ABSTRACT. 

Objective. Streptococcus pneumoniae remains the most common cause of occult bacteremia, bacterial pneumonia, and meningitis in young febrile children. We sought to determine the utility of a pneumococcal urine antigen assay among young febrile children at varying risk of invasive pneumococcal disease.

Methods. We prospectively enrolled 5 groups of children, 3 months to 5 years of age, who presented to an urban pediatric emergency department or hospital-based clinic between January 1, 2000, and April 1, 2001. The groups enrolled included 1) children with pneumococcal bacteremia, 2) febrile children with pneumonia, 3) febrile nonbacteremic children with leukocytosis, 4) febrile nonbacteremic children with normal white blood cell (WBC) counts, and 5) afebrile children with no evidence of current or recent bacterial infection.

Results. Of 346 children enrolled, positive assay results were found in 23 (95%) of 24 with pneumococcal bacteremia (95% confidence interval [CI]: 77%–100%), 47 (76%) of 62 with lobar pneumonia (95% CI: 63%–85%), 28 (15%) of 181 nonbacteremic children with fever (95% CI: 11%–22%) with no difference among patients with elevated WBC counts (18%; 95% CI: 11%–27%) compared with those with normal WBC counts (11%; 95% CI: 5%–21%), and 6 of 79 patients without fever (8%; 95% CI: 3%–16%).

Conclusions. This S pneumoniae antigen detection assay demonstrated high sensitivity for proven (bacteremic) and suspected (focal pneumonia) invasive pneumococcal infections. The rate of false-positive test results among febrile children without identified pneumococcal infection is approximately 15%. Although not ideal, this combination of sensitivity and specificity compares favorably with other available tests, such as the WBC or absolute neutrophil count used to screen children for clinically unsuspected pneumococcal infections. Pediatrics 2003;112:1279–1282; Streptococcus pneumoniae, pneumonia, fever, detection, urine.

ABBREVIATIONS. CI, confidence interval; WBC, white blood cell; ED, emergency department.

From the Divisions of *Emergency Medicine and ‡Infectious Diseases, Children’s Hospital, Boston, Massachusetts.

Received for publication Feb 13, 2003; accepted May 8, 2003.

Reprint requests to (M.I.N.) Department of Emergency Medicine, Children’s Hospital, 300 Longwood Ave, Boston, MA 02115. E-mail: mark.neuman@ tch.harvard.edu

PEDIATRICS (ISSN 0031 4005). Copyright © 2003 by the American Academy of Pediatrics.

METHODS

We prospectively enrolled 5 groups of children, 3 months to 5 years of age, who presented to an urban pediatric emergency department (ED) or hospital-based clinic between January 1, 2000, and April 1, 2001. The groups enrolled included 1) children with pneumococcal bacteremia, 2) febrile children with pneumonia, 3) febrile children with focal consolidation on chest radiograph, 4) febrile nonbacteremic children with leukocytosis, 5) febrile nonbacteremic children with a normal WBC count, and 6) afebrile children with no evidence of current bacterial infection. We have previously reported the results of this assay among patients with bacteremia as well as those serving as afebrile control subjects and include the results in this study for comparison.1 Any patient who was seen in the ED or hospital-based clinic was considered eligible for enrollment. Enrollment criteria, which differed for each of the 5 groups, are summarized below.

Bacteremic Group

Patients with bacteremia were identified by the microbiology laboratory and reported to 1 of the study investigators. Once a diagnosis of bacteremia had been made, the patient’s medical record was reviewed, and clinical and laboratory information
including discharge diagnosis (from the ED) was established. Patients with a discharge diagnosis of fever without an obvious source (excluding otitis media) were considered to have occult bacteremia. During the study period, all urine specimens that were sent to the microbiology laboratory for culture were plated on culture media, and the remainder were stored in a refrigerator (3°C–5°C) for a period of 48 hours after collection. When patients were identified as having pneumococcal bacteremia, their urine specimen, if available, was retrieved within this 48-hour window. When no urine was available in the laboratory for those patients with pneumococcal bacteremia who were admitted or returned for reevaluation, the families were approached and urine was collected for the study after written informed consent was obtained.

Lobar Pneumonia

Febrile children (age ≤5 years) with a focal consolidation or lobar infiltrate on chest radiograph were considered eligible for enrollment. Fever was defined as temperature ≥38.0°C documented in the ED or reported by a caregiver. When notified of positive radiographic findings, 1 of the investigators approached the patients and their families and asked them to participate in the study. After written informed consent was obtained, a urine sample was collected during this ED visit. In addition, 1 of the investigators reviewed daily ED logs to identify children who had pneumonia and were not enrolled at the time of their visit. When the patient had submitted a urine sample as a part of routine care during the visit and there was remaining urine that could be retrieved, the patient was included in the study.

Febrile Nonbacteremic Patients With Leukocytosis and Febrile Nonbacteremic Patients With Normal WBC Counts

For the purposes of this study, leukocytosis was defined as WBC ≥20 000/mm³, and normal WBC count was defined as WBC ≤10 000/mm³. Daily logs of all children who were 3 to 36 months of age and identified to have a WBC ≥20 000/mm³ or WBC ≤10 000/mm³ were reviewed by 1 of the study investigators (M.I.N.). For patients who were found to have either, the ED record was reviewed to determine whether the patient had fever (temperature ≥39.0°C documented in the ED or report of fever by a caregiver) and whether a urine specimen was obtained as part of the evaluation of his or her fever. At our institution, most febrile boys younger than 6 months and most febrile girls younger than 2 years without a known source for fever will have a urine specimen obtained for urinalysis and urine culture. Patients was considered eligible for inclusion in the study when they had fever and a urine specimen remaining from the original visit after clinical tests were completed. Any child who met definition for the bacteremia or pneumonia group was not eligible for inclusion into these groups.

Afebrile Children

This group consisted of patients without fever and with no identified focal bacterial infection (other than urinary tract infection). Any patient who was seen in the ED or hospital-based clinic during the hours that 1 of the study investigators was available for enrollment. We excluded from the control group patients with a recent (previous 4 weeks) diagnosis of bacteremia, pneumonia, meningitis, septic arthritis, acute otitis media, or other suspected infection caused by S pneumoniae. Any patient who met eligibility criteria was approached by 1 of the 2 study investigators and asked to provide a urine sample after written informed consent was obtained. Informed consent was obtained from the parents or guardians of all children who submitted a urine sample solely for the purposes of our study.

Laboratory Methods

Blood for culture was inoculated into Bactec (Sparks, MD) aerobic Peds Plus bottles. One milliliter of blood is the routinely recommended volume for inoculation at our institution. All urine specimens were stored in the microbiology laboratory (3°C–5°C), and the rapid urine pneumococcal antigen assay (Binax NOW) was run in batches (every 2 weeks). Study investigators performed the assay in a blinded manner (blinded to diagnosis and study group). The test device contains an immunochromatographic membrane that is used to detect soluble pneumococcal antigen in human urine. Rabbit anti-S pneumoniae antibody is adsorbed onto the nitrocellulose membrane as a sample line. Goat anti-rabbit immunoglobulin G, the control line, is adsorbed onto the same membrane as a second stripe. Urine was added to the test plate, followed by 3 drops of a buffer solution (citrate/phosphate buffer with sodium lauryl sulfate, Tween 20, and sodium azide). Pneumococcal antigen present in the urine reacts to bind anti-S pneumoniae conjugated antibody. The resulting antigen-conjugate complexes are captured by immobilized anti-S pneumoniae anti-body, forming the sample line. Immobilized goat anti-rabbit immunoglobulin G captures excess visualizing conjugate, forming the control line.

Assays were interpreted at 15 minutes as per recommendations by the manufacturer. A negative sample produced a single pink-to-purple-colored control line. A positive sample produced 2 pink-to-purple-colored lines. Any visible sample line was regarded as a positive result. When no lines were seen or just the sample line was seen, the assay was regarded as invalid and was repeated using the same urine sample. The study investigators performed all assays. Before patient enrollment, control swabs provided by the manufacturer were used to perform quality assurance measurements. The manufacturer provided support only in the form of test kits and positive and negative control swabs. Quality control measurements were performed before analysis of each sample batch.

Statistical Analysis

The main outcome of the study is the diagnostic test characteristics of this pneumococcal antigen assay among children at varying risk of invasive pneumococcal disease. Descriptive statistics for the sample are presented as median and interquartile range as the data in each of the categories was determined to be not normally distributed (SPSS 9.0; SPSS, Chicago, IL). CIs surrounding proportions were calculated using STATA 7.0 (College Station, TX). For evaluating interrater reliability on test interpretation, 10 samples were analyzed independently by each of the 2 study investigators. The agreement as a result of chance was evaluated using the κ statistic. The study was approved by the Institutional Review Board.

RESULTS

A total of 346 patients were enrolled during the study period: 24 children with pneumococcal bacteremia, 62 with pneumonia, 110 with fever and leukocytosis, 71 with fever and normal WBC count, and 79 without fever or recent infectious complaints. No samples were deemed invalid. There was complete agreement between study investigators on interpreting the results of the assay (κ: 1.0) among the 10 sample specimens.

The characteristics of the enrolled children are presented in Table 1. The median age of the cohort was 1.1 year (interquartile range: 0.7–2.6 years). The median age of children in the afebrile control group (2.8 years) differed from that of the other groups (1.2 years) because no patients were catheterized solely for the purpose of obtaining study samples. In addition to the group of febrile children with leukocytosis, elevated WBC counts were observed among patients within the pneumonia group (median: 23 700/ mm³) and the bacteremia group (median: 18 000/ mm³).

Results of antigen testing among the 5 groups are shown in Table 2. Children with bacteremia were most likely to have a positive result (96%), followed by those with lobar pneumonia (76%). The control populations had positive results of 15% and 8% in the febrile and afebrile groups, respectively. No statistically significant difference was noted among fe-
brile nonbacteremic normal WBC and febrile nonbacteremic leukocytosis groups.

Twenty patients with otitis media were included in the 2 febrile nonbacteremic groups. Five (33%) of 15 patients (95% CI: 12%–62%) with otitis media and leukocytosis tested positive with this assay, and 1 (20%) of 5 patients (95% CI: 1%–72%) with otitis media and normal WBC count tested positive. When patients who had a diagnosis of otitis media were removed from these groups, there was no significant change in the rate of positive assay: 13% (95% CI: 8%–19%) overall were positive with 15% (95% CI: 8%–24%) and 11% (95% CI: 4%–21%) positive among febrile children with and without leukocytosis respectively.

**DISCUSSION**

Although studies using this urine antigen assay among adults with pneumococcal pneumonia, bacteremia, and meningitis have shown excellent sensitivity and specificity, studies in children have yielded varying results. In our preliminary investigation, we found this assay to be highly sensitive in identifying children with pneumococcal bacteremia, as well as specific among children without fever or recent infectious complaints. However, studies of children conducted in regions with high rates of nasopharyngeal colonization with S. pneumoniae have concluded that the assay may not be useful as a result of the high rate of assay positivity among children with colonization but without invasive disease.

We found that among febrile children with lobar infiltrates on chest radiograph, 76% had pneumococcal antigen detected in their urine using this assay. This is higher than the 35% of children with pneumonia (not defined as having focal consolidation) having a positive assay reported by Dowell et al. Our results are more similar, however, to the rates reported by Michelow et al of 55% positive among children with lower respiratory infections (the majority had focal consolidation on chest radiograph) and 88% (7 of 8) positive by urine antigen testing among children with culture-confirmed pneumococcal disease. We believe that this provides strong direct evidence that the majority of febrile young children with a lobar infiltrate on chest radiograph have pneumococcal rather than viral pneumonia. This also suggests that the high rate of clinically unsuspected pneumonia previously reported among highly febrile young children with leukocytosis likely represents pneumococcal infections.

Unfortunately, although the rate of false-positive tests was low among afebrile children, the clinical usefulness of the test is limited by the finding that 15% of the febrile children without identified invasive pneumococcal disease also tested positive for pneumococcal antigen using this assay. It is not entirely clear why the pneumococcal urine antigen test performs less well among febrile children than among afebrile children without infectious complaints. We surmise that some of these false-positive tests may have been attributable to spontaneously resolved or clinically unrecognized pneumococcal infections or false-negative blood cultures, but this is unproved. It is also possible that children with nasal colonization with pneumococci have an increased risk of having pneumococcal antigen reach the urinary tract. This could be especially likely in the setting of an upper respiratory tract viral infection, which may disrupt the usual nasal mucosal barriers. This disruption in mucosal barriers could then result in pneumococci or pneumococcal antigens transiently entering the bloodstream and subsequently being cleared in the urinary tract without necessarily causing pneumococcal infection.

Nonetheless, the sensitivity and specificity demonstrated among our test groups do suggest that this pneumococcal antigen test may be preferable to the WBC count as a screening tool to identify occult pneumococcal bacteremia and clinically unsuspected pneumonia. Certainly, in the child who is having urine collected, this test can serve as an alternative to venipuncture or finger prick for WBC count when used to screen febrile children who are considered to be at risk of pneumococcal disease. Practitioners who wish to use this assay as a screen for occult pneumococcal bacteremia must be wary about interpreting the results among children with otitis media.

Several limitations are inherent to our study design. Because of the relatively small sample size, our 95% CIs around the estimates for rate of positive assay result are wide. Sensitivity and specificity may depend, in part, on pneumococcal nasopharyngeal

---

**TABLE 1.** Baseline Clinical and Laboratory Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Median Age (Years)</th>
<th>Gender (% Male)</th>
<th>Median Temperature (°C)</th>
<th>Median WBC (×1000/mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteremia (n = 24)</td>
<td>1.0 (0.7–2.0)</td>
<td></td>
<td>39.1 (37.1–39.9)</td>
<td>18.0 (15.7–26.4)</td>
</tr>
<tr>
<td>Fever, pneumonia (n = 62)</td>
<td>1.7 (0.8–3.3)</td>
<td>53</td>
<td>39.1 (37.9–39.7)</td>
<td>23.7 (16.9–28.6)</td>
</tr>
<tr>
<td>Fever, high WBC (n = 110)</td>
<td>0.8 (0.5–1.2)</td>
<td>32</td>
<td>39.1 (38.3–39.7)</td>
<td>23.1 (21.0–27.0)</td>
</tr>
<tr>
<td>Fever, normal WBC (n = 71)</td>
<td>1.2 (0.6–1.8)</td>
<td>27</td>
<td>39.2 (38.5–40.0)</td>
<td>7.0 (5.4–8.7)</td>
</tr>
<tr>
<td>Afebrile (n = 79)</td>
<td>2.8 (0.9–4.5)</td>
<td>56</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA indicates not applicable.
Values in parentheses represent interquartile range.

**TABLE 2.** Analysis of Assay Results

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Positive Assay (%; 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteremia</td>
<td>24</td>
<td>23 (96; 77–100)</td>
</tr>
<tr>
<td>Fever, lobar pneumonia</td>
<td>62</td>
<td>47 (76; 63–85)</td>
</tr>
<tr>
<td>Fever, no invasive infection</td>
<td>181</td>
<td>28 (15; 11–22)</td>
</tr>
<tr>
<td>Fever, high WBC</td>
<td>110</td>
<td>20 (18; 11–27)</td>
</tr>
<tr>
<td>Fever, normal WBC</td>
<td>71</td>
<td>8 (11; 5–21)</td>
</tr>
<tr>
<td>Afebrile</td>
<td>79</td>
<td>6 (8; 3–16)</td>
</tr>
</tbody>
</table>

and 88% (7 of 8) positive by urine antigen testing among children with culture-confirmed pneumococcal disease. We believe that this provides strong direct evidence that the majority of febrile young children with a lobar infiltrate on chest radiograph have pneumococcal rather than viral pneumonia. This also suggests that the high rate of clinically unsuspected pneumonia previously reported among highly febrile young children with leukocytosis likely represents pneumococcal infections.

Unfortunately, although the rate of false-positive tests was low among afebrile children, the clinical usefulness of the test is limited by the finding that 15% of the febrile children without identified invasive pneumococcal disease also tested positive for pneumococcal antigen using this assay. It is not entirely clear why the pneumococcal urine antigen test performs less well among febrile children than among afebrile children without infectious complaints. We surmise that some of these false-positive tests may have been attributable to spontaneously resolved or clinically unrecognized pneumococcal infections or false-negative blood cultures, but this is unproved. It is also possible that children with nasal colonization with pneumococci have an increased risk of having pneumococcal antigen reach the urinary tract. This could be especially likely in the setting of an upper respiratory tract viral infection, which may disrupt the usual nasal mucosal barriers. This disruption in mucosal barriers could then result in pneumococci or pneumococcal antigens transiently entering the bloodstream and subsequently being cleared in the urinary tract without necessarily causing pneumococcal infection.

Nonetheless, the sensitivity and specificity demonstrated among our test groups do suggest that this pneumococcal antigen test may be preferable to the WBC count as a screening tool to identify occult pneumococcal bacteremia and clinically unsuspected pneumonia. Certainly, in the child who is having urine collected, this test can serve as an alternative to venipuncture or finger prick for WBC count when used to screen febrile children who are considered to be at risk of pneumococcal disease. Practitioners who wish to use this assay as a screen for occult pneumococcal bacteremia must be wary about interpreting the results among children with otitis media.

Several limitations are inherent to our study design. Because of the relatively small sample size, our 95% CIs around the estimates for rate of positive assay result are wide. Sensitivity and specificity may depend, in part, on pneumococcal nasopharyngeal
We report on the sensitivity and specificity of this assay among different groups of children. As with any diagnostic tool, the positive and negative predictive values, as well as the posttest probability of disease, depend on the prevalence of disease in the cohort being tested. Thus, as more patients become immunized, if there is a decrease in the occurrence of pneumococcal disease as expected, then there will be more false-positive assay results as compared with true positive results, resulting in a decreased positive predictive value. Any decision to treat a child presumptively on the basis of a positive test result must take this fact into consideration.

This *S. pneumoniae* antigen detection assay demonstrated high sensitivity for the defined invasive pneumococcal infections (bacteremia and lobar pneumonia) as well as a high specificity among febrile children without infection. The rate of false-positive test results among febrile children is not ideal, being in the range of 15%. This sensitivity and specificity compare favorably to measurements of WBC count or absolute neutrophil count in the detection of patients at risk for occult bacteremia.

ACKNOWLEDGMENTS

This work was presented in part at the American Academy of Pediatrics Annual Meeting, 2001, San Francisco, CA; was funded by National Institutes of Health training grant T32 HD40128-01; and was supported in part by Binax, Inc (Portland, ME).

REFERENCES