-acquired pneumonia in children treated in hospital

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Abstract. Viral and bacterial antigen and antibody assays were prospectively applied to study the microbial aetiology of community-acquired pneumonia in 195 hospitalised children during a surveillance period of 12 months. A viral infection alone was indicated in 37 (19%), a bacterial infection alone in 30 (15%) and a mixed viral-bacterial infection in 32 (16%) patients. Thus, 46% of the 69 patients with viral infection and 52% of the 62 patients with bacterial infection had a mixed viral and bacterial aetiology. Respiratory syncytial virus (RSV) was identified in 52 patients and *Streptococcus pneumoniae* in 41 patients. The next common agents in order were non-classified *Haemophilus influenzae* (17 cases), adenoviruses (10 cases) and *Chlamydia* species (8 cases). The diagnosis of an RSV infection was based on detecting viral antigen in nasopharyngeal secretions in 79% of the cases. Pneumococcal infections were in most cases identified by antibody assays; in 39% they were indicated by demonstrating pneumococcal antigen in acute phase serum. An alveolar infiltrate was present in 53 (27%) and an interstitial infiltrate in 108 (55%) of the 195 patients. The remaining 34 patients had probable pneumonia. C-reactive protein (CRP), erythrocyte sedimentation rate and total white blood cell count were elevated in 25%, 40% and 36% of the patients, respectively. CRP was more often elevated in patients with bacterial infection alone than in those with viral or mixed viral-bacterial infections. No other correlation was seen between the radiological or laboratory findings and serologically identified viral, bacterial or mixed viral-bacterial infections. By using a comprehensive serological panel, the causative agent could be found in over 50% of patients with pneumonia. We conclude that RSV and pneumococcus are the two most common organisms

causing pneumonia in children. Our results suggest that mixed viral-bacterial aetiology is common in lower respiratory tract infections affecting children.

Key words: Pneumonia – Respiratory syncytial virus – Pneumococcus – Enzyme immunoassay – Radioimmunoassay

Introduction

Antibody assays are widely used for the aetiological diagnosis of viral or mycoplasmal pneumonia. Moreover, recently developed method for direct detection of viral antigen in samples from the respiratory tract have proven useful in respiratory syncytial virus (RSV), parainfluenza, influenza and adenovirus infections, especially in young children [7, 10, 18]. The assessment of bacterial aetiology, however, is difficult in children with pneumonia or other respiratory syndromes. Less than 5% of bacterial pneumonias in children are bacteraemic with a positive blood culture [4, 33, 39, 48]. Invasive procedures, such as lung puncture, transtracheal aspiration or broncho-alveolar lavation, are justified in severe cases only. Detection of pneumococcal (PNC) or *Haemophilus influenzae* type b antigens in urine or serum appear to be insensitive indicators of pneumonia when caused by these organisms [12, 39, 40, 50]. Bacterial antibodies to the capsular polysaccharides of PNC have been studied in children with acute otitis media (AOM) [23, 24] and in recipients of pneumococcal vaccine [25, 30], but only occasionally in children with lower respiratory infections [4, 20, 33]. However, the use of polysaccharide antibody assays may not be suitable, because children under 2 years of age respond poorly to these polysaccharide antigens [25, 30]. Determination of an antibody response to the protein antigen, pneumolysin, has been shown to be useful in adults, but it has been quite insensitive in children with PNC pneumonia [4, 13, 16].

Viral respiratory infection may precede bacterial invasion and infection of the airways [6, 26]. Serological

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Abbreviations: AOM = acute otitis media; CF = complement fixation; EIA = enzyme immunoassay; IF = immunofluorescence; NPS = nasopharyngeal secretion; PNC = pneumococcal; PV = parainfluenza; RSV = respiratory syncytial virus

methods have enabled the detection of common mixed viral-bacterial respiratory infections in children [4, 11, 20–22, 33].

The aim of the present study was to evaluate the role of viral, bacterial and mixed viral-bacterial aetiology of community-acquired pneumonia in hospitalised children by means of viral and bacterial antibody and antigen assays.

Material and methods

During a surveillance period of 12 months, from 1 September 1981 to 31 August 1982, 195 children (117 less than and 78 over 2 years of age) were treated for pneumonia in the Department of Paediatrics, at Kuopio University Hospital, which provides care for all paediatric patients in a geographically defined area. The average population of children under 2 years of age and under 15 years of age in that area during the study period was 5818 and 45122, respectively. Thus the incidence of community-acquired pneumonia treated in hospital was 20/1000 per year in children under 2 years of age and 4/1000 per year in the whole child population. The diagnosis of pneumonia was based on the presence of pulmonary infiltration as evaluated independently by two radiologists.

The radiographs were later re-evaluated and the findings classified into three groups; definite alveolar or interstitial pneumonia, or probable pneumonia. The diagnosis of AOM was established, if the eardrum was red or cloudy with limited motility.

On admission, acute phase nasopharyngeal (NPS), serum and urine samples were obtained and stored at -20°C until studied (Table 1). Tests for direct viral antigen detection by radioimmunoassay (RIA) in NPS were performed for RSV, parainfluenza (PV) types 1, 2 and 3, adenoviruses and influenza A and B virus, as previously described [42–44]. PNC and *H. influenzae* type b polysaccharide antigens were assayed in acute phase serum samples using a commercially available latex particle agglutination test (Bactigen, Wampole Laboratories, NJ) and PNC antigen also in tenfold concentrated urine by counterimmuno-electrophoresis with PNC Omniserum (Statens Serum Institute, Copenhagen, Denmark). In addition, neutral capsular polysaccharides of PNC types 7 and 14 were detected by a latex particle agglutination test [28]. The concentration of C-reactive protein (CRP; immunonephelometric method), erythrocyte sedimentation rate (ESR; Westergren method) and white blood cell count (WBC) were determined from acute phase blood samples.

Convalescent phase sera were obtained 3–4 weeks later (Table 2). Enzyme immunoassay (EIA) was used for measuring pneumococcal C-polysaccharide and type-specific capsular polysaccharide antibodies (the 11 most common PNC types), as well as for measuring antibodies to non-classified *H. influenzae*, *Moraxella* (*Branhamella*) *catarrhalis* and *Chlamydia* species [17, 23, 25, 29, 37]. A threefold or greater rise in IgM, IgG or IgA antibody titres between the paired sera was regarded as significant [17, 20, 33]. The antibody assays were validated by studying paired sera from 200 healthy children and adults; a threefold or greater rise in titre was present in less than 1% between these paired sera [20]. In addition, IgG antibodies to the PNC protein antigen pneumolysin were measured by EIA and a twofold or greater rise was regarded as significant [13, 16]. This assay was validated by studying paired sera from 186 healthy children; a twofold or greater rise in titre was present in 1%–2% between these paired sera. A scaled-down conventional complement fixation (CF) assay was used to measure antibodies to RSV, PV types 1, 2 and 3, adenoviruses, influenza A and B virus, coronaviruses, mumps virus, herpes simplex virus, cytomegalovirus, enteroviruses, *M. pneumoniae* and the chlamydial group antigen. A fourfold or greater rise in titre was considered significant [9]. The serum pairs showing an antibody rise to *Chlamydia* species either by EIA or CF were further tested with a micro-immunofluorescence (IF) test specific for *C. trachomatis* and *C. pneumoniae* [52].

Table 1. Methods for detection of antigen of respiratory pathogens in nasopharyngeal secretion, blood or urine

Agent and antigen	Specimen	Assay	Reference
RSV	NPS	RIA	[42]
Parainfluenza virus type 1, 2 or 3	NPS	RIA	[42, 44]
Adenovirus	NPS	RIA	[42]
Influenza A or B virus	NPS	RIA	[43]
<i>S. pneumoniae</i>			
Polyvalent capsular PS	Acute serum	LA	^a
Polyvalent capsular PS	Acute urine ^c	CIE	^b
PS of types 7 and 14	Acute urine ^c	LA	[28]
<i>H. influenzae</i>			
Type b capsular PS	Acute serum	LA	^a

LA, Latex agglutination; CIE, counterimmuno-electrophoresis; PS, polysaccharide

^a Bactigen, Wampole Laboratories, Cranbury, NJ, USA

^b Omniserum, Statens Serum Institute, Copenhagen, Denmark

^c Urine samples were tenfold concentrated

Table 2. Methods for determination of antibodies to respiratory pathogens in paired serum samples

Agent and antigen	Assay	Diagnostic response	References
Respiratory viruses ^a	CF	Fourfold	[9]
<i>S. pneumoniae</i>			
Type-specific capsular polysaccharide ^b	EIA	Threefold	[24, 30]
C-polysaccharide	EIA	Threefold	[23]
Pneumolysin (IgG)	EIA	Twofold	[13, 16]
<i>H. influenzae</i>	EIA ^c	Threefold	[17]
<i>M. catarrhalis</i>	EIA ^d	Threefold	[29]
<i>M. pneumoniae</i>	CF	Fourfold	[9]
<i>Chlamydia</i> species	CF	Fourfold	[9]
	EIA ^e	Threefold	[37]
<i>C. trachomatis</i> ^f	IF	Fourfold	[52]
<i>C. pneumoniae</i> ^f	IF	Fourfold	[52]

^a Standard CF serology, on a micro-scale, using antigens of RSV, PV 1, 2 and 3, influenza A and B, adenovirus, enteroviruses and coronaviruses

^b Types 1, 3, 4, 6, 7, 8, 9, 14, 18, 19 and 23

^c Whole bacterial cell antigen of ten nontypable *H. influenzae* strains

^d Whole bacterial cell antigen of ten *M. catarrhalis* strains

^e Purified lipopolysaccharide from an Re mutant of salmonella which shows crossreactions with chlamydial group antigen

^f Only patients showing a seroresponse to chlamydial group antigen tested

An infection was considered to have viral aetiology, if any virus was implicated by CF serology, or if any of the seven viral antigens studied was found in NPS by RIA. The patient was considered to have a bacterial infection, if a significant antibody response to any of the five bacteria was demonstrated, or if PNC of *H. influenzae* type b antigen was found in serum or urine. Infections caused by both viruses and bacteria are called mixed viral-bacterial infections, and those caused by two viruses or two bacteria dual viral or dual bacterial infections.

The chi-square test was used for statistical analysis of the data.

Results

A definite alveolar infiltrate was noted in 27% and a definite interstitial infiltrate in 55% of the 195 patients (Table 3). Alveolar infiltrates were rare, 20% in infants as opposed to 30%–31% in older children. AOM was seen in 37% of patients younger than 2 years of age and in 24% of the older children (Table 3). CRP concentrations, ESR levels and WBC counts were elevated in 25%, 40% and 36% of patients, respectively (Table 3). CRP concentrations and ESR levels were more often elevated in older children than in infants while WBC counts were independent of age.

Serological evidence of a viral or bacterial aetiology of an infection was present in 99 (51%) of the 195 children with pneumonia (Table 4). A viral infection was seen in 37 patients (19%); 35 had a single and 2 a dual viral infection (Table 5). A mixed viral-bacterial infection was seen in 32 patients; 5 had two bacterial findings. A bacterial infection alone was seen in 30 patients (32%) (Table 5). A single bacterial infection was diagnosed in 25 cases and dual bacterial infection in 5 cases (including 1 case with three bacterial findings). Thus, the number of patients with viral infection, either alone or combined with bacterial infection, was 69 (35%). The number of

patients with a bacterial infection, whether single, mixed viral-bacterial or dual bacterial, was 62 (32%). RSV was the most common aetiological agent identified in 52 patients (Table 4). RSV infections were more common in infants than in older children. A single RSV infection was present in 27 cases, a mixed infection with bacteria in 23 cases and a dual infection with adenoviruses in 2 cases (Table 5). A great majority, 79%, of the 52 RSV findings were obtained by antigen detection tests (Table 6).

Streptococcus pneumoniae was the second most frequently found aetiological agent identified in 41 patients (Table 4). No age dependence was seen. A single PNC infection was present in 19 cases, a mixed infection with viruses in 18 cases and a dual infection with other bacteria in 5 cases (Table 5). Over half, 63%, of the 41 PNC infections were found by antibody assays; in 39% the diagnosis was based on the presence of antigen in acute phase serum (Table 6). Antibody responses to the protein antigen pneumolysin were found in only 3 cases, whereas responses to type-specific capsular polysaccharide antigens were noted in 15 cases and to C-polysaccharide in 6 cases.

A seroconversion to non-classifiable *H. influenzae* was demonstrated in 17 patients; all serum and urine

Table 3. Clinical, radiological and laboratory data of 195 patients with pneumonia

Findings	Age of patients				P
	1–11 months (n = 61)	12–23 months (n = 56)	≥ 24 (n = 78)	All ages (n = 195)	
Alveolar infiltrate	12 (20%)	17 (30%)	24 (31%)	53 (27%)	NS
Interstitial infiltrate	34 (56%)	29 (52%)	45 (58%)	108 (55%)	NS
Acute otitis media	23 (38%)	20 (36%)	19 (24%)	62 (32%)	NS
C-reactive protein > 40 mg/l	6 (10%)	12 (21%)	30 (38%)	48 (25%)	< 0.001
Erythrocyte sedimentation rate > 30 mm/h	14 (23%)	27 (48%)	36 (46%)	77 (40%)	< 0.01
White blood cell count > 15 × 10 ⁹ /l	20 (33%)	21 (38%)	29 (37%)	70 (36%)	NS

NS, Non significant

Table 4. Serological evidence of specific viral or bacterial infection in 195 patients with pneumonia

Infection	Age of patients				P
	1–11 months (n = 61)	12–23 months (n = 56)	≥ 24 months (n = 78)	All ages (n = 195)	
Serological evidence of specific infection	35 (57%)	29 (52%)	35 (45%)	99 (51%)	NS
RSV	23*	14	15	52	
<i>S. pneumoniae</i>	13	11	17	41	
<i>H. influenzae</i>	6	6	5	17	
Adenoviruses	1	5	4	10	
<i>Chlamydia</i> species	1	5 ^a	2	8 ^a	
Parainfluenza type 1, 2 or 3 virus	2	1	2	5	
<i>M. catarrhalis</i>	1	2	1	4	
<i>M. pneumoniae</i>	1	2	0	3	
No serological evidence of specific infection	26 (43%)	27 (48%)	43 (55%)	96 (49%)	NS

* $P < 0.05$ versus patients older than 12 months

^a Four cases were positive in micro-IF test; *C. trachomatis* in one case, *C. pneumoniae* in one case and non-classifiable *Chlamydia* species two cases

Table 5. The occurrence of infections caused by specific agents as single, mixed viral-bacterial or dual viral or dual bacterial infections

Organisms	Single infection	Mixed viral-bacterial infection	Dual viral or dual bacterial infection
RSV (<i>n</i> = 52)	27	23	2
Adenoviruses (<i>n</i> = 10)	1	7	2
Parainfluenza viruses (<i>n</i> = 5)	2	3	0
All viruses (<i>n</i> = 69)	35	32 ^a	2
<i>S. pneumoniae</i> (<i>n</i> = 41)	19	18	4
<i>H. influenzae</i> (<i>n</i> = 17)	3	11	3
<i>M. catarrhalis</i> (<i>n</i> = 4)	2	2	0
<i>M. pneumoniae</i> (<i>n</i> = 3)	0	1	2
<i>Chlamydia</i> species (<i>n</i> = 8)	1	5	2
All bacteria (<i>n</i> = 62)	25	32 ^a	5 ^b
All viruses and/or bacteria	60	32	7

^a Five cases with one viral and two bacterial findings included

^b One case with three bacterial findings

Table 6. Results of antibody and antigen assays in 99 patients with serological evidence of viral or bacterial pneumonia

Organism	Number	A positive result in	
		antibody assay	antigen assay
RSV	52	30	41
Adenoviruses	10	9	3
Parainfluenza type 1, 2 or 3 virus	5	4	4
Other viruses	2	2	Not studied
<i>S. pneumoniae</i>	41	26 ^a	18 ^b
<i>H. influenzae</i>	17	17	0
<i>Chlamydia</i> species	8	8 ^c	Not studied
Other bacteria	6	6 ^d	Not studied

^a Pneumococcal antibodies were positive to capsular type-specific antigens in 15 cases, to C-polysaccharide in 6 cases to pneumolysin in 3 cases

^b Pneumococcal antigen was present in serum in 16 cases and in urine in 2 cases

^c All cases were positive by EIA and negative by CF

^d Seroconversion to *M. catarrhalis* in four cases and to *M. pneumoniae* in two cases

samples were negative for the type b antigen (Table 4). AOM was present in 10 of these 17 patients. *Moraxella catarrhalis* was indicated in 4 cases; in 1 patient with AOM and in 3 with no AOM. An antibody response to *Chlamydia* species was present in 8 patients, but in a micro-IF test only one *C. trachomatis* and one *C. pneumoniae* infection was identified. AOM was present in 6 of these 8 patients. *M. pneumoniae* infection was indicated in only 3 patients (Table 4). *H. influenzae*, *M. catarrhalis*, *M. pneumoniae* and *Chlamydia* species infections occurred infrequently as single infections; 82% of *H. influenzae*, 50% of *M. catarrhalis*, 88% of *Chlamydia* species and 100% of *M. pneumoniae* infections were either mixed viral-bacterial or dual bacterial infections (Table 5).

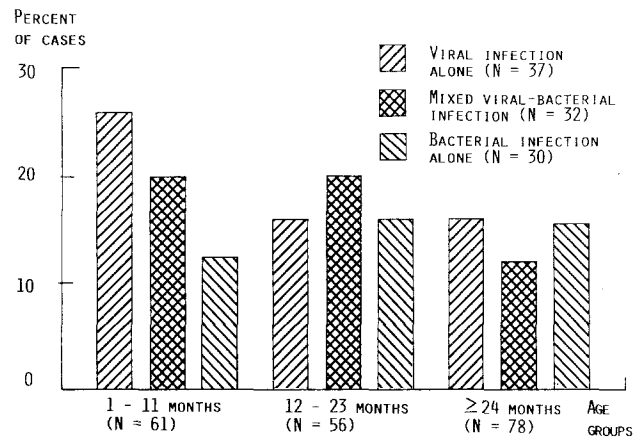


Fig. 1. Age distribution of viral, bacterial and mixed viral-bacterial infections in 195 patients with community-acquired pneumonia treated in hospital

The frequent occurrence of mixed viral-bacterial infections was an important finding in the present study (Fig. 1). They were common in all age groups, being observed in 19%–20% of the patients under 2 years, and in 12% of the older children. In fact, such a mixed aetiology was found in 46% of all the viral and in 52% of all the bacterial infections. CRP was more often elevated in patients with bacterial infection alone than in those with viral or mixed viral-bacterial infections. Radiological or other laboratory findings showed no significant correlation with the presence of bacterial, viral or mixed viral-bacterial infections (Table 7). This was also seen when the infections caused by specific agents were analysed separately.

Discussion

The results confirm the importance of viruses, especially RSV, as aetiological agents of pneumonia in children [4, 5, 33, 36]. Serological evidence of a viral infection was seen in 35% of cases; 75% of viral infections were attributable to RSV. In earlier studies, the importance of RSV has been underestimated because antibody assays and viral cultures are insensitive, especially in infants [10, 18, 51]. Accordingly the majority, 78%, of RSV infections in the present study were diagnosed by detecting viral antigen in NPS. To be optimal, the diagnostic panel for respiratory viral infection should include culture, direct antigen detection and IgG antibody assay [32]. Two highly sensitive methods, an immunoperoxidase assay for culture and a time-resolved fluorimmunoassay for antigen detection have been developed after this study [2, 53].

Adenoviruses were found in 15% of the viral pneumonias in the present study. These viruses are known to cause severe pneumonias in children with long-lasting airway damage [47]. On the other hand, a cultural finding of adenovirus from a patient with pneumonia may not always be aetiological associated with the disease, as a latent adenovirus infection may be reactivated by other infections [4]. Antigen detection or seroconversion, which were used in the present study, can be considered as an indication of the aetiology.

Table 7. Radiological and laboratory data of 99 patients with serological evidence of viral, bacterial or mixed viral-bacterial infection

Findings	Bacterial infection alone (n = 30)	Mixed viral-bacterial infection (n = 32)	Viral infection alone (n = 37)	P
Alveolar infiltrate	12 (40%)	9 (28%)	9 (24%)	NS
Interstitial infiltrate	13 (43%)	17 (53%)	19 (51%)	NS
C-reactive protein > 40 mg/l	14 (47%)	9 (28%)	7 (19%)	<0.05
Erythrocyte sedimentation rate > 30 mm/h	19 (63%)	16 (50%)	13 (35%)	NS
White blood cell count > 15 × 10 ⁹ /l	13 (43%)	9 (28%)	11 (30%)	NS

NS, Non significant

Infections due to RSV, influenza or parainfluenza viruses, as well as to the bacterial pathogen as *M. pneumoniae*, appear as epidemics. This study was done during an RSV epidemic, but no epidemics caused by influenza viruses or *M. pneumoniae* were present. A clear local outbreak caused by PV type 2 occurred during the study period, but this agent is mostly associated with croup and other non-pneumonic syndromes [19]. All five patients with PV pneumonia in this study had an infection caused by PV types 1 or 3.

The role of bacteria other than *M. pneumoniae* in the aetiology of pneumonia is difficult to assess. Blood culture gives a definite aetiological diagnosis, but it is positive in less than 5% of children with pneumonia [4, 33, 39, 48]. Bacterial culture or antigen detection in sputum has been useful in adults, but in children these methods indicate the carriage rather than the causative agents of pneumonia [14, 15, 31, 49]. Optimally the determination of bacterial aetiology should be based on the isolation of bacteria from lung tissue. In children with severe pneumonia in developing countries bacteria have been identified from these samples in 79% of cases [46]. In our country, however, lung puncture or other invasive methods are only seldom justified in children with community-acquired pneumonia.

In the present study we measured antibodies in paired sera to three PNC components and to non-classifiable *H. influenzae*, to *M. catarrhalis*, to *M. pneumoniae* and to *Chlamydia* species. In addition, PNC and *H. influenzae* type b antigens were assayed in acute blood and urine. By this diagnostic panel, bacterial aetiology was indicated in 32% of children with pneumonia; *S. pneumoniae* comprised 66% of bacterial infections. The most sensitive methods for the aetiological diagnosis of PNC in the present study were antibody assays to type-specific capsular polysaccharides in paired sera and antigen assays in acute phase serum. Antibody assays are quite insensitive in aetiological diagnosis of bacterial pneumonia, and especially children aged less than 2 years respond poorly to polysaccharide antigens [23, 27, 30]. Antigen detection in urine, as also previously suggested, is an insensitive means to demonstrate PNC aetiology [12, 39, 40, 50]. Demonstration of an antibody response to a PNC protein antigen pneumolysin, although specific and sensitive in adults, was positive in only three children [13, 16]. The low sensitivity of pneumolysin antibody assays has been observed in children before and it is possible

that antibodies to pneumolysin are bound to circulating immunocomplexes which must be dissociated before testing [4, 45].

All serum and urine samples were negative for *H. influenzae* type b antigen. However, antibody responses to non-classified strains were common, 27% of all bacterial findings. Thus, our results support the role of non-capsulated *H. influenzae* as a real aetiological agent in pneumonia in children [33, 35, 54]. On the contrary, infections caused by *M. catarrhalis* were rare, 2%.

Antibody responses to *Chlamydia* species were in eight children, but a micro-IF test verified only one case as *C. trachomatis* and one as *C. pneumoniae* infection. Antibody responses measured by micro-IF are often delayed in primary infection; serum samples should be obtained 6 weeks or more apart [41, 55]. Only one chlamydial infection occurred during infancy, indicating that infantile pneumonia caused by *C. trachomatis* is uncommon in our country, as in other parts of Europe [4, 37]. Recent serological studies have stressed the importance of *C. pneumoniae* infections in both developing and industrialised countries [1, 41]. Interestingly, six out of the eight children with chlamydial infection also had AOM. Both *C. trachomatis* and *C. pneumoniae* have been isolated from middle ear fluid samples of patients with AOM [3, 8, 34]. However, culture studies are needed to clarify the ultimate role of *C. pneumoniae* in pneumonia in children.

A mixed viral-bacterial aetiology was surprisingly common; it was present in 16% of the pneumonic patients of this study. A mixed aetiology constituted 46% of all viral and 52% of all bacterial infections. The frequent occurrence of such mixed infections has been observed in adults with pneumonia [27] and in children with lower respiratory tract infection [11, 20–22]. As previously suggested, there was no specific viral-bacterial connection, a certain virus preceding a certain bacterial infection; the viral diagnosis does not help clinicians in the selection of antibiotics [20]. Our results showed that neither radiological findings nor laboratory parameters such as CRP, ESR or WBC, although commonly used for this purpose, could discriminate between viral, bacterial or mixed viral-bacterial aetiology of pneumonia [38].

In conclusion, the study confirms the view that viruses, especially RSV, are common aetiological agents in pneumonia in children. The study also shows that in

many patients PNC antigens can be found in acute serum, and that many patients produce seroresponses to these antigens. The sensitivity of all antigen and antibody assays for PNC aetiology, however, appears to be low. Similarly, their correlation with commonly used non-specific indicators of a bacterial infection as elevated CRP, ESR and WBC is poor. Thus, owing to methodological failures we still underestimate the role of bacteria in pneumonia affecting children. Further studies are needed to improve the non-invasive methods for the diagnosis of bacterial aetiology of pneumonia in children.

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