Characteristics of *Streptococcus pneumoniae* and Atypical Bacterial Infections in Children 2–5 Years of Age with Community-Acquired Pneumonia

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The characteristics of community-acquired pneumonia associated with *Streptococcus pneumoniae* infection were compared with those associated with atypical bacterial infection and with mixed *S. pneumoniae*-atypical bacterial infection in 196 children aged 2–5 years. *S. pneumoniae* infections were diagnosed in 48 patients (24.5%); atypical bacterial infections, in 46 (23.5%); and mixed infections, in 16 (8.2%). Although white blood cell counts and C-reactive protein levels were higher in patients with pneumococcal infections, no other clinical, laboratory, or radiographic characteristic was significantly correlated with the different etiologic diagnoses. There was no significant difference in the efficacy of the different treatment regimens followed by children with *S. pneumoniae* infection, whereas clinical failure occurred significantly more frequently among children with atypical bacterial or mixed infection who were not treated with a macrolide. This study shows the major role of both *S. pneumoniae* and atypical bacteria in the development of community-acquired pneumonia in young children, the limited role of clinical, laboratory, and radiological features in predicting etiology, and the importance of the use of adequate antimicrobial agents for treatment.
are seen as atypical bacteria that cause a form of CAP with a very slow and benign course and radiological findings of interstitial lung disease [22, 23]. However, recent reports have suggested that atypical bacteria may cause CAP with characteristics similar to those associated with pneumococcal infections [24, 25]. We report the results of a study in which the clinical, laboratory, and radiological data for CAP associated with S. pneumoniae infections were compared with data for CAP associated with atypical bacterial or with mixed infection due to S. pneumoniae and atypical bacteria.

PATIENTS AND METHODS

Study patients. The study involved 196 children who were 2–5 years of age and who were admitted to Pediatric Department I, University of Milan (Milan, Italy). The patients were hospitalized on the basis of clinical decisions made by the physicians on duty. Previously healthy male and female children who were 2–5 years of age and who had signs, symptoms, and chest radiograph findings consistent with CAP were considered eligible for inclusion. The exclusion criteria were severe concomitant disease (e.g., neoplasms, kidney or liver disease, immunosuppression, cardiovascular disease, and malabsorption syndrome), nosocomially acquired infection, and use of antibiotics in the 48 h before enrollment.

Methods. Each patient’s medical history, rectal temperature, respiratory rate, and auscultation findings were systematically recorded at the time of admission. Only 1 investigator (N.P.) was responsible for the collection of patient data during the study period.

Blood samples were obtained for evaluation of the WBC count, serum C-reactive protein (CRP) level, and erythrocyte sedimentation rate (ESR) by means of standard methods.

Serum samples were obtained to determine titers of antibodies to S. pneumoniae and atypical bacteria (M. pneumoniae and C. pneumoniae). An anti-S. pneumoniae ELISA was used to measure IgG antibodies to the 9 most common pneumococcal serotypes (1, 4, 5, 6B, 9V, 14, 18C, 19F, and 23F), as described elsewhere [26–28]. The pneumococcal antibody titer in the serum samples was quantitated against the human pneumococcal standard reference serum, lot 89-SF (provided by Carl E. Frash, Center for Biologics Evaluation and Research, US Food and Drug Administration, Rockville, MD). Titers of IgM and IgG to M. pneumoniae were determined by a standardized ELISA (Pantec), and titers of IgM, IgG, and IgA to C. pneumoniae were determined by microimmunofluorescence (Labsystems). Nasopharyngeal aspirates were also obtained, immediately immersed in a sucrose-phosphate-glutamate transport medium, and stored at −70°C until they were assayed for the presence of M. pneumoniae and C. pneumoniae DNA by PCR, as described elsewhere [29, 30].

Chest radiographs (erect posteroanterior and lateral view) were obtained at admission and then were blindly reviewed by an experienced radiologist. Seven radiological features (hyperinflation, peribronchial wall thickening, perihilar linear opacities, reticulonodular infiltrate, segmental or lobar consolidation, bilateral consolidations, and pleural effusion) were recorded as present or absent [10, 31]. Alternative radiographic interpretations were not mutually exclusive, and >1 category could be checked in each radiograph.

In the absence of results of serological tests and PCR analysis, the children were treated on the basis of the judgment of their attending pediatricians. During hospitalization, the results of a detailed physical examination of the respiratory apparatus, any changes in clinical symptoms, and the clinical response to therapy were recorded every day by 1 investigator (N.P.) who was blinded to both the etiologic assignment and the antimicrobial treatment given. The patients were discharged from the hospital if they were afebrile and if their clinical condition had been stable for 48 h. After hospitalization, the children were asked to return to the study center immediately if they experienced any recurrent or worsening signs and symptoms.

The medical history, general physical condition, and clinical symptoms of each child were blindly reevaluated by 1 investigator (N.P.) 4–6 weeks after enrollment. At the same time, a second serum sample was obtained to assay convalescent-phase S. pneumoniae, M. pneumoniae, and C. pneumoniae antibody titers. During this evaluation, the clinical response to therapy was defined as a “cure” (complete resolution of signs and symptoms of CAP) or a “failure” (persistence or progression of signs and symptoms of CAP after 3 days of therapy or development of new clinical findings consistent with active infection) [15, 25]. Treatment with antibiotics was considered evaluable if it had been administered in accordance with the recommended posology and duration [32].

Evaluation of infections. Acute pneumococcal infection was diagnosed if the patient showed a ≥3-fold increase in the concentration of type-specific capsular IgG to ≥1 of the 9 tested serotypes in a comparison of paired serum samples [16]. Acute M. pneumoniae and/or C. pneumoniae infection was diagnosed by the recommended serological means of determining the prevalence of infection in epidemiological studies—that is, ELISA was used to detect M. pneumoniae, and microimmunofluorescence was used to detect C. pneumoniae [21, 24, 33, 34]. Nested PCR was used to confirm the results of serological testing because, even if nested PCR method usually has sensitivity and specificity lower than those associated with methods of determination of antibody titers, it may confirm the presence of infection in children who did not have an immunologic response either because of the immaturity of their immune system or because of poor antigenic stimulation [21, 24, 33, 34]. According to criteria described elsewhere, the presence of
infection was defined by a significant antibody response in paired serum samples (for *M. pneumoniae*: specific IgM $\geq 1:100$, specific IgG $\geq 1:400$, or a 4-fold increase in IgG titer; for *C. pneumoniae*: specific IgM $\geq 1:16$, specific IgG $\geq 1:512$, or a 4-fold increase in IgG titer) or by a positive result of PCR analysis of nasopharyngeal aspirates [10, 16, 25, 33].

The reference laboratories were Wyeth Lederle Laboratories (Rochester, NY; for *S. pneumoniae* serological testing), Pediatric Department I, University of Milan (Milan, Italy; for *M. pneumoniae* serological testing and PCR analysis), and the Institute of Respiratory Diseases, Istituto di Ricerca e Cura a Carattere Scientifico Maggiore Hospital, University of Milan (Milan, Italy; for *C. pneumoniae* serological testing and PCR analysis). The study protocol was approved by the Institutional Review Board at the University of Milan and was conducted in accordance with the revised Helsinki Declaration of 1983. Written informed consent was obtained from the parents or legal guardians of each child.

**Analysis.** Data were analyzed using SAS for Windows version 12 (SAS Institute). All of the patients were included in the analysis. $P < .05$ was considered to be statistically significant for all of the statistical tests. Parametric data were compared by analysis of variance with terms for treatment and test for multiple comparisons; when the data were not normally distributed or were nonparametric, the Kruskal-Wallis test was used. Categorical data were analyzed by contingency analysis and by either the $\chi^2$ test or Fisher’s exact test.

**RESULTS**

**Demographics.** Table 1 shows the characteristics of the study population. On the basis of the serological and PCR findings, 110 patients (56.1%) had acute infections due to *S. pneumoniae*, atypical bacteria, or mixed *S. pneumoniae*–atypical bacteria.

Acute *S. pneumoniae* infection associated with the 9 tested serotypes was diagnosed in 48 children (24.5%). Forty patients had a seroresponse to only 1 serotype; these serotypes and their prevalences were as follows: 1 (30.0%), 14 (27.5%), 9V (10.0%), 4 (7.5%), 18C (7.5%), 6B (5.0%), 19F (5.0%), 23F (5.0%), and 5 (2.5%). Serological evidence of $>1$ serotype was found in 8 cases (16.7%).

Acute atypical bacterial infection was identified in 46 children (23.5%). Acute *M. pneumoniae* infection was found in 30 patients (15.3%), having been determined serologically in all 30 patients and having been confirmed by PCR in 21 patients (70.0%). In none of the patients was *M. pneumoniae* DNA detected without any evidence of seroconversion. Acute *C. pneumoniae* infection was found in 6 patients (3.1%); it was serologically determined in all 6 patients and was confirmed by PCR in 3 patients (50.0%). None of the patients had *C. pneumoniae* DNA detected without any evidence of seroconversion. Ten patients (5.1%) showed evidence of acute mixed *M. pneumoniae*–*C. pneumoniae* infection. In all of these patients, acute *M. pneumoniae* infection was serologically determined; *M. pneumoniae* DNA was also detected in 6 patients (60.0%). Acute *C. pneumoniae* infection was serologically determined in 7 of the patients who had mixed infection, and it was confirmed by PCR in 4 patients (57.1%). In 3 patients, *C. pneumoniae* DNA was detected without any evidence of seroconversion.

Acute mixed *S. pneumoniae*–atypical bacterial infection was diagnosed in 16 children (8.2%). Eleven patients had a seroresponse to only 1 serotype; these serotypes and their prevalences were as follows: 1 (27.2%), 14 (18.2%), 18C (18.2%), 4 (9.1%), 5 (9.1%), 6B (9.1%), and 9V (9.1%). Serological evidence of $>1$ serotype was found in 5 patients (31.3%). Of the infections due to atypical bacteria, acute *M. pneumoniae* infection was diagnosed by serological testing in 14 patients and was confirmed by PCR in 8 patients (57.1%). Acute *C. pneumoniae* infection was identified in 2 patients; both cases were confirmed by serological testing and PCR.

**Clinical findings.** The clinical characteristics of the study population at the time of enrollment are summarized in Table 2. Sex, age distribution, clinical presentation, duration of illness, and hospitalization were similar in the different etiologic groups.

**Laboratory evaluation.** Table 3 shows the laboratory data by etiologic group. Although there was a large overlap in individual values, total WBC counts, neutrophil percentages, and CRP concentrations were significantly higher in the children with *S. pneumoniae* infection than in both of the other groups ($P < .05$), for which the data were similar. The distribution of ESRs was similarly wide in each group.

**Radiographic characteristics.** The radiographic characteristics of the study population are shown in Table 4. There...
were no significant correlations between the radiological findings (considered separately or together) and the etiology of infection.

**Clinical response 4–6 weeks after enrollment.** At the time of admission, antibiotics were prescribed for all 196 children. The patients were treated with a wide range of antimicrobial regimens. Table 5 compares the clinical outcomes of the evaluable children according to known bacterial etiology and antimicrobial therapy.

Among the 48 children with *S. pneumoniae* infection, 4 (8.3%) were considered unevaluable in the analysis of clinical response because the antibiotic therapy was administered either for too short a time (for 3 patients treated with ceftriaxone) or in an inadequate dose (for 1 patient treated with amoxicillin plus clavulanate). Also, among the 46 children with atypical bacterial infection, 4 (8.7%) were considered unevaluable in the analysis of clinical response because the antibiotic therapy had been administered either for too short a time (for 2 patients treated with ceftriaxone and 1 treated with erythromycin) or in an inadequate dose (for 1 patient treated with amoxicillin plus clavulanate). Of the 16 children with mixed *S. pneumoniae*–atypical bacterial infection, only 1 patient (6.2%) was considered unevaluable in the analysis of clinical response because antibiotic therapy (ceftriaxone plus clarithromycin) had been administered for too short a time.

Among the 44 children with *S. pneumoniae* infection who were evaluated, no significant difference in the efficacy of the different regimens was observed. However, the proportion of patients for whom a cure was achieved was higher among those treated with β-lactam monotherapy or the combination of a β-lactam plus a macrolide.

Among the 42 children with acute atypical bacterial infection who were evaluated, the administration of β-lactam monotherapy was significantly more often associated with clinical failure than was the combination of a β-lactam plus a macrolide (47.6% vs. 0; *P* = .030) or macrolide monotherapy (47.6% vs. 7.1%; *P* = .023). There was no significant difference between the latter regimens with regard to clinical outcome.

Among the 15 evaluated children with acute mixed *S. pneumoniae*–atypical bacterial infection, administration of β-lactam monotherapy was associated with more-frequent clinical failures than was the combination of a β-lactam plus a macrolide (50.0% vs. 0; *P* = .060) or macrolide monotherapy (50.0% vs. 0; *P* = .166), although the differences were not significant.

Treatment with β-lactam monotherapy was associated with a significantly higher number of clinical failures among the children with atypical bacterial infection and among those with mixed *S. pneumoniae*–atypical bacterial infection than

### Table 2. Clinical characteristics of 196 children at the time of enrollment in a study of pediatric community-acquired pneumonia.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Infection due to</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Streptococcus pneumonia (n = 48)</em></td>
</tr>
<tr>
<td>Male sex</td>
<td>25 (52.1)</td>
</tr>
<tr>
<td>Age, mean years ± SD</td>
<td>3.666 ± 0.899</td>
</tr>
<tr>
<td>Onset&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Gradual</td>
</tr>
<tr>
<td></td>
<td>Acute</td>
</tr>
<tr>
<td>Similar illness in the child’s family</td>
<td>6 (12.5)</td>
</tr>
<tr>
<td>Cough&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31 (64.6)</td>
</tr>
<tr>
<td>Tachypnea&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12 (25.0)</td>
</tr>
<tr>
<td>Fever&lt;sup&gt;d&lt;/sup&gt;</td>
<td>42 (87.5)</td>
</tr>
<tr>
<td>Rales</td>
<td>45 (93.8)</td>
</tr>
<tr>
<td>Wheezing</td>
<td>6 (12.5)</td>
</tr>
<tr>
<td>Hospitalization, mean days ± SD</td>
<td>6.857 ± 3.523</td>
</tr>
<tr>
<td>Illness&lt;sup&gt;e&lt;/sup&gt;, mean days ± SD</td>
<td>12.325 ± 6.065</td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of patients, unless otherwise indicated. No difference was significant.

<sup>a</sup> Onset was defined as acute if all symptoms and signs appeared within no more than 2 days; otherwise, onset was defined as gradual.

<sup>b</sup> Defined as presence of cough, regardless of duration or type.

<sup>c</sup> Defined as a respiratory rate of >30 breaths/min.

<sup>d</sup> Defined as an axillary temperature of ≥37.8°C.

<sup>e</sup> Defined as the number of days of illness until symptoms and signs were absent.
among the children with *S. pneumoniae* infection (P = .0003 and P = .034, respectively).

**DISCUSSION**

This study emphasizes that both *S. pneumoniae* and atypical bacteria play a major role in causing CAP in young children. Using some of the most-advanced laboratory techniques for the identification of these pathogens in a large study population, we also showed the limited role of clinical, laboratory, and radiological features in predicting the etiology of CAP in pediatric patients.

Blood culture was not a part of the standard diagnostic workup in our study. However, no more than 20% of pediatric patients with pneumococcal CAP usually have positive blood culture results [35–37]. Given the methods that we used to diagnose pneumococcal infections, it is reasonable to suppose that most of our pneumococcal cases were nonbacteremic CAP, which are those usually found in routine practice. This is particularly important because data describing the characteristics of nonbacteremic pneumococcal CAP in the pediatric population are limited. In the children with pneumococcal infection who we evaluated, the classic presentation of bacteremic pneumococcal CAP appeared to be less common. This is in agreement with the results of previous studies showing that the clinical characteristics of pneumococcal CAP in young children may range from mild, nonspecific respiratory symptoms to severe respiratory distress and life-threatening disease [38, 39]. One of these studies also demonstrated that antibody measurements, compared with blood culture, can detect less severe cases [39].

Most of the data on the contribution of the different *S.
In this regard, the first pathogen to infect the child and the coccal CAP showed signs of coinfection with demonstrated that 10% and 23% of patients with pneumococcal CAP had mixed infections. Our data are in line with those published by Toikka et al. and Heiskanen-Kosma et al., who respectively considered that these are, in any case, pneumococcal infections, and they cannot be used to predict CAP etiology precisely.

Of note, we found a significant number of cases of CAP associated with mixed S. pneumoniae–atypical bacterial infections. Our data are in line with those published by Toikka et al. and Heiskanen-Kosma et al., who respectively demonstrated that 10% and 23% of patients with pneumococcal CAP showed signs of coinfection with M. pneumoniae. In this regard, the first pathogen to infect the child and the role of each of these organisms are the important unanswered questions. The same is probably the case for combined viral-bacterial infections, which appeared even more frequently than did combined infections with S. pneumoniae and atypical bacteria. Thus, some of the cases of CAP with an apparent single etiology in our series may also be combined with viruses, with at least the possibility that the virus was the primary pathogen. This may, in turn, explain why many of the findings make distinguishing etiology on the basis of clinical presentation difficult.

Results of routine laboratory tests also seem to be nonspecific parameters that may be affected by a number of physical, chemical, or microbial stimuli. Although they are significantly higher in association with pneumococcal infections, CRP levels and WBC counts cannot be used to discriminate between different etiologic agents because of the high degree of overlapping of the individual values in the different groups.

Radiological findings have the same limitations regarding sensitivity and specificity as do other diagnostic medical tests, and they cannot be used to predict CAP etiology precisely. Segmental or lobar consolidation, which usually is considered an indicator of pneumococcal infection, and reticulonodular infiltrate, which is thought to be characteristic of atypical bacterial infection, were also demonstrated in all of the other etiologic groups. These findings are particularly important because many pediatricians base their therapeutic decisions on findings of chest radiography, even though their appropriateness may be debatable.

The impossibility of routinely differentiating between infec-

### Table 5. Comparison of clinical outcomes for 196 evaluable children with community-acquired pneumonia, according to known bacterial etiology and antimicrobial therapy received.

<table>
<thead>
<tr>
<th>Treatment, clinical response</th>
<th>Streptococcus pneumoniae (n = 44)</th>
<th>Atypical bacteria and mixed S. pneumoniae (n = 15)</th>
<th>Atypical bacteria (n = 42)</th>
<th>Mixed S. pneumoniae and atypical bacteria (n = 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-Lactam monotherapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cure</td>
<td>28 (63.6)</td>
<td>21 (50.0)</td>
<td>4 (26.7)</td>
<td></td>
</tr>
<tr>
<td>Failure</td>
<td>27 (96.4)</td>
<td>11 (52.4)</td>
<td>2 (50.0)</td>
<td></td>
</tr>
<tr>
<td><strong>β-Lactam plus macrolide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cure</td>
<td>9 (20.5)</td>
<td>7 (16.7)</td>
<td>6 (40.0)</td>
<td></td>
</tr>
<tr>
<td>Failure</td>
<td>9 (100.0)</td>
<td>7 (100.0)</td>
<td>6 (100.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Macrolide monotherapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cure</td>
<td>14 (33.3)</td>
<td>1 (5.0)</td>
<td>5 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Failure</td>
<td>6 (85.7)</td>
<td>12 (92.9)</td>
<td>5 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Data are no. (%) of patients. Unless indicated, differences were not significant.

- a compared with atypical bacterial infection treated with β-lactam monotherapy.
- b compared with mixed S. pneumoniae-atypical bacterial infection treated with β-lactam monotherapy.
- c compared with atypical bacterial infection treated with β-lactam plus a macrolide.
- d compared with atypical bacterial infection treated with a macrolide.
tions due to *S. pneumoniae*, atypical bacteria, and mixed *S. pneumoniae* and atypical bacteria in clinical practice has consequences regarding making decisions about use of antibiotic therapy for children. Given the high probability that, in a young child, CAP is caused by at least 1 of these infections, therapy should cover all of the possibilities. Although the power of the study, when broken down by treatment group, may be somewhat lacking, we found that the use of macrolides either alone or in combination for the treatment of atypical bacterial infections leads to a better clinical outcome [25, 47]. On the other hand, macrolides are not always active in vitro against *S. pneumoniae*, and resistance of up to 50% has been reported in many geographic areas [48–50]. This means that, especially when strains with an ermB mechanism of resistance are prevalent, the risk in failure in the treatment of pneumococcal infections is possible [48–50]. Although the outcome of pneumococcal infections generally was good even when macrolides were prescribed to the patients in this study, a number of cases of postpneumonia bacteremia due to resistant strains of *S. pneumoniae* treated with macrolides recently have been reported [51–53]. On the basis of these considerations, the combination of a β-lactam plus a macrolide could be suggested in the first-line treatment of CAP in immunocompetent children aged 2–5 years.

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References

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