Measurement of the IgG2 response to Pneumococcal capsular polysaccharides may identify an antibody deficiency in individuals referred for immunological investigation

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ABSTRACT

IgG2 is the most efficient subclass for providing protection against pneumococcal pathogens. We hypothesised that some individuals may be unable to mount an effective pneumococcal capsular polysaccharide (PCP) IgG2 response despite having a normal PCP IgG concentration (PCP IgG2 deficient). The median pre-vaccination PCP IgG2 concentration was significantly lower in individuals referred for immunological investigation compared to healthy controls (2.8 mg/L range, 95% CI 1.1–88 vs. 29.5 mg/L, 95% CI 13.5–90, \( p = 0.0002 \)). PCP IgG:IgG2 ratios were significantly higher for the referral population than for healthy controls suggesting the increased production of PCP specific subclasses other than IgG2. The percentage of individuals with PCP IgG2 deficiency was significantly higher in referral groups compared to controls (31% vs. 5%; \( p = 0.0009 \)) and in an individual with PCP IgG2 deficiency, the balance of PCP specific IgG subclass antibodies post vaccination changed from IgG2>IgG1>IgG3>IgG4 to IgG1>IgG3>IgG2>IgG4. The median PCP IgG2 concentration in those with PCP IgG2 deficiency was significantly lower in the referral groups compared to controls (7.8 mg/L, 95% CI 1.1–12 vs. 12.7 mg/L, 95% CI 11.8–13.1; \( p = 0.006 \)). The data suggests a defect in the production PCP IgG2 may be present in individuals with normal PCP IgG referred for immunological investigation.

KEYWORDS

Pneumococcal; IgG2; antibody deficiency; IgG; immunodeficiency

Introduction

Measurement of vaccine response is an essential test for assessment of a suspected antibody deficiency, particularly for polysaccharide antigens. Interpretation requires the measurement of the increase in antibody concentration after vaccination and the final post-vaccination concentration.\textsuperscript{[1]} The
current definition of a defective antibody response is the inability to mount an adequate IgG vaccine response; at present, this is the only antigen specific immunoglobulin assessment included in guidelines.\textsuperscript{1–3}

It has been reported that IgG2 is the most efficient subclass for stimulating phagocytosis of pneumococcal antigens,\textsuperscript{4,5} which is thought to be via both complement dependent and independent mediated mechanisms.\textsuperscript{5,6} Antigen specific IgG2 production is defective in children with otitis media,\textsuperscript{7–9} where lower pre-vaccination concentrations of pneumococcal specific IgG2 antibodies (PCP IgG2) correlate with positive cultures of Streptococcus pneumoniae (\textit{S. pneumoniae}).\textsuperscript{7} Low PCP IgG2 levels correlate with recurrent bacterial infection.\textsuperscript{7,10,11} Deficient PCP IgG2 activity has been mainly studied in the background of abnormally low PCP IgG.\textsuperscript{8}

Response to pneumococcal vaccination may reach normal IgG concentrations due to production of IgG1 and IgG3 subclasses,\textsuperscript{12,13} despite defective IgG2 production. In the present study, we report the frequency and characterization of abnormally low PCP IgG2 in the presence of normal PCP IgG (herein defined as “PCP IgG2 deficiency”) in two healthy control populations and three populations of individuals who were referred for immunological investigation due to suspected immunodeficiency.

\textbf{Material and methods}

\textit{Description of clinical samples}

Five groups of serum samples were collected and stored at −80°C:

(1) Control Group A consisted of healthy subjects (paediatric and adult) with unknown vaccination status. Pediatric subjects (\(n = 49\), median age 5 years, range 1–14) were admitted to hospital St Josef-Hospital, Bochum, Germany for minor surgery.\textsuperscript{14} Adult blood donor samples (\(n = 79\), median age 55 years, range 18–90 years) were collected in donor centres by Biomex Solutions (Heidelberg, Germany) and purchased from Quest Biomedical (Solihull, UK).\textsuperscript{15}

(2) Control Group B consisted of matched pre and post-vaccination samples from healthy adults vaccinated with Pneumovax (\(n = 10\), 20 samples\textsuperscript{16}).

(3) Patient Group A consisted of post-vaccination samples from patients vaccinated with Prevnar. Samples were obtained from patients admitted to Hospital Universitario Marqués de Valdecilla, Santander, Spain for routine immunological investigation between June and September 2015 (\(n = 29\), median 5 years, range 1–14 years).

(4) Patient Group B consisted of post-vaccination samples from patients vaccinated with Prevnar and Pneumovax. Samples were obtained from
patients admitted to Hospital Universitario Marqués de Valdecilla, Santander, Spain for routine immunological investigation between June and September 2015 (n = 25, median 48 years, range 21–74 years).

(5) Patient Group C consisted of pre- and post-vaccination samples from patients vaccinated with Pneumovax. Samples were obtained from patients admitted to Hospital Universitario Central de Asturias, Oviedo, Spain for routine immunological investigation between June and September 2015 (n = 16 patients, 32 samples, median 54 years, range 19–81).

For control group B, post vaccination samples were taken 28 days post vaccination (16) and for patient groups A–C samples were taken during routine immunological investigation 3–8 weeks post vaccination.

**Measurement of antigen-specific antibodies**

Qualitative measurement of PCP IgG subclasses 1–4 was performed using ELISA with the appropriate anti IgG subclass secondary antibody coupled to HRP for detection. The results were reported in optical density (OD) Units.

PCP IgG and IgG2 antibody concentrations were measured using commercially available VaccZyme™ PCP IgG and IgG2 ELISAs (The Binding Site Group Limited, Birmingham, UK).

The lower limit of published age-specific normal reference ranges[17] were used as concentration cut offs. For Control Group A (unknown vaccination status), ranges derived from subjects without antecedent vaccination were used. Ranges derived from subjects with antecedent vaccination were used for all other groups. Normal PCP IgG and IgG2 were defined as pre and post vaccination concentrations above the lower limit of these ranges and abnormal PCP IgG and IgG2 levels as concentrations below the lower limit of these ranges. PCP IgG2 deficiency was defined as normal concentrations of PCP IgG and abnormal concentrations of PCP IgG2.

**Ethics approval**

Informed written consent was obtained from all participants where required and studies were performed according to the Declaration of Helsinki. Remnants of specimens collected for routine diagnostic testing were used in accordance with the Declaration of Helsinki.

**Statistical analysis**

ANOVA, Fishers exact and Mann Witney U tests were performed using GraphPad Prism (Version 5.04 for Windows, La Jolla, California, USA). A p value <0.05 was considered statistically significant.
Results

Comparison of PCP IgG2 pre-vaccination concentrations

Pre-vaccination samples were available from Control Group B (n = 10) and Patient Group C (n = 16). The median PCP IgG2 pre-vaccination concentration was significantly lower in patients admitted for routine immunological investigation compared to healthy adults (2.8 mg/L range 95% CI 1.1–88 vs. 29.5 mg/L, 95% CI 13.5–90, p = 0.0002).

Comparison of PCP IgG: IgG2 ratios

The ratio of PCP IgG concentration to PCP IgG2 concentration (PCP IgG: IgG2 ratio) was calculated for individuals in control group A and the post-vaccination concentrations for all patient groups (Figure 1A). The median PCP IgG: IgG2 ratio for individuals in control group A was 2.6 (95% CI, 1.8–3.9). The median ratio was significantly higher in patient groups compared to control group A (A, 4.5 (95% CI, 1.4–20) p < 0.0001; B, 3.3 (95% CI, 1.0–14.8) p < 0.0001 and C, 3.1 (95% CI, 1.9–31) p = 0.0007). The percentage of individuals with a normal PCP IgG concentration was 72%, 55%, 72%, and 50% for control group A and patient groups A-C, respectively. The median PCP IgG: IgG2 ratio in individuals with normal concentrations of PCP IgG in control group A was 2.5 (95% CI, 1.8–4; Figure 1B). Again, the ratios in all three patient groups were significantly higher than in control group A (A, 4.5 (95% CI, 2.4–28) p < 0.0001; B, 3.2 (95% CI, 2.3–18.6) p < 0.0001; and C, 3.1 (95% CI, 1.9–31) p = 0.006).

Figure 1. Comparison of PCP IgG: IgG2 ratios between healthy controls and patients referred for immunological investigation. (A) The PCP IgG: IgG2 ratios were calculated in all samples (control group A, n = 128; patient groups A, n = 29; B, n = 25 and C, n = 16) and (B) for individuals with normal concentrations of PCP IgG (control group A, n = 92; patient groups A, n = 16; B, n = 18 and C, n = 8).
**Post-vaccination IgG subclass composition**

The percentage contribution of all four IgG subclasses in the response to Pneumovax was measured in control group B with normal PCP IgG2 (PCP IgG:IgG2 ratio 2.7) and an individual with PCP IgG2 deficiency (PCP IgG: IgG2 ratio 31, Figure 2). In control group B, the main subclass produced was PCP IgG2 (54%, Figure 2A). The combined response of PCP IgG1 and IgG3 accounted for 46% of the overall response. In contrast, in a patient with PCP IgG2 deficiency (Figure 2B) the combined response of PCP IgG1 and IgG3 accounted for 95% of the overall response.

**Incidence of PCP IgG2 deficiency**

The number and percentage of individuals in each group with PCP IgG2 deficiency is shown in Table 1. The percentage of individuals with PCP IgG2 deficiency was significantly higher in the three patient groups compared to the control groups (p = 0.0003).

**PCP IgG2 concentrations in PCP IgG2 deficient individuals**

There was a significantly higher PCP IgG2 response in PCP IgG2 deficient patients from the control group compared to those from the patient populations (12.7 mg/L, 95% CI 11.8–13.1 vs. 7.8 mg/L, 95% CI 1.1–12; p = 0.006; Figure 3A). There was no significant difference in PCP IgG concentrations (48.7 mg/L, 95% CI 44–53.2 vs. 39.4 mg/L, 95% CI 20.0–60.0; p=0.711).

![Figure 2](image.png)

**Figure 2.** Distribution of PCP IgG subclass antibodies produced in response to Pneumovax in individuals with normal PCP IgG. (A) The subclass distribution in healthy individuals vaccinated with Pneumovax (n = 10, Control Group B) and (B) the subclass distribution in a patient with PCP IgG2 subclass deficiency who had been vaccinated with Pneumovax. Post-vaccination optical densities (ODs) were determined for each subclass and corrected for the blank OD and the OD at pre-vaccination. The data for each subclass is represented as a percentage.
Measurement of antigen-specific IgG alone may provide an incomplete assessment of a patient’s ability to respond to polysaccharide vaccination. We hypothesised that individuals referred for immunological investigation with normal concentrations of PCP IgG may have defective production of PCP IgG2. In this study a higher frequency of PCP IgG2 deficiency was detected in individuals referred for immunological investigation compared to healthy controls (31% vs. 5%). In a PCP IgG2 deficient individual, the PCP IgG:IgG2 ratios were significantly higher in those referred for immunological investigation than in controls, suggesting an increase in the production of PCP specific subclasses other than IgG2 in the referral population. In support of this there was a change in distribution of PCP IgG subclass antibodies from a combined percentage of IgG1 and IgG3 of 46% in the control group to 95% IgG1 and IgG3 in PCP IgG2 deficient individuals. The data suggests that there is lower production of PCP IgG2 in individuals with PCP IgG2 deficiency referred for immunological investigation compared to those with PCP IgG2 deficiency in the control group.

Individuals vaccinated with Prevnar had a higher PCP IgG:IgG2 ratio and higher percentage of individuals with PCP IgG2 deficiency than those vaccinated with Pneumovax (Patient groups A>B>C). The PCP IgG:IgG2 ratios in patient group B were lower than group A but higher than group C. Patients

**Table 1.** Frequency of PCP IgG2 deficiency in control groups and patient groups. PCP IgG and PCP IgG2 were measured in all groups. In control B, pre- and post-vaccination samples were used and patients groups A–C just post vaccination samples used. The number and percentage of subjects with PCP IgG2 deficiency were calculated in each cohort.

| Cohort | Control groups | Patient groups |
|--------|----------------|----------------|
|        | Control A | Control B | Patient A | Patient B | Patient C |
| % with PCP IgG2 deficiency | 5/92 (5%) | 0/20 (0%) | 8/16 (50%) | 4/18 (22%) | 1/8 (13%) |
| Total | 5/112 (5%) | 13/42 (31%) | |

**Figure 3.** PCP IgG2 concentrations in individuals with PCP IgG2 Deficiency are lower in individuals referred for immunological investigation compared to the control group. (A) PCP IgG2 concentrations and (B) PCP IgG concentrations were determined in individuals with PCP IgG2 deficiency in both control groups (n = 5) and individuals referred for immunological investigation (n = 13).
in group B received a Pneumovax vaccination after the Prevnar vaccination whereas the patients in group C only received Pneumovax. The response to Prevnar produces predominately IgG1 with lower IgG2 which may account for the differences.\textsuperscript{[18]} Appropriate cut offs should be developed to take this into account although care should be taken since the two groups were not age matched, patient group A have a median age of 5 years compared to 48 and 54 years for groups B and C, the response to Pneumovax may produce higher concentrations of pneumococcal antibodies since its serotype coverage is larger than Prevnar and may be influenced by any differences in the time of sample collection.

Although screening for PCP IgG2 deficiency would not be recommended (since asymptomatic individuals in a normal healthy population will be identified) the data presented here suggests that a PCP IgG2 concentration cut-off of approximately 12 mg/L may differentiate between the PCP IgG deficiency observed in a normal population and the symptomatic form observed in those referred for immunological investigation.

Given the relatively high rates of normal PCP IgG observed in the course of immunological investigation of the referral population, we have proposed an algorithm for the assessment of antibodies raised in response to pneumococcal antigens (Figure 4). PCP IgG testing could serve as an initial screen for antibody deficiency in individuals referred for immunological investigation. Subsequent measurement of PCP IgG2 in individuals with a normal PCP IgG concentration may identify those with a PCP IgG2 deficiency, which would have gone undetected by PCP IgG assessment alone. In the present study, 13 additional individuals (31%) with an antibody deficiency would have been identified using the proposed algorithm.

![Figure 4](image)

\textbf{Figure 4.} Proposed algorithm for determining pneumococcal antibody concentrations and response to pneumococcal vaccinations in individuals referred for immunological investigation.
Assessment for PCP IgG2 deficiency may be relevant in individuals with a clinical status that worsens or in those with a change in the pattern and frequency of infections. PCP IgG2 measurement may also serve as an early marker of disease progression, e.g., in IgA deficient patients who may progress to Common variable immunodeficiency (CVID), or as a marker of disease severity in certain antibody deficiencies. IgG2 specific response to meningococcal polysaccharide A+C vaccine has been shown to be a potential marker of increased infection in IgA deficient patients.\textsuperscript{19}

In conclusion, we show that a significant percentage of individuals who produce a normal PCP IgG response are unable to generate PCP IgG2 antibodies. The incidence of PCP IgG2 deficiency was higher in a population referred for immunological investigation, compared to healthy controls. Larger studies are required to understand the true clinical impact of this type of antibody deficiency.

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**Conflicts of interest**

ARP, DS, GW, and SH are employees of the Binding Site Group who manufacture the PCP IgG and IgG2 ELISA.

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