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LETTER TO EDITOR

Measurement of Pneumococcal Polysaccharide Vaccine Responses for Immunodeficiency Diagnostics: Combined IgG Responses Compared to Serotype Specific IgG Responses

Willemijn J. M. Janssen · Andries C. Bloem · Perijne Vellekoop · Gertjan J. Driessen · Marianne Boes · Joris M. van Montfrans

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Introduction

Common variable immunodeficiency (CVID) is a heterogeneous primary immunodeficiency mainly characterized by defective antibody production and markedly reduced serum levels of IgG, IgA and frequently IgM [1]. One hallmark in the diagnosis is a poor response to vaccination [2], which is often gauged by measuring IgG responses to 23-valent pneumococcal polysaccharide (PnPS) vaccine. The majority of childhood vaccination programs now include vaccination against Pneumococcal disease using a 7-, 10- or 13- valent conjugated Pneumococcal vaccine, that has been shown to provide protection against invasive Pneumococcal disease by the serotypes in these vaccines [3]. However, vaccination using a conjugate Pneumococcal (Pn-C) vaccine may interfere with the diagnostic use of the 23-valent polysaccharide vaccine [4].

Different methods for the quantification of the Pn-PS vaccination response are available and in use by diagnostic laboratories, such as IgG measurement of separate serotypes by luminex [5–7] or ELISA [6, 8], and a commercially available combined Pn-PS IgG measurement by ELISA. We here compare the usefulness of the combined PnPS IgG measurement with specific serotype measurement in a defined cohort of immunodeficiency patients and control patients. Our data show that the ELISA-based combined measurement of IgG Pn-PS responses can mask the presence of immunodeficiency in up to 42 % of patients. We conclude that the luminex-based serotype specific method is superior in detection of Pn-PS vaccination responses and thereby for immunodeficiency diagnostics.

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W. J. M. Janssen · P. Vellekoop · M. Boes · J. M. van Montfrans (✉)
Department of Pediatric Immunology and Infectious Diseases, University Medical Center Utrecht/Wilhelmina Children's Hospital, KC 03.063.0, Lundlaan 6, PO Box 85090, 3508 AB Utrecht, The Netherlands
e-mail: J.vanMontfrans@umcutrecht.nl

A. C. Bloem
Department of Medical Immunology, University Medical Center Utrecht, F03.818, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

G. J. Driessen
Department of Paediatric Infectious Disease and Immunology, Erasmus MC, Sophia Children's Hospital, PO Box 2060, 3015 GJ Rotterdam, The Netherlands

Methods

Subjects

We included CVID patients (n=11) and specific antibody deficient (SAD) patients (normal IgG, IgM and IgA, with disturbed specific antibody production; n=13), diagnosed according to the ESID criteria, in a retrospective cohort study at the University Medical Centre Utrecht from 2010 to 2011. As control group, we enrolled children referred to our hospital with recurrent infections mostly of the upper respiratory tract (infections only group; IO; n=22), who proved to lack a diagnosis of primary immunodeficiency and that were otherwise healthy (see Table I).

Patients were vaccinated with 23-valent Pn-PS vaccine Pneumovax®. All vaccinations were part of our institutional work up scheme for evaluation of recurrent infections.

Luminex and ELISA

Measurement of specific serotype IgG responses by luminex and combined IgG responses by ELISA were performed according to standard operation procedures 4 (3–6) weeks after

Table I Baseline characteristics

	CVID and SAD patients (n=24)	Infection only (n=22)	
Male (n)	14	5	P<0.08
Female (n)	10	17	
Age (means, range)	12 years (4–67)	11 years (3–35)	P<0.106
Number of patients pre-vaccinated with 7-valent Pn-C vaccine (n)	3	5	P<0.887
Specific serotype IgG response (mg/ml): amount of serotypes sufficient/total			
CVID patient 1 (prevnar)	1/4	IO 1 (prevnar)	3/4
CVID patient 2	6/11	IO 2	10/11
CVID patient 3	5/11	IO 3	6/11
CVID patient 4	3/11	IO 4	9/11
CVID patient 5	3/11	IO 5 (prevnar)	4/4
CVID patient 6	1/11	IO 6	11/11
CVID patient 7	0/11	IO 7	9/11
CVID patient 8	0/11	IO 8	11/11
CVID patient 9	0/11	IO 9	11/11
CVID patient 10	0/11	IO 10	10/11
CVID patient 11	0/11	IO 11	10/11
SAD patient 1 (prevnar)	0/4	IO 12 (prevnar)	4/4
SAD patient 2	7/11	IO 13 (prevnar)	4/4
SAD patient 3	7/11	IO 14	8/11
SAD patient 4	6/11	IO 15	7/11
SAD patient 5	5/11	IO 16	10/11
SAD patient 6	5/11	IO 17	9/11
SAD patient 7	4/11	IO 18	10/11
SAD patient 8	4/11	IO 19 (prevnar)	2/4
SAD patient 9	3/11	IO 20	11/11
SAD patient 10	2/11	IO 21	6/11
SAD patient 11	1/11	IO 22	10/11
SAD patient 12	0/11		
SAD patient 13 (prevnar)	0/4		

Baseline characteristics of patient- and control group. The patient group consists of CVID (n=11) and SAD (n=13). The control group consists of patients with recurrent infections, but normal evaluation for immune deficiency. Prevnar: pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F individually conjugated to diphtheria CRM197 protein. Specific serotype IgG response after diagnostic vaccination with Pneumovax®: Patients <6 years reached a post vaccination IgG titer of >1 mg/ml for <50 % of serotypes. Patients ≥6 years reached a post vaccination IgG titer of ≥1 mg/ml for <75 % of serotypes

vaccination and data were analysed using SPSS (Mann Whitney-test, Chi square for statistics). For luminex assays, we measured the IgG response of 11 serotypes: 1, 3, 4, 5, 6B, 7 F, 9 V, 14, 18C, 19 F and 23 F [5]. Luminex reagents were purchased from Jackson Immuno Research, Valley Biomedical, Merck, and Statens Serum Institut Denmark. For the ELISA method, we measured the combined IgG response to the 23 serotypes present in the Pneumovax® vaccine. The Binding Site Group Ltd kindly donated ELISA kits used in this study.

Results and Discussion

Patients enrolled in this study were divided in two groups; immunodeficient (CVID and SAD combined) or infections only (IO). The diagnosis of CVID/SAD was based on the presence or absence of hypogammaglobulinemia and the percentage of serotypes found responsive (IgG>1 mg/ml) after diagnostic vaccination with Pneumovax® [9]. In patients previously vaccinated with a Pn-C 7 vaccine, the luminex results of the serotypes not present in the Pn-C vaccine (serotypes 1, 3, 5 and 7 F) were evaluated. We further made use of the following benchmarks to correct for young age: for ages 4–6 years, an abnormal Pn-PS result was defined as <50 % of serotypes evaluated reaching a post vaccination IgG titer of >1 mg/ml. For age ≥6 years, an abnormal result was defined as <75 % of serotypes evaluated reaching a post vaccination IgG titer of ≥1 mg/ml (Table I). For the combined IgG ELISA, we used an IgG reference value of 40 mg/L [10].

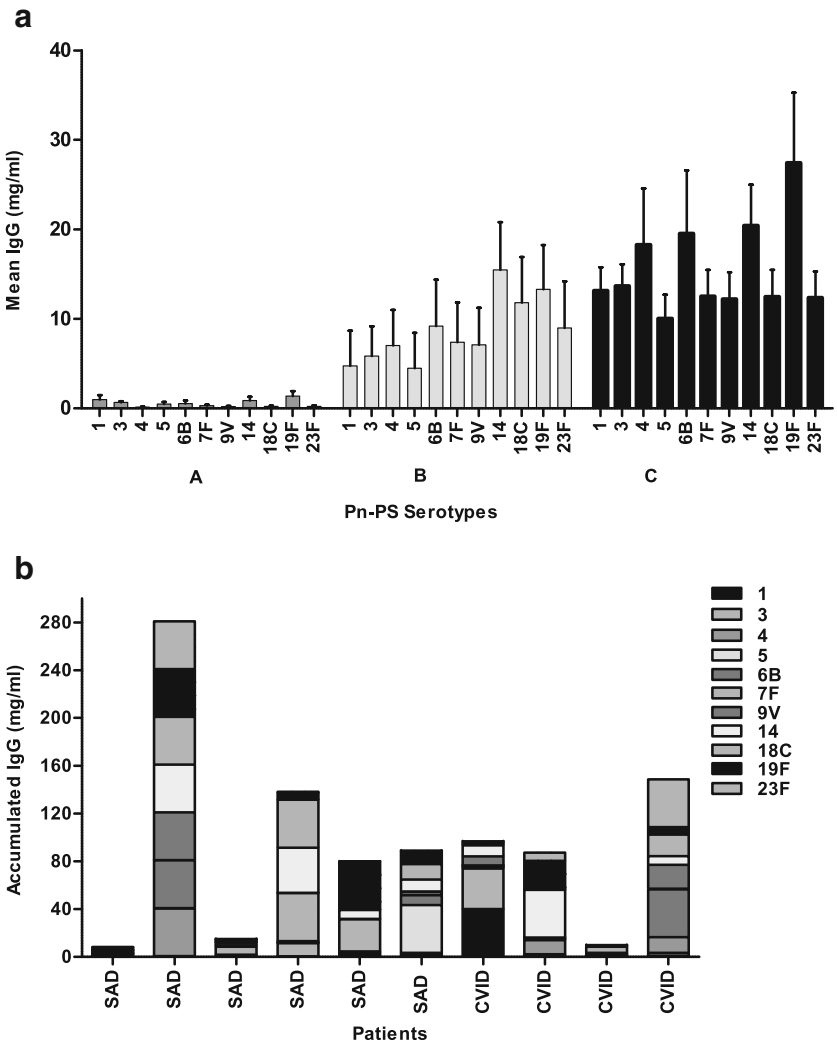

Fig. 1 **a** Mean Pn-PS vaccination response in CVID and SAD patients and infection only group. Mean IgG (mg/ml) responses per serotype as measured by luminex. *a* CVID and SAD patients with insufficient response by combined ELISA (<40 mg/ml; $n=14$), *b* CVID and SAD patients with sufficient response by combined ELISA (≥40 mg/ml; $N=10$), *c* infections only group ($n=22$). Pn-PS: pneumococcal polysaccharide. **b** Pneumococcal polysaccharide antibody responses in CVID and SAD patients with normal combined ELISA results. Specific serotype responses as measured by luminex for patients ($n=10$) with sufficient response by ELISA (≥40 mg/L). Accumulated bars indicate the absolute value of IgG response (mg/ml). Patients <6 years reached a post vaccination IgG titer of >1 mg/ml for <50 % of serotypes. Patients ≥6 reached a post vaccination IgG titer of ≥1 mg/ml for <75 % of serotypes. Pn-PS: pneumococcal polysaccharide

We asked how the combined IgG response measurement by ELISA compares to the serotype specific IgG response measurement by luminex. To this end, we first assessed the vaccination responses by luminex and categorised these according to their readings from combined serotype ELISA data: below 40 mg/L (Fig. 1a) or above (Fig. 1b). We then found that 10 out of 24 (42 %) patients (by luminex) had a sufficient response by ELISA (sensitivity 58 %). There were no false positive test results (<40 mg/L) for the healthy subjects (Table II).

We next focused our study on patient samples that were tested by ELISA as sufficient (>=40 mg/L), (Fig. 1b). We observed that individual immunodeficiency patients exhibit diverse presentations in serotype-specific IgG responses as described previously [11]. Most Pn-PS IgG responses were dominated by only few serotype-specific IgG that varied amongst individuals. Moreover, 3/10 patients showed an insufficient response on all 11 serotypes measured by luminex, while being tested as sufficient response by ELISA, probably through high IgG titers on the remaining serotypes not measured by luminex. Thus, false negative results from the ELISA method are caused by selective responsiveness to pneumococcal serotypes, that conceal defective immune responsiveness to a majority of pneumococcal serotypes.

Table II Cross table of specific serotype and combined IgG response measurement

	Patient ($n=24$)	Infection only ($n=22$)	
ELISA IgG response > =40 mg/L (n)	10/24 (42 %)	22/22 (100 %)	$P<0.00$
ELISA IgG response <40 mg/L (n)	14/24 (58 %)	0 (0 %)	

Crosstable of CVID/SAD patients and Infection Only group as determined by luminex specific serotype IgG response versus ELISA combined IgG response with cut off 40 mg/L. Sensitivity 58 %, specificity 100 %

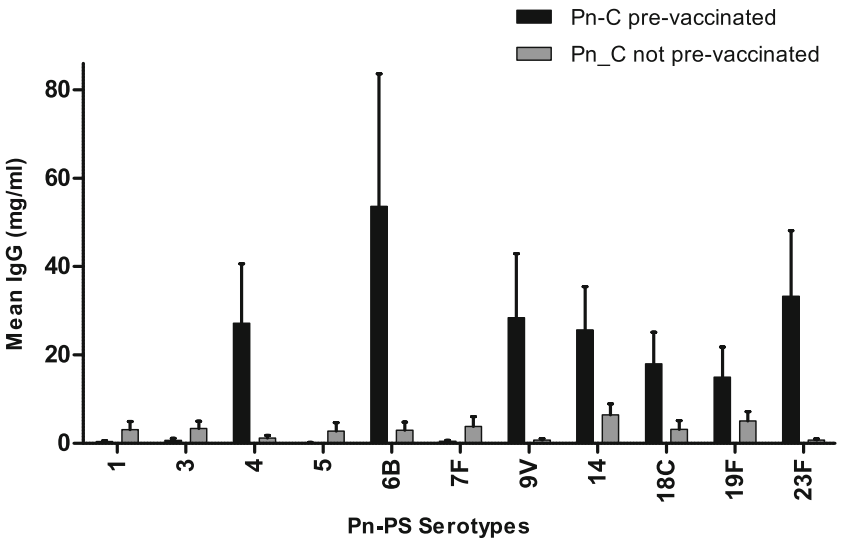

Fig. 2 Mean Pneumococcal polysaccharide antibody response per serotype in CVID and SAD patients, with and without Prevnar pre-vaccination. Mean IgG (mg/ml) responses upon Pn-PS vaccination per serotype as measured by luminex, for patients with and without pneumococcal conjugate (Pn-C) pre-vaccination (n=3 and n=21, respectively). Pn-PS: pneumococcal polysaccharide

We finally hypothesized that pre-vaccination with Pn-C 7 vaccine (e.g. Prevnar®), before vaccination with Pneumovax®, may additionally contribute to false negative ELISA test results, through protein-conjugate mediated priming of the IgG response [12]. Patients that had been pre-vaccinated with Prevnar indeed routinely had increased Pn-PS responses to the serotypes present in Prevnar when compared non-pre-vaccinated patients (Fig. 2).

The use of a combined Pn-PS ELISA for diagnosis of CVID/SAD patients is especially troublesome in Pn-C pre-vaccinated individuals, even more for future testing since a majority of childhood vaccination programmes now include Pn-C vaccination. The luminex method tests serotypes separately and allows evaluation of serotypes not present in Pn-C vaccines. For CVID/SAD patients, we observed a 42 % false-negative rate when the combined Pn-PS ELISA is used. We therefore advocate the use of a Pn-PS luminex method that allows measuring serotypes not present in any of the Pneumococcal conjugate vaccines for diagnostic purposes of antibody deficiency patients.

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