

response<sup>5,10</sup> have shown promising results; however, this small study did not reveal any POCUS findings more prevalent in severely malnourished children with TB. Additionally, limitations with POCUS image visualization and participants' acceptability with the procedure were demonstrated in this study of young, severely ill children.

POCUS is generally defined as an ultrasound examination done by a nonradiologist with the scanning and interpretation of images done in real time. If accurate and feasible, POCUS is an attractive alternative to chest radiography to help with diagnosing TB, especially in resource-limited or remote settings, because POCUS is relatively inexpensive, portable, does not require radiation protection, and can be fully implemented by a single, trained operator.<sup>2</sup> POCUS may be particularly helpful for detecting specific features of TB in children living with HIV,<sup>5,6</sup> raising the question of whether POCUS may also be helpful in another immunosuppressed population at high risk for TB: children with SAM.<sup>2</sup> A recent study of 139 Bissau-Guinean children with presumptive TB, with 60% of the children having SAM, found that of the children diagnosed with TB those with SAM were more likely to have an abnormal POCUS finding of any type compared to those without SAM (98% vs. 82%, respectively).<sup>4</sup> Recent pediatric studies assessing POCUS findings in relation to TB diagnosis, including the study from Guinea-Bissau,<sup>4</sup> have enrolled children with suspected TB.<sup>5–7</sup> Almost all children admitted with SAM were eligible for inclusion in our study, regardless of whether they were explicitly considered to be TB suspects. This difference in enrollment criteria may explain the lack of differences in POCUS findings by TB diagnosis for this population of severely malnourished children, in contrast to these other recent pediatric studies of POCUS in TB suspects. We suspect that using POCUS to assess for TB in children with SAM may be more discriminatory in selected patients with SAM (eg, only those with HIV, prolonged cough or fever), rather than all children with SAM. Adequately powered studies to assess this should be a priority.

Regardless of how helpful POCUS may be for diagnosing TB in children, routine implementation is only feasible if adequate images can reliably be obtained by scanners (visualization) and children cooperate enough to obtain the images (acceptability). We found visualization to be “satisfactory” for the chest in 65% of children and for the abdomen in 68%. A study of South African children suspected to have TB found a higher proportion of POCUS evaluation of the chest to be “good” at 83%. Compared to our study, those children were older overall (median 18 months, IQR: 10 to 26 months vs. median 26 months, IQR: 15 to 59 months, respectively).<sup>10</sup> Another study of South African children suspected to have TB reported POCUS evaluation of the abdomen to be “satisfactory” in 55%,<sup>5</sup> similar to our study.

Only 52% of children had “good” acceptability with the POCUS procedure in this study. This is similar to the South African study of POCUS of the abdomen, which reported 65% of children scanned to have “good” acceptability. That study noted that improved POCUS visualization and acceptability were associated with older age.<sup>5</sup> Visualization and acceptability may limit the use of POCUS in U5s, the age group most vulnerable to poor outcomes from TB.<sup>1</sup> This scoping assessment of POCUS findings associated with TB in our study entailed a long examination that may have contributed to the lower acceptability. An abbreviated examination, focusing on a select number of high-yield ultrasound findings (when they have been identified), may improve acceptability and overall implementation.

The limitations of this study include a small sample size with only 31 TB cases (confirmed or unconfirmed). Also, with no postdischarge follow-up and no use of tests of infection (eg, tuberculin skin test), some TB cases may have been misclassified.<sup>8</sup> Nonetheless, these data add to the growing evidence based on the use of POCUS for child TB case finding, particularly for U5s with SAM—a TB high-risk population in which there is very little POCUS data published.

## CONCLUSIONS

This study did not reveal any POCUS findings that were more prevalent in U5s with SAM and coprevalent TB, compared to those without coprevalent TB. Larger studies are needed to optimally assess POCUS findings that may differentiate those with TB in this vulnerable population. Those studies should explore the appropriate placement of POCUS in TB diagnostic algorithms (eg, all admitted with SAM versus selected patients with higher pretest probability).

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## EVALUATION OF ACUTE AND CONVALESCENT ANTIBODY CONCENTRATION AGAINST PNEUMOCOCCAL CAPSULAR POLYSACCHARIDES FOR THE DIAGNOSIS OF PNEUMOCOCCAL INFECTION IN CHILDREN WITH COMMUNITY-ACQUIRED PNEUMONIA

OPEN

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**Abstract:** We evaluated whether the quantification of IgG to pneumococcal capsular polysaccharides is an accurate diagnostic test for pneumococcal infection in children with pneumonia in Nepal. Children with pneumococcal pneumonia did not have higher convalescent, or higher fold change, IgG to pneumo-

coccal polysaccharides than children with other causes of pneumonia. Caution is needed in interpreting antibody responses in pneumococcal infections.

**Key Words:** pneumococcal, serology, pneumonia, children, diagnostics

Accepted for publication October 23, 2023

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Funded by a European Society for Paediatric Infectious Diseases Small Grant to M.J.C. With support from Wellcome (Clinical Research Training Fellowship to M.J.C., 104439/Z/14/Z) and the NIHR (Academic Clinical Lectureship). The wider study was supported by Gavi, the Vaccine Alliance through its support of the PneumoNepal Project (<https://pneumonepal.org/>). A.J.P. was chair of the UK Department of Health and Social Care's Joint Committee on Vaccination and Immunisation (JCVI) during the study period and was a member for WHO's Strategic Advisory Group of Experts on Immunization during this period. Detection of respiratory viruses was done by Micropathology Ltd, Warwick, UK and the authors are grateful for the support of Dr. Colin Fink and Dr. Marie Voice in particular. The views expressed in this report do not necessarily represent the views of the Joint Committee on Vaccination and Immunisation, NIHR, or WHO. This work received funding from the Wellcome Trust (now Wellcome). The other authors have no conflicts of interest to disclose.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website ([www.pidj.com](http://www.pidj.com)).

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DOI: 10.1097/INF.00000000000004185

**M**odeling of data from randomized controlled trials has suggested that approximately one-third of children with pneumonia and radiographic consolidation have pneumococcal infection in settings without routine infant pneumococcal conjugate vaccination (PCV).<sup>1</sup> However, microbiologic techniques have limited accuracy to diagnose pneumococcal pneumonia for individual patients due to the inaccessibility of lung for sampling, pretreatment with antibiotics and prevalent nasopharyngeal (NP) carriage of pneumococci in healthy children.<sup>2</sup> Paired acute and convalescent serology to pneumococcal capsular polysaccharides (PS)<sup>3</sup> or proteins<sup>4</sup> from children with pneumococcal pneumonia may have diagnostic utility for pneumococcal pneumonia, but previous studies either do not use controls from the same disease population and/or use arbitrary thresholds for defining a positive result. We evaluated the accuracy of serology to pneumococcal PS for the diagnosis of pneumococcal infection in children hospitalized with pneumonia in Kathmandu, Nepal during 2015–2017. Ten-valent PCV (*Synflorix*, GSK) was introduced in the Nepal infant immunization schedule in 2015, with no catch-up campaign. In children hospitalized with pneumonia in this setting, 73% and 78% of invasive pneumococcal disease isolates were of serotypes covered by 10-valent PCV during 2005–2013 before 10-valent PCV introduction,<sup>5</sup> and NP carriage of any pneumococci was 36% and of pneumococcal serotypes within 10-valent PCV was 14% during 2014–2015.<sup>6</sup>

## METHODS

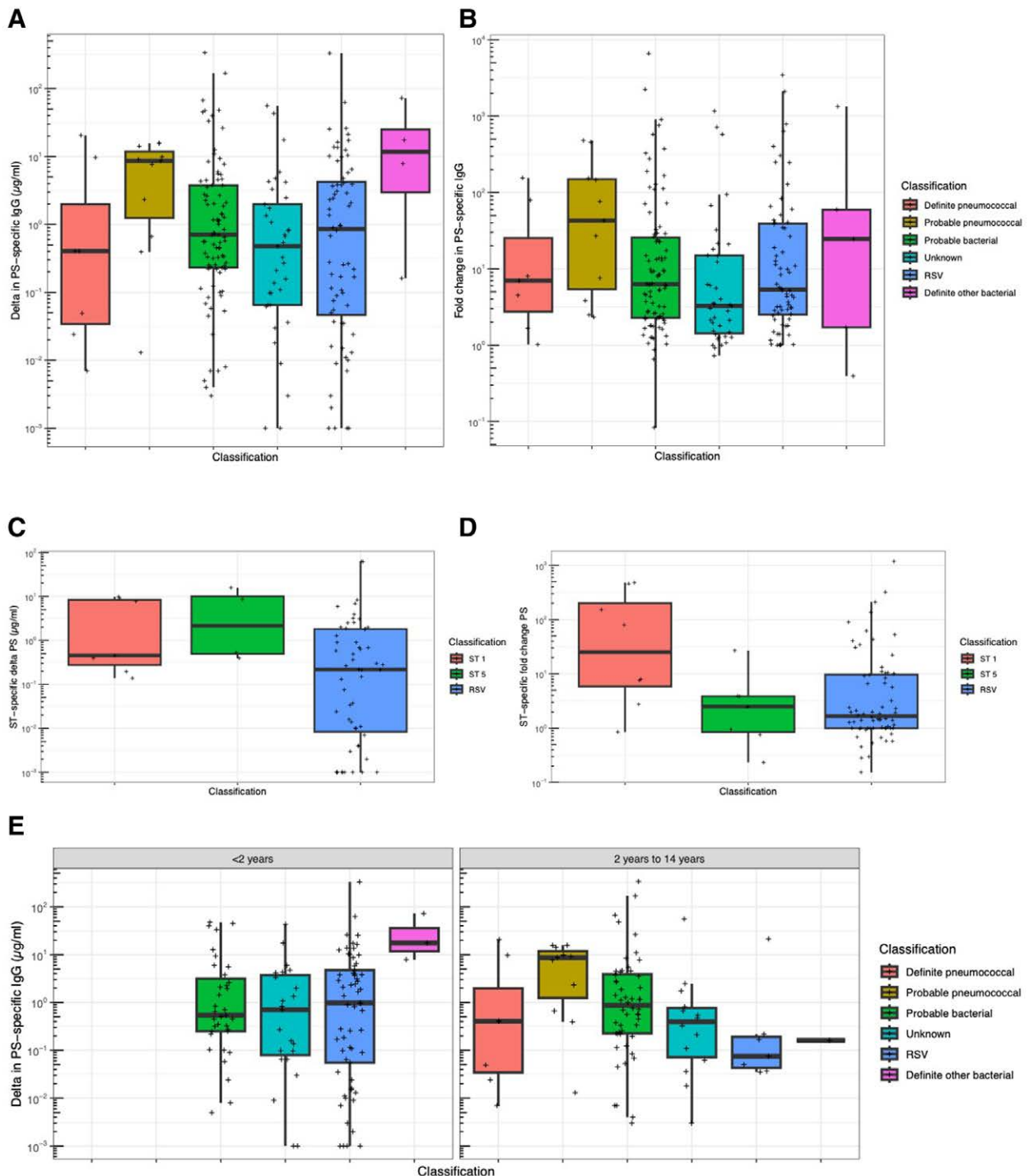
We sequentially recruited children 2 months to 14 years of age admitted to Patan Hospital, Kathmandu with a clinical

diagnosis of pneumonia. All children had chest radiographs, full blood count and C-reactive protein (CRP) measurement, culture of blood (Bactec PedsPlus culture bottles, BD, Franklin Lakes, NJ; aerobic culture in 5% CO<sub>2</sub> at 35–37°C) and NP sampling with flocced swabs (ThermoFisher Scientific, Waltham, MA) for pneumococcal culture and polymerase chain reaction detection of respiratory viruses (NxTAG Luminex Respiratory Pathogen Panel, Luminex Corp Austin, TX) within 48 hours of admission. Serotyping of pneumococci used the Quellung method (Statens Serum Institut, Denmark).<sup>6</sup> Convalescent sampling for serum of recruited children was done in 6–8 weeks following admission.

Samples were included for serologic testing if paired acute and convalescent samples were available. We defined a series of comparator groups by a priori probability of having true pneumococcal pneumonia. Of note, NP carriage of serotype 1 or 5 pneumococci (but not other serotypes) has a high positive predictive value for invasive pneumococcal disease in this setting.<sup>5</sup> Participants were grouped as definite pneumococcal pneumonia (pneumococci cultured from blood or pleural fluid), probable pneumococcal pneumonia (CRP concentration  $\geq 60$  mg/L and NP carriage of serotype 1 or 5), probable bacterial pneumonia (CRP concentration  $\geq 60$  mg/L and no NP carriage of serotype 1 or 5), unknown pneumonia etiology, respiratory syncytial virus (RSV) pneumonia only (CRP concentration  $< 60$  mg/L and NP carriage of RSV) and definite other bacterial pneumonia (other bacterial pathogen cultured from blood). All participant's samples with definite and probable pneumococcal pneumonia, RSV pneumonia and definite other bacterial pneumonia, a randomized selection of probable bacterial pneumonia and unknown pneumonia were included. Serum concentration of IgG to pneumococcal PS contained in the 13-valent PCV was measured using a fluorescence-based multiplex immunoassay (FMIA, RIVM, Netherlands).<sup>7</sup> We investigated whether change in absolute PS-specific IgG concentration (delta concentration) or fold change in PS-specific IgG concentration between acute and convalescent samples, or maximum PS-specific IgG convalescent concentration, was associated with pneumococcal pneumonia. As a sensitivity analysis, we also evaluated serotype 1 and serotype 5 specific values in children with pneumococcal pneumonia caused by serotypes 1 and 5, with children with RSV pneumonia only as a comparator group.

## RESULTS

Between 2015 and 2017, 897 children were sequentially recruited to the overall study. Of all children recruited, median age was 1.5 years (interquartile range, IQR, 0.7–3.1 years) and 528 (59%) children were male. Of these children, 454 (51%) returned for convalescent sampling (median 47, IQR 37–62, days after acute sampling) and 221 (49%) children with paired serum samples entered further analysis. Of these 221 children, median age was 1.7 (IQR 0.7–3.7) years, 133 (60%) children were male and 58 (26%) had received  $\geq 2$  doses of 10-valent PCV according to caregiver information (Table, Supplemental Digital Content 1, <http://links.lww.com/INF/F309>). On admission chest radiographs, 80 (36%) children had alveolar consolidation or effusion. Median CRP concentration at admission was 58 (IQR 6.8–109) mg/L. Eight children were classified as definite pneumococcal pneumonia (median age 5.0, IQR 3.8–6.6, years), 11 children as probable pneumococcal pneumonia (median age 4.7, IQR 3.3–7.7, years), 90 children as probable bacterial (median age 2.7, IQR 1.4–5.5, years), 68 children as RSV pneumonia (median age 0.7, IQR 0.4–1.5, years), 5 children as other bacterial pneumonia (median age 0.7, IQR 0.7–0.9, years) and 39 children as unknown (median age 1.3, IQR 0.8–2.9, years). Of children with definite pneumococcal pneumonia, 5 children had pneumococci isolated from blood (2 each of serotypes



**FIGURE 1.** Serum IgG to pneumococcal polysaccharides in children with acute pneumonia. In all plots, the y axis is on a  $\log_{10}$  scale and points represent the greatest of change in concentration from acute to convalescent samples (expressed as “delta”), or greatest fold change for and of the 13 polysaccharides assayed in an individual child. A: Maximum change in concentration between acute and convalescent samples for each diagnostic group. B: Maximum fold change in concentration between acute and convalescent samples for each diagnostic group. C: Maximum change in concentration between acute and convalescent samples for children with pneumonia associated with serotype 1 pneumococci, with pneumonia associated with serotype 5 pneumococci or RSV pneumonia (as a comparator). D: Maximum fold change in concentration between acute and convalescent samples for children with pneumonia associated with serotype 1 pneumococci, with pneumonia associated with serotype 5 pneumococci or RSV pneumonia (as a comparator). E: Maximum change in concentration between acute and convalescent samples for each diagnostic group stratified by age group.



1 and 5 and 1 serotype 6C) and 3 children had pneumococci isolated from pleural fluid (1 serotype 19A, 1 serotype 6B and 1 not serotyped).

There were no significant differences in the acute to convalescent change in concentration (delta concentration) of IgG to pneumococcal PS by classification of pneumonia etiology (Kruskal–Wallis test,  $P = 0.44$ , Fig. 1A; multiple pairwise comparisons with Wilcoxon test and Benjamin-Hochberg adjustment for multiple comparisons,  $P > 0.15$  for all comparisons), and there were no significant differences in acute to convalescent fold change of IgG to pneumococcal PS by classification of pneumonia etiology ( $P = 0.45$ , Fig. 1B;  $P > 0.10$  for pairwise comparisons). When analysis was limited to patients with serotype 1 (8 children) and serotype 5 (7 children) definite or probable pneumococcal pneumonia, there were no differences in delta concentration to the relevant PS in comparison with children with RSV pneumonia ( $P = 0.27$  and  $P = 0.36$ , respectively, Fig. 1C). In children with serotype 5 definite or probable pneumococcal pneumonia, there was no difference in fold change of IgG to pneumococcal PS5 ( $P = 0.79$ ), but children with serotype 1 definite or probable pneumococcal pneumonia had significantly higher fold change of IgG to pneumococcal PS1, in comparison with children with RSV pneumonia ( $P = 0.01$ , Fig. 1D). No children <2 years of age had definite or probable pneumococcal pneumonia. In children  $\geq 2$  years of age, there were no significant differences in delta concentration of IgG to pneumococcal PS by classification of pneumonia etiology (Fig. 1E,  $P > 0.15$  for pairwise comparisons). Similar results were obtained by maximum convalescent IgG concentration (Figure, Supplemental Digital Content 2, <http://links.lww.com/INF/F309>).

## DISCUSSION

Analyses of data from Belgium,<sup>3</sup> Finland<sup>4</sup> and Brazil<sup>4</sup> have suggested that paired serologic testing may accurately diagnose pneumococcal pneumonia in children. Tuerlinckx et al<sup>3</sup> quantified PS-specific IgG and IgA concentrations in acute and convalescent serum from Belgian children with pneumonia. Among children with culture-proven pneumococcal pneumonia, 83% of paired samples met the predefined “positive” threshold of  $\geq 3$ -fold increase in PS-specific IgG concentration. Among children with nonproven pneumococcal pneumonia, 55% of paired samples met this threshold; no other control samples were analyzed. As with data from Nepal, PS-specific IgG concentration in these Belgian data was associated with increasing age, with only 50% of definite pneumococcal pneumonia cases and 13% of suspected/possible pneumococcal cases <2 years of age meeting the positive threshold. Different age distributions between the Nepal (median 1.7 years) and Belgian studies (median 4.0 years), or different distributions of first colonization with pneumococcal serotype, may have contributed to differences in apparent prevalence of “positive” pneumococcal serology.

Given the diversity of pneumococcal PS, assay of IgG to pneumococcal proteins may improve the sensitivity of serologic testing. Andrade et al<sup>4</sup> evaluated the use of paired serology to 8 pneumococcal proteins to discriminate between pneumococcal pneumonia in Brazil (13 children, median age 14 months, non-PCV vaccinated) and a viral pharyngitis control group in Finland (23 children, median age 37 months, PCV vaccinated). Receiver-operator characteristic curves yielded areas under the curve of 0.67–0.93. However, the use of controls from a different population and disease may have confounded these results. We

previously extended this work by examining the production of IgG to 5 conserved pneumococcal proteins in the antibody in lymphocyte supernatant assay in Nepali children,<sup>8</sup> finding that lymphocyte production of IgG to pneumococcal proteins discriminated between pneumococcal and nonpneumococcal pneumonia with areas under the curve of 0.60–0.85. However, when stratified into children  $\geq 2$  years of age, there were no significant differences in protein-specific IgG production between pneumococcal and nonpneumococcal pneumonia. As with PS-specific IgG concentration, production of protein-specific IgG was associated with increasing age.

In the absence of a Gold standard, we used comparator groups from the same population of children with other pneumonia etiologies to assess a diagnostic test. We have previously shown that NP carriage of serotype 1 or 5 pneumococci may enrich this cohort for pneumococcal pneumonia.<sup>6</sup> Despite this, the small number of children with definite or probable pneumococcal pneumonia limited our study power, particularly in children <2 years of age. In addition, we sampled convalescent serum at a median 47 days, while other studies sampled convalescent serum at 3–4 weeks<sup>3</sup> or 2–5 weeks,<sup>4</sup> following admission. Our data may therefore represent antibody concentrations that are already declining. Measurement of IgG to specific pneumococcal PS has been used to assess population immunity<sup>9</sup> and combined with functional antibody studies to assess correlates of PCV-mediated protection.<sup>10</sup> However, in this cohort, it was not useful to diagnose pneumococcal pneumonia for individual patients.

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