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Usefulness of Urinary Antigen Detection by an Immunochromatographic Test for Diagnosis of Pneumococcal Pneumonia in Children

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We evaluated an immunochromatographic assay detecting pneumococcal antigen in urine samples from children diagnosed with pneumococcal pneumonia. The sensitivity and specificity of the immunochromatographic test with nonconcentrated urine (NCU) were 86.7 and 62.9%, respectively; with concentrated urine (CU), they were 100 and 11.7%, respectively. Pneumococcal antigen was also detected in 42.5% of NCU and 87.1% of CU samples from nasopharyngeal carriers. This is a nonspecific test for the diagnosis of pneumococcal pneumonia in children, particularly the very young.

Streptococcus pneumoniae is presumed to be the main bacterial cause of community-acquired lower respiratory infections among children (2, 9, 12). The severity of pneumococcal diseases heightens the importance of the identification of children with pneumococcal infections (10). Detection of *S. pneumoniae* antigen in urine samples is an alternative for the diagnosis of pneumococcal pneumonia. We assessed the utility of a rapid immunochromatographic membrane test (ICT) (Binax Now *S. pneumoniae* urinary antigen test; Binax, Portland, Maine) for detecting C-polysaccharide (PnC) *S. pneumoniae* antigen in urine samples from children diagnosed with pneumococcal pneumonia. We also studied whether the status of pneumococcal nasopharyngeal carriage could interfere with the performance of the ICT.

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Patient groups. In all cases of pneumonia, clinical and radiological changes compatible with acute pneumonia were present. Urine samples included in the study were classified according to the following groups of patients: for group 1, we studied 15 urine samples from 15 children (nine male and six female) with pneumonia caused by *S. pneumoniae*. The mean age in this group was 66.3 months (range, 6 to 192 months). In four cases, the pneumonia was bacteremic, in which *S. pneumoniae* was isolated by Pedi-Bact blood culture (BioMérieux SA, Marcy-l'Etoile, France), and identification was based on the usual criteria (14). In the remaining 11 cases, the pneumonia was nonbacteremic, in which the diagnostic criterion was the detection of pneumococcal capsular antigen (PCA) in urine by counterimmunoelectrophoresis (CIE) (4).

Group 2 consisted of 35 urine samples from 35 patients (17 male and 18 female) with pneumonia caused by *Mycoplasma pneumoniae*. The mean age was 79.8 months (range, 27 to 186

months). Children were diagnosed by specific antibody (immunoglobulin M [IgM] and IgG) detection by agglutinating gelatin particles sensitized with *M. pneumoniae* cell membrane components (Serodia Myco II particle agglutination test; Fujerebio Inc., Tokyo, Japan). The diagnostic criterion was a fourfold rise in titer to 1/160 or greater. The detection of PCA in urine samples by CIE was negative in all cases.

The third group comprised 40 urine samples from 40 healthy children with nasopharyngeal pneumococcal carriage (27 male and 13 female). The mean age in this group was 50.5 months (range, 3 to 168 months). Pneumococcal carriage status was determined by nasopharyngeal culture. Nasopharyngeal specimens were obtained by using a calcium alginate fiber-tipped swab that was then placed in transport medium. Swabs were immediately plated onto Trypticase agar medium containing 5% sheep blood and incubated in the presence of 5% CO_2 at 35°C for 48 h (14). Pneumococcal isolates were identified by their susceptibility to a 5-μg-optochin disk and by positive latex agglutination (Slidex Pneumo-kit; BioMérieux SA). Cultures were semiquantified by recording the number of colonies grown.

Finally, group 4 contained 41 urine samples from 41 healthy children without pneumococcal nasopharyngeal carriage (27 male and 14 female). Their mean age was 72.9 months (range, 2 to 168 months).

Children from groups 3 and 4 were considered ineligible if they had a recent history of otitis media, acute respiratory infection, documented pneumococcal infection, or immunization with any pneumococcal vaccine. In both groups of patients urine and nasopharyngeal samples from each patient were collected on the same day. The detection of PCA in urine samples by CIE was negative in all cases.

Sample treatment for antigen detection. Urine specimens were collected and frozen at −20°C until use and thawed immediately before being used. Urine samples were boiled for 5 min and centrifuged at 1,000 × *g* for 15 min to prevent nonspecific reactions (6). The antigen present in urine was concentrated 25-fold by selective ultrafiltration (Urifil-10 concentrator; Millipore Corporation, Bedford, Mass.). This sam-

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ple treatment protocol is not described in the manufacturer's procedures.

Binax Now *S. pneumoniae* antigen test. The ICT was performed according to the manufacturer's instructions.

Detection limit. In order to determine the lowest concentration of PnC detectable by the ICT, serial dilutions of purified PnC (Statens Serum Institut, Copenhagen, Denmark) were used. In addition, we assessed the performance of the test by using serial dilutions of the pneumococcal vaccine PNU-Immune (Cyanamid GmbH, Wolfratshausen, Germany), which contains 25 μg of each of the 23 most prevalent pneumococcal polysaccharide types.

Statistical analysis. Differences in the ICT results among patients in groups 3 and 4 were analyzed by Fisher's exact test (one-tailed). To assess the distribution of the number of colonies grown in nasopharyngeal culture among ICT-positive and ICT-negative pneumococcal carrier children, the chi-square test was performed; nonparametric tests (Mann-Whitney) were applied to compare the median age in these two groups. For all the statistical analyses a 0.05 significance level was used. Data were analyzed with SPSS 10.0 software (SPSS Inc., Chicago, Ill.).

The lowest concentrations of purified PnC and of the mixture of the 23 most prevalent pneumococcal polysaccharide types were 0.5 and 11.5 ng/ml, respectively.

S. pneumoniae was detected in 13 of 15 nonconcentrated urine (NCU) samples from patients with pneumococcal pneumonia (86.7%) and in 14 of 14 concentrated urine (CU) samples (100%). For patients with a diagnosis of *M. pneumoniae*, a positive result by ICT was obtained in 13 of 35 NCU samples (37.1%) and in 30 of 34 CU samples (88.2%). The specificity of the ICT with NCU was 62.9%; use of CU decreased this specificity to 11.7%.

Pneumococcal antigen was also detected in 17 of 40 NCU samples (42.5%) and 27 of 31 CU samples (87.1%) from nasopharyngeal carriers and in 4 of 41 NCU samples (9.7%) and 18 of the 26 CU samples (69.2%) from non-nasopharyngeal carriers. There were significant differences between the numbers of positive results for carrier children and those for noncarriers ($P < 0.001$). These results show that there is an association between colonization and the test's ability to detect urinary antigen.

For pneumococcal carrier children no significant association has been found between a positive result by ICT and the number of recorded colonies grown in nasopharyngeal culture ($P > 0.900$). However, we found that positive results for ICT were more likely to occur in young infants than in older children. The median age for nasopharyngeal carriers with positive results was 29 months; for carriers with negative results, it was 52 months, the difference being statistically significant ($P < 0.001$). We determined pneumococcal serotypes for 23 pneumococci isolated from nasopharyngeal carriers. No particular association has been found between serotype and positive ICT result.

Several authors have evaluated the usefulness of the ICT in the diagnosis of pneumococcal pneumonia in adults (3, 5, 13) and found that it shows excellent sensitivity and specificity. In contrast, far fewer studies have been performed to assess the clinical usefulness of this test among children (1, 7, 8). Dowell et al. (7) evaluated the test by using 88 children with radiolog-

ically confirmed pneumonia and 198 control subjects, finding that the difference in the results was not significant. However, a positive result for the urine antigen detection test was strongly associated with pneumococcal colonization. Similarly, Adegbola et al. (1), studying the presence of pneumococcal antigen in urine from Gambian children, found that the positive and negative predictive values for carriage were 96 and 22%, respectively. Recently, Hamer et al. (8) also evaluated the ICT, finding that healthy children with nasopharyngeal carriage of *S. pneumoniae* had significantly more positive results than did noncarrier children.

We have found that the capability to detect pneumococcal urinary antigen in carrier children was significantly higher for younger than for older children. It remained unclear how the detection of pneumococcal antigen in urine from healthy carrier children is possible. The higher level of pneumococcal urinary antigen in younger than in older children may be explained by differences in immunological status, such as IgA. In children under 2 years of age the concentration of IgA is very low or else virtually nothing (11). As is well known, the secretory IgA intercepts the antigens at mucosal surface levels, facilitating their luminal degradation and clearance (11).

From our point of view, although without microbiological confirmation, the positive results for group 2 should be attributed to the nasopharyngeal colonization of the children. No cross-reaction between PnC and any *Mycoplasma* antigen has been described previously. On the other hand, we cannot rule out a coinfection with *S. pneumoniae* and *M. pneumoniae*; however, the blood culture and the detection of PCA in urine by CIE were negative in all cases.

The positive results obtained in our study with in vitro serial dilutions of the pneumococcal vaccine should be considered when pneumococcal infection is detected in patients who have been more recently vaccinated. Sørensen and Henrichsen (15), working with 14-valent pneumococcal vaccine (Pneumovax; Merck, Sharp & Dohme), reported that the amount of PnC represents close to 15% of the total polysaccharide content of the vaccine.

In summary, ICT is a rapid test with a high capability of detecting PnC antigen (0.5 ng/ml). However, given that *S. pneumoniae* urinary antigen could be detected in nasopharyngeal carrier children, the ICT is a nonspecific test for detecting pneumococcal pneumonia in young children.

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