Community-Acquired Bacteremic Pneumococcal Pneumonia in Children: Diagnosis and Serotyping by Real-Time Polymerase Chain Reaction Using Blood Samples

Massimo Resti, Maria Moriondo, Martina Cortimiglia, Giuseppe Indolfi, Clementina Canessa, Laura Becciolini, Elisa Bartolini, Fernando Maria de Benedictis, Maurizio de Martino, and Chiara Azzari; for the Italian Group for the Study of Invasive Pneumococcal Disease

1Department of Pediatrics, University of Florence, and Anna Meyer Children’s Hospital, Florence, and 2Department of Pediatrics, Salesi Children’s Hospital, Ancona, Italy

(See the editorial commentary by Ramirez and Melo-Cristino, on pages 1050–1052.)

Background. The aim of this study was to use real-time polymerase chain reaction (RT-PCR) on blood samples to diagnose and serotype pneumococcal infection in a large cohort of Italian children hospitalized for community-acquired pneumonia.

Methods. We conducted an observational study from April 2007 through June 2009 of children aged 0–16 years with a diagnosis of community-acquired pneumonia admitted to 83 pediatric hospitals in Italy.

Results. Seven hundred fifty-three children were studied. RT-PCR found pneumococcal infection in 80 (10.6%) of 753 patients. In 292 patients, culture and RT-PCR were simultaneously performed. Streptococcus pneumoniae was identified in 47 of 292 patients; 45 (15.4%) tested positive by RT-PCR and 11 (3.8%) tested positive by culture. RT-PCR was significantly more sensitive than culture in revealing bacteremic pneumonia (odds ratio, 30.6; 95% confidence interval, 5.8–97.5; P < .001). Complicated pneumonia was found in 162 (21.5%) of 753 children; 152 (93.8%) of these 162 had parapneumonic effusion, and 51 (33.6%) had empyema. Children with complicated pneumonia were significantly older. Pneumococcal bacteremia was found by RT-PCR to occur significantly more frequently in children with complications (38 [23.5%] of 162) than in children with uncomplicated pneumonia (44 [7.4%] of 591; odds ratio, 3.8; 95% confidence interval, 2.30–6.30; P < .001). RT-PCR allowed serotyping from blood in 92.5% of patients. More than two-thirds of the pneumonia cases were due to nonpneumococcal conjugate vaccine 7 serotypes. Serotype 1 was the most frequent serotype (26 [32.5%] of 80) and was significantly associated with complications (50.0% in patients with complicated pneumonia vs 18.2% in patients with uncomplicated pneumonia; odds ratio, 4.5, 95% confidence interval, 1.48–14.03; P = .005) and older age. Serotype 19A was second in frequency (15.0%) and was significantly associated with younger age.

Conclusions. RT-PCR allows diagnosis and serotyping of pneumococcal bacteremic community-acquired pneumonia in children and is an important tool for evaluating serotype distribution in culture-negative samples.

Streptococcus pneumoniae is the most common cause of community-acquired pneumonia (CAP) in children and adults [1–7]. Available data on the incidence and serotype distribution of circulating S. pneumoniae have been obtained by serologic determination of the capsular type after culture or by molecular typing of isolates [8–10]. However, the effectiveness of these methods is limited because of the need of bacterial growth, which in turn depends on a sufficient load of viable bacteria in biological samples. Children with pneumonia are at low risk of bacteremia [11, 12]; furthermore, the use of antibiotic therapy on an outpatient basis at the beginning of a febrile episode significantly reduces the sensitivity of culture [13].
Molecular methods applied on blood samples or other body fluids can be efficiently used for diagnosing and serotyping invasive pneumococcal disease [13–17]. These methods do not require viable bacteria, need small sample volumes, and appear more sensitive than cultural methods; therefore, they could be a useful tool in the diagnosis and serotyping of pneumococcal pneumonia [18]. The aim of this study was to diagnose and serotype pneumococcal bacteraemic pneumonia using real-time polymerase chain reaction (RT-PCR) directly on blood samples in a large cohort of Italian children hospitalized with CAP and to compare the sensitivity of molecular methods versus culture-based methods.

**PATIENTS AND METHODS**

**Study design.** This observational study was conducted from April 2007 through June 2009 in children admitted with a diagnosis of CAP to pediatric hospitals or pediatric wards of general hospitals in Italy. Hospitals from all Italian regions were invited to participate.

**Case definition.** Pneumonia was suspected on the basis of clinical signs, such as tachypnea and abnormal breath sounds, and was confirmed by chest radiographic examination or computed tomography [19]. In collaboration with radiologists, a pediatric pulmonologist with experience in radiology who was unaware of the clinical and laboratory findings of the patients evaluated radiographs and assigned standardized and mutually exclusive diagnoses that included focal, segmental, or lobar consolidation with or without pleural effusion, interstitial pneumonia, atelectasis, or necrotizing pneumonia.

Complicated CAP was defined as the presence of ≥1 of the following: parapneumonic effusion defined as loculated pleural fluid on a chest radiograph film, a chest ultrasonogram, or computed tomography; any pleural fluid parameters consistent with empyema [19]; and/or atelectasis or necrotizing pneumonia [20]. Children were considered to be fully vaccinated if they had completed the national vaccination schedule, including 3 doses of the 7-valent conjugate anti–pneumococcal conjugate vaccine (PCV-7) at 3, 5, and 12 months or a single dose after the first year of life. Children were considered to be incompletely vaccinated if they had started but not completed the vaccine schedule.

**Patients and blood samples.** All patients aged 0–16 years admitted with a diagnosis of pneumonia to the participating centers during the study period were included in the study. The exclusion criteria were severe concomitant diseases (eg, kidney, liver, or cardiovascular disease, immunodepression, malabsorption syndrome, or neoplasia) and nosocomial acquired infections. To exclude the latter, children who had been admitted to the hospital or had been evaluated in the day hospital or emergency department in the previous 14 days were excluded from the study. Written informed consent was obtained from all parents or guardians. The study was approved by the local institutional review board.

Whole blood was obtained as soon as possible after hospital admission. Samples for RT-PCR were obtained from all patients; clinicians were allowed to choose when to also request blood cultures. For culture purposes, 4–6 mL of blood samples (up to 3 sets) were immediately sent to the local laboratory. Standardized procedures were used for collection and shipment of blood samples to local laboratories. Blood samples for molecular tests were sent at room temperature to the central laboratory (Immunology Laboratory, Anna Meyer Children’s University Hospital, Florence, Italy) with use of a fast freepost carrier. The samples were delivered by the following day, and the molecular tests were performed within 2 h after delivery; 200 µL of whole blood (not spun down) was used for both diagnosis and serotyping by RT-PCR.

**Diagnosis of bacteraemic pneumococcal pneumonia.** Diagnosis of laboratory-confirmed bacteraemic pneumococcal pneumonia was performed in the presence of culture positive for *S. pneumoniae* and/or RT-PCR positive for the *lytA* gene, as described elsewhere [15]. The cycle threshold (*C* <value>2 value) is the PCR cycle number (of 45) at which the measured fluorescent signal exceeds a calculated background threshold, identifying amplification of the target sequence. If there was no increase in fluorescent signal before the 45th cycle, the sample was assumed to be negative.

PCR specificity on whole blood had been preliminarily evaluated by testing blood samples drawn from 87 age-matched healthy controls. Pneumococcal DNA was never found in the blood of healthy children, either carriers or noncarriers of *S. pneumoniae* [21].

**Quantitative analysis of pneumococcal DNA.** To quantify the amount of bacterial DNA present in each sample, standards were prepared as described elsewhere [12] with use of suspensions of *S. pneumoniae* in phosphate-buffered saline. The amount of bacterial DNA was obtained by direct extrapolation of the *C* <value>2 values to the amount of DNA [12, 22] as read from the concentration versus the *C* <value>2 standard curve.

**Serotyping by RT-PCR.** Twenty-one primer/probe sets targeting different regions of the *CpsA* gene specific for 21 serotypes were used as described elsewhere [15]. If no increase in fluorescent signal was observed before the 45th cycle for any of the sets but both *lytA* in RT-PCR and *CpsA* in end point PCR [14, 15] were positive, the sample was reported as nontypeable. PCV-7 serotypes, PCV-7–related serotypes, or nonvaccine serotypes (including 19A) were defined according to Hsu et al [23].

**Statistical analysis.** Data were processed with the SPSSX 11.0 statistical package (SPSS); *P <0.05* was considered to be statistically significant. Results were expressed as mean levels and standard deviations or as median and interquartile range.
as appropriate. The χ² test was used to assess group differences in categorical variables. Odd ratios and 95% confidence intervals (CIs), when possible, were calculated. For continuous variables, Student’s t test was used with logarithmic transformation of nonnormal distributed variables.

RESULTS

Seven hundred fifty-three children with pneumonia (417 male children [55.4%]; median age, 3.8 years; interquartile range, 2.1–6.5 years) were included in the study; 177 (23.5%) were aged <2 years. Patients were recruited from 83 centers in 19 of 20 Italian regions. The only region that did not include any patient represents 0.2% of the Italian population aged <16 years.

Complicated and noncomplicated pneumonia. Complicated pneumonia was present in 162 patients (21.5%). The distribution of complications is given in Table 1. Complications were significantly associated with older age, lobar consolidation, increased C-reactive protein level, and neutrophil blood counts. Incidence of complications was not modified by preadmission antibiotic treatment (Table 2).

Diagnosis of bacteremic pneumococcal pneumonia by RT-PCR and/or culture. All 753 patients have been tested; 461 were tested by RT-PCR alone, and 292 were tested both by RT-PCR and culture. In the latter group, S. pneumoniae was found in 47 patients (16.1%); it was identified by RT-PCR in 45 patients (15.4%) and by culture in 11 patients (3.8%). Overall, S. pneumoniae was identified by RT-PCR only in 36 (76.6%) of 47 patients, by culture only in 2 (4.3%) of 47 patients, and by both tests in 9 (19.1%) of 47 patients. RT-PCR was significantly more sensitive than culture in achieving laboratory diagnosis of bacteremic pneumonia (odds ratio, 30.6; 95% CI, 5.8–97.5; P < .001, by χ² test; Cohen’s κ, 0.3; P < .001, by McNemar’s test).

Antibiotic treatment had been received before hospital admission by 6 (54.5%) of 11 patients whose conditions were diagnosed by cultures and by 55 (68.7%) of 80 patients whose conditions were diagnosed by RT-PCR (P = .925). The mean age of culture-positive or RT-PCR–positive patients was similar (5.0 years [range, 0.45–14.5 years] vs 3.7 years [range, 0.03–14.5 years]). Clinicians tended to request cultures in the more severe cases. Indeed, culture tests were requested by clinicians in 217 patients with uncomplicated pneumonia (36.7%) and in 75 patients with complicated pneumonia (46.2%) (odds ratio, 1.5; 95% CI, 1.03–2.14; P = .034). The RT-PCR result was positive in 45 patients (15.4%) in whom both culture and RT-PCR were performed and in 37 patients (7.6%) in whom culture was not performed.

Overall, 82 (10.9%) of the 753 patients had positive results for S. pneumoniae. Among them, 20 were aged <2 years (Table 3). No difference in the rate of bacteremic pneumonia was found between younger or older patients with either method (Table 3). Besides the 11 samples found positive for S. pneumoniae by culture, 3 of the 292 cultures tested positive for Staphylococcus epidermidis (n = 2) and Staphylococcus hominis (n = 1); they were reported as “bacterial contamination” by the laboratory microbiologist.

Association between complication and pneumococcal bacteremia. Demographic, clinical, and laboratory characteristics of the children according to demonstration of pneumococcal bacteremia by RT-PCR are given in Table 1. Increased levels of C-reactive protein and/or white blood cell count were associated with pneumococcal bacteremia. When complications were present in the children, RT-PCR was positive in 35 patients (46.2%) and RT-PCR was positive in 46 patients (47.4%) in whom culture was requested; RT-PCR was positive in 32 patients (45.1%) in whom culture was performed; and RT-PCR was positive in 44 patients (42.4%) in whom culture was performed and RT-PCR was requested. The rate of bacteremic pneumonia was not modified by preadmission antibiotic treatment (Table 2).

Table 1. Baseline Demographic, Clinical, and Laboratory Characteristics of the Children Enrolled According to the Isolation of Streptococcus pneumoniae in Blood Samples by Real-Time Polymerase Chain Reaction (RT-PCR)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Proportion (%) of patients</th>
<th>S. pneumoniae in blood samples by RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>C-reactive protein level, mean ± SD, mg/dL</td>
<td>...</td>
<td>17.80 ± 12.45</td>
</tr>
<tr>
<td>White blood cell count, mean ± SD, cells/µL</td>
<td>...</td>
<td>21,199 ± 9053</td>
</tr>
<tr>
<td>Neutrophil count, median ± SD, neutrophils/µL</td>
<td>...</td>
<td>16,301 ± 11,102</td>
</tr>
<tr>
<td>Age, median (range), years</td>
<td>...</td>
<td>4.2 (14 days to 14.5 years)</td>
</tr>
<tr>
<td>Sex, ratio F:M</td>
<td>...</td>
<td>40:40</td>
</tr>
</tbody>
</table>

Patients with pneumonia

|               | 753/753 (100.0) | 80/753 (10.6) | 673/753 (89.4) | ... | NS |

Patients with complicated pneumonia

|               | 162/162 (21.5) | 36/80 (43.3) | 126/673 (18.7) | 3.55 (2.14–5.90) | <.001 |
| Parapneumonic effusion | 152/162 (93.8) | 35/80 (43.7) | 117/673 (17.4) | 3.70 (2.21–6.17) | <.001 |
| Parapneumonic effusion with empyema | 51/162 (31.5) | 19/80 (23.7) | 32/673 (4.8) | 6.24 (3.19–12.17) | <.001 |
| Necrotizing pneumonia | 8/162 (4.9) | 3/80 (3.7) | 5/673 (0.7) | 5.21 (0.95–25.58) | .056 |
| Atelectasis | 8/162 (4.9) | 1/80 (1.2) | 7/673 (1.0) | ... | NS |

NOTE. Data are proportion (%) of patients, unless otherwise indicated. CI, confidence interval; NS, not significant; OR, odds ratio; SD, standard deviation.

a Defined as cloudy, bloody, or purulent appearance; white blood cell count >60,000 × 10⁶ cells/L; pH < 7.1; lactate dehydrogenase level >1000 IU/L; glucose level <40 mg/dL; positive Gram stain and/or culture and/or RT-PCR results [19].

b Including cases in which computed tomography showed pneumatoceles, cavitations, or liquefaction of the lung tissue.
count and/or neutrophil blood count were associated with complicated cases and with pneumococcal origin (Tables 1 and 2). Both culture and RT-PCR positivity were significantly associated with complications (Table 2).

**Quantitative analysis of pneumococcal DNA.** The median bacterial load was 2.16 log_{10} copies/mL (range, 1–5.88 log_{10} copies/mL). The bacterial load was significantly higher in culture-positive patients (median, 2.70 log_{10} copies/mL [range, 1.60–5.88 log_{10} copies/mL] vs 2.25 log_{10} copies/mL [range, 1.60–2.88 log_{10} copies/mL]; 95% CI, 0.06–1.16; P < .031) and tended to be higher in patients aged ≥2 years (Table 3); no difference in bacterial load was found between patients with complicated and noncomplicated pneumonia (Table 2). The C_{T} value ranged between 24 and 40.

**Serotyping.** RT-PCR allowed serotyping in 73 (91.2%) of 80 patients with bacteremic pneumonia, including 35 (97.2%) of 36 patients with complicated pneumonia and 38 (86.4%) of 44 with noncomplicated pneumonia. The distribution of serotypes in the whole population and in patients with complications is summarized in Figures 1 and 2, respectively. The 11 serotypes found in patients with positive cultures were the following: serotype 1 and 3 (2 isolates each) and serotypes 5, 6A, 8, 9V, 14, 18C, and 19A (1 isolate each). No discordance was found between molecular and culture serotyping.

Serotype 1 was the most frequent (26 [32.5%] of 80), followed by serotypes 19A (12 [15.0%] of 80) and 3 (10 [12.5%] of 80). Serotype 1 was significantly associated with complications (18 [50.0%] of 36 patients with complicated pneumonia vs 8 [18.2%] of 44 patients with uncomplicated pneumonia; odds ratio, 4.5; 95% CI, 1.48–14.03; P < .005) and age (median

### Table 2. Demographic and Laboratory Features According to the Presence of Complications

<table>
<thead>
<tr>
<th>Feature</th>
<th>Complicated pneumonia</th>
<th>Noncomplicated pneumonia</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean, years; median (IQR), years</td>
<td>6.0; 5.0 (1.9–6.2)</td>
<td>4.7; 3.7 (3.0–7.1)</td>
<td>...</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>C-reactive protein level, mean ± SD, mg/dL</td>
<td>21.0 ± 13.1</td>
<td>10.7 ± 9.5</td>
<td>...</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Neutrophil blood count, mean ± SD, neutrophils/µL</td>
<td>14,691 ± 8191</td>
<td>12,722 ± 8740</td>
<td>...</td>
<td>.024</td>
</tr>
<tr>
<td>Pneumococcal DNA quantitative analysis, mean (range), log_{10} copies/mL</td>
<td>2.32 (1–2.88)</td>
<td>2.30 (1.60–5.88)</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>Preadmission antibiotic treatment, no of patients (n = 401)</td>
<td>80</td>
<td>321</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>No preadmission antibiotic treatment, no of patients (n = 352)</td>
<td>82</td>
<td>270</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>Radiologic feature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lobar consolidation (n = 94)</td>
<td>31/94 (33.0)</td>
<td>63/94 (67.0)</td>
<td>1.95 (1.18–3.19)</td>
<td>.007</td>
</tr>
<tr>
<td>Other radiologic features (n = 659)</td>
<td>131/659 (19.9)</td>
<td>527/659 (80.0)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Culture result</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>7/11 (63.6)</td>
<td>4/11 (36.4)</td>
<td>5.6 (1.4–23.5)</td>
<td>.009</td>
</tr>
<tr>
<td>Negative</td>
<td>67/281 (23.8)</td>
<td>214/281 (76.2)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>RT-PCR result</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>36/80 (45.0)</td>
<td>44/80 (55.0)</td>
<td>3.6 (2.1–5.9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Negative</td>
<td>126/673 (18.7)</td>
<td>547/673 (81.3)</td>
<td>...</td>
<td></td>
</tr>
<tr>
<td>Result of either culture or RT-PCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>38/82 (46.3)</td>
<td>44/82 (53.7)</td>
<td>3.8 (2.3–6.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Negative</td>
<td>124/671 (18.5)</td>
<td>547/671 (81.5)</td>
<td>...</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** Data are proportion (%) of patients, unless otherwise indicated. CI, confidence interval; IQR, interquartile range; NS, not significant; OR, odds ratio; RT-PCR, real-time polymerase chain reaction; SD, standard deviation.

### Table 3. Clinical and Laboratory Characteristics of the Children According to Age (<2 years vs 2–16 years)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>&lt;2 years (n = 177)</th>
<th>2–16 years (n = 576)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complicated pneumonia (n = 162)</td>
<td>18/177 (10.2)</td>
<td>144/576 (25.0)</td>
<td>2.94 (1.70–5.14)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Rate of pneumococcal bacteremic pneumonia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>By culture</td>
<td>4/89 (4.6)</td>
<td>7/233 (3.0)</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>By RT-PCR</td>
<td>19/177 (10.7)</td>
<td>61/576 (10.6)</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>20/177 (11.3)</td>
<td>62/576 (10.8)</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>Bacterial load, median (range), log_{10} copies/mL</td>
<td>1.98 (1.0–2.98)</td>
<td>2.38 (1.0–5.88)</td>
<td>...</td>
<td>.08</td>
</tr>
</tbody>
</table>

**NOTE.** Data are proportion (%) of patients, unless otherwise indicated. CI, confidence interval; NS, not significant; OR, odds ratio; RT-PCR, real-time polymerase chain reaction.
Seven of 80 patients had nontypeable disease; among them, 3 could not be serotyped because of the paucity of the blood samples, and in 4 children, \textit{S. pneumoniae} was demonstrated not to belong to the 21 serotypes studied.

In younger children (<2 years) the distribution of pneumococcal serotypes was more homogenous, with frequencies ranging from 5.3% (serotypes 4, 5, and 18) to 15.8% (serotypes 19A and 19F). Serotype 19A was significantly associated with younger age (median age, 3.1 years; age range, 10 months to 3.7 years; vs. median age, 5.4 years; age range, 11 months to 14.3 years for non-19A serotypes; 95% CI, 0.21–2.61; \( P = .023 \)).

Vaccination status. Two hundred seventy (35.8%) of 753 patients were fully vaccinated, 387 (51.4%) were nonvaccinated, and 41 (5.4%) were incompletely vaccinated. Data regarding

\textbf{Figure 1.} Distribution of \textit{Streptococcus pneumoniae} serotypes in 80 patients with pediatric bacteremic pneumococcal community-acquired pneumonia. Figure 2. Percentages of \textit{Streptococcus pneumoniae} serotypes in children with and without complicated pneumonia.
vaccination were not available for 55 (7.3%) of 753 children. Among the 73 children with *S. pneumoniae* serotyped via molecular methods, 25 were vaccinated, 3 were incompletely vaccinated, and 40 were nonvaccinated. The data were not available for 5. Pneumonia due to PCV-7 serotypes or PCV-7–related serotypes (13 of 73 serotyped cases; 17.8%) was only found in the group of nonvaccinated children. No difference in the rate of complicated pneumonia was present among the groups; 87 complications (22.5%) were observed in 387 nonvaccinated patients, and 58 complications (18.6%) were observed in 311 (∼P = .252) fully or partially vaccinated children.

**DISCUSSION**

The present study, performed on a large cohort of >750 Italian children hospitalized for CAP in 83 hospitals in Italy, demonstrates a percentage of pneumococcal bacteremic pneumonia of >10% when RT-PCR is used directly on blood samples for diagnosis. RT-PCR appears significantly more sensitive than culture for diagnosis of bacteremic pneumococcal pneumonia.

RT-PCR is extremely sensitive, and its specificity is an important topic. In the present research, 2 different target genes (*lytA* and *Cps*) known to be the most specific [14, 15, 24] were used to increase the confidence in positivity. Moreover, we previously demonstrated that, using *lytA* as the target gene and RT-PCR, pneumococcal DNA is never found in the blood of healthy children, regardless of their carrier state [21]. Therefore, it seems that RT-PCR sensitivity does not come at the cost of reduced specificity.

Even though only 292 cases could be simultaneously studied by culture and RT-PCR, the present research demonstrates a frequency of pneumococcal bacteremic pneumonia higher (3.8%) than described elsewhere (1.6%–2%) [11, 25]. The difference may be dependent on the fact that, in the design of the study, clinicians were allowed to choose when to also request blood cultures, in addition to RT-PCR. As indicated, clinicians requested blood cultures more frequently in severe cases. The selection bias is in favor of culture sensitivity (and, therefore, it obviously goes toward the null hypothesis of the study, which was aimed to demonstrate the higher sensitivity of RT-PCR vs the culture-based method); nonetheless, the higher sensitivity of RT-PCR has been demonstrated. Actually, RT-PCR demonstrated a frequency of pneumococcal bacteremic pneumonia of ~10% when all pneumonia cases are included, which increased up to 16% in the group of patients in whom molecular and cultural tests were performed simultaneously. In the clinical approach, on-the-field, molecular tests appear to have a sensitivity 4 times that of culture. The advantage may in part be attributable to the preadmission use of antibiotics, which undoubtedly reduces culture sensitivity [13] yet seems to have a lower effect on RT-PCR sensitivity, even though the difference does not reach statistical significance. The limited number of positive cultures did not permit statistical analyses; however, the distribution of culture-positive isolates in different serotypes suggests that the advantage of PCR is not serotype specific.

The C-reactive protein level, white blood cell count, and/or neutrophil blood count were significantly associated with pneumococcal cause and complications. However, given the wide range and overlapping of values, those biomarkers can hardly be used to predict cause or complications in a single patient.

Quantitative analysis demonstrated, as expected, that bacterial load is significantly higher in culture-positive patients and tended to be higher in older children. On the contrary, no difference in bacterial load was found between patients with complicated versus noncomplicated pneumonia. It may be suggested that factors other than bacterial load, depending on the single serotype aggressiveness or on the host response, are more important in causing complications.

Serotype distribution demonstrated a large preponderance of serotype 1, followed by serotypes 19A and 3. The data obtained by serotyping directly from blood samples reached results consistent with those obtained by serotyping of isolates in western countries [26]. Serotype 1 appeared significantly associated with complications and older age. The important role of serotype 1 in all pneumonia cases and especially in complicated ones had been suggested by other reports [19, 26–28]; the present data show for the first time, to our knowledge, that in Italy serotype 1 alone currently causes more than one-third of pneumococcal bacteremic pneumonia cases and 50% of complicated cases. Serotype 19A prevalence is 15%, with a significantly higher prevalence in younger children. Serotype 14, which represented one of the most frequent serotypes in Italy in the past decade [29, 30] now has a prevalence of <5%. A shift toward nonvaccine serotypes has been described after mass vaccination [31] and is associated with a decrease in the number of cases due to vaccine serotypes [3, 32]. This is not the case in Italy, where vaccination is limited to some regions, overall covering <30% of the country. Therefore, it is unlikely that a shift attributable to PCV-7 vaccination is the only cause for the serotype 1 and 19A increase. A spontaneous increase in serotype 1 frequency, as demonstrated in the prevaccination era in Belgium [33] and the secular trend demonstrated for different serotypes in the United States [34, 35], may probably contribute to the phenomenon observed in Italy. PCV-7 serotypes were never found in fully or partially vaccinated children. However, at present, vaccines other than PCV-7 are needed in Italy. Actually, >65% of all pneumonia cases and >75% of complicated cases are due to serotypes that are not included in the PCV-7 but are included in the 13-valent formulation.

The present study showed a percentage of complicated CAP of >20%, consistent with results obtained in other western countries. In recent years, the percentage of complicated CAP
has appeared to be growing in the world [25, 36, 37], increasing from 14% to 26% in the United States [19], and a role of aggressive serotypes of S. pneumoniae has been hypothesized. Our data demonstrate that pneumococcal bacteremia was 3 times more frequent in complicated CAP and that serotype 1 is prevalent. This finding is confirmed by the separate analysis on the group (177 patients) of younger children (aged <2 years), which showed that even though the rate of bacteremic pneumococcal pneumonia is similar to that of older children, the rate of complication is significantly lower. This is probably attributable to high frequency of more aggressive pneumococcal serotypes in older children, such as serotype 1, which was never found in children aged <2 years.

Another study [7] suggested that an expansion of a prevaccination clone more than new clones is responsible for the diffusion of complicated CAP due to serotype 1. Sequencing of bacterial DNA from the 26 cases due to serotype 1 is now ongoing in our laboratory to evaluate whether a similar condition is present in Italy.

Among the causes of CAP, S. pneumoniae, although presumably the most common bacterial cause, still remains underestimated because nonbacteremic pneumococcal CAP is probably the cause in most cases [6, 25, 38]. Surrogate biomarkers have been studied, but the search for specific antibodies or urine antigens has often produced frustrating results [39–42]. Even though RT-PCR performed on blood samples cannot identify all pneumococcal pneumonia, being limited to finding bacteremic cases, it is undoubtedly an important tool in obtaining a reliable monitoring of circulating serotypes. Moreover, because of the high sensitivity of this method, using molecular serotyping directly on blood samples allows the acquisition of a higher number of bacteremic pneumococcal pneumonia in a narrower time window. Actually, given the frequency of culture-positive results demonstrated in the present study, a population of >3000 patients with pneumonia would probably have been necessary to get 80 positive cases with culture-based methods. RT-PCR is simple and known to be less expensive [15] than culture-based methods. In respect to multiplex sequential PCR, it could also be a further improvement [15] for countries in the developing world, where molecular methods have demonstrated [17, 43] a sensitivity that is significantly higher than that of culture-based methods.

ITALIAN GROUP FOR THE STUDY OF INVASIVE PNEUMOCOCCAL DISEASE

Agostiniani R, Pistoia; Allievi P, Carbagnate Milanese; Allà G, Ragusa; Amigoni A, Ravenna; Baldo E, Rovereto; Barlocco G, Grosseto; Bernardi P, Bologna; Bernardini R, Empoli; Biban P, Borgotrento; Bossi G, Pavia; Bottone U, Massa; Cardinale A, Montevarchi; Cardona A, Foligno; Castelli Gattinara G, Roma; Celandroni A, Pontedera; Chiossi M, Cuneo; Colleselli P, Vicenza; Correra A, Napoli; D’Ascola G, Arezzo; D’Aquino A; Udine; Dollfus L, Terni; Domenici R, Lucca; Flacco V, Furbetta M, Perugia; Gaetti MT, Jesi; Gagliardi L, Viareggio; Ghiori F, Firenze; Giani L, Montepulciano; Giglio P, Gubbio; Guala A, Verbania; Icardi G, Genova; Lanciano; Lanari M, Imola; Lippi E, Firenze; Lizzoli C, Magenta; Lombardi E, Firenze; Macchia PA, Pisa; Magnni M, Esine; Memmigini G, Carrara; Mesirca P, Montebelluna; Mieleletti E, Livorno; Migliozzi L, Senigallia; Nunziata E, Aversa; Osimani P, Ancona; Paravati D, Crotone; Pecile P, Udine; Pepe G, Gallipoli; Peris A, Firenze; Perri P.F, Macerata; Pescoluderung B, Bolzano; Pezzati M, Firenze; Poggi G.M., Firenze; Poggiolesi C., Firenze; Prato R, Foggia; Principi N, Milano; Rapisardi G, Firenze; Ridi F, Firenze; Riva A, Brescia; Rizzo L, Portoferrio; Roman B, Vimercate; Toffolo A, Oderzo; Strano M, Borgo San Lorenzo; Trapani S, Firenze; Vassarri P, Prato; Vascotto M., Siena; Vecchi V, Rimini; Ventura A, Trieste; Verini M., Chieti; Zorzì C., Camposanpiero.

Acknowledgments

We are sincerely grateful to Giancarlo Perferi and Claudia Valieriani for their technical support.

Financial support. The research has been partially supported by the Italian Department of Health, Italian Center for Disease Control and Prevention (grant 117–19.01.07-#6728 to C.A.).

Potential conflicts of interest. All authors: no conflicts.

References

10. Pai R, Gertz RE, Beall B. Sequential multiplex PCR approach for de-