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Aetiological agents, interleukin-6, interleukin-8 and CRP concentrations in children with community- and hospital-acquired pneumonia

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Abstract

Objective: To determine the pathogens causing pneumonia in community-acquired pneumonia (CAP) and hospital-acquired pneumonia (HAP) and to investigate serum levels of interleukin-6 (IL-6), interleukin-8 (IL-8) and CRP in pneumonia caused by different aetiological agents.

Study design: Eighty-seven children (mostly <5 years of age) were recruited in a prospective study, 55 of them with CAP without prior antibiotic treatment and 32 with HAP. Thirty healthy outpatient children served as controls.

Results: The causative micro-organisms were determined by serological and microbiological methods in 40 cases with CAP (72.7%) and 30 with HAP (93.7%). In CAP, *M. pneumoniae* was the most common causative agent (43.6%), followed by *S. pneumoniae* (20%) and *C. pneumoniae* (18.1%). Bacteria alone were the sole causative agents in only 21.8% of cases with HAP. *Pseudomonas aeruginosa* (34.3%) and *K. pneumoniae* (32.5%) were the most frequently isolated. Although IL-6 and IL-8 levels were raised, there was no statistical difference between the CAP and HAP groups, or between bacterial and mycoplasma infections; neither was there a difference in CRP levels between these two groups.

Conclusion: The causes of pneumonia differ between CAP and HAP. Levels of IL-6, IL-8 and CRP are raised in pneumonia but are unhelpful in differentiating the various aetiologies.

Introduction

Pneumonia is a major cause of childhood mortality and is one of the most common reasons for hospital admission. The incidence of childhood pneumonia is 4–5 cases/100 children/year in 2–3-year-olds, 2 cases/100 children/year in 5–9-year-olds and one case/100 children/year above the age of 10 years.[1] Identifying the cause of pneumonia is difficult because invasive procedures such as broncho-alveolar lavage (BAL), lung biopsy and pleural fluid aspiration are often required.

Community-acquired bacterial pneumonia (CAP) is commonly caused by *Mycoplasma pneumoniae* and *Streptococcus pneumoniae*.[2–5] *M. pneumoniae* and *Chlamydia pneumoniae* are the predominant atypical pathogens in children over 5 years of age[6,7] and their prevalence seems to be increasing.[8] *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are the most commonly isolated bacterial agents in hospital-acquired pneumonia (HAP).[5,6,9–11]

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Early diagnosis of severe infections and prompt initiation of appropriate antimicrobial therapy are essential for the successful treatment of CAP and HAP. Management decisions, usually made at presentation, are based on clinical and radiological features. Treatment of pneumonia, even in severe cases, is almost always empirical.

Cytokines are widely recognised as mediators of the inflammatory response. Their role in the pathogenesis of pneumonia is still poorly understood. Interleukin-6 (IL-6) and interleukin-8 (IL-8) are important mediators in infections with bacteria and atypical pathogens, but there have been few studies on the role of serum IL-6, IL-8 and serum CRP in distinguishing bacterial from atypical pneumonia.[12,13]

The aim of this study was to determine the pathogens involved in CAP and HAP and to measure the levels of IL-6, IL-8 and C-reactive protein (CRP).

Patients and Methods

The prospective study was conducted by the Department of Pediatrics, University of Cukurova, Adana from December 2000 to March 2002. Eighty-seven children aged 1–156 months (mean 22.1, SD 32.3) were recruited; 55 were admitted with CAP without prior antibiotic treatment and 32 were diagnosed with HAP. The control group comprised 30 healthy age- and sex-matched children from the outpatient department without recent infection or any history of chronic disease.

In a previously healthy child, CAP was defined by the presence of fever ($\geqslant 38^{\circ}$C), cough or crackles and tachypnoea (according to WHO criteria: 0–2 mths, >60/min; 3–12 mths, >50/min; 13–59 mths, >40/min; >60 mths, >30/min) and infiltrates on the chest radiograph. HAP was defined as a new infection of lung parenchyma developing more than 48 hours after admission. Among the cases with HAP, six had congenital heart disease, three a neurometabolic disorder and three cerebral palsy; additionally, three had tracheostomies. Children with pre-existing pulmonary or immunological diseases were excluded. Those who had received an antibiotic in the week before recruitment were also excluded. Ten such patients recruited in the CAP group were excluded.

Investigations

Venous blood, deep tracheal aspirate and pleural fluid samples were cultured.

Laboratory investigations

Serology. The serum levels of IgG and IgM antibodies to *M. pneumoniae* and *C. pneumoniae* and IL-6, IL-8 and CRP levels were determined in patients and controls.

Serum was stored at -70°C until tested. The diagnoses of chlamydia and mycoplasma infections were based on an IgG antibody response measured by micro-immunofluorescence (MIF) and an IgM antibody response measured by micro-enzyme-linked immunosorbent assay (microELISA) (Euroimmune-kit, GMBH Laboratories). Acute serum specimens were collected at the time of the initial visit, and the convalescent sera 4 weeks later. A four-fold difference in antibody titres between acute and convalescent sera or a single IgG titre $\geqslant 512$ were considered to be sufficient immunological evidence of infection.

IL-6 and IL-8 levels were determined by microELISA (IMMUNOTECH® and CYTIMMUNE®, respectively). CRP (normal <6 mg/dl) was determined by rate nephelometry (Boehring Nephelometry® 100 machine).

Cultures. Blood cultures were performed in all patients: 1–3 ml of blood were inoculated in peds plus/F haemoculture bottles and incubated in Bactec 9240 (Becton Dickinson) for 1 week. Deep tracheal aspiration cultures were performed in 43 of 87 patients. Samples were obtained by deep tracheal aspiration by nasogastric

tube after i.v. midazolam (0.2 mg/kg) infusion. Cultures of pleural fluid were performed in eight patients (CAP 3, HAP 5). Pleural fluid was centrifuged at 1000 rpm for 10 min and spread onto blood agar and MacConkey agar. The sputum obtained by deep tracheal aspiration was spread onto blood agar and MacConkey agar directly or after dilution at a ratio of 1/10, 1/100 or 1/1000. Replicated micro-organisms were determined as cfu/ml.

Statistical analysis

Statistical analysis was conducted using SPSS 10 software. Independent samples *t*-test, one-way ANOVA, Mann–Whitney U and univariate analysis of variance tests were performed.

Ethics

The protocol was approved by the Regional Ethical Committee of Cukurova University and informed consent was obtained from parents or legal guardians.

Results

Table 1 illustrates the patients' demographic and clinical details. Infiltrates (parahilar, reticulo-nodular, diffuse bilateral) were found to be the most common radiological appearance in both CAP and HAP, followed by lobar pneumonia, pleural fluid and air-trapping, respectively (Table 1).

The causative micro-organisms were determined by serological and microbiological methods in 70 cases with pneumonia

TABLE 1. *Clinical details of children with pneumonia and controls.*

	Patients (n=87)		
	CAP, n=55	HAP, n=32	Controls, n=30
Age, mths, mean (SD)	23.2 (24.5)	30.6 (41.6)	28.3 (23.3)
Gender, M/F	33/22	20/12	15/15
Symptoms (%):			
Cough	54 (98.2)	22 (68.8)	1 (3.3)
Dyspnoea	49 (89.0)	21 (65.6)	0
Fever	42 (76.4)	20 (62.5)	0
Vomiting	10 (18.2)	5 (15.6)	0
Weakness	31 (56.4)	15 (46.9)	1 (3.3)
Examination (%):			
Retractions	45 (81.0)	22 (68.8)	0
Flaring	42 (76.0)	20 (62.5)	0
Cyanosis	14 (25.5)	11 (34.4)	0
Crackles	49 (89.1)	29 (90.6)	0
Diminished air entry	8 (14.5)	7 (21.9)	0
Wheeze	23 (41.8)	8 (25.0)	0
Signs (SD):			
Temperature, °C	38.9 (0.7)	38.7 (0.5)	36.4 (0.4)
Pulse rate/min	148 (22)	145 (28.0)	105 (15)
Respiratory rate/min	50 (15)	49 (16.0)	23 (4)
Radiographic appearance (%):			
Infiltrates (parahilar, reticulo-nodular, diffuse bilateral)	39 (70.9)	20 (62.0)	
Lobar pneumonia	11 (20.9)	8 (25.0)	
Pleural fluid	3 (5.4)	5 (15.6)	
Air-trapping	4 (7.2)	2 (6.2)	
Oxygen treatment	24 (43.6)	21 (65.6)	
Intensive care	16 (29.0)	18 (56.0)	
Mechanical ventilation	2 (3.6)	10 (31.2)	

(80.4%), 40 with CAP (72.7%) and 30 with HAP (93.7%). In cases with CAP, *M. pneumoniae* was the most common causative agent in all age groups (24/55, 43.6%), followed by *S. pneumoniae* (11/55, 20%), *C. pneumoniae* (10/55, 18.1%), *Staphylococcus warneri* (3/55, 5.4%), *Streptococcus anginosus* (2/55, 3.6%), *Streptococcus agalactia* (2/55, 3.6%) and *Stenotrophomonas maltophilia* (2/55, 3.6%). *M. pneumoniae* was detected in 60% (3/5) of CAP cases aged $\geqslant$5 and *C. pneumoniae* in 40% (2/5) (Table 2).

Bacteria alone were the sole causative agent in only 21.8% (7/32) of cases with HAP. *P. aeruginosa* was isolated most frequently (11/32, 34.3%) and other agents encountered were *K. pneumoniae* (10/32, 32.5%), *A. baumannii* (8/32, 25%) and *Serratia marcescens* (4/32, 12.5%). In 13 (40.6%) of 32 cases with HAP, various cultures yielded more than one micro-organism. Mixed bacterial and/or chlamydia and/or mycoplasma infection were found in 62.5% of these cases. While mycoplasma was found to be the sole causative agent in two cases (6.2%), none of the cases of HAP was caused by chlamydia alone. Mixed infection with mycoplasma and chlamydia was determined in five (15.6%) of the remaining cases (Table 2).

There was no statistically significant difference in mean serum IL-6 levels between CAP and HAP cases or between bacterial or mycoplasma infections. However, serum IL-6 levels of both CAP and HAP cases were significantly higher than in the control group ($p<0.001$). Mean serum IL-6 levels did not differ significantly between cases of sole bacterial, chlamydial or mycoplasma infection. Similarly, there was no statistically significant difference in mean serum IL-8 levels between CAP and HAP cases with bacterial, chlamydial or mycoplasma infections. Serum IL-8 levels in CAP and HAP cases were also significantly higher than in the control group ($p<0.01$). In cases of sole bacterial, chlamydial or mycoplasma infection, mean serum IL-8 levels did not differ significantly between the different micro-organisms (Table 3).

Serum CRP levels in CAP and HAP cases were raised, but there was no statistically significant difference between the two groups. In pneumonia cases with sole bacterial, chlamydial or mycoplasma infection, mean serum CRP levels did not differ significantly between the different micro-organisms (Table 3).

Discussion

Viruses, bacteria and mycoplasmas are the most common agents in the aetiology of pneumonia.[2,3,11,14] Atypical bacterial and viral pneumonias usually can not be distinguished from one another by clinical or radiological assessments. White blood cell count, CRP and other non-specific methods are often used to differentiate bacterial from

TABLE 2. *Aetiology of pneumonia in CAP and HAP.*

Bacteriological and serological diagnosis	CAP, n=55 (%)	HAP, n=32 (%)
No aetiology	15 (27.2)	2 (6.2)
Bacterial infection	7 (12.7)	7 (25.0)
Mycoplasma infection	11 (20.0)	2 (6.2)
Chlamydial infection	4 (7.2)	0
Mixed infections:		
Mixed bacterial	5 (9.0)	6 (18.7)
Bacterial + chlamydial	0	0
Bacterial + mycoplasma	7 (12.7)	6 (18.7)
Chlamydial + mycoplasma	4 (7.2)	5 (15.6)
Bacterial + mycoplasma + chlamydial	2 (3.6)	4 (12.5)

viral pneumonia but in many cases there is no clear difference.[13]

Some authors consider blood culture to be the gold standard for diagnosing childhood pneumonia but this method has very low sensitivity.[15] Endotracheal aspiration, bronchoscopy, transtracheal aspiration and lung puncture are other invasive methods but are usually used only for difficult cases or for research. They have a high diagnostic yield of up to 70%.[15]

The most common radiological abnormality detected was infiltration in 39 cases (70.9%) of CAP and 20 cases (60%) of HAP. In a study of 98 pneumonia patients, 61% had infiltrations.[4] Korpi *et al.* found that alveolar and interstitial infiltrations were the most common radiological changes (61%) in bacterial pneumonia.[16]

A micro-organism was determined in 40 patients (72.7%) with CAP. The responsible agents were recovered in blood and pleural cultures in 21 patients (38.1%). In a similar study of CAP, a bacterial agent was determined in 69% of the serological samples and 30% of the sputum culture samples.[17] Turner *et al.* detected bacterial agents in only 19%.[4] In this study, a pure bacterial infection was diagnosed in only seven of the 55 CAP cases (12.7%). The most commonly isolated agent in children with CAP was *S. pneumonia* (11/55, 20%), and more than one agent were detected in eight cases (8/55, 14.5%). These results are similar to other studies.[17,18]

Nosocomial infections, especially nosocomial pneumonia, are the most common causes of mortality in intensive care units. In this study, we were able to determine the aetiology of 30 of the 32 cases (93%) of HAP. *P. aeruginosae*, *K. pneumoniae*, *A. baumannii* and *S. marcescens* were the four most commonly encountered agents, which is similar to other reports.[5,6,9,10,19] *M. pneumoniae* was detected in only 6.2% of cases.

M. pneumoniae and *C. pneumoniae* are the most common causes of atypical pneumonia, especially in CAP in children over 5 years of age.[6,20–23] In this CAP study, which was in children mostly <5 years of age, isolated *M. pneumoniae* and *C. pneumoniae* infections were detected, respectively, in 11 of 55 cases (20%) and four of 55 cases (7.2%).

IL-6 and IL-8 are primary mediators of inflammation. High levels are detected in broncho-alveolar lavage (BAL) samples from CAP patients.[25,26] Puren *et al.* found that serum IL-6 levels in patients with pneumonia were higher than in controls and that this was also the case in other pulmonary infections.[27] In this study, there was no statistically significant difference in IL-6 levels between bacterial, chlamydial or mycoplasma infections. Bouten *et al.* reported that IL-8 levels were raised in BAL fluid from patients with CAP, but when compared with the control group there was no significant difference.[26] In other studies, however, raised IL-8 levels

TABLE 3. *Serum IL-6, IL-8 and CRP levels in CAP and HAP.*

Groups (*n*)	Serum Il-6 (pg/ml) Mean (median, range)	Serum IL-8 (pg/ml) Mean (median, range)	CRP (mg/dl) Mean (median, range)
CAP (55)	160.1 (46.8, 0–3404)⋆	182.7 (46.2, 0–2217)†	56.8 (23, 0–211)
HAP (32)	375.0 (58.4, 0–3184)⋆⋆	173.8 (44.0, 0–1811)‡	78.7 (64.5, 0–216)
Controls (30)	1.0 (1.0, 0–16.2)	36.7 (42.3, 0–209)	
Chlamydia in CAP (4)	28.8 (38.3, 0–68)	33.5 (36.6, 20–43)	74.8 (57.5, 0–184)
Mycoplasma in CAP (12)	214.9 (51.6, 0–829)	437.2 (158.4, 8.8–2226)	58.9 (48.5, 0–195)
Bacterial in CAP (9)	248.7 (37.6, 5.2–3404)	83.7 (67.0, 29.3–143)	49.5 (15.3, 0–211)

⋆ $p=0.01$ (CAP *vs* controls); ⋆⋆ $p=0.001$ (HAP *vs* controls); † $p=0.01$ (CAP *vs* controls); ‡ $p=0.01$ (HAP *vs* controls).

were detected in pneumonia patients.[25,28–30] In this study, serum IL-8 levels were higher in both pure bacterial and pure chlamydial cases compared with other *C. pneumoniae* cases, but the difference was not statistically significant. Kraisberg *et al.* reported that serum IL-8 levels in pneumococcal pneumonia were higher than in chlamydial, legionella and influenza A pneumonia.[31]

Serum CRP concentration has a higher predictive value than the leucocyte or neutrophil counts in febrile children with suspected pneumonia.[32] However, raised CRP levels are found in both bacterial and viral pneumonia.[33,34] In our study, there was no statistically significant difference in CRP levels between CAP and HAP patients.

In conclusion, the most commonly encountered agents in patients with CAP were *M. pneumoniae*, *S. pneumoniae* and *C. pneumoniae* and in HAP were *P. aeruginosa* and *K. pneumoniae*. IL-6 and IL-8 are not useful in the differential diagnosis of sole bacterial, *M. pneumoniae* or *C. pneumoniae* infections.

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