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ORIGINAL PAPER

Etiology of community-acquired pneumonia in hospitalized children based on WHO clinical guidelines

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Abstract Community-acquired pneumonia (CAP) is a major cause of death in developing countries and of morbidity in developed countries. The objective of the study was to define the causative agents among children hospitalized for CAP defined by WHO guidelines and to correlate etiology with clinical severity and surrogate markers. Investigations included an extensive etiological workup. A potential causative agent was detected in 86% of the 99 enrolled patients, with evidence of bacterial (53%), viral (67%), and mixed (33%) infections. *Streptococcus pneumoniae* was accounted for in 46% of CAP. Dehydration was the only clinical sign associated with bacterial pneumonia. CRP and PCT were significantly higher in bacterial infections. Increasing the number of diagnostic tests identifies potential causes of CAP in up to 86% of children, indicating a high prevalence of viruses and frequent co-infections. The high proportion of pneumococcal infections re-emphasizes the importance of pneumococcal immunization.

Keywords Community-acquired pneumonia · Child · WHO guidelines · Pneumococcal infection · Antibiotic · Immunization

Abbreviations

CAP	Community-acquired pneumonia
WHO	World Health Organization
CRP	C-reactive protein
PCT	Procalcitonin
NPA	Nasopharyngeal aspirates
BTS	British Thoracic Society
NS	Nasopharyngeal swabs
WBC	White blood cell
URS	Upper respiratory symptoms
hMPV	Human metapneumovirus

Introduction

Childhood community-acquired pneumonia (CAP) is a common and serious health care problem, responsible for one fifth of children's deaths around the world (2 million), 70% occurring in developing countries [45]. In Europe and North America, the annual incidence of CAP is approximately 40 cases per 1,000 and therefore also represents a major cause of morbidity [19]. There are two major difficulties in the diagnosis of CAP. The first one is how to define pneumonia, particularly in the young age group in

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which both pneumonia and viral lower respiratory tract infections are common conditions. The World Health Organization (WHO) has defined pneumonia and subsequent treatment on strict clinical signs (Table 1) [1, 7, 17]. A prospective study showed that the WHO guidelines can also be applied in developed countries, as recommended by the guidelines of the British Thoracic Society (BTS) [2, 11]. The second difficulty is to identify the causative agent of pneumonia in children. This remains challenging for a number of reasons: endo-tracheal aspirates cannot be obtained routinely, nasopharyngeal swabs (NS) only show colonization by normal flora including the bacteria most commonly causing pneumonia, and bacteremia is demonstrated in less than 10% of bacterial CAP. Therefore, the etiology of CAP remains often unknown, with the consequence that many children get an antibiotic treatment for non-bacterial infections, contributing to the increase of bacterial resistance to antibiotics [9, 41]. The aims of this study, performed before the routine implementation of the heptavalent pneumococcal vaccine in Switzerland, were to define, as strictly as possible, the causative agents of pneumonia among children aged from 2 months to 5 years hospitalized for CAP on the basis of WHO clinical criteria, and to determine which factors could help clinicians to discriminate bacterial from viral pneumonia.

Material and methods

Study design and population

Participation to this prospective and descriptive study was offered to all consecutive children aged 2 months to 5 years old presenting with CAP according to the WHO criteria (Table 1) consecutively admitted to the children's unit of the university hospitals of Lausanne and Geneva between March 2003 and December 2005. They were classified according to initial severity. Children with actively treated asthma, an underlying chronic disease, immunosuppression, or wheezing were excluded. Children with wheezing were specifically excluded because wheezing is considered to be a symptom of asthma and/or viral bronchiolitis and not of pneumonia, and therefore these children are not treated with antibiotics. Cough and fever, associated signs and symptoms, the use of antibiotics in the month before admission, and the main features of the clinical course were recorded. The Ethical committees of both institutions approved the study protocol. Signed consent was obtained from informed parents.

Table 1 Clinical definition of pneumonia according to the WHO

	Fever[a]	Tachypnea[b]	Chest indrawing	Not able to drink and/or central cyanosis
Stage I (non-severe)	+	+		
Stage II (severe)	+	+	+	
Stage III (very severe)	+	+	+	+

[a] Fever≥38°C axillary

[b] Respiratory rate >50/min (2–11 months) and >40/min (1–5 years)

Routine samples for inflammatory parameters

On admission, a blood sample was taken for total white blood cell count (WBC) with manually verified differential count. Serum CRP and PCT (Lumitest®, Brahms, Berlin, Germany) were measured.

Radiology

A senior radiologist, blinded to clinical and laboratory findings, reviewed all chest radiographs and assigned a standardized description of the X-ray as proposed by the WHO working group [5]: the presence of consolidation or pleural effusion with parenchymal infiltrate defined pneumonia.

Microbiology

On admission, before starting an antibiotic treatment, peripheral blood cultures, and during the winter epidemic, a rapid antigen detection test for RSV (Coris or Becton Dickinson), were performed. Within 8 h after admission, nasopharyngeal aspirates (NPA) were obtained after respiratory physiotherapy for viral and bacterial culture, PCR analysis, and viral antigen detection. PCR analysis was performed for *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. RT-PCR assays were performed for 13 viruses including Influenza A and B, RSV A and B, Rhinovirus, Parainfluenza 1–3, enterovirus, human metapneumovirus, coronavirus OC43, E229, and NL 63 as previously described [15, 16, 25]. In the present study, all the assays were used as qualitative tools. PCR for *M. pneumoniae* and *C. pneumoniae* were done by a multiplex real-time PCR according to Welti et al. [44]. Viral antigen detection was performed for Adenovirus, Para-influenza 1–3, RSV, and Influenza A and B by indirect immunofluorescence (Argene Antibodies) or ELISA.

Serology

Serum samples were taken at admission and approximately 3 weeks after hospitalization, then stored at −20°C and subsequently transported on dry ice to the laboratories. The

laboratory was unaware of clinical data. Antibodies for Influenza A and B; Parainfluenza 1, 2, and 3; RSV; Adenovirus; and *M. pneumoniae* were detected by the complement fixation method. Paired samples were tested in the same run. Acute infection was defined by a fourfold rise or a titer higher than 1/80. Serologic tests for *C. pneumoniae* were done by using a micro-immunofluorescence test (MRL Diagnostics). A fourfold rise between the acute and convalescent sera or an initial titer higher than fourfold the positive cut off value (1:16) were considered as indicative of a recent infection, as previously described [26]. Serum IgG antibodies to pneumolysin were measured by indirect ELISA on antigen-coated Immulon plates (Thermo Labsystem), following 60 min incubation at 37°C. Results were compared to a pool of purified human immunoglobulins (Endobulin, Baxter) used as standard and expressed in ELISA Units/ml. Samples with a rise greater than twofold between acute or convalescent sera and/or acute serum antibody titers greater than 300 EU/ml were scored positive. These limits have been previously demonstrated to be a reliable method for the serological diagnosis of pneumococcal exposure [22, 35].

Detection of *Streptococcus pneumonia* by PCR

The blood samples were taken at the time of admission and sera were frozen at −70°C in order to be analyzed in a second phase. The detection of *S. pneumoniae* was performed by real-time PCR with the Taqman method. The specific target was the pneumolysin gene. Briefly, 200 µl of blood specimen were pre-lysed with 80 µl of lysis buffer (Bacterial lysis buffer, Roche Diagnostics) and with 30 µl of a cocktail of enzymes (Lyt030 enzymmix, Roche Diagnostic). After an incubation period of 30 min at 37°C, the sample was automatically extracted with a MagNa Pure compact instrument using the MagNa Pure compact isolation kit I. Five microliters of the eluted DNA were then amplified in triplicate with a Taqman ABI Prism 7700 Sequence Detector (Applied Biosystems). Any significant curve before the cycle 40, was considered as a positive signal, the positive cut off was defined as 1,000 copy/ml [8, 27, 43].

Etiologic classification criteria

The criteria for viral infection were a positive viral culture, viral antigen, positive viral serology, or viral PCR. A bacterial infection was considered if a blood culture, a pleural fluid culture, a pneumococcal PCR, or a serologic assay was positive. Atypical and typical bacteria were grouped together. Subjects with criteria of both viral and bacterial infections were considered to have infections of mixed etiology.

Treatment

Patients were treated with a course of parenteral antiobiotics first (cefuroxime (n=46), ceftriaxone (n=22), amoxiclavulanic acid (n=38), floxapen (n=1)), followed by oral antibiotics (amoxiclavulanic acid or cefuroxime) for a total duration of 2 to 25 days. Parenteral cefuroxime was initially associated with clarithromycin in four patients or switched for vancomycin and amoxyclavulanic acid or for amoxycillin and clindamycin in two patients with pleural effusion.

Statistical analysis

Data were analyzed using Epi Info 6.04d (Center for Disease Control and Prevention, Atlanta, GA, USA). Standard parametric and non-parametric statistical tests were done according to distribution and variance of studied variables. The level of statistical significance was defined as $p \leq 0.05$. Kappa statistics were computed using PAIRS module, version 1.33, of Pepi for Windows software.

Results

Population characteristics

A total of 111 children were eligible during the study period. Ninety-nine children were finally enrolled (six patients were excluded because of the impossibility to obtain samples and, after initial consent, five parents decided to interrupt the study). Among these patients, 51 were females (52%). The average age was 29 months. According to WHO criteria, nine patients (9%) requiring hospitalization had non-severe pneumonia; 67 (68%) patients, severe pneumonia; and 23 (23%), very severe pneumonia. All subjects had fever or a recent history of fever and tachypnea. Many patients showed a number of other symptoms: 96 had cough (97%), 80 (81%) had feeding difficulties, 45 (45%) had vomiting, and 17 (17%) abdominal pain. Other symptoms were tiredness or apathy (n=7); thoracic pain (n=5); or upper respiratory symptoms (URS): rhinitis (n=6), sore throat (n=3), and ear pain (n=2). The average temperature at admission was 39.1°C. Breath sounds were abnormal in 78 patients (79%) and 25 (26%) were dehydrated. Antibiotics were given to 27 patients (27%) during 30 days before hospitalization. Although there was a large variation in the number of monthly hospitalizations, no clear seasonal pattern was observed. The average duration of hospitalization was 2 days ranging from a few hours to 25 days. Twenty-six children (26%) required supplemental oxygen therapy, nine patients (9%) needed a gastric feeding tube, and nine (9%)

a thoracic drainage. Three patients were transferred to the pediatric intensive care unit, two of them requiring non-invasive assisted ventilation (Table 2).

Overall results

Table 3 shows the number of samples obtained for each test. Viral and atypical bacterial serology, were the most difficult to obtain (follow-up blood sample), as well as nasopharyngeal aspirates (NPA), notably in children without upper respiratory tract involvement. A potential causative agent was detected in 85 (86%) of the 99 patients. Evidence of bacterial infection alone was demonstrated in 19 patients (19%) and viral infection alone in 33 (33%) cases. Mixed bacterial-viral infection was found in 33 (33%) patients (Fig. 1).

Bacterial infection

Only one patient had a positive blood culture (*S. pneumoniae*), while three other blood cultures were contaminated by a *coagulase-negative Staphylococci*. One patient had a *Group A β-hemolytic Streptococcus* in pleural fluid culture. Overall, bacteria with or without co-infecting pathogens were identified in 53% of the cases (52 patients). Pneumococcal pneumonia was diagnosed in 45 patients

Table 2 Demographic and clinical results of 99 patients hospitalized for community-acquired pneumonia and correlation with etiology

Characteristics	Total	Bacterial	Viral	Mixed	Unknown pathogen	*P* Value
No. of patient (%)	99	19 (19)	33 (33)	33 (33)	14 (14)	
Age, months (0.25; 0.75)[a]	29.4 (17; 48)	23.4 (19; 48)	25.1 (15; 38)	42.9 (24; 52)	22.4 (11; 34)	0.033[c,e]
Gender (m/f)	48/51	11/8	17/16	14/19	6/8	0.692[d]
Antibiotics before hospitalization	27	8	8	9	2	0.327[d]
Pneumonia stage I[b] (non-severe)	9	2	5	2	0	0.650[d]
Pneumonia stage II[b] (severe)	67	13	19	24	11	
Pneumonia stage III[b] (very severe)	23	4	9	7	3	
Temperature at admission (0.25; 0.75)[a]	39.1 (38.5; 39.8)	39.0 (38.5; 39.8)	39.2 (38.7; 39.8)	39.1 (38.3; 39.6)	38.8 (38.5; 39.5)	0.308
Symptoms of pain[b,f]	22	2	6	13	1	0.026[d,g]
Vomiting[b]	45	8	13	18	6	0.634[d]
URS[b]	11	1	4	3	3	0.505[d]
Dehydration[b]	25	9	2	8	6	0.003[d,h]
Oxygen requirement[b]	26	5	11	7	3	0.693[d]
Radiological consolidation[b]	77	16	25	26	10	0.911[d]
CRP mg/l[a]	167 (60; 200)	200 (100; 204)	88 (21; 194)	200 (117; 250)	142 (23; 192)	0.009[c,i]
PCT ng/ml[a]	6.0 (1; 14)	11.5 (5; 18)	2.0 (0.5; 7.5)	11.0 (3; 18)	3.0 (0.5; 4)	0.018[c,j]
WBC count (G/L)[a]	15.0 (9; 21)	15.0 (11; 19)	12.0 (7; 21)	16.0 (10; 21)	15.5 (11; 18)	0.591[c]
Band forms (G/L)[a]	1.85 (0.6–3.8)	2.65 (1.3; 3.8)	1.4 (0.5; 2.6)	1.82 (1.0; 4.0)	1.31 (0.6; 3.1)	0.417[c]
Duration of hospitalization, days[a] (0.25; 0.75)	2 (2; 4)	3 (2; 9)	2 (1; 4)	2 (2; 4)	2 (1; 3)	0.255[d]

[a] The median value was used (25th quantile; 75th quantile)

[b] Number of patients

[c] The Kruskal–Wallis one-way analysis of variance was used

[d] The Chi-square analysis was used

[e] Further Kruskal–Wallis analyses showed that, after exclusion of unknown pathogen, results were significant between viral and mixed pneumonia (P=0.017) but not between bacterial and mixed (P=0.143) or viral and bacterial (P=0.425)

[f] Abdominal, dorsal or thoracic pain

[g] Further Chi-square analyses showed that, after exclusion of unknown pathogen, results were significant between mixed and bacterial group (P=0.027), but not between mixed and viral group (P=0.057), neither between bacterial and viral group (P=0.461)

[h] Further Chi-square analyses showed that difference was significant between viral and bacterial (P=0.0004) or mixed (P=0.039) group but not between bacterial and mixed group (P=0.087)

[i] Further Chi-square analyses showed that, after exclusion of unknown pathogen, results were significant between all bacterial infections (bacterial + mixed) and viral infections (P=0.003), but not between bacterial and mixed (P=0.803), neither between bacterial and viral (P=0.023).

[j] Further Chi-square analyses showed that, after exclusion of unknown pathogen, results were significant between all bacterial infections (bacterial + mixed) and viral infections (P=0.009), but not between bacterial and mixed (P=0.805), neither between bacterial and viral (P=0.016)

Table 3 Microbiological armamentarium and number of samples performed

	Blood culture	NPA (viral culture)	NPA (viral AG)	NPA (viral PCR)	NPA (*M. pneumoniae, C. pneumoniae* PCR)	Viral serology[a]	Serology for *M. pneumoniae and C. pneumoniae*[a]	Pneumoc. serology[b]	Pneumoc PCR
No. of exams (total=99) (%)	98 (99)	85 (86)	80 (81)	93 (94)	89 (90)	71 (72)	71 (72)	98 (99)	97 (98)
No. of positive (%)	1 (1)	15 (17)	12 (15)	59 (63)	8 (9)	17 (24)	12 (17)	40 (41)	21 (22)

[a] Convalescent serology

[b] Acute and/or convalescent serology

(46% overall), based on PCR (21/45, 47%), rising antibody titer (14/45, 31%), and/or high antibody titers at admission (30/45, 66%). Despite a negative PCR, 53% (24/45) of patients had serological evidence of acute pneumococcal infection. The single child with a pneumococcal bacteremia had a positive PCR and serology. *S. pneumoniae* was diagnosed alone in 12 patients (63% of bacterial etiology). Mixed pneumococcal-viral infections occurred in 28 patients (85% of mixed pneumonia), with a large spectrum of concomitant viruses (Table 4).

Viral infection

Overall, 66 among the 99 children (67%) had evidence of an acute viral infection (Fig. 2; Table 4). PCR was very sensitive to detect several viruses. In no patient with a negative viral PCR, could a viral infection be detected using another method. There was a significant relationship between age and etiological category (Table 2).

Clinical or inflammatory parameters and etiology

Excluding patients with undetermined etiology, univariate analyses showed that dehydration was the only clinical sign significantly more represented in the bacterial group of pneumonia. Indeed, 47% of children with bacterial pneumonia, while only 24% in the mixed group and 6% in the viral pneumonia group (P=0.003), showed signs of dehydration, (Table 2). Other symptoms were not significantly associated with etiology. In particular, URS were not significantly more frequent in the viral group. The proportion of patients in each clinical severity category (WHO stages) did not differ significantly according to etiology (P=0.698). Analysis of laboratory data showed that children with bacterial pneumonia overall had higher inflammatory parameters: CRP and PCT values were significantly higher in the bacterial pneumonia group when mixed and bacterial pneumonia were analyzed together (Table 2). However, inflammatory indices were not statistically associated with clinical severity. For a cut off level of 60 mg/l the sensitivity of CRP was 88% but the specificity was low (44%). For a cut off level of 5 ng/ml the sensitivity of PCT was 72% and the specificity 58%. Total white blood cell count and band forms were not significantly associated with etiology. The proportion of children treated with antibiotics before hospitalization, did not differ according to the etiological category (Chi square, P=.33). The average duration of hospitalization was related to clinical severity.

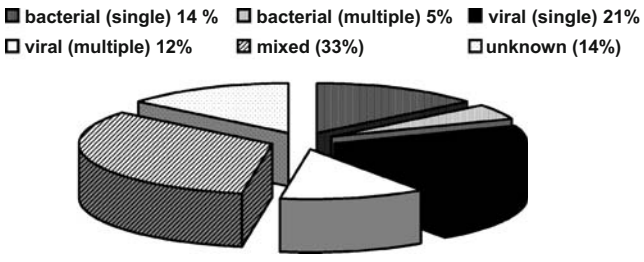

Fig. 1 Aetiology of pneumonia in 99 hospitalized children. Five patients, in the bacterial group, had a dual bacterial pneumonia (four including *S. pneumoniae* and *Mycoplasma pneumoniae*, one including *S. pneumoniae* and *Group A streptococcus*)

Clinical and radiological data

On admission, breath sounds were described as completely normal in 21 (21%) patients. In 78 patients with abnormal auscultation, the most constant sign was diminished breath sounds (59 patients). Chest X-ray was abnormal in 97 of 98 patients (99%). Radiological consolidation was present in 77 (79%) patients. Overall percent agreement between the presence of diminished breath sounds and radiological consolidation was 60.2% with a Kappa coefficient of 0.11 (95% CI—0.07 to 0.29), confirming very poor agreement. There was no association between radiological description and severity or etiology of pneumonia.

Discussion

One hundred and eleven immunocompetent children, aged between 2 months and 5 years old, hospitalized for pneumonia, were studied prospectively to elucidate the

Table 4 Pathogens identified in 99 hospitalized children with community-acquired pneumonia

Pathogen		No Co-infection	Co-infection with bacteria[a]	Co-infection with viruses[a]	Total no. of episodes, %
Bacteria	*S. pneumoniae*	12	10	28	45 (46)
	S. Group A β hemolytic	0	1	1	1 (1)
	M. pneumoniae	2	5	5	11(11)
	C. pneumoniae	0	6	6	7 (7)
Viruses	Influenza A or B	0	8	10	14 (14)
	Parainfluenza 1–3	2	8	8	13 (13)
	Rhinovirus	7	11	10	20 (20)
	hMPV	5	5	7	13 (13)
	RSV A or B	6	3	7	13 (13)
	Enterovirus	1	8	11	13 (13)
	Adenovirus	0	6	5	7 (7)
	Coronavirus	0	4	7	7 (7)

[a] The categories of co-infection with bacteria or with viruses are not mutually exclusive

factors that could help the clinician to differentiate viral from bacterial pneumonia. Ninety-nine patients met the inclusion criteria in the strict respect of the WHO definition of pneumonia. A multiple diagnostic approach combining microbiology, serology, and biochemical assays was used to define the spectrum of pathogens that cause pneumonia in children unimmunized against *S. pneumoniae*. Overall, a potential causative agent was detected in 86% of the children, confirming previous studies showing that the more tests are done, the more potential causes emerge [23, 24, 31, 39]. Evidence of bacterial infection was demonstrated in 53% of patients.

S. pneumoniae was documented in 45% of the cases, corroborating previous reports such as the one by Michelow et al. [32]. A virus was found in 67% of the children. In 33% of the cases, a concurrent bacterial and viral infection was found. Antibiotic treatment before hospitalization doesn't seem to be a factor of misdiagnosis. Our study showed that in children fulfilling the WHO clinical definition of pneumonia and severity criteria, diagnosis was confirmed by radiological consolidation in 78% of cases. The poor agreement between auscultation and radiological consolidation confirms the poor sensitivity and specificity of auscultation to diagnose pneumonia in young children [11]. As previously described [14, 28] chest X-ray and blood cultures were not helpful in discriminating etiological categories of pneumonia. Duration of hospitalization was correlated with clinical severity essentially because WHO criteria define children with stage III pneumonia as requiring oxygen supplementation. Pain was the only clinical sign significantly associated with bacterial pneumonia. Acute-phase surrogate markers such as CRP and PCT, were significantly higher in bacterial infection regardless of the presence or absence of co-infecting viruses, corroborating several previous analyses [20, 33, 37] even if these findings are controversial in recent literature [13]. Further analysis is needed to determine the exact utility of these tests, as the sensibility and specificity with a bacterial cut off level were quite low. Finally, our data could not show a significant correlation between clinical severity and etiology of pneumonia [24].

As reported previously [12, 21, 40, 42], our data confirm the high prevalence of viruses and showed the frequent occurrence of co-infection in childhood CAP. The viruses most commonly found were rhinovirus (30%) and hMPV (20%), confirming the recent findings that these viruses frequently involved in pediatric CAP [3, 11, 36, 38]. RSV was less represented than in other reports [6], probably because all patients with typical bronchiolitis were strictly excluded. Several points still have to be elucidated: whether viruses are the direct cause of pneumonia, whether they

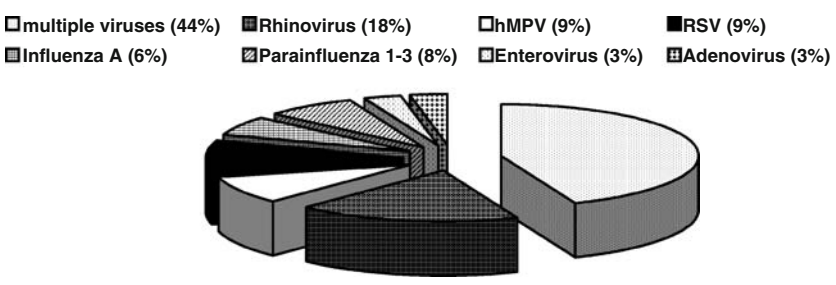

Fig. 2 Distribution of viruses associated with pneumonia. A virus was found in 66 (67%) of the 99 patients hospitalized for pneumonia

create conditions favoring secondary bacterial infections, or whether their presence plays a relatively minor role in CAP pathogenesis.

The main limitation to our study is the lack of validation and standardization of all microbiological cut off values, especially those concerning *S. pneumoniae* [4]. One of the major obstacles to improve the understanding of CAP is probably that we are still underdiagnosing pneumococcal and possibly other bacterial pneumonia. The use of a single antigen (pneumolysin) for the serological diagnosis of pneumococcal pneumonia may be a limitation, as well as the difficulty, in our study, to obtain convalescent serum samples (available for only 73% of the children). Therefore, it remains difficult to distinguish a real acquired infection from a recent colonization. As recently described by Nakayama et al. [34], the use of PCR to detect bacterial pneumonia could be a useful additional diagnostic method. However, the fact that 46% of young children with CAP requiring hospitalization demonstrated evidence of an acute pneumococcal infection, is in accordance with the demonstrated impact of pneumococcal immunization [10, 18, 29, 30]. Finally, our results do not support the hypothesis that clinical severity depends on the causal agent. However, for ethical and practical reasons we chose to study only hospitalized children. Therefore, we have a limited number of patients with stage I pneumonia whom we usually treat in the ambulatory setting. A further study should include this group in order to confirm, with a greater number of patients, the non-association between clinical severity at admission and etiology.

In conclusion, this study used strict WHO clinical criteria to select suitable subjects to undergo an extensive microbiological etiological workup using a complete set of modern diagnostic tools. Our data confirm a high prevalence of viruses and the frequent occurrence of co-infection in childhood CAP. Dehydration and surrogate markers of inflammation such as CRP and PCT were significantly higher in children with bacterial pneumonia. Therefore, further analyses should be performed in order to find out which children should be treated with antibiotics and in which children antibiotics could be withheld safely. The high proportion of *S. pneumoniae* in severe CAP re-emphasized the importance of pneumococcal immunization and antibiotics for treatment of CAP in young children. Other studies should also be performed to evaluate the epidemiology of moderate to severe bacterial pneumonia after introduction of pneumococcal immunization.

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Conflicts of interest All the authors certify that they have no financial relationship with the organization that partly sponsored the research and no conflicts of interest.

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