

## ORIGINAL ARTICLE

# The clinical relevance of IgM and IgA anti-pneumococcal polysaccharide ELISA assays in patients with suspected antibody deficiency

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## Summary

Unlike immunoglobulin (Ig)G pneumococcal polysaccharide (PnPS)-antibodies, PnPS IgA and IgM-antibodies are not routinely determined for the assessment of immunocompetence. It is not yet known whether an isolated inability to mount a normal IgM or IgA-PnPS response should be considered a relevant primary antibody deficiency (PAD). We studied the clinical relevance of anti-PnPS IgM and IgA-assays in patients with suspected primary immunodeficiency in a large teaching hospital in 's-Hertogenbosch, the Netherlands. Serotype-specific-PnPS IgG assays were performed; subsequently, 23-valent-PnPS IgG assays (anti-PnPS IgG assays), and later anti-PnPS IgA and IgM assays, were performed in archived material (240 patients; 304 samples). Eleven of 65 pre- and six of 10 post-immunization samples from good responders to PnPS serotype-specific IgG testing had decreased anti-PnPS IgA and/or IgM titres. Of these, three pre- and no post-immunization samples were from patients previously classified as 'no PAD'. Determination of anti-PnPS IgA and IgM in addition to anti-PnPS IgG did not reduce the need for serotype-specific PnPS IgG testing to assess immunocompetence [receiver operating characteristic (ROC) analysis of post-immunization samples: anti-PnPS IgA + IgG area under the curve (AUC) = 0.80, 95% confidence interval (CI) = 0.63–0.97; anti-PnPS IgM + IgG AUC 0.80, 95% CI = 0.62–0.98; anti-PnPS IgA + IgG + IgM AUC = 0.71, 95% CI = 0.51–0.91; anti-PnPS IgG AUC = 0.93, 95% CI = 0.85–1.00]. Our data show that patients classified as having an intact antibody response based on measurement of serotype-specific PnPS IgG can still display impaired anti-PnPS IgM and IgA responses, and that the additional measurement of anti-PnPS IgA and IgM could not reduce the need for serotype-specific IgG testing. Future studies are needed to investigate the clinical relevance of potential 'specific IgA or IgM antibody deficiency' in patients with recurrent airway infections in whom no PAD could be diagnosed according to the current definitions.

## KEYWORDS

antibody deficiency, anti-pneumococcal polysaccharide IgA, anti-pneumococcal polysaccharide IgG, anti-pneumococcal polysaccharide IgM, ELISA, pneumococcal polysaccharide response, pneumococcal vaccination response, Pneumovax, serotype-specific assay

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## INTRODUCTION

Specific antibody deficiency (SPAD) is defined as the inability to mount an immunoglobulin (Ig)G antibody response to purified *Streptococcus pneumoniae* capsular polysaccharide antigens in the presence of normal immunoglobulin concentrations and normal antibody responses to protein antigens [1]. SPAD was first reported in a small group of patients in the early 1980s [2,3]. Patients with SPAD suffer from recurrent ear–nose–throat (ENT) and airway infections with encapsulated bacteria. Pneumococcal polysaccharide (PnPS) antibodies can be measured as the cumulative titre of antibodies to all 23 serotypes present in the PnPS vaccine (hereafter called ‘anti-PnPS IgG assay’), or as individual serotype-specific antibodies (hereafter called ‘serotype-specific PnPS IgG testing’) [4–6]. Such serotype-specific PnPS IgG testing is expensive, not widely available, and interpretation of the results has proved to be challenging [7,8]. The anti-PnPS IgG assay has been shown to be a reliable screening test for poor [9] as well as for good [10] serotype-specific PnPS IgG responders to PnPS vaccine in conjugated pneumococcal (Pn-C) vaccine-naïve patients. This reduces the number of patients needing serotype-specific PnPS IgG testing, thus reducing the costs while maintaining the quality of the diagnostic assessment for potential SPAD.

The cumulative PnPS antibody response can also be measured for IgM- and IgA-type antibodies, but this is not routinely performed for the assessment of immunocompetence or risk of pneumococcal infection [11–14]. Anti-PnPS IgA and IgM antibody responses have been investigated in healthy donors [12–14], patients with common variable immunodeficiency disorders (CVID) [11,15], patients with primary antibody deficiency (PAD) [16] and children with transient hypogammaglobulinaemia of infancy (THI) [17]. The anti-PnPS IgA and IgM assays identify CVID patients with greater risk of infectious and non-infectious (autoimmunity, enteropathy) complications [11,15,16,18] and predict the disease course in young children diagnosed with antibody deficiency [19]. However, it is unknown whether an isolated inability to mount a normal IgM or IgA PnPS response should be considered a clinically relevant PAD. Theoretically, such specific IgM or IgA antibody deficiencies could be clinically relevant, because IgM and IgA are predominant immunoglobulin isotypes in the upper and lower airways with different effector mechanisms to IgG [20,21].

In this study, we investigated the clinical relevance of anti-PnPS IgM and IgA assays in addition to the anti-PnPS IgG assay when analysing patients for potential PAD in a general hospital population. Our first objective was to investigate whether there were patients in our cohort with recurrent ENT and/or respiratory tract infections labelled as ‘no PAD’ based on a good response in serotype-specific PnPS IgG assays [10] with a reduced anti-PnPS IgA and/or IgM response. Secondly, we investigated whether adding anti-PnPS IgA

and/or IgM assays to the anti-PnPS IgG assay could reduce the need for serotype-specific PnPS testing.

## MATERIALS AND METHODS

### Study design

Anti-PnPS IgA and IgM assays were performed on 304 blood samples, obtained from 240 patients in regular patient care who were analysed for the potential presence of primary immunodeficiency (PID) in the Jeroen Bosch Hospital (JBZ) in 's-Hertogenbosch, the Netherlands, between February 2012 and December 2018. Of these, 61 samples were from 49 patients who were previously vaccinated with the Pn-C vaccine. Residual samples were stored at  $\leq -80^{\circ}\text{C}$  and later retrieved from the laboratory to perform anti-PnPS IgA and IgM assays between September and November 2019; anti-PnPS IgG assays were previously performed (and published) between August and September 2018 [10]. Most patients ( $n = 84$ ) were diagnosed with unclassified primary antibody deficiency (unPAD): deficiency of IgG, and/or combination(s) of deficiency of IgG-subclass(es), IgM, IgA or specific antibodies. Thirteen patients were diagnosed with common variable immunodeficiency disorders (CVID), four with selective IgA deficiency (sIgAdef), three with selective IgM deficiency (sIgMdef), two with transient hypogammaglobulinaemia of infancy (THI), four with another type of PID than PAD, one with human immunodeficiency virus infection (HIV) and eight with secondary immunodeficiency. In 87 patients it was concluded that they did not have a PID; in 34 patients there was no definitive diagnosis because of incomplete data. High-resolution CT (HRCT) scans were available for 68 patients; these were scored by a thoracic radiologist according to the ‘chest CT in ADS’ criteria [22]. The study was granted ethical approval by the local medical ethics committee and written informed consent was obtained from all adults and parents of the children.

### Methods

#### ELISA for the quantification of anti-PnPS IgG, IgM and IgA

Commercially available ELISA kits [VaccZyme<sup>TM</sup> pneumococcal capsular polysaccharide enzyme-linked immunosorbent assay (ELISAs), The Binding Site Group Limited, UK] were used to measure anti-PnPS IgG, IgM and IgA, according to the manufacturer's instructions. Absorption of interfering anti-cell wall polysaccharide (anti-CWPS) antibodies was incorporated into these assays. Cut-offs used for Pn-C vaccine-naïve patients were the lower limit of the normal

range (LLNR), as determined by Parker *et al.* in healthy adults (pre-immunization: anti-PnPS IgG 10 µg/ml, anti-PnPS IgA 6 U/ml, anti-PnPS IgM 16 U/ml; post-immunization: anti-PnPS IgG 77 µg/ml, anti-PnPS IgA 78 U/ml, anti-PnPS IgM 60 U/ml) [13].

## Quantification of serotype-specific anti-PnPS IgG antibodies

The Luminex multiplex immunoassay was used to measure serotype-specific IgG antibodies against PnPS, as previously described [10], including CPS 22F adsorption to block anti-CWPS antibodies [4]. For assessing the response to PnPS vaccination a blood sample was drawn 4–8 weeks after intramuscular vaccination with one dose of 23-valent PnPS vaccine (Pneumovax 23; Merck, Sharp & Dohme BV, Haarlem, the Netherlands). A good response to PnPS vaccination was defined according to the international consensus response criteria [23].

## Statistical analysis

Data were analysed using SPSS version 27.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 6.0 software (GraphPad Software, La Jolla, CA, USA) for Mac. The Mann–Whitney *U*-test was used for unpaired comparisons of anti-PnPS IgG, IgA and IgM titres in: (1) pre- and post-immunization samples (often both were not available from the same patient), (2) poor and good responders to PnPS vaccination, as determined by the serotype-specific assay, (3) patients with and without PAD and (4) patients with and without bronchiectasis. Separate analyses were performed for patients who were previously immunized with Pn-C vaccine. Spearman's correlation coefficient (*r*) was estimated to determine the linear association between the anti-PnPS IgG, IgM or IgA titres and serum immunoglobulins. The results were interpreted according to the degree of association as strong ( $r = 0.7–1$ ), moderate ( $r = 0.5–0.7$ ) or low ( $r = 0.3–0.5$ ) after taking significant correlation values ( $P < 0.05$ ) into consideration. In order to be able to compare anti-PnPS IgA and IgM titres (U/ml) with anti-PnPS IgG titres (µg/ml), these values in our data set were standardized by converting them into *Z*-scores. To determine whether the sum of anti-PnPS IgG, IgA and/or IgM titres could better predict whether that patient was a good or a poor responder to PnPS vaccination as assessed by the serotype-specific assay, compared to the anti-PnPS IgG titre alone, receiver operating characteristics (ROC) curves were plotted and the areas under the curve (AUCs) were calculated. This was performed separately for pre- and 4–8 weeks post-immunization titres. All tests were two-tailed and *P*-values  $< 0.05$  were considered statistically significant.

**TABLE 1** Baseline characteristics

	Pn-C pre-vaccinated patients			Vaccine-naïve patients		
	Pre-immunization	4–8 weeks post-immunization	> 8 weeks post-immunization	Pre-immunization	4–8 weeks post-immunization	> 8 weeks post-immunization
Number of samples	54	2	5	175	26	42
Age at time of measurement (years, median, IQR range)	4.7 (2.7–6.1)	*	4.6 (4.5–6.8)	42.2 (21.8–61.2)	49.0 (36.5–65.8)	49.4 (16.2–68.0)
Gender (% female)	31%	#	20%	64%	62%	45%

Abbreviations: IQR, interquartile range; Pn-C, pneumococcal conjugated.

\*Statistical analysis not possible because of too few samples ( $n = 2$ ). These patients were aged 6.7 and 3.9 years at the time of measurement.

#See under \*\*; statistical analysis not possible because of too few samples ( $n = 2$ ). These patients were both female.

## RESULTS

The baseline characteristics of the 304 blood samples, obtained from 240 patients, are summarized in Table 1. The age-specific responses to PnPS vaccination are shown in Supporting information, Figure S1 for vaccine-naïve patients and in Supporting information, Figure S2 for pre-immunization titres of Pn-C pre-vaccinated patients. In vaccine-naïve patients, the anti-PnPS IgM titres pre-immunization and > 8 weeks post-immunization were lower in patients aged 61–80 years, compared to patients aged 0–20 years ( $P = 0.0001$  and  $P = 0.0002$ , respectively). Age did not influence the anti-PnPS IgA or IgG response in this vaccine-naïve patient cohort with suspected PID. In Pn-C pre-vaccinated patients with suspected PID there was no significant difference between the pre-immunization anti-PnPS IgM, IgA and IgG titres at 1–2 years of age and  $\geq 8$  years of age ( $P = 0.546$ ,  $P = 0.497$  and  $P = 0.999$ , respectively). Because of too few data for post-immunization titres in Pn-C pre-vaccinated patients, this analysis could not be performed for post-immunization titres in this group.

### Comparison of all cumulative antibody tests in all samples

Spearman's correlation analysis revealed a moderate correlation between anti-PnPS IgG and anti-PnPS IgA ( $r = 0.52$ ,  $P < 0.0001$ ), while a poor correlation was observed between anti-PnPS IgM and anti-PnPS IgA ( $r = 0.39$ ,  $P < 0.0001$ ) and anti-PnPS IgG and anti-PnPS IgM ( $r = 0.23$ ,  $P < 0.0001$ ; Supporting information, Figure S3). There was a moderate correlation between anti-PnPS IgM and the serum IgM level ( $r = 0.54$ ,  $P < 0.0001$ ; Supporting information, Figure S4). Poor correlations were found between anti-PnPS IgA and

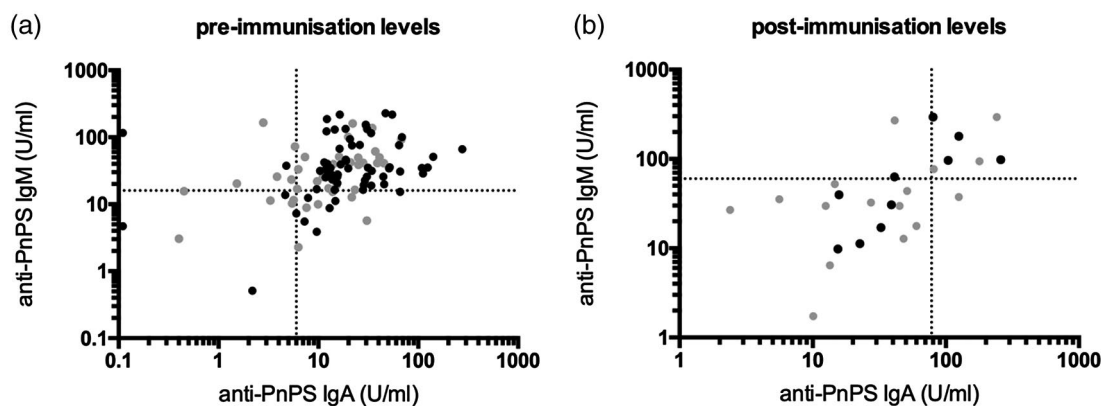
serum IgA ( $r = 0.38$ ,  $P < 0.0001$ ) and anti-PnPS IgG and serum IgG ( $r = 0.03$ ,  $P = 0.665$ ). As expected, IgA- and IgM-deficient patients did not produce anti-PnPS IgA or IgM, respectively.

### Patients previously classified as no-PAD based on their IgG response only

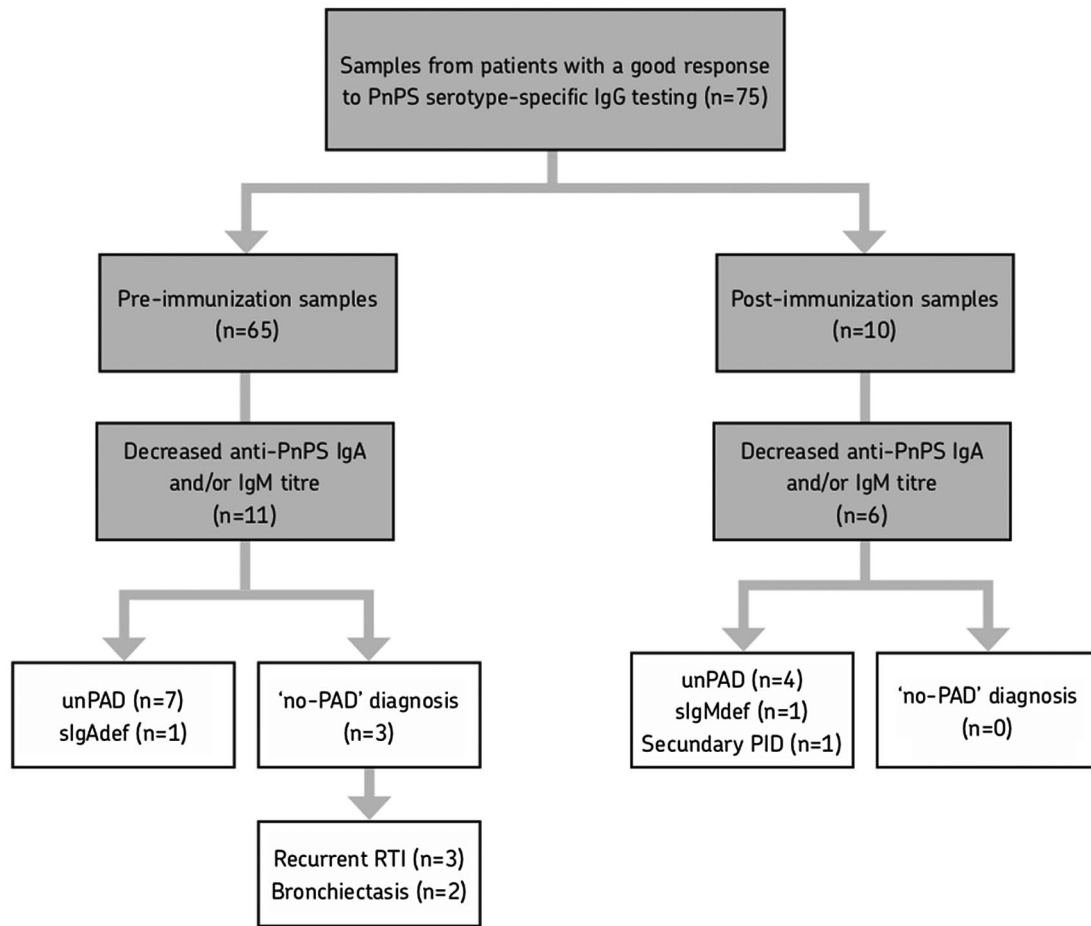
To investigate whether patients from our cohort with recurrent airway infections who had been classified as 'no PAD', based on normal serotype-specific PnPS IgG vaccination response and normal serum immunoglobulin levels, could have defective anti-PnPS IgA and/or IgM responses, pre- and post-immunization anti-PnPS IgA and IgM titres were divided into four groups (IgA/IgM both decreased, only IgA decreased, only IgM decreased and IgA/IgM both normal; Figure 1a,b). Eleven of 65 pre-immunization samples and six of 10 post-immunization samples from patients with a good response to PnPS serotype-specific IgG testing had decreased anti-PnPS IgA and/or IgM titres. Of these, three pre-immunization samples and none of the post-immunization samples were from patients who were previously classified as 'no PAD' (Figure 2). The data, therefore, indicate that up to 60% (six of 10) of patients with an adequate anti-PnPS IgG response still can display defects in the ability to generate a sufficient anti-PnPS IgM and/or IgA response.

### The added value of anti-PnPS IgA and IgM assays in Pn-C vaccine-naïve patients

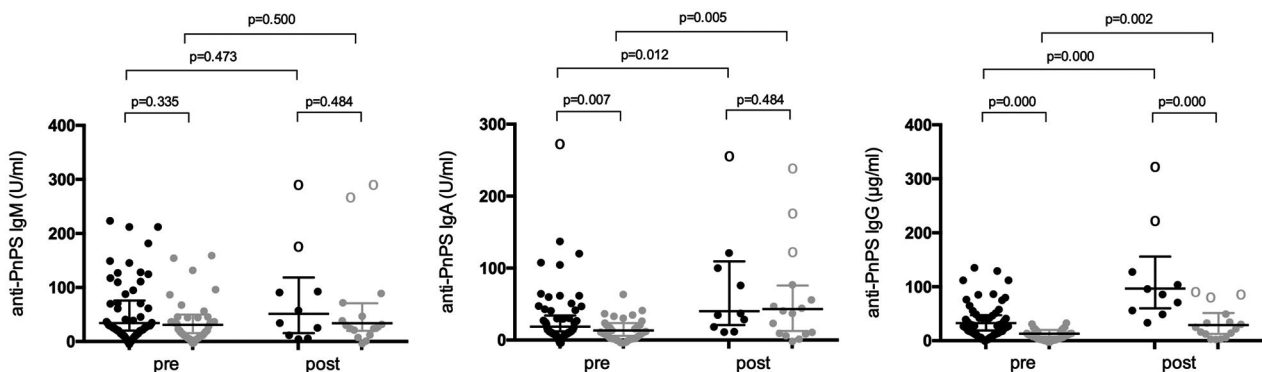
The anti-PnPS IgG, IgA and IgM concentrations pre-immunization and in response to PnPS vaccination in all



**FIGURE 1** Pre- (a) and post-immunization (b) anti-pneumococcal polysaccharide (PnPS) immunoglobulin (Ig)A and IgM titres distinguished four immunological groups: (1) IgA/IgM both decreased, (2) only IgA decreased, (3) only IgM decreased and (4) IgA/IgM both normal [the lower limit of the normal range (LLNR) cut-offs: 6 U/ml for pre- and 78 U/ml for post-immunization anti-PnPS IgA; 15 U/ml for pre- and 60 U/ml for post-immunization anti-PnPS IgM, according to Parker *et al.* [13]]. Poor serotype-specific PnPS IgG responders are coloured grey; good serotype-specific PnPS IgG responders are coloured black. In Fig. 1a, the anti-PnPS IgA values of two samples have been rounded from 0.0 to 0.1 U/ml to make these points visible in the logarithmic scale



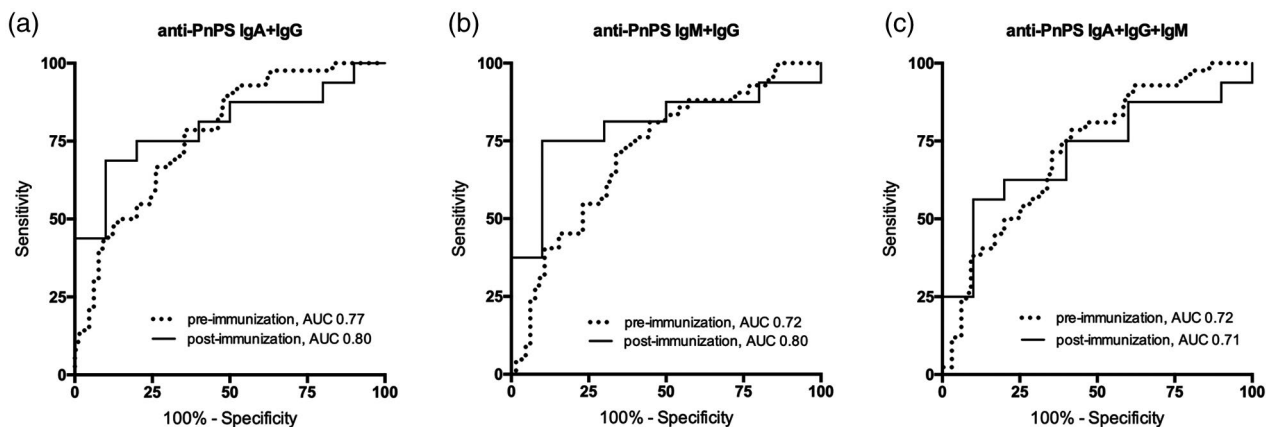
**FIGURE 2** Patients previously classified as 'no PAD' based on their immunoglobulin (Ig)G response, with abnormal results in the anti-pneumococcal polysaccharide (PnPS) IgA and/or IgM assays. Abbreviations: PAD, primary antibody deficiency; PID, primary immunodeficiency; RTI, respiratory tract infections; sIgAdef, selective IgA deficiency; sIgMdef, selective IgM deficiency; unPAD, unclassified primary antibody deficiency



**FIGURE 3** Pre- and post-immunisation cumulative anti-pneumococcal polysaccharide (PnPS) immunoglobulin (Ig)M, IgA and IgG titres for good (black dots) and poor (grey dots; outliers marked as open circles) responders as assessed by serotype-specific PnPS IgG testing. *P*-values were calculated with Mann–Whitney *U*-tests

Pn-C vaccine-naïve patients are shown in Figure 3, categorized as either good or poor responders as assessed by the serotype-specific IgG assay in the same samples. In good and poor IgG responders, the concentration increase from pre- to 4–8 weeks post-immunization was significant for anti-PnPS

IgG and IgA, but not for anti-PnPS IgM. Even when outliers were omitted (open circles in Figure 3), the anti-PnPS IgA and IgG response remained significant in poor IgG responders (anti-PnPS IgA: 13.3 versus 27.4 U/ml;  $P = 0.05$ , anti-PnPS IgG: 12.7 versus 21.1 µg/ml;  $P = 0.02$ ). Also, in



**FIGURE 4** Receiver operating characteristic (ROC) curves of sensitivity versus specificity for the sum of Z-scores of pre- and 4–8 weeks post-immunization pneumococcal immunoglobulins *versus* serotype-specific immunoglobulin (Ig)G response to vaccination: (a) anti-PnPS IgG + IgA Z-scores, (b) anti-PnPS IgG + IgM Z-scores, (c) anti-PnPS IgG + IgA + IgM Z-scores

patients from whom both pre- and post-immunization samples were available, the concentration increase from pre- to 4–8 weeks post-immunization was significant for anti-PnPS IgG and IgA, but not for anti-PnPS IgM (Supporting information, Figure S5). Only the anti-PnPS IgG fold increase could reliably discriminate between poor or good responders to serotype-specific PnPS IgG vaccination [ROC analysis; AUC = 0.90, 95% confidence interval (CI) = 0.76–1.00], while the anti-PnPS IgM and IgA could not (ROC analysis; anti-PnPS IgM: AUC = 0.59, 95% CI = 0.33–0.85 and anti-PnPS IgA: AUC = 0.75, 95% CI = 0.50–1.00; Supporting information, Figure S6).

Next, we evaluated whether adding anti-PnPS IgM and/or IgA assays could reduce the requirement for serotype-specific analyses, compared to conducting only the anti-PnPS IgG assay. The sum of the Z-scores of anti-PnPS IgA and IgG, anti-PnPS IgM and IgG and anti-PnPS IgA, IgG and IgM were separately compared for pre- and 4–8 weeks post-immunization titres with the serotype-specific PnPS IgG vaccination response. The results of the ROC curve analyses are shown in Figure 4a–c. The sum of the Z-scores of post-immunization anti-PnPS IgA + IgG and anti-PnPS IgM + IgG could best discriminate between good and poor responders as determined by the serotype-specific PnPS IgG vaccination response (ROC analysis; AUC = 0.80, 95% CI = 0.63–0.97 and 0.80, 95% CI = 0.62–0.98, respectively). However, the discriminative power of using the anti-PnPS IgG assay alone was higher (ROC analysis; pre-immunization: AUC = 0.84, 95% CI = 0.76–0.91 and post-immunization: AUC = 0.93, 95% CI = 0.85–1.00 [10]).

### Comparison of patients with and without PAD

Pre-immunization anti-PnPS IgG, IgA and IgM titres were significantly lower in Pn-C vaccine-naïve patients with

PAD compared to those without PAD (Supporting information, Table S1A). This comparison could not be made for post-immunization anti-PnPS IgG, IgA and IgM titres, because only one patient did not have PAD. Pn-C vaccine-naïve patients with PAD had significantly more often pre-immunization anti-PnPS IgG and IgM titres below the LLNR compared to patients without PAD (Supporting information, Table S1B). In addition, a number of PAD patients had post-immunization anti-PnPS IgG (16 of 23, 70%), IgA (18 of 23, 78%) and IgM (15 of 23, 65%) titres below the LLNR. In Pn-C pre-vaccinated patients anti-PnPS IgG, IgA and IgM titres were not statistically different between patients with and without PAD (Supporting information, Table S1C). This comparison could not be made for post-immunization titres, because there were only two post-immunization samples in the Pn-C pre-vaccinated patient group.

### Comparison of Pn-C vaccine-naïve patients with and without bronchiectasis

The prevalence of bronchiectasis was identical in patients with post-immunization anti-PnPS IgA or IgM titres above and below the LLNR (75% in all categories). Also, both pre- and post-immunization IgA and IgM titres were not lower in patients with bronchiectasis compared to those without bronchiectasis (Supporting information, Table S2A and S2B).

### Comparison of Pn-C pre-vaccinated with Pn-C vaccine-naïve patients

Pn-C pre-vaccinated patients had significantly higher pre-immunization anti-PnPS IgM titres (median = 56 U/ml, range = 8–270 U/ml) compared to Pn-C vaccine-naïve

patients (median = 35 U/ml, range = 1–305 U/ml,  $P = 0.001$ ). Anti-PnPS IgG and IgA pre-immunization titres were not significantly different between Pn-C pre-vaccinated and PnC-vaccine-naïve patients (Supporting information, Table S3).

## DISCUSSION

### Principal findings

In this study we have expanded our analysis of PnPS antibody levels and response to vaccination by also including, as well as to IgG, IgM and IgA anti-PnPS antibodies. Our data show that patients classified as having an intact antibody response based on measurement of serotype-specific PnPS IgG still can display impaired anti-PnPS IgM and IgA responses. Isolated decreased anti-PnPS IgM, and in particular the anti-PnPS IgA response, might have clinical relevance. Decreased anti-PnPS IgA and IgM responses have been reported in healthy adult blood donors [12,13], but have also been associated with a greater rate of respiratory infections in patients with CVID [11,15] and PAD [16]. In patients with recurrent ENT, or airway infections in whom no PAD could be diagnosed according to the current standards, the clinical relevance of isolated decreased anti-PnPS IgA and/or IgM responses has not yet been investigated. In this study we measured anti-PnPS IgA and IgM levels in order to determine whether – in addition to ‘specific IgG antibody deficiency’ – ‘specific IgA or IgM antibody deficiency’ might be a clinically relevant form of antibody deficiency. None of the patients with a decreased anti-PnPS IgM or IgA response had been classified as ‘no PAD’ based on serotype-specific PnPS IgG testing and serum immunoglobulin levels. Therefore, we could not determine its clinical relevance based on our data.

To gain further insight into the clinical relevance of anti-PnPS IgA and IgM assays, we investigated whether adding these assays to the anti-PnPS IgG assay could reduce the need for the more expensive and difficult to interpret serotype-specific PnPS IgG testing [10]. ROC analysis showed that the discriminative power of the anti-PnPS IgG assay alone to detect good responders was superior to any other combination. Therefore, based on our data, it does not seem useful for a clinician in a general hospital to request anti-PnPS IgA and IgM assays in addition to anti-PnPS IgG assay in order to reduce the need for serotype-specific PnPS IgG testing.

### Comparison with existing literature

Previous studies have reported conflicting results concerning the correlation between PnPS responses for all three immunoglobulin isotypes with their respective serum

levels. Similar to our results, poor correlations were found in a healthy population by Parker *et al.* [13] and a PAD cohort by De Carlos *et al.* [16]. In contrast, Cavaliere *et al.* found a significant correlation in a CVID cohort [11]. By definition, CVID patients have decreased IgM and/or IgA concentrations, and a significant proportion would have decreased anti-PnPS IgM and IgA responses. This might explain the good correlation between PnPS responses and their respective serum immunoglobulins in CVID patients, which is not expected in ‘milder’ PAD patients or a healthy population.

In contrast to Cavaliere *et al.* we did not find a higher bronchiectasis prevalence in patients with impaired anti-PnPS IgA and IgM responses [11]. However, while our cohort included patients with unPAD and milder forms of CVID with an ‘infection-only’ phenotype, Cavaliere *et al.* mainly included severely affected CVID patients with immune dysregulation complications. Also, our results might be biased towards a higher bronchiectasis prevalence, because HRCT scans were only performed in patients in whom pathology was expected.

Previous studies have reported on the influence of age on the anti-PnPS IgM and IgA response in healthy adults and highlighted the importance of age-specific reference ranges. Park *et al.* reported that older adults (> 60 years) had lower anti-PnPS IgM and IgA responses compared to younger adults [13,24,25]. We found that with increasing age the pre-immunization titres of anti-PnPS IgM antibodies were lower, but in our cohort of patients with suspected PID we did not find lower anti-PnPS IgM and IgA responses with increasing age. Our results, however, represent a mixture of patients with and without PAD with both normal and impaired anti-PnPS IgG, IgA and IgM responses. Future studies in large healthy adult populations are needed to improve the evidence on age-specific reference ranges for pre- and post-immunization anti-PnPS IgM and IgA titres.

The higher pre-immunization anti-PnPS IgM titres in the pneumococcal conjugate vaccine (PCV)-primed paediatric group, compared to the unprimed adult group, can be due to lower age and the different immunogenicity of PCV. In contrast to the unconjugated PnPS vaccine, PCV induces a T-dependent, more pronounced memory response. A single dose of PCV is able to induce a significant IgM response measurable 1 month after vaccination [26]. It would be interesting to investigate this issue in prospective cohort studies comparing PCV primed and unprimed groups.

### Limitations

Our study has several limitations. First, the time-point to take post-immunization samples 4–6 weeks after vaccination may

be adequate for IgG and IgA antibodies, but IgM antibodies could already have been declining. This could explain our finding that, in vaccine-naïve patients, good serotype-specific PnPS IgG responders showed a significant anti-PnPS IgA and IgG rise, but not a significant anti-PnPS IgM rise. Parker *et al.* also reported a high percentage of healthy individuals with decreased anti-PnPS IgM concentrations 4–6 weeks post-vaccination [13]. In contrast, Schütz *et al.* found that in healthy adults anti-PnPS IgM titres reached its maximum 3–4 weeks post-immunization, and remained at a plateau for 3 months [14].

Secondly, anti-PnPS IgA and IgM were only measured in blood, not in mucosal tissues or secretions. While the PCV and PnPS vaccine, after priming with PCV, have been shown to be able to induce protective mucosal IgA antibodies [27,28], it is unknown whether this also occurs after immunization with PnPS vaccine alone. Most infectious pathogens enter the host via mucosal surfaces, where mucosal IgA represents the hallmark of immune responses [29]. In future studies it would be interesting to investigate anti-PnPS IgA responses in both blood and mucosal tissues to learn more about the clinical relevance of a defective anti-PnPS IgA response in the circulation.

## Conclusion and implications for future research

Our study shows that patients classified as having an intact PnPS antibody response, based on measurement of IgG antibodies, can still display defective anti-PnPS IgA and IgM responses. In addition, we show that the additional measurement of anti-PnPS IgA and IgM could not reduce the need for serotype-specific PnPS IgG testing. However, our sample size was too small to draw any definitive conclusions on the clinical relevance of our findings. Future studies are needed in patients with recurrent ENT or airway infections in whom no PAD could be diagnosed according to the current standards, to investigate whether – in addition to ‘specific IgG antibody deficiency’ – ‘specific IgA or IgM antibody deficiency’ can also be a clinically relevant form of antibody deficiency.

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## CONFLICT OF INTEREST

VaccZyme™ Anti-PCP human IgA and IgM ELISA kits were donated by the Binding Site Group Ltd, Birmingham, UK. Binding Site Group Ltd had no role in the experimental design or the analysis of the data. There are no other financial or commercial relationships to declare.

## AUTHOR CONTRIBUTIONS

L. M. A. J., E. d. V. and G. T. R. designed the study. L. M. A. J. wrote the manuscript. M. H., E. d. V., J. L. M. and A. C. A. P. L. acquired the data. L. M. A. J. carried out statistical analyses. E. d. V. and G. T. R. helped with the interpretation of the data. All authors reviewed the results and contributed to the final version of the manuscript. All authors approved the manuscript as submitted.

## DATA AVAILABILITY STATEMENT

The data sets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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