Published in final edited form as:

Pediatr Allergy Immunol. 2019 May ; 30(3): 392–395. doi:10.1111/pai.13026.

Using only a subset of pneumococcal serotypes is reliable for the diagnosis of specific antibody deficiency in children: a proof-of-concept study

Maleewan Kitcharoensakkul, MD^a, Andrew L. Kau, MD, PhD^b, Leonard B. Bacharier, MD^a, Charles W. Goss, PhD^c, and Avraham Beigelman, MD, MSCI^a

^aDivision of Allergy, Immunology and Pulmonary Medicine, Department of Pediatrics, St. Louis Children's Hospital, Washington University School of Medicine, St. Louis, Missouri, USA

^bCenter for Women's Infectious Disease Research and the Division of Allergy and Immunology, Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri, USA

^cDivision of Biostatistics, Washington University School of Medicine. St. Louis, Missouri, USA

Keywords

Pneumococcal titers; specific antibody deficiency

To the Editor,

Specific or selective antibody deficiency (SAD) is one of the most commonly identified immune defects among patients with recurrent sinopulmonary infections.¹ SAD is defined as an impaired response to polysaccharide antigens and can be detected in approximately 15% of children older than 2 years old referred for evaluation of recurrent infections.^{2, 3} The diagnosis of SAD requires the documentation of an adequate response to polysaccharide antigens measured by immune response to the 23-polyvalent pneumococcal vaccine (Pneumovax®, 23-PPV) and exclusion of other causes of immunodeficiency.^{4, 5} Measurement of antibody responses to all serotypes in 23-PPV may not be available in all facilities, and thus a limited set of pneumococcal serotypes are often used to diagnose SAD.⁶ Based on previous studies that demonstrated differences in the immunogenicity among pneumococcal serotypes,^{7, 8} we hypothesized that a narrower panel of serotype-specific anti-pneumococcal antibody titers would provide comparable diagnostic utility for SAD diagnosis as the complete panel of 23 serotypes. We conducted a retrospective proof-of-concept study to test this hypothesis.

Corresponding author: mkitcharoensakkul@wustl.edu.

Authors contribution:

Maleewan Kitcharoensakkul: study design, acquisition of data, analysis and interpretation of data, drafting and revising the manuscript

Andrew L Kau: study design, analysis and interpretation of data, and revising the manuscript

Charles W Goss: analysis and interpretation of data, and revising the manuscript

Leonard B Bacharier: study design, analysis and interpretation of data and revising the manuscript

Avraham Beigelman: study design, analysis and interpretation of data and revising the manuscript

Potential conflict of interests: None

We used the Clinical Investigation Data Exploration Repository (CIDER) to identify patients 2–18 years old who had been evaluated for recurrent infections in the allergy and immunology clinic at St. Louis Children’s Hospital, and who had 2 sets of pneumococcal titers to 23 serotypes drawn 4–12 weeks apart between January 2005 and December 2014. To define an adequate response to 23-PPV, we utilized cutoffs recommended by a task-force of the American Academy of Allergy, Asthma & Immunology: a post-immunization level > 1.3 mcg/mL, or at least a 4-fold rise in a titer, compared to the pre-immunization level to 50% of serotypes tested for 2–5 years of age, or 70% of serotypes tested for children 6 years and older¹. In our clinics, patients with an inadequate immune response are boosted with a 23-PPV, and a second set of antibody levels were obtained 4–8 weeks following the booster. Exclusion criteria included treatment with an immunosuppressive agent in the 3 months prior to the testing, history of solid organ or bone marrow transplants, and diagnosis of another immunodeficiency. We thoroughly reviewed the patients’ chart to ascertain that the SAD patients did not have clinical or laboratory features that may raise concerns for other immunodeficiencies that could potentially present with impaired response to vaccines. These included: DiGeorge syndrome (absence of lymphopenia, and dysmorphic features); Ataxia Telangiectasia (absence of delayed gross motor, telangiectasias, abnormal lymphocyte count, and abnormal immunoglobulin levels); Hyper IgE syndrome (absence of severe eczema accompanied by skeletal abnormalities in patients with elevated IgE levels); Wiskott-Aldrich syndrome (absence of thrombocytopenia, small platelet size, neutropenia and abnormal immunoglobulin levels); and NEMO deficiency (absence of ectodermal dysplasia and typical infections for NEMO patients). Patients were diagnosed with SAD if they had an inadequate response to 23-PPV, but demonstrated normal total immunoglobulin levels and had documented protective titers to tetanus toxoid and *Haemophilus influenza* type b. The pneumococcal quantitative levels of antibody were measured at Mayo Clinic Laboratory using a Luminex® multiplex bead-based immunoassay⁹. The study and waiver of consents were approved by the Washington University School of Medicine Institutional Review Board.

T-tests, Chi-square test or Fisher’s exact test were used for comparison of baseline characteristics between groups (SAD vs normal responders). Univariable and multivariable statistical analyses were performed using logistic regression analysis (SAS version 9.4) to assess the ability of post-immunization antibody level (>1.3mcg/mL) to each serotype to predict the presence of SAD. A forward stepwise logistic regression approach with entry and removal criteria of $P = 0.10$ was used to select the final multivariable model. The predictive abilities of the logistic regression models were assessed using sensitivity, specificity, and area under the ROC (Receiver Operating Characteristic) curve (AUC).

We identified 164 children who had 2 sets of pneumococcal antibody levels drawn 4–12 weeks apart. Of 164, 40 children were excluded due to: diagnosis of other primary immunodeficiency including hypogammaglobulinemia, combined immunodeficiency, selective IgA deficiency or common variable immunodeficiency (n=26), history of transplantation/chemotherapy (n=12), history of prednisolone use in the preceding 3 months (n=1), or gastrointestinal loss from protein-losing enteropathy (n=1). Hence, 124 patients were included in the final analysis.

The mean age, of the 124 patients that were included in the study, at the time of first set of antibody measurement drawn, was 7.7 ± 4 years. Half of the patients were male and 96% were Caucasians. Of these 124 patients, 23 (19%) demonstrated inadequate responses to 23-PPV and were diagnosed with SAD, and 101 (81%) were defined as normal responders. Patient characteristics (age, race, gender, and mean duration between the two 23-PPV tests) did not differ between SAD patients and normal responders (Table 1).

Multiple univariable analyses that evaluated the ability of a post-immunization antibody level of >1.3 mcg/ml to each serotype in predicting SAD revealed a wide range of predictive abilities for each individual serotype (AUCs range from 0.57 to 0.81, Figure 1). A multivariable stepwise regression revealed that the post-immunization levels of 5 specific serotypes: 4, 5, 11A, 18C and 20 were negatively associated with the presence of SAD, $P < 0.05$ (Table 2), and indicated that among children with post-immunization antibody level > 1.3 mcg/ml to each of these serotypes, the predicted odds of a SAD diagnosis decreased by 88–99%. The estimated ROC AUC, using the post-immunization levels to the combination of these 5 serotypes alone to predict whether or not the subject has SAD, was 0.96 (95% CI 0.92–1.0).

In this proof-of-concept study, approximately 14% of children referred to the allergy/immunology clinic at a tertiary care center for recurrent infections and had two sets of pneumococcal titers drawn, were diagnosed with SAD. This finding is consistent with prior studies conducted in a similar setting.^{2,3} We demonstrated a subset of five serotypes, 4, 5, 11A, 18C and 20, predict SAD to a degree comparable to the full 23-serotype panel. This finding suggests that after additional independent prospective confirmation this smaller subset of 23 serotypes may be utilized in the clinical practice to diagnose SAD.

This study has certain limitations. Serotypes 4, 5 and 18C are present in the Prevnar® vaccine, and we were not able to retrospectively obtain the immunization records in most patients although all patients were recorded as immunizations were up-to-date. We used the Luminex® multiplex bead-based immunoassay⁹, which currently is the most common method to measure these antibody levels, but other measurement methods, such as the opsonophagocytic assays, are being developed.¹⁰ There could be a concern of an over-diagnosed SAD in our population, as we used the 4-fold rise, as one of the criteria determining adequate response. However, the frequency of SAD in our cohort is not different than others.^{2,3} Moreover, we performed a sensitivity analysis evaluating the diagnosis of SAD by using a criteria of a 2-fold increases and this did not lead to changes in diagnosis in SAD in our patients. We did not exclude IgG subclass deficiency for a diagnosis of SAD, as the clinical significance of this diagnosis remains controversial,¹ the treatment of IgG subclass deficiency relies on the determination of vaccine responses.¹ Our regression model may be over-fitted due to a small sample size of patients with SAD; however, given the excellent predictive abilities demonstrated for each serotype with the univariate models, the findings most likely would remain significant in a larger sample size. Our findings were obtained in a pediatric population, and the performance of this model in adults with suspected SAD needs to be specifically evaluated. Finally, as this was a proof-of concept study, additional prospective validation in an independent cohort, and ideally in a different

commercial laboratory, is required before this smaller panel could be incorporated into the clinical care.

In summary, this proof-of-concept study suggests that use of a narrower panel consisting of 5 anti-pneumococcal antibody levels (4, 5, 11A, 18C and 20) may provide a comparable utility for the diagnosis of SAD as the complete 23 serotype panel. These results should be confirmed in a prospective larger cohort.

Acknowledgments

Financial support: Research reported in this publication was supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1 TR002345 and CTSA Grant number UL1 TR000448, and the Children's Discovery Institute of Washington University and St. Louis Children's Hospital. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health

References

1. Orange JS, Ballou M, Stiehm ER, et al. Use and interpretation of diagnostic vaccination in primary immunodeficiency: a working group report of the Basic and Clinical Immunology Interest Section of the American Academy of Allergy, Asthma & Immunology. *J Allergy Clin Immunol.* 2012; 130: S1–24. [PubMed: 22935624]
2. Boyle RJ, Le C, Balloch A, Tang ML. The clinical syndrome of specific antibody deficiency in children. *Clin Exp Immunol.* 2006; 146: 486–492. [PubMed: 17100769]
3. Sanders LA, Rijkers GT, Kuis W, et al. Defective antipneumococcal polysaccharide antibody response in children with recurrent respiratory tract infections. *J Allergy Clin Immunol.* 1993; 91: 110–119. [PubMed: 8423268]
4. Bonilla FA, Khan DA, Ballas ZK, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. *J Allergy Clin Immunol.* 2015; 136: 1186–1205 e1181–1178. [PubMed: 26371839]
5. Perez E, Bonilla FA, Orange JS, Ballou M. Specific Antibody Deficiency: Controversies in Diagnosis and Management. *Front Immunol.* 2017; 8: 586. [PubMed: 28588580]
6. Estrada J, Najera M, Pounds N, Catano G, Infante AJ. Clinical and Serologic Response to the 23-valent Polysaccharide Pneumococcal Vaccine in Children and Teens with Recurrent Upper Respiratory Tract Infections and Selective Antibody Deficiency. *Pediatr Infect Dis J.* 2016; 35: 205–208. [PubMed: 26517331]
7. Hidalgo H, Moore C, Leiva LE, Sorensen RU. Preimmunization and postimmunization pneumococcal antibody titers in children with recurrent infections. *Ann Allergy Asthma Immunol.* 1996; 76: 341–346. [PubMed: 8612116]
8. Laferriere C The immunogenicity of pneumococcal polysaccharides in infants and children: a meta-regression. *Vaccine.* 2011; 29: 6838–6847. [PubMed: 21816198]
9. Pickering JW, Martins TB, Greer RW, et al. A multiplexed fluorescent microsphere immunoassay for antibodies to pneumococcal capsular polysaccharides. *Am J Clin Pathol.* 2002; 117: 589–596. [PubMed: 11939734]
10. Burton RL, Antonello J, Cooper D, et al. Assignment of Opsonic Values to Pneumococcal Reference Serum 007sp for Use in Opsonophagocytic Assays for 13 Serotypes. *Clin Vaccine Immunol.* 2017; 24.

Figure 1.
Plot of Area under the Receiver Operating Characteristic (ROC) curve for each pneumococcal serotype for diagnosis of specific antibody deficiency in children.

Table 1

Comparison of characteristics of children with specific antibody deficiency and normal responders

Variables	Normal responders (n=101)	Specific Antibody Deficiency (n=23)	P-value
Age at time of initial evaluation (yrs)	7.6 ± 3.9	8.1 ± 4.6	0.6
Male	52 (51%)	15 (65%)	0.2
Caucasian	96 (95%)	23 (100%)	1.0
Duration between repeated antibody measurements (weeks)	8.5 ± 2.2	8.3 ± 2.8	0.7

Table 2

Final regression model describing the utility of post-immunization serotype-specific pneumococcal antibodies level >1.3 mcg/ml for the diagnosis of specific antibody deficiency in children

Serotypes	OR estimates (95% CI)	P-value
Serotype 4	0.009 (<0.001 – 0.15)	0.0009
Serotype 5	0.097 (0.015 – 0.62)	0.01
Serotype 11A	0.093 (0.011 – 0.77)	0.03
Serotype 18C	0.12 (0.017 – 0.82)	0.02
Serotype 20	0.022 (0.001 – 0.43)	0.01

* Calculated as a multivariable stepwise regression model