Novel Approaches to the Identification of \textit{Streptococcus pneumoniae} as the Cause of Community-Acquired Pneumonia

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Current diagnostic tests lack sensitivity for the identification of the bacterial etiology of pneumonia. Attempts during the past 2 decades to improve sensitivity of detection of bacterial constituents in blood by use of antibody-antigen complexes and polymerase chain reaction have been disappointing. Recent data using pneumococcal conjugate vaccines as probes suggest that increased levels of both C-reactive protein and procalcitonin may be useful adjuncts to chest radiographs in the selection of patients with presumed bacterial pneumonia for inclusion in clinical trials. Among pneumococcal diagnostics currently under investigation, quantitative real-time polymerase chain reaction of respiratory secretions, as well as urinary antigen detection and pneumococcal surface adhesin A serological analysis for adults, are candidates for use in future clinical trials of antibiotics.

Acute respiratory infections are the leading infectious cause of death globally among both infants and adults [1]. Although these deaths are, in large part, attributable to community-acquired pneumonia (CAP) caused by \textit{Streptococcus pneumoniae} [2], our ability to identify pneumococci as the specific etiological agent of pneumonia is very limited.

Although culture of lung puncture aspirate may increase the yield of pneumococci, relative to blood culture alone, its sensitivity for diagnosing pneumococcal pneumonia is <20\% [2, 3]. Additionally, other invasive techniques, such as bronchoalveolar lavage and protected specimen brush sampling, are not practical to use to define the role of pneumococci in patients with CAP who are routinely enrolled in clinical trials.

This article reviews advances in the specific diagnosis of pneumococcal infections in CAP. It starts with an overview of the insights gained from trials of conjugate pneumococcal vaccine on the role of chest radiographs and acute inflammatory responses in helping to define pneumococcal CAP and then looks at molecular and serological advances in the diagnosis of pneumococcal CAP for both children and adults.

\textbf{PNEUMOCOCCAL CAP EXTENDS BEYOND CONSOLIDATION SEEN ON CHEST RADIOGRAPHS}

Evidence of alveolar consolidation seen on chest radiograph is usually used to define the subset of patients with acute respiratory infections that have progressed to CAP. Although it is recognized that chest radiographs may not manifest alveolar consolidation early in the course of disease, particularly in pediatric studies, it has been difficult, in the absence of a sensitive diagnostic assay, to define a role for pneumococci in patients with pneumonia who do not have classic changes seen on chest radiographs [4].

Although pneumococcal conjugate vaccine trials involving children have demonstrated a decrease in the incidence of World Health Organization–defined consolidation seen on chest radiograph (chest radiograph
end point consolidation) among 25%–37% of children receiving ≥3 doses of 7- or 9-valent conjugate vaccine [2, 5, 6], the vaccine has also been used as a probe to investigate the role of C-reactive protein (CRP) and procalcitonin in defining a pneumococcal etiology. This is done by comparing the incidence of study end points between vaccine recipients and control individuals, after blinded determination of CRP and procalcitonin levels in archived specimens taken during the randomized clinical trials [7, 8]. In children with CAP and chest radiograph end point consolidation, an elevated CRP level identified an enriched population of patients with pneumonia among whom the fraction of pneumococcal-vaccine preventable pneumonia increased (in the intent-to-treat analysis) from 21% to 38% at the fraction of pneumococcal-vaccine preventable pneumonia enriched population of patients with pneumonia among whom the fraction of pneumococcal-vaccine preventable pneumonia increased (in the intent-to-treat analysis) from 21% to 38% at a CRP level of >120 mg/L, and the fraction of pneumococcal-vaccine preventable pneumonia increased from 21% to 46% as the procalcitonin level increased to 5 ng/mL [7]. These levels of acute-phase reactants are too high to be clinically sensitive but demonstrate increasing specificity of the pneumococcal fraction of chest radiograph end point pneumonia as levels of acute-phase reactant increase. Among patients with the combination of high levels of CRP and high levels of procalcitonin, 64% of chest radiograph end point–confirmed CAP cases were preventable by the conjugate vaccine [7].

With the vaccine probe approach, the use of a CRP value >40 mg/L has been shown to identify a considerable burden of pneumococcal pneumonia in children with nonspecific changes seen on chest radiograph [8]. These data suggest that, at least in studies involving children, an increased CRP level in situations where any changes are seen on chest radiograph may be an enriched clinical trial population.

**NONQUANTITATIVE PCR ASSAYS FOR S. PNEUMONIAE**

The first generation of PCR assays of blood samples from adults with CAP had sensitivities less than or equal to those of blood cultures [9]. Throat swab specimens were probed by PCR of the ply gene (which encodes pneumolysin) in patients with pneumonia. Of 417 patients, 229 (55%) had positive results, but 73 (18%) of 126 control individuals had positive results [10]. The lack of specificity is now believed to be caused by the presence of the ply gene in some species of streptococcal mouth flora. In children, the use of pneumolysin-based nonquantitative PCR on blood samples is contradictory. Dagan et al. [11] showed a lack of sensitivity and specificity, whereas a study by Michelow et al. [12] suggested 100% sensitivity and 95% specificity, compared with blood culture. The data overall suggest a lack of specificity for the ply gene. A recent report shows improved specificity with use of a probe for the hlyA gene [13].

**QUANTITATIVE REAL-TIME PCR**

Recent studies involving adults have investigated quantitative differences in pneumococcal colonization of the nasopharynx and sputum by using quantitative real-time PCR. Using the ply gene as a reporter and a cutoff of >3.7 × 10^4 genomic equivalents per mL of good-quality sputum, sensitivity was 90% and specificity was 80% [14]. Using the same target at >10^5 copies of ply gene per mL of sputum was more sensitive than was culture, especially for patients receiving antibiotics [15, 16]. A more specific target, the Spn9802 gene fragment, at >10^5 copies/mL in nasopharyngeal aspirate specimens is reported to correlate with clinical disease [17]. The Spn9802 gene fragment compared favorably with quantitative hlyA-based real-time PCR in distinguishing patients with pneumonia from control individuals [17]. There is some evidence that disease presentation and severity may correlate with real-time PCR levels of pneumococci in clinical specimens. In patients with pneumococcal meningitis, the concentration of pneumococcal DNA in blood and CSF specimens correlated with mortality [18]. Finally, in the diagnosis of pneumococcal empyema in children, real-time PCR is more sensitive than is culture [19, 20].

Real-time quantitaive PCR of respiratory specimens with specific primers for genes such as hlyA is thus a promising method for the identification of patients who should be enrolled in antibiotic trials of CAP. This approach appears to be more useful than is culture for identifying pneumococcal empyema.

**DIFFERENTIAL PATTERNS OF GENE EXPRESSION**

The patterns of gene expression in pneumococci vary between colonizing and invasive bacteria. In mice, opaque phenotypes of *S. pneumoniae* correlate with invasive disease, whereas transparent phenotypes correlate with colonization [21]. The phenotype is a function of capsular expression [22] and the expression of other virulence genes. Virulence genes other than capsular genes showed greater differential expression than did capsular genes, in lung and blood specimens of mice but not in nasal secretions [23]. These observations, however, were complicated by strain-specific differences in expression that were also found. Thus, differential gene expression may be a diagnostic modality of the future—a major hurdle that first must be overcome is the need to improve the sensitivity of in situ gene expression [23]. There are also large differences in host immune protein gene expression between different niches associated with pneumococcal infection in mice [23]. Thus, host protein expression may identify specific etiological agents, such as pneumococci. In mice, pneumococcal infection may be differentiated from influenza by patterns of host gene expression [24, 25], and the colonization of human pharyngeal epithelial cells with pneumococci can similarly be detected by
changes in the cells’ gene expression [26]. The possibility therefore exists that, in the future, patterns of pneumococcal gene expression detected in the blood may identify invasive strains.

PNEUMOCOCCAL SEROLOGICAL ANALYSIS

Serological analysis is not useful for immediate treatment decisions, but the persistence of an antibody response despite preceding antibiotic exposure makes serological analysis appealing for the etiologic diagnosis of pneumococcal disease in epidemiologic studies. Pneumococcal surface adhesin A (PsaA) is present in the cell walls of all pneumococcal serotypes and some oral streptococci [27]. Anti-PsaA ELISA is a possible method of establishing a diagnosis of pneumococcal pneumonia. A serotype 4 outbreak in a nursing home led to hospitalization of 18 patients during a 2-week period. Blood culture results were positive for 3 patients; 6 other patients showed evidence of seroconversion to PsaA [28]. In patients with bacteremic pneumococcal pneumonia, a 2-fold increase in ELISA units of anti-PsaA from paired convalescent- and acute-phase serum samples had 85% sensitivity and 83% specificity [29]. The same assay achieved a sensitivity of 89% and a specificity of 98% among Kenyan adults with a 1.3-fold cutoff [30]. Unfortunately, among children with invasive pneumococcal disease, sensitivity was poor, and interpretation required higher cutoff values [31]. Comparison with convalescent-phase serum samples is necessary because single measurements of PsaA concentrations in acute-phase serum specimens lack sensitivity [30, 32]. This is a major limitation of the assay, because clinicians are unlikely to obtain convalescent-phase serum samples. Patients enrolled in clinical trials, however, are routinely seen at the end of treatment and/or at 21–30 days after initiation of therapy. PsaA seroconversion is currently an underutilized opportunity to make pneumococcal diagnoses in clinical trials. A major limitation of the assay for this purpose is that there is no US Food and Drug Administration (FDA)–licensed or commercially available assay.

Pneumococcal antigen-antibody immune complexes have been used in experimental assays to indicate pneumococcal infection. These assays are not standardized, and their utility in clinical studies remains to be established [33, 34]. Assays of pneumococcal antibody using ELISA and including absorption steps with C-polysaccharide and whole 22F pneumococci have been standardized by an international effort [35]. The possibility of measuring antibody-specific assays has become practical with use of simultaneous multiple-assay platforms, such as Luminex (Luminex Corporation), but, again, their use remains to be clinically established in the trial setting. In conclusion, the development of an FDA-licensed serological assay would be a useful adjunct to the diagnosis of pneumococcal pneumonia in clinical trials.

PNEUMOCOCCAL URINARY ANTIGEN TESTING

Serotype-specific latex-agglutination urinary assays for pneumococcal serotypes are limited because there are 91 identified pneumococcal serotypes [36, 37]. An ELISA for urinary pneumolysin detection in children has been recently developed. Compared with the immunochromatographic membrane assay (ICT) Binax Now S. pneumoniae (Binax), the urinary pneumolysin ELISA achieved lower sensitivity for pneumococcal infection in children with and without nasopharyngeal carriage, but markedly improved specificity [38]. The low sensitivity of 56.6% in adults likely does not suggest much added benefit in this age group [38]. The detection of pneumococcal C-polysaccharide with the ICT Binax NOW S. pneumoniae assay allows a bedside diagnosis of pneumococcal disease within 15 min after receipt of a urine sample [39]. The assay is reported to have a sensitivity of 74%, specificity of 94%, positive predictive value of 79%, and negative predictive value of 92% for adults not infected with HIV who have microbiologically confirmed pneumococcal disease [40]. The ICT may also be useful for special populations, such as HIV-infected patients [40] and critically ill patients [41]. Specificity issues have to be viewed in light of the limitations of traditional microbiological testing. In a latent-class analysis, the ICT performed favorably, with better sensitivity than that of blood and sputum culture and higher specificity than that of lytA or ply (nonquantitative) PCR of respiratory samples at comparable sensitivity [42]. The lower sensitivity of the ICT after antibiotic use that was found in some studies [37, 43] was not confirmed in others [12, 44]. Discordant results, with negative results of culture for pneumococci and positive results of ICT, were highly associated with previous use of antibiotics [45]. The measured fraction of patients with pneumonia of unknown etiology might be reduced by 23% [40], and the measured fraction of pneumococcal etiology could be increased up to 2-fold by use of this assay. The limitations of this assay include unacceptably low specificity in young children, because of correlation with pneumococcal carriage [39, 46], although not in adults [47], and possible cross-reactions with some α-hemolytic oral streptococci (Streptococcus mitis and Streptococcus oralis).

The persistence of positive test results after recovery from pneumococcal disease is reported for up to 90% of patients 7 days after diagnosis [48] and for 40%–48% after 4–6 weeks [40, 49]. The persistence of positive assay findings is a particular problem for children (occurring for 70% at 1 month) if urine specimens are concentrated 25-fold with use of ultrafiltration [45]. The added time, effort, and cost, together with some loss in specificity from the use of concentrated urine, likely outweigh the sensitivity gained, but the increased sensitivity achieved by urine concentration might be useful for epidemiological studies.

Severe disease has been associated with higher detection rates with use of the ICT [50] and higher semiquantitative titers.
which might be an indication either of the severity of pneumococcal disease or simply of a higher pneumococcal burden in severe disease. Bacteremic infections tend to have higher rates of positivity [50, 52]. Promising results have been reported for testing of pleural fluid for the diagnosis of empyema [44, 53]. Finally, ICT has been used for management decisions. Targeted amoxicillin use among Russian military recruits with nonsevere pneumonia who had positive assay results achieved similar clinical success rates as did clarithromycin use among those with negative results [54]. Similarly, in a Swedish study, a positive result of ICT was associated with higher success rates of initial β-lactam monotherapy, compared with a negative result of ICT [55].

CONCLUSIONS

In clinical trials for CAP, S. pneumoniae is the most common and virulent bacterial pathogen against which empirical treatment is directed. We suggest that the following tests for S. pneumoniae be included in future clinical trials of both mild pneumonia in outpatients and severe pneumonia in hospitalized patients.

1. Culture of blood and of good-quality sputum
2. S. pneumoniae urinary antigen detection
3. Quantitative assays of CRP and procalcitonin
4. Serological analysis of acute- and convalescent-phase serum samples, pending an FDA-licensed assay for PsaA.

It is possible that, in the near future, specific primers for real-time PCR will allow the application of this method to respiratory secretions, and perhaps to blood and other bodily fluids, for quantitative differentiation of pneumococcal pneumonia from nasopharyngeal carriage.

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