

Validation of current joint American Academy of Allergy, Asthma and Immunology and American College of Allergy, Asthma and Immunology guidelines for antibody response to the 23-valent pneumococcal vaccine using a population of HIV-infected children

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Background: Measuring antibody responses to 23-valent pneumococcal polysaccharide vaccines (PPV) is crucial to evaluation of humoral immune function. However, data are limited comparing responses in immunodeficient subjects. **Objective:** A case-controlled study comparing changes in PPV antibody titer in immunocompetent and immunodeficient children was performed to validate current guidelines. **Methods:** A cohort of 95 immunocompetent children and 22 HIV-infected children, ages 2 to 15 years, was vaccinated with PPV, and before and after vaccination, IgG titers against capsular polysaccharides for 12 pneumococcal serotypes were measured. Results were used to calculate the receiver operator characteristic curve, sensitivity, and specificity of various interpretation criteria for their accuracy in detecting suboptimal responders.

Results: Immunodeficient subjects had lower CD4%, antidiphtheria, antitetanus titers, and mean post-PPV titers (2.5 ± 1.7 vs 4.5 ± 2.1 $\mu\text{g/mL}$; $P < .001$) compared with immunocompetent subjects. The interpretation, using individual post-PPV titer of ≥ 4 -fold increase over prevaccination as a positive response, yielded the highest accuracy as measured by area under the curve value (receiver operator characteristic curve = .755). For this criterion, the numbers of serotypes with positive responses of ≤ 5 of 12 serotypes measured yielded 72.7% sensitivity and 56.8% specificity in detecting antibody-deficient subjects.

Conclusion: Current guidelines using ≥ 4 -fold increase in post-PPV titers has sufficient sensitivity and specificity to identify antibody deficiency. The minimal positive responses should be at least 50% of serotypes tested.

Clinical implications: Measurement of PPV antibody response based on the current guidelines accurately identify children with humoral immunodeficiency. (J Allergy Clin Immunol ■■■■;■■■;■■■-■■■.)

Key words: *Pneumococcal antibody, humoral deficiency, children, polysaccharide vaccine, HIV*

Humoral immunodeficiency is the most common type of primary immunodeficiency and accounts for approximately $\frac{1}{2}$ of all primary immunodeficiency cases.^{1,2} Because known genetic mutations account for only a small portion of patients with humoral immunodeficiency, measurement of antibody responses against vaccines becomes a crucial tool in making the diagnosis.^{1,3-6} Vaccines for this purpose are divided into protein-based vaccines such as diphtheria or tetanus toxoids, for evaluating T-cell-dependent immune responses, and polysaccharide-based vaccines such as pneumococcal and meningococcal vaccines for evaluating T-cell-independent immune responses. Because of the inconsistency of eliciting immune response of meningococcal vaccine,⁷ the use of 23-valent pneumococcal polysaccharide vaccine (PPV) is widely recommended.

Currently, no study has been performed to evaluate immunologic responses to PPV in immunodeficient subjects. Although guidelines for PPV interpretation have recently been proposed, many debates still exist regarding test accuracy and what should be considered appropriate responses.^{1,8} To address these questions better, data on PPV responses in humoral immunodeficient subjects are required. However, the availability of appropriate immunodeficient subjects for study is low because most of these patients have been receiving intravenous immunoglobulin treatment, were previously vaccinated with PPV, or are younger than 2 years. On the other hand, children with

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Abbreviations used

PCV7: 7-Valent pneumococcal conjugate vaccine
 PPV: 23-Valent pneumococcal polysaccharide vaccine
 ROC: Receiver operator characteristic curve

HIV infection are known to have a wide array of humoral immunodeficiencies such as polyclonal hypergammaglobulinemia, decreased responsiveness to vaccinations, and *ex vivo* antigen stimulation.⁹⁻¹⁵ In addition, all previous studies in HIV-infected subjects have shown no clinical benefit or poor immunologic responses after PPV vaccination.¹⁶⁻¹⁹

In this study, we evaluated immunologic responses of PPV in immunocompetent children and compared them with responses of HIV-infected children with evidence of existing secondary immunodeficiency. The results were used to validate the current recommendations for interpretation of PPV responses and to establish recommendations that would have the highest accuracy in identifying subjects with humoral immunodeficiency.

METHODS

The study was performed in the Pediatric Specialty Clinic at the University of South Florida/All Children's Hospital (St Petersburg, Fla). The study protocol was approved by the Institutional Review Board of All Children's Hospital.

Study subjects

Subjects were children between 2 to 15 years of age who had never received 7-valent pneumococcal conjugate vaccine (PCV7) or who had not received PPV in the previous 5 years, and who had not taken immunosuppressive medication or intravenous immunoglobulin in the past 6 months.

Immunocompetent (control) subjects. Ninety-five children referred to the clinic for immunologic evaluation because of suspected immunodeficiency on the basis of criteria described elsewhere were enrolled.³ The subjects were thoroughly evaluated and found to be immunologically normal by all measured parameters. Immunologic laboratory evaluations were performed including immunoglobulin panel (IgG, IgA, and IgM), specific antibodies to diphtheria vaccinations, and HIV antibody by ELISA method. In selected individuals, further immunologic evaluations were performed as appropriate. Children who were found to have abnormal immunologic evaluations were excluded. To reduce the possibility of undiagnosed immunodeficiency, the subjects continued to be followed in this referral clinic until the immune deficiency had been excluded or clinically improved.

Immunodeficient (HIV-infected) subjects. Perinatally acquired HIV-infected children were selected on the basis of their immune status at the time of vaccination. To be included in the study, the subjects had to meet at least 1 of the 2 following criteria: (1) cell-mediated immunodeficiency with documented Centers for Disease Control immunology category 2 or 3 (CD4 count < 25% on more than 1 occasion), or (2) low antibodies to diphtheria or tetanus (<0.15 IU/mL) despite appropriate vaccinations. Of 85 perinatally acquired HIV-infected children in the clinic, 22 children fulfilled the inclusion criteria and were used as subjects with pneumococcal-impaired responses.

Vaccine, blood samples, and laboratory studies

A pneumococcal vaccine, Polyvalent Pneumovax*23 (Merck & Co, Inc, West Point, Pa), containing 25 µg each of the purified capsular polysaccharide of 23 pneumococcal serotypes was used to immunize the subjects intramuscularly with a 0.5-mL injection in the anterolateral thigh. Venous blood samples were collected before the PPV vaccination (pre-PPV) and at 1 to 3 months after vaccination (post-PPV) to be analyzed for specific IgG against pneumococcal capsular polysaccharides of 12 most prevalent pneumococcal serotypes (including serotype 1, 3, 4, 6B, 7F, 8, 9V, 12F, 14, 18C, 19F, and 23F). Pre-PPV and post-PPV titers were determined by ELISA. In short, ELISA plates were prepared by incubating 1 mg/L of different serotypes of pneumococcal antigens (serotypes: 1, 3, 4, 6B, 7F, 8, 9V, 12F, 14, 18C, 19F, and 23F; obtained from ATCC; Merck & Co, Inc, Manassas, Va) at 20°C overnight in 96-well Microtiter polystyrene plates (Immulon 1B; Dynex, Chantilly, Va). In each well, 100 µL of 1:100 diluted patient serum in serum-conjugate buffer (newborn calf serum, Brij-35 detergent, and PBS) was mixed with 50 µL of 2 µg/mL lyophilized pneumococcal cell wall polysaccharide (Statens Serum Institute, Copenhagen, Denmark) and 50 µL of 4 µg/mL Ps22F (ATCC) and incubated overnight at 4°C. These adsorptions were used to remove nonserotype-specific and nonopsonic antibodies.²⁰ The plates were washed, and 100 µL of 1:2000 Alkaline Phosphatase Labeled Goat Anti-Human IgG (Sigma, St Louis, Mo) in serum conjugate buffer was added. They sat at room temperature for 2 hours. After washes, the plates were incubated with 100 µL substrate (Sigma) for exactly 20 minutes. Absorbance was measured at 405 nm by using a Microplate Reader (Bio-Tek Elx808 Ultra; Bio-Tek Instruments, Inc, Winooski, Vt). In each experiment, 4 controls were used, which included 2 control sera from 20 pool samples (1 serum from 10 pool subjects before vaccination served as low pneumococcal antibody level, and a second serum from 10 pool subjects after PPV served as high antibody level); 1 serum from a healthy individual with known antibody level served as a medium antibody level, and US Food and Drug Administration reference serum 89SF (kindly provided by Dr Carl Frasch, Rockville, Md, with known concentrations of the listed serotypes expressed as mg/L as follows: 1 [6.3], 3 [2.4], 4 [4.1], 6B [16.9], 7F [5.2], 8 [5.1], 9V [7.8], 12F [1.7], 14 [27.8], 18C [4.5], 19F [13], 23F [8.1]). Each sample was analyzed in duplicate; the coefficient of variation was less than 12%.

Statistical analysis

Baseline demographic, immunologic, and pre-PPV and post-PPV titers data were compared between immunodeficient and immunocompetent subjects by Student *t* test. Two-sided tests were used, and a *P* value less than .05 was considered statistically significant.

To determine which evaluation criterion was the best in identifying immunodeficient subjects, we calculated receiver operator characteristic curves (ROC) for each evaluation criterion. The ROC represented a curve plotted between the true-positive rate (sensitivity) and the false-negative rate (1-specificity) using every possibility (from 0 to 12 serotypes) of positive response cutoff points for each criterion. The criterion yielding the highest ROC value (area under curve value that was closest to 1) was considered the most accurate. The selected criteria were divided into 2 categories. The criteria for the first category used arbitrary levels of individual post-PPV titer ≥ 1.3 , ≥ 1.5 , and ≥ 2.0 µg/mL, and in the second category, the criteria compared between pre-PPV and post-PPV titers, with ≥ 2 -fold, ≥ 3 -fold, or ≥ 4 -fold increase of post-PPV titers considered positive responses.

To determine how many serotypes with positive responses were needed for an appropriate PPV response, we calculated the sensitivity and specificity for every cutoff point of positive response (from 0 to 12

TABLE I. Demographics

	Immunodeficient	Immunocompetent	P value
N	22	95	
Mean age, y $\pm$ SD	5.7 $\pm$ 2.2	4.8 $\pm$ 2.9	.18
Male:female	7:15	48:47	.11
Race			.03
White	11 (50%)	71 (74.7%)	
African American	10 (45.5%)	18 (18.9%)	
Other	1 (4.5%)	6 (6.3%)	
Clinical data			
Sinopulmonary infections	18 (81.8%)	70 (73.7%)	.426
Opportunistic infections	7 (31.8%)	0 (0%)	<.001
Prophylaxis antibiotics	6 (27.2%)	5 (5.2%)	.001
Immunologic data			
CD4, % $\pm$ SD	31.3 $\pm$ 9.3	39.1 $\pm$ 8.9	<.01
CD4 counts, cells/mm ³ $\pm$ SD	1108.8 $\pm$ 562.5	1340.9 $\pm$ 643.9	.14
IgG, mg/dL $\pm$ SD	1845.6 $\pm$ 991.5	741.3 $\pm$ 296.3	<.001
IgA, mg/dL $\pm$ SD	252.7 $\pm$ 268.3	91.8 $\pm$ 69.3	<.001
IgM, mg/dL $\pm$ SD	159.8 $\pm$ 88.6	101.8 $\pm$ 42.4	<.001
Antidiphtheria, IU/mL $\pm$ SD	0.9 $\pm$ 1.5	1.8 $\pm$ 1.8	<.05
Antitetanus, IU/mL $\pm$ SD	1.2 $\pm$ 1.4	2.3 $\pm$ 1.7	<.01
Pre-PPV titer, μ g/mL $\pm$ SD	1.5 $\pm$ 1.2	1.3 $\pm$ 0.7	.30
Post-PPV titer, μ g/mL $\pm$ SD	2.5 $\pm$ 1.7	4.5 $\pm$ 2.1	<.001

serotypes). Sensitivity was defined as the portion of immunodeficient (HIV-infected) subjects who had abnormal responses (true positive). Specificity was defined as the portion of immunocompetent subjects that had good responses (true negative). An acceptable number of serotypes with positive responses should yield at least 50% sensitivity and specificity in detecting immunodeficiency subjects.

To use the results to interpret PPV responses for children who previously received 7-valent pneumococcal conjugate vaccine (PCV7), we presumed that there was negligible amount of pneumococcal-specific IgG to non-PCV7 serotypes produced after PCV7 vaccination. We tested pneumococcal titers of 5 serotypes that were not included in PCV7—serotypes 1, 3, 7F, 8, and 12F—and calculated the ROC of each interpretation criterion to identify the most accurate criterion. We then identified the numbers of positive responses needed for an appropriate PPV response for each interpreted criterion. All analyses were performed by use of SPSS for Windows, version 14.0 (SPSS Inc, Chicago, Ill).

RESULTS

Of the 117 children between 2 and 15 years of age included in the study, 95 were immunocompetent children, and 22 were vertically HIV-infected immunodeficient children with Centers for Disease Control category 2 or 3 or low antibody titer to diphtheria/tetanus. There was no significant difference in age between immunocompetent and immunodeficient subjects (Table I). There was no difference in history of sinopulmonary infection between the 2 groups. Immunodeficient HIV-infected subjects had more opportunistic infections and used more prophylaxis antibiotic than the immunocompetent subjects. In the immunodeficient HIV-infected group, there was a lower CD4 percentage (31.3% $\pm$ 9.3% vs 39.1% $\pm$ 8.9%; P < .01), antidiphtheria (0.9 $\pm$ 1.5 vs 1.8 $\pm$ 1.8

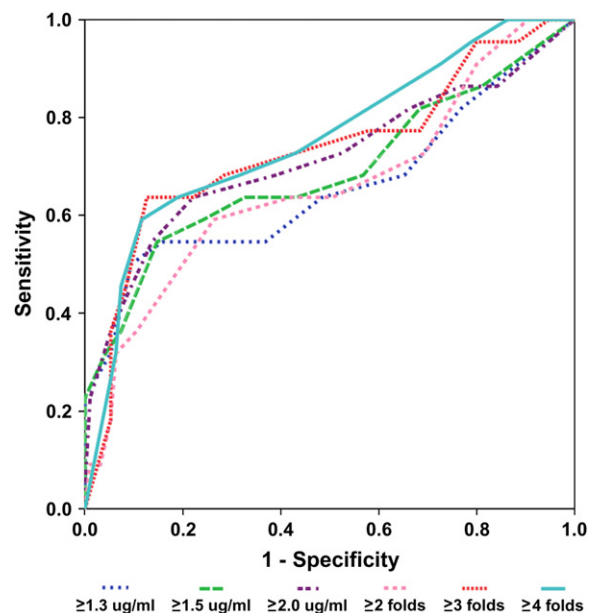


FIG 1. ROC representing accuracy of each evaluation criterion in detecting immunodeficient HIV-infected subjects. Value of area under curve for each evaluation criterion was as follows: $\geq 1.3 \mu\text{g/mL}$ = 0.656; $\geq 1.5 \mu\text{g/mL}$ = 0.688; $\geq 2.0 \mu\text{g/mL}$ = 0.714; ≥ 2 -fold = 0.659; ≥ 3 -fold = 0.733; ≥ 4 -fold = 0.755.

1.8 IU/mL; P < .05), antitetanus antibody titers (1.2 $\pm$ 1.4 vs 2.3 $\pm$ 1.7 IU/mL; P < .01), and post-PPV titers (2.5 $\pm$ 1.7 vs 4.5 $\pm$ 2.1; P < .001) compared with immunocompetent subjects. IgG, IgA, and IgM levels were higher in immunodeficient HIV-infected subjects than in the immunocompetent subjects (P < .001).

TABLE II. Sensitivity and specificity of the PPV test on the basis of numbers of serotypes with positive responses

No. of responses*	ImmDef (%)	ImmCom (%)	Sensitivity	Specificity	No. of responses*	ImmDef (%)	ImmCom (%)	Sensitivity	Specificity
Criteria using post-PPV titer ≥ 1.3 $\mu\text{g/mL}$					Criteria using post-PPV titer raising ≥ 2-fold above baseline				
=0 serotypes	4.5	0	4.5	100	=0 serotypes	9.1	1.1	9.1	98.9
≤ 1 serotypes	9.1	0	9.1	100	≤ 1 serotypes	9.1	3.2	9.1	96.8
≤ 2 serotypes	22.7	0	22.7	100	≤ 2 serotypes	18.2	5.3	18.2	94.7
≤ 3 serotypes	22.7	0	22.7	100	≤ 3 serotypes	31.8	6.3	31.8	93.7
≤ 4 serotypes	31.8	5.3	31.8	94.8	≤ 4 serotypes	36.4	10.5	36.4	89.5
≤ 5 serotypes	50.0	9.5	50.0	90.5	≤ 5 serotypes	45.5	16.8	45.5	83.2
≤ 6 serotypes	54.5	15.8	54.6	84.2	≤ 6 serotypes	59.1	26.3	59.1	73.7
≤ 7 serotypes	54.5	25.3	54.6	74.7	≤ 7 serotypes	63.6	42.1	63.6	57.9
≤ 8 serotypes	54.5	36.8	54.6	63.2	≤ 8 serotypes	63.6	50.5	63.6	49.5
≤ 9 serotypes	63.6	48.4	63.6	51.6	≤ 9 serotypes	72.7	69.5	72.7	30.5
≤ 10 serotypes	68.2	65.3	68.2	34.7	≤ 10 serotypes	90.9	80.0	90.9	20.0
≤ 11 serotypes	81.8	76.8	81.8	23.2	≤ 11 serotypes	100	90.5	100	9.5
≤ 12 serotypes	100	100	100	NA	≤ 12 serotypes	100	100	100	NA
Criteria using post-PPV titer ≥ 1.5 $\mu\text{g/mL}$					Criteria using post-PPV titer raising ≥ 3-fold above baseline				
=0 serotypes	4.5	0	4.6	100	=0 serotypes	18.2	5.3	18.2	94.7
≤ 1 serotypes	9.1	0	9.1	100	≤ 1 serotypes	36.4	5.3	36.4	94.7
≤ 2 serotypes	22.7	0	22.7	100	≤ 2 serotypes	45.5	8.4	45.5	91.6
≤ 3 serotypes	31.8	4.2	31.8	95.8	≤ 3 serotypes	63.6	12.6	63.6	87.4
≤ 4 serotypes	36.4	7.4	36.4	92.6	≤ 4 serotypes	63.6	22.1	63.6	77.9
≤ 5 serotypes	54.5	14.7	54.6	85.3	≤ 5 serotypes	68.2	28.4	68.2	71.6
≤ 6 serotypes	59.1	24.2	59.1	75.8	≤ 6 serotypes	72.7	43.2	72.7	56.8
≤ 7 serotypes	63.6	32.6	63.6	67.4	≤ 7 serotypes	77.3	57.9	77.3	42.1
≤ 8 serotypes	63.6	43.2	63.6	56.8	≤ 8 serotypes	77.3	68.4	77.3	31.6
≤ 9 serotypes	68.2	56.8	68.2	43.2	≤ 9 serotypes	95.5	80.0	95.5	20.0
≤ 10 serotypes	81.8	68.4	81.8	31.6	≤ 10 serotypes	95.5	88.4	95.5	11.6
≤ 11 serotypes	86.4	81.1	86.4	19.0	≤ 11 serotypes	100	94.7	100	5.3
≤ 12 serotypes	100	100	100	NA	≤ 12 serotypes	100	100	100	NA
Criteria using post-PPV titer ≥ 2.0 $\mu\text{g/mL}$					Criteria using post-PPV titer raising ≥ 4-fold above baseline				
=0 serotypes	4.5	0.0	4.6	100	=0 serotypes	31.8	6.3	31.8	93.7
≤ 1 serotypes	22.7	1.1	22.7	99.0	≤ 1 serotypes	45.5	7.4	45.5	92.6
≤ 2 serotypes	36.4	5.3	36.4	94.7	≤ 2 serotypes	59.1	11.6	59.1	88.4
≤ 3 serotypes	45.5	9.5	45.5	90.5	≤ 3 serotypes	63.6	18.9	63.6	81.1
≤ 4 serotypes	54.5	13.7	54.5	86.3	≤ 4 serotypes	68.2	31.6	68.2	68.4
≤ 5 serotypes	63.6	22.1	63.6	77.9	≤ 5 serotypes	72.7	43.2	72.7	56.8
≤ 6 serotypes	68.2	38.9	68.2	61.1	≤ 6 serotypes	86.4	65.3	86.4	34.7
≤ 7 serotypes	72.7	52.6	72.7	47.4	≤ 7 serotypes	90.9	72.6	90.9	27.4
≤ 8 serotypes	81.8	66.3	81.8	33.7	≤ 8 serotypes	95.5	78.9	95.5	21.1
≤ 9 serotypes	86.4	76.8	86.4	23.2	≤ 9 serotypes	100	86.3	100	13.7
≤ 10 serotypes	86.4	84.2	86.4	15.8	≤ 10 serotypes	100	92.6	100	7.4
≤ 11 serotypes	100	100	100	0	≤ 11 serotypes	100	98.9	100	1.1
≤ 12 serotypes	100	100	100	NA	≤ 12 serotypes	100	100	100	NA

ImmDef, Immunodeficient subjects; ImmCom, immunocompetent subjects.

*Numbers of serotypes with positive responses in each selected criterion.

Accuracy of interpretation criteria based on ROC value

Fig 1 shows the ROC that represents the accuracy of each evaluation criterion when applied to studied subjects. Of the 2 categories of criteria evaluated, the category that based positive responses on comparing pre-PPV and post-PPV titers showed a higher ROC value than the category that used arbitrary levels of post-PPV titers, and thus was more accurate in detecting immunodeficient HIV-infected subjects. Among each interpreted category, criteria

using post-PPV titers ≥ 2.0 $\mu\text{g/mL}$ and criteria using ≥ 4 fold increase of post-PPV titers over baseline yielded the highest accuracy (ROC = .714 and .755, respectively).

Number of serotypes with positive response needed for a normal response

To determine the number of serotypes with positive response that should be considered a normal response, we calculated the sensitivity and specificity of the test in its ability to detect immunodeficient HIV-infected subjects

using every possible positive response (from to 12) for each criterion (Table II). If a positive response was determined by ≥ 4 -fold increase of post-PPV titers above baseline and positive responses in at least 6 out of 12 serotypes tested were required in an individual for a normal response, the PPV test was 72.7% sensitive and 56.8% specific in identifying immunodeficient HIV-infected subjects. If post-PPV titer ≥ 2 $\mu\text{g/mL}$ was considered as a positive response and at least 6 out of 12 serotypes with positive responses were required for a normal response, the PPV test was 63.6% sensitive and 77.9% specific.

To interpret PPV responses in children who previously received PCV7, we calculated 4 serotypes of post-PPV titers that were not included in PCV7 (ie, 3, 7F, 8, and 12F). The results showed that criteria using ≥ 4 -fold increase of post-PPV titers above baseline yielded the most ROC value (data not shown). ROC results were not significantly different when more than 4 serotypes were used for calculation or when a different set of 4 non-PCV7 serotypes was selected. A minimal number of positive responses that should have been considered an appropriate response was 50% (2 out of 4 serotypes) of all serotypes tested. If post-PPV titers ≥ 4 fold increase from baseline were considered a normal response, and 2 out of 4 serotypes with positive responses were required for a normal response, the PPV test was 59.1% sensitive and 86.3% specific in detecting immunodeficient subjects.

DISCUSSION

In this study, we carefully selected children with secondary immunodeficiencies to represent pneumococcal-impaired response subjects. By using these subjects, we were able to determine the accuracy of current guidelines for interpretation of PPV responses in children. The HIV-infected subjects enrolled in this study showed a broad range of humoral immune defects including low antidipteria, antitetanus, and post-PPV titers. Subjects with low CD4 counts were chosen because low CD4 counts were found to correlate with impaired immune responses to PPV.^{19,21} High levels of IgG, IgA, and IgM found in these subjects also represented polyclonal B-cell activation from their underlying humoral immunodeficiency.^{22,23}

To interpret PPV tests, current recommendations require the comparison of pneumococcal titers before and after vaccination. A 2-fold to 4-fold increase in individual titers from baseline is recommended as a normal response.²⁴ Hedlund et al²⁵ showed that elderly patients with a ≥ 4 -fold increase in post-PPV titers had a lower risk of recurrent pneumonia compared with individuals with lower fold increase. Our ROC data confirmed that the criteria using increased post-PPV titers ≥ 4 -fold from baseline yielded the highest area under the curve and thus provided greater accuracy in separation between immunocompetent and deficient subjects.

Another widely recommended interpreting criterion is an arbitrary level of post-PPV titers that correlates with

clinical protection as positive responses. In children, the pneumococcal antibody of 200 ng antibody nitrogen/mL (converted to 1.3 $\mu\text{g/mL}$) correlated with decreased nasopharyngeal colonization with specific pneumococcal serotypes.²⁶ In adults, systemic pneumococcal infection was rare when pneumococcal antibody was >250 to 300 ng of antibody nitrogen/mL (converted to 1.56–1.88 $\mu\text{g/mL}$).²⁴ Based on our ROC data, we found that criteria using arbitrary levels of post-PPV titers as positive responses were less accurate than criteria comparing fold increase in post-PPV titers from baseline. Among criteria using arbitrary levels of post-PPV titers, we found using post-PPV titers of ≥ 2.0 was superior to using titers of ≥ 1.5 or ≥ 1.3 $\mu\text{g/mL}$ in detecting immunodeficient HIV-infected subjects. However, results of pneumococcal antibody levels vary greatly among different laboratories depending on techniques used and whether the tests included preabsorbing steps to remove nonspecific antibodies. We felt that the appropriate arbitrary level of positive responses obtained from this study (≥ 2.0 $\mu\text{g/mL}$) may not be appropriate for use in interpreting results from other laboratories.

Another important aspect of interpretation is how many serotypes with positive response a child should have to be considered normal. A recent consensus panel recommended that individuals between the ages of 2 and 5 years should respond to at least 50% of the serotypes tested.¹ The data from our study support this recommendation. We found that on the basis of this criterion, the PPV test was 72.7% sensitive and 56.8% specific in identifying immunodeficient HIV-infected subjects. Sensitivity would have improved if higher numbers of serotypes with positive response were used; however, specificity would have dropped below an acceptable level (below 50%).

Lastly, pneumococcal conjugate vaccine is currently recommended in children younger than 5 years. This vaccine is conjugated with diphtheria protein; thus, the immune response is T-cell-dependent.²⁷ Because most young children have received the vaccination, interpretation of the PPV test presents a greater challenge. Epidemiologic studies have shown the clinical benefit of PCV7; however, the protection of PCV7 occurred only in the pneumococcal serotypes included in the vaccines.²⁸ This suggests the conjugate vaccine induced very little, if any, cross-immunologic response to other serotypes not included in the vaccine. We found measuring 4 pneumococcal serotypes not included in the PCV7 could be performed to evaluate children who had previously received PCV7. In addition, these data suggested that using 4 to 6 serotypes might be sufficient for pneumococcal antibody evaluation without significant loss of sensitivity or specificity.

This study has a few limitations. First, data obtained from the HIV-infected population may not accurately represent patients with primary immunodeficiency because each has a different pathogenesis and mechanism of disease. Second, a previous study suggested pneumococcal vaccination might be less effective in black compared with white subjects.²⁹ The higher proportion of black subjects in the HIV group in this study may be a

contributing factor to the lower antibody responses seen in HIV subjects. Third, the difference in the use of prophylaxis antibiotics and in the incidence of opportunistic infections among the studied groups might affect baseline pneumococcal antibodies in the studied subjects. However, low baseline pneumococcal antibody levels and no difference in baseline titers between the HIV and control groups suggest these factors did not interfere significantly with the pneumococcal test.

In conclusion, we found the pneumococcal vaccine to be an accurate, sensitive, and specific test for diagnosis of subjects with humoral immunodeficiency secondary to HIV infection. Furthermore, these parameters can be applied to patients who are being evaluated for primary immunodeficiency. We confirm the recent proposed recommendation of considering ≥ 4 -fold increase in pneumococcal titers after PPV immunization for at least 50% of serotypes tested (ie, 6 out of 12 serotypes) as adequate responses in children between 2 and 15 years of age. For children who had previously received PCV7, positive responses to 2 out of 4 serotypes not included in the PCV7 should be considered adequate.

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