Validation of Immunoglobulin G Enzyme-Linked Immunosorbent Assay for Antibodies to Pneumococcal Surface Adhesin A in the Diagnosis of Pneumococcal Pneumonia among Adults in Kenya

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Epidemiologic studies of pneumococcal pneumonia, including vaccine efficacy trials, are hampered by a lack of sensitive and specific diagnostic tests. Pneumococcal surface adhesin A (PsaA) is a genetically conserved, surface-expressed protein common to all serotypes of Streptococcus pneumoniae and is highly immunogenic. Detection of anti–PsaA immunoglobulin G by recombinant PsaA enzyme-linked immunosorbent assay was evaluated for diagnosis of pneumococcal pneumonia in paired serum samples from 4 adult populations: 47 healthy control subjects, 56 clinic control subjects without pneumococcal disease syndromes, 109 patients with pneumococcal pneumonia, and 93 pneumonia patients with no evidence of pneumococcal etiology. By considering a 2-fold increase in antibody concentration as positive, sensitivity was 0.70, and specificity was 0.98. With a 1.3-fold increase, these were 0.89 and 0.98, respectively. The test's performance was not affected by the patients' human immunodeficiency virus status or by the pneumococcal serotype. The combination of high sensitivity and high specificity makes this an ideal assay for epidemiologic studies of pneumococcal pneumonia.

Pneumococcus is the most commonly implicated bacterial cause of death among the 1.4 million fatal cases of lower respiratory tract infection that are estimated to occur in adults each year [1]. It is not possible to estimate the burden of pneumococcal disease in adults more precisely because of the insensitivity of the reference standard diagnostic technique, blood culture [2]. Diagnostic insensitivity also undermines epidemiologic studies of pneumococcal pneumonia, particularly vaccine efficacy studies, which must be large in order to identify a sufficient number of cases. However, methods that have been proposed to increase the sensitivity of diagnosis frequently lack the high specificity required to obtain an unbiased estimate of vaccine efficacy [3, 4].

Before doing an epidemiologic study of risk factors for pneumococcal pneumonia in adults, we evaluated several diagnostic methods, including lung aspirate cultures, serotype-specific capsular antigen detection in urine, and immune complex EIAs with the use of common pneumococcal antigens [2, 3, 5, 6]. Although lung aspirate cultures and antigen detection provided diagnoses with high specificity, neither technique identified more than half of all patients with pneumococcal pneumonia.

Pneumococcal surface adhesin A (PsaA), a metal-binding lipoprotein located in the cell wall of Streptococcus pneumoniae, is involved in intracellular transport of metal ions [7–9]. Immunodot blot analyses of monoclonal antibodies to PsaA demonstrate binding with pneumococci of all 90 serotypes and suggest universal expression in S. pneumoniae [10]. Polymerase chain reaction (PCR) restriction fragment–length polymorphism analysis of the gene psaA, which encodes PsaA, reveals only minor variation in restrictions sites, limited to 1 of 10 restriction enzymes in 2 of 23 pneumococcal isolates of different serotypes [11]. Primers for psaA amplify an identically sized PCR product in isolates of all 90 pneumococcal serotypes [12]. Purified PsaA, obtained by expression of psaA in recombinant Escherichia coli [9, 13], is currently being evaluated as a potential vaccine antigen against pneumococcal disease. Intranasal delivery of recombinant PsaA (rPsaA) is highly immunogenic in mice, without the need for adjuvant, and protects against pneumococcal colonization [13, 14].
By using purified native PsaA from a clinical isolate of serotype 22F, sensitivity of the ELISA for anti-PsaA was previously estimated at 0.67–0.85 in 2 small samples of paired serum samples from American adults with bacteremic pneumococcal pneumonia. Specificity estimates were 1.00 in 30 healthy control subjects and 0.83 and 0.97 in 2 sample groups of ≤30 adults with pneumonia caused by _Legionella pneumophila, Chlamydia_ or _Mycoplasma pneumoniae_ [15, 16]. Here, we report the results of a study that used rPsaA ELISA in the diagnosis of pneumococcal disease. We evaluated the assay in 4 populations of adults from the coast of Kenya.

**Subjects and Methods**

*Study populations.* All subjects were residents of Coast Province, Kenya, and were ≥15 years old. Four populations were studied. Groups 1 and 2 were healthy control subjects and sick control subjects, respectively. Group 1 comprised 47 volunteers among employees of an agricultural company in Kilifi who attended work on the day of recruitment. Group 2 comprised 56 consecutive patients who presented to the outpatient clinic of Kilifi District Hospital, Kilifi, who did not have a diagnosis commonly associated with pneumococcal disease (pneumonia, meningitis, or septicemia).

Group 3, the pneumococcal pneumonia group, was from a study of 281 patients attending Kilifi District Hospital, Kilifi, or Coast Province General Hospital, Mombasa. These patients had an acute (<14 days) history of illness and radiographic evidence of pneumonia [2]: 129 had evidence of pneumococcal etiology, and 109 of these had sufficient volume of serum in pairs for analysis by ELISA for anti-PsaA. Pneumococcal etiology was assigned on the basis of blood or lung aspirate cultures in 32 patients, by serotype-specific urine antigen detection for 10 serotypes (1, 4–7, 9, 12, 14, 19, and 22) in 46 patients, and by both methods in 31 patients. The urine antigen assay had a specificity of 0.98 when validated in a similar population [5]. The final group was “other pneumonia.” In the group of 281 patients with pneumonia described above, 93 had no evidence of pneumococcal etiology, by culture or urine antigen detection, and had paired serum samples of appropriate timing and in sufficient volume for ELISA for anti-PsaA.

Serum samples were taken from patients with pneumonia at admission to hospital, at discharge, and at a follow-up clinic 3 weeks after discharge. Samples from control subjects were collected at recruitment and 2 weeks later. Serum samples from patients with pneumonia and from control groups were stored at −70°C for 2–5 years and 1–4 months, respectively. All samples from the pneumococcal pneumonia group were tested. In the other pneumonia group, serum pairs were tested only if the acute sample was taken <10 days after the onset of symptoms and the convalescent sample was taken ≥3 days after the acute sample. When 2 convalescent samples were available, only the sample taken soonest after day 14 of the illness was assayed.

**ELISA for anti-PsaA antibodies.** This study used the method of Tharp et al. [15, 16]. Flat-bottom microtiter plates (Immunon II HB; Dynatech) were coated with 2.5 μg/mL purified PsaA in 0.01 M PBS (pH 7.2) for 16 h at 4°C. PsaA was produced by recombination and expression in _E. coli_ of the _psaA_ gene derived from the serotype 2 strain D39 [9]. Plates were washed 4 times with PBS (pH 7.2) with 0.05% Tween 20 after each stage except blocking, when they were simply emptied. Plates were blocked for 1 h at 37°C with 1% bovine serum albumin (BSA; EIA grade; Sigma Chemical) in PBS. Test serum samples were diluted in 1% BSA–0.05% Tween 20 in PBS and incubated for 1 h at 37°C.

Test serum samples were assayed in duplicate in 8 2-fold dilutions, and all specimens from 1 patient were assayed on the same plate. A reference serum of high anti-PsaA concentration and an immunoglobulin control serum (Sandoglobulin; Sandoz) were assayed on every plate in 7 2-fold dilutions. Bound antibody was labeled with mouse monoclonal anti-human IgG Fc conjugated to horseradish peroxidase (clone PH6043; Hybridoma Reagent Laboratories) in a 2-h incubation at 37°C. Bound enzyme was visualized with tetramethylbenzidine (TMB 1 component microwell peroxidase substrate; Kirkegaard and Perry Laboratories) for 30 min at room temperature, and the reaction was stopped with 0.18 M sulfuric acid. Plates were read by ELISA reader at a wavelength of 450 nm with a reference filter of 620 nm. Results were analyzed by a 4-parameter logistic log curve fit program (ELISA version 1.11; CDC) and expressed in ELISA units (EU) as a percentage of the concentration of the reference serum. The coefficient of variation was estimated by repeated assay of 1 serum sample on 31 days as 14.6%.

**Antibiotic assay.** Sterile 6-mm filter paper disks were placed at the center of a Columbia agar plate inoculated evenly with a fully susceptible _S. aureus_ strain (National Collection of Type Cultures 6571). A 20-μL aliquot of urine was dropped onto the disk, and the inhibition zone surrounding the disk was read after overnight incubation. The assay was reliably sensitive to concentrations of benzyl penicillin ≥0.75 μg/mL.

**Human immunodeficiency virus (HIV) tests.** Serum samples were first screened with an HIV-1 antibody ELISA (Enzgnost HIV-1 [Behring] or Vironostika HIV-1 [Organon Teknika]), and those with positive or equivocal results were retested with a second ELISA (Recombigen HIV-1 EIA; Cambridge Biotech). Discordant results were resolved by Western blotting.

**Statistical analysis.** Sensitivity was calculated as the proportion of subjects in the pneumococcal pneumonia group with a test value higher than the defined cutoff, and specificity was calculated as the proportion of subjects in the control groups with a test value lower than the defined cutoff. For proportions (e.g., sensitivity and specificity), exact binomial 95% confidence intervals (CIs) were calculated. Sensitivity and specificity were calculated for every observed value and were plotted on receiver operating characteristic (ROC) curves. All analyses were conducted with STATA software (StataCorp). In stratified analyses, the sensitivities and specificities at different cutoff values were calculated separately for the subpopulations of pneumococcal pneumonia patients and control subjects that fell into each stratum.

**Results**

Table 1 lists median age, proportion of males, and proportions of subject who were HIV antibody positive in each study group. Paired serum samples from healthy control subjects were separated by a median of 14 days (range, 14–16 days); those from sick control subjects were separated by a median of 13
Table 1. Comparison of subjects in 4 study populations, by median age, male sex, and human immunodeficiency virus (HIV) serostatus.

<table>
<thead>
<tr>
<th>Study group</th>
<th>n</th>
<th>Age, median years (range)</th>
<th>Male, no. (%)</th>
<th>HIV positive, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy control subjects</td>
<td>47</td>
<td>32 (18–56)</td>
<td>32 (68)</td>
<td>11 (23)</td>
</tr>
<tr>
<td>Sick control subjects</td>
<td>56</td>
<td>29 (16–65)</td>
<td>18 (32)</td>
<td>8 (14)</td>
</tr>
<tr>
<td>Pneumococcal pneumonia</td>
<td>109</td>
<td>30 (15–61)</td>
<td>73 (67)</td>
<td>51 (47)</td>
</tr>
<tr>
<td>Other pneumonia group</td>
<td>93</td>
<td>33 (15–70)</td>
<td>55 (59)</td>
<td>50 (54)</td>
</tr>
</tbody>
</table>

Figure 1. Frequency distributions of anti–pneumococcal surface adhesin A (PsaA) responses expressed as ratios of convalescent to acute concentrations on a log_{10} scale in each of 4 study populations. A, Healthy control subjects; B, sick control subjects; C, pneumococcal pneumonia group; D, other pneumonia group.

days (range, 13–15 days). Each of the 109 patients with pneumococcal pneumonia was sampled at admission, a median 6 days after the onset of illness. Most patients with pneumococcal pneumonia were also sampled at discharge (n = 86), a median 5 days after admission, and at a follow-up appointment (n = 100), a median 27 days after admission. Every convalescent sample was assayed, but, unless otherwise specified, only the higher value for each subject was used in the analysis. These samples were taken a median of 28 days (range, 7–114 days) after the onset of the illness. We used the temporal pattern of anti-PsaA concentrations in the patients with pneumococcal pneumonia to define the criteria for selecting a single convalescent serum from patients in the other pneumonia group. For those serum samples selected, the median interval between the onset of illness and the sample was 27 days (range, 4–106 days).

The ratio of concentrations of anti-PsaA in convalescent and acute or second and first serum samples was calculated for each subject. Figure 1 shows the distribution of these ratios. There is little overlap in the distributions of the pneumococcal pneumonia group and the 2 control groups. One of the sick control subjects had a fold increase of 2.2, another of 1.3. Both these patients were HIV negative. The first had a clinical diagnosis of urinary tract infection, the other of influenza. All other responses in both the control groups were <1.3. Pearson correlation coefficients for the comparison of first and second serum samples were 0.98 for healthy control subjects and 0.97 for sick control subjects.

Figure 2 shows an ROC curve that uses the sick control group to estimate specificity and the pneumococcal pneumonia group to estimate sensitivity. The assay is optimized at a ratio cutoff >1.3, which yielded a sensitivity of 0.89 and a specificity of 0.98 (table 2). At a more conventional cutoff, a ≥2-fold increase,
Among patients in the pneumococcal pneumonia group, 14 had high concentrations of anti-PsaA in convalescent serum samples but did not show a 2-fold increase because the acute sample was also high. By applying a composite criterion of positivity, which included either a ≥2-fold increase for all subjects or a ≥1.3-fold increase for subjects with concentrations of anti-PsaA in convalescent serum samples that exceeded the concentration of anti-PsaA for the control on the same plate, 9 of the 14 patients were reclassified as positive. The sensitivity was 0.78, and the specificity remained 0.98. The mean (±SD) concentration of anti-PsaA in the immunoglobulin control serum was 34 ± 6 EU. The within-plate design eliminated most of the variation around this comparison.

The pneumonia patient group and the sick control group differed with respect to sex and HIV status (table 1). In particular, exclusion of potential pneumococcal syndromes reduced the prevalence of HIV in sick control subjects. However, sensitivity and specificity estimates were unchanged when stratified by these variables (table 2). By restricting the pneumococcal pneumonia group to patients with positive blood or lung aspirate cultures, there was a modest improvement in the sensitivity estimate (table 2). Of the culture-positive patients, 47 (73%) were bacteremic.

In the pneumococcal pneumonia group, 38 patients had serotype 1 disease, 15 had disease caused by serogroup 7, and 11 had disease caused by serotype 5. Other serotypes identified (number of patients) were 14 (8), 19 (7), 6 (5), 12 (5), 4 (3), 9 (3), 22 (3), and 38 (2). One infection was not typed, and the following serotypes were responsible for 1 case each: 3, 8, 11, 17, 18, 23, 24, and 29. The proportion of patients with a 2-fold increase in anti-PsaA concentration was independent of serotype ($\chi^2$, 13.7; df, 18; $P = .75$).

The sensitivity estimates in this study were augmented by the fact that 2 convalescent serum samples were available for each patient but only the higher of the 2 was analyzed. An estimate of sensitivity in situations where only 1 convalescent serum sample is available can be obtained by analyzing every convalescent serum sample (and its paired acute serum sample) as a separate observation. By taking this approach, sensitivity at the 1.3-fold and 2-fold thresholds were 0.80 and 0.58, respectively. To describe the kinetics of the anti-PsaA response, the anti-PsaA concentrations of all serum samples from the pneumococcal patient group were plotted against the patients’ reported duration of illness over the first 31 days (figure 3). To smooth out this curve, it is expressed as a rolling 3-day mean. With a log$_2$ scale on the ordinate, the gridlines indicate the vertical distance required to achieve a 2-fold increase.

A disadvantage of analyzing paired serum samples is that the diagnosis is available only after convalescence. The data were also analyzed to determine whether a high concentration of anti-PsaA in acute serum samples alone was predictive of pneumococcal pneumonia. An acute serum sample with a concentration of anti-PsaA that exceeded the concentration of anti-PsaA in the control on the same plate has a sensitivity of 0.13 and specificity in sick control subjects of 0.98. Reduction of this concentration threshold improved sensitivity but with an unacceptable loss of specificity (table 2). The area under the ROC curve for the analysis of acute serum concentrations was 0.65 (95% CI, 0.56–0.73).

In paired samples from the other pneumonia group, 31 (33%) had a 2-fold increase in anti-PsaA concentration, and 47 (51%) had a 1.3-fold increase. Urine taken before the administration of antibiotics in hospital was available for 101 patients with pneumococcal pneumonia and 84 patients with other types of pneumonia. Antimicrobial activity was found in the urine of 51 (51%) patients with pneumococcal pneumonia and 50 (60%) patients with other types of pneumonia. There was a significant association between the prevalence of antimicrobial activity in patients with other types of pneumonia and the magnitude of the anti-PsaA response; for those with a fold increase <1.3, 1.3–1.9, or ≥2.0, the prevalence of antimicrobial activity was 49% (21/43), 60% (9/15), and 77% (20/26), respectively ($\chi^2$ test for trend, 4.65; $P = .03$).

In 31 pneumonia episodes for which the only evidence of pneumococcal etiology was a 2-fold increase in anti-PsaA, there was evidence of an alternative etiology in 9 (29%): 4 had a positive blood or lung aspirate culture for another pathogen, 3 had viral pneumonia or *M. pneumoniae*, and 2 had tuberculosis. This was indistinguishable statistically from the proportion (21%) with evidence of a second etiology among 76 patients with a 2-fold increase in anti-PsaA and either culture...
Table 2. Sensitivity and specificity estimates for the ELISA for antibodies against pneumococcal surface adhesin A (PsaA) in different groups of samples and for different positivity criteria.

<table>
<thead>
<tr>
<th>Serum sample tested, study subsample, and/or increase in anti-PsaA concentration</th>
<th>No. of subjects tested</th>
<th>Sensitivity (95% CI)</th>
<th>No. of subjects tested</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute and the higher of 2 convalescent serum samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1.3-fold increase</td>
<td>109</td>
<td>0.89 (0.82–0.94)</td>
<td>56</td>
<td>0.98 (0.90–1.00)</td>
</tr>
<tr>
<td>≥2-fold increase</td>
<td>109</td>
<td>0.70 (0.60–0.78)</td>
<td>56</td>
<td>0.98 (0.90–1.00)</td>
</tr>
<tr>
<td>Composites</td>
<td>109</td>
<td>0.78 (0.69–0.85)</td>
<td>56</td>
<td>0.98 (0.90–1.00)</td>
</tr>
<tr>
<td>Men only, ≥2-fold increase</td>
<td>73</td>
<td>0.68 (0.57–0.79)</td>
<td>50</td>
<td>0.98 (0.89–1.00)</td>
</tr>
<tr>
<td>Women only, ≥2-fold increase</td>
<td>36</td>
<td>0.72 (0.55–0.86)</td>
<td>53</td>
<td>1.00</td>
</tr>
<tr>
<td>HIV-positive subjects only, ≥2-fold increase</td>
<td>51</td>
<td>0.71 (0.56–0.83)</td>
<td>19</td>
<td>1.00</td>
</tr>
<tr>
<td>HIV-negative subjects only, ≥2-fold increase</td>
<td>58</td>
<td>0.69 (0.55–0.80)</td>
<td>84</td>
<td>0.99 (0.94–1.00)</td>
</tr>
<tr>
<td>Culture-positive subjects only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1.3-fold increase</td>
<td>63</td>
<td>0.97 (0.89–1.00)</td>
<td>56</td>
<td>0.98 (0.90–1.00)</td>
</tr>
<tr>
<td>≥2-fold increase</td>
<td>63</td>
<td>0.84 (0.73–0.92)</td>
<td>56</td>
<td>0.98 (0.90–1.00)</td>
</tr>
<tr>
<td>Acute and both convalescent serum samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1.3-fold increase</td>
<td>186</td>
<td>0.80 (0.74–0.86)</td>
<td>56</td>
<td>0.98 (0.90–1.00)</td>
</tr>
<tr>
<td>≥2-fold increase</td>
<td>186</td>
<td>0.58 (0.51–0.65)</td>
<td>56</td>
<td>0.98 (0.90–1.00)</td>
</tr>
<tr>
<td>Acute serum samples only</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-PsaA in serum greater than anti-PsaA in immunoglobulin control serum</td>
<td>109</td>
<td>0.13 (0.07–0.21)</td>
<td>56</td>
<td>0.98 (0.90–1.00)</td>
</tr>
<tr>
<td>Anti-PsaA in serum ≥0.5× anti-PsaA in immunoglobulin control serum</td>
<td>109</td>
<td>0.28 (0.20–0.38)</td>
<td>56</td>
<td>0.93 (0.83–0.98)</td>
</tr>
</tbody>
</table>

NOTE: CI, confidence interval; HIV, human immunodeficiency virus.

a Composite criterion of positivity is either ≥2-fold increase in anti-PsaA concentration between acute and convalescent serum samples or >1.3-fold increase in anti-PsaA concentration between acute and convalescent serum samples among patients with a convalescent serum that exceeds the concentration of anti-PsaA found in the immunoglobulin control serum on the same plate.

b Because stratification of the sick control group yielded strata with few observations, subjects from both control groups were combined for subgroup analyses of specificity.

Discussion

Although anti-PsaA concentrations vary widely among healthy adults in Kenya, they vary little within healthy adults over the time period relevant for a diagnostic study (i.e., 2 weeks). Setting a diagnostic cutoff as low as 1.28 on the ratio of convalescent to acute serum samples will correctly classify all healthy control subjects as negative. However, healthy control subjects may not be the ideal test of a diagnostic assay that, in practice, will be applied entirely to sick persons [17]. Because the insensitivity of existing diagnostic methods makes it difficult to exclude patients with pneumococcal disease from a sick control group, we excluded all patients with syndromes associated with pneumococcal disease—pneumonia, meningitis, and sepsis. The convalescent:acute ratios for the 56 sick control subjects conformed to a log normal distribution between 0.76 and 1.30, with a single outlying value at 2.2. This exceptional value may be due to the inadvertent inclusion of a patient with pneumococcal pneumonia in the control group, to misclassification or laboratory error, or to an increase in cross-reactive antibody raised against an alternative antigenic stimulus.

Several proteins identified in Enterococcus faecalis, oral streptococci, and pathogenic equine streptococci share structural similarities with PsaA, and their genes have high sequence identities with psaA [10, 11, 18, 19]. Comparison of amino acid sequences of PsaA with proteins SsaB, FimA, ScaA, and EfaA from Streptococcus sanguis, Streptococcus parasanguis, Streptococcus gordonii, and E. faecalis, respectively, demonstrated identities of 57%–81% [11]. Monoclonal antibodies of different epitope specificities raised against PsaA have variable reactivity with E. faecalis, and several species of viridans streptococci also have non-specific cross-reactivity with Streptococcus equisimilis, group G streptococci, and Staphylococcus aureus [10]. It is not known whether natural antibodies raised against any of these organisms are cross-reactive with purified rPsaA antigen, although it remains possible. The coincidence of an anti-PsaA response to a natural nonpneumococcal stimulus, together with a clinical illness of the magnitude of pneumonia, is likely to be rare, and the specificity recorded here gives strong support for this view. Cross-reactivity, if it exists, does not significantly compromise the specificity of the anti-PsaA ELISA in paired samples.

Assaying the reference and test serum samples in 7 and 8 dilutions, respectively, and fitting a 4-parameter logistic log function to the reference serum results gave the ELISA for anti-PsaA precision in the measurement of concentration. This allowed a cutoff value to be defined on a continuous scale of convalescent:acute ratios, not merely at integers on the log scale. Although a 2-fold increase is a conventional and, in this case, conservative cutoff, the sensitivity of the assay can be

or urine antigen evidence of S. pneumoniae (χ², 0.69; df, 1; P = .41); 3 had a positive blood or lung aspirate culture for an additional pathogen, 6 had viral pneumonia, 2 had M. pneumoniae, and 5 had tuberculosis.
enhanced further in Kenyan adults without compromising specificity by selecting a ratio increase \(\geq 1.3\). Ideally, the choice of a cutoff should be determined locally after assay of serum samples from sick control subjects.

In previous evaluations of ELISA, using native PsaA in samples of bacteremic patients with pneumococcal pneumonia, sensitivity was estimated at 0.67 (95% CI, 0.50–0.84) and 0.85 (95% CI, 0.72–0.99), specifying a 1.41-fold increase in optical density and a 2-fold increase in concentration, respectively, as the definition of a positive sample [15, 16]. In the present study, by use of rPsaA, the sensitivity in culture-positive patients approximated 1.00 as the cutoff ratio was reduced to 1.3. At this point, only 2 culture-positive patients remained negative by ELISA for PsaA. Neither person was bacteremic: serotype 1 was cultured from the lung aspirate of 1 person, and serogroup 12 was cultured from the lung aspirate of the other. These results are unlikely to be due to failure of expression of PsaA in the infecting organism. Reactivity of anti-PsaA was previously shown against all pneumococcal serotypes [10] and among >100 isolates of \(S.\ pneumoniae\) tested for surface binding with anti-PsaA antibodies. Only 1 strain (serotype 16F) has been found to lack PsaA expression [7, 10]; the gene encoding PsaA was also absent from this strain but was present in 20 other strains of serotype 16F tested [12].

The high sensitivity estimates reported here apply to the assay of 2 convalescent samples per patient. However, removal of this additional advantage by reporting all convalescent serum samples independently still produced a useful range of sensitivity estimates (0.58–0.80). The rolling mean function of anti-PsaA against duration of illness (figure 3) gives some indication of the optimal time for sampling, to maximize the sensitivity of a single convalescent sample. There are 2 limitations to this approach. First, the data rely on the patients’ reports of the duration of illness. Kenyan adults may underestimate the duration of illness to increase the probability of hospital admission. This is likely to account for the apparent fall in mean anti-PsaA concentrations during the reported first 4 days of illness. Second, each point is a mean of a constantly changing set of samples and does not represent serial daily examinations of the same cohort. Nonetheless, the curve suggests that anti-PsaA concentrations increase steeply during the second week of illness, but, even among patients who present after 10–14 days, the concentration of anti-PsaA may still increase by \(\geq 2\)-fold.

The sensitivity of the ELISA for anti-PsaA is much higher than the current standard, blood cultures, leading to a considerably greater diagnostic yield. Among 202 patients with pneumonia tested by the ELISA for anti-PsaA, 47 had positive blood cultures, 107 had an anti-PsaA response \(\geq 2\), and 143 had an anti-PsaA response \(\geq 1.3\). The high specificity of the assay suggests that few of these additional diagnoses will be false positives. Additional credence is given to the new anti-PsaA ELISA diagnoses by the fact that the assay appears to concentrate the proportion of pneumonia patients recently exposed to antibiotics. In the etiology study from which these samples were drawn, the yield of blood and lung aspirate cultures was halved by the presence of antimicrobial activity in urine [2], and we anticipate that serologic diagnostic methods that are unaffected by antibiotics would identify persons whose diagnoses were obscured previously by the inhibitory effect of antibiotics on culture.

There are 3 disadvantages to the ELISA for anti-PsaA. First, convalescent serum samples are required for diagnosis, and the assay therefore offers no useful information in the acute clinical setting. Second, the assay cannot be used to define the etiology of patients who die during the first 2 or 3 days after presentation or who do not cooperate with convalescent serum sampling. Third, the wide range in ambient concentrations of anti-PsaA requires that serum samples be assayed at a wide range of dilutions, to ensure sufficient points for comparison with the reference curve, making the test relatively cumbersome in the laboratory. Set against the costs and difficulties of observing additional cases of pneumococcal pneumonia in a vaccine efficacy trial, these disadvantages seem insignificant. In the 2 groups of patients with pneumonia studied here, the addition of diagnoses based on a 2-fold increase in anti-PsaA concentration to those determined by blood culture increased the total number of pneumococcal cases detected from 47 to 113, a 2.4-fold increase in diagnostic efficiency. In designing a vaccine efficacy study, this would reduce size of the study groups required by \(\sim 60\%\).

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