

Pneumococcal Antibody Levels in Children With PID Receiving Immunoglobulin



WHAT'S KNOWN ON THIS SUBJECT: Although immunoglobulin replacement is recognized as effective in children with primary immunodeficiency, pneumococcal infection may occur. There is no available prospective clinical study evaluating levels of protective serospecific antibodies in patients and products.



WHAT THIS STUDY ADDS: Protective ($0.2 \mu\text{g/mL}$) antibody levels for the most frequent pneumococcal serotypes were measured in children treated for primary immunodeficiencies. A linear relationship was demonstrated between peak and trough levels of serospecific antipneumococcal antibodies in patients and infused immunoglobulins.

abstract

OBJECTIVES: Clinical data are lacking on optimal levels of specific antipneumococcal antibodies (PnPsAbs) in patients with primary immunodeficiency (PID) receiving intravenous immunoglobulin (IVIG) replacement. Objectives were to conduct a prospective multicenter study providing data on total immunoglobulin G (IgG) and peak/trough levels of PnPsAbs specifically targeting the 16 most prevalent pneumococcal serotypes in IVIG-treated children with PID; to compare trough PnPsAb levels with those measured in healthy adults and the IVIG product; and to evaluate PnPsAb protection correlates with thresholds based on World Health Organization.

METHODS: Patients received 7 consecutive IVIG infusions. Total IgG and PnPsAb levels were determined on plasma samples obtained before and after infusion.

RESULTS: Twenty-two children with PID were treated with IVIG (mean weekly dose: 0.10 g/kg). The mean trough and peak levels of total IgG were 7.77 and 13.93 g/L , respectively. Trough and peak geometric mean concentrations and distribution curves differed between serotypes and showed wide dispersion (0.17 – $7.96 \mu\text{g/mL}$). In patients (89% – 100%), antibodies against most serotypes reached trough levels $\geq 0.2 \mu\text{g/mL}$, a threshold considered protective against invasive pneumococcal infection. For several serotypes, trough levels reached ≥ 1.0 to $1.3 \mu\text{g/mL}$, the level found in adults. Trough geometric mean concentrations correlated well with the PnPsAb contents of the IVIG product.

CONCLUSIONS: In IVIG-treated children with PID, protective PnPsAb levels for most pathogenic serotypes were obtained. A correlation was observed between PnPsAb levels in patients and in the IVIG product. This offers the potential to improve infection prevention by adapting the IVIG product and dose according to epidemiology. *Pediatrics* 2014;133:e154–e162

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KEY WORDS

pediatric patients, immunodeficiency, specific antipneumococcal IgG, intravenous immunoglobulin, seroepidemiology

ABBREVIATIONS

CI—confidence interval

ELISA—enzyme-linked immunosorbent assay

GMC—geometric mean concentration

IgG—immunoglobulin G

IPD—invasive pneumococcal disease

IVIG—intravenous immunoglobulin

PID—primary immunodeficiency

PnPsAb—antipneumococcal capsular polysaccharide antibodies

WHO—World Health Organization

Dr Tuerlinckx designed the study, contributed to data acquisition, conducted the analysis, and reviewed and revised the manuscript; Drs Florin, Ferster, and Chantrain contributed to study design and data acquisition, carried out the initial analyses, and reviewed and revised the manuscript; Drs De Schutter, Haerynck, and Philippet contributed to study design and data acquisition, and reviewed and revised the manuscript; Dr Strengers reviewed and revised the manuscript; Dr Laub contributed to study design, conducted the analysis, and drafted the initial manuscript; and all authors approved the final manuscript as submitted

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Streptococcus pneumoniae is a leading cause of mortality and morbidity worldwide.¹ Its major virulence factor is the capsular polysaccharide. Extreme age and antibody immunodeficiency are the primary risk factors for pneumococcal infection. The World Health Organization (WHO) estimates that 1.6 million people die of pneumococcal diseases each year.² Although most of the 90 known capsular serotypes can cause serious disease, a limited number cause the majority of cases of invasive pneumococcal disease (IPD). Immunity to *S pneumoniae* is mediated by phagocytosis of the bacteria in the presence of complement and serotype-specific antibody. Evaluation of serologic responses to different pneumococcal vaccines in large clinical trials provides correlates of protection required to release new vaccines.^{3,4} For predicting protection against IPD disease, WHO recommends a reference threshold of 0.2 to 0.35 $\mu\text{g}/\text{mL}$ for pneumococcal conjugate vaccines (all serotypes)⁵; for polysaccharide vaccines, the current guideline is 1.3 $\mu\text{g}/\text{mL}$.^{6,7}

Primary immunodeficiencies (PIDs) are genetically inherited disorders characterized by deficiencies in individual components of the innate or adaptive immune system, with a clinical result of an increased susceptibility to infection. To date, >120 distinct genes have been characterized, accounting for >150 forms of PID. Antibody deficiency (the most frequent) characteristically leads to recurrent ear-nose-throat and respiratory tract infections by encapsulated bacteria, mainly *S pneumoniae*.⁸ Human polyvalent intravenous immunoglobulins (IVIGs) are the mainstay long-term therapy for reducing the severity and frequency of infections. IVIG infusion results rapidly in an immunoglobulin G (IgG) concentration peak, followed by an IgG decrease over time. The IgG level just before the next

infusion (ie, the trough level) is monitored to evaluate the adequacy of a particular regimen. Yet there is no consensus on the optimum target IgG levels required to minimize the infection risk.^{8–10} Despite the importance of IVIGs in inducing passive protection against invasive bacteria, few studies have quantified antipathogen antibody levels in medicinal IVIGs^{11,12} or patients.^{13–15} No clinical correlation with protective trough plasma titers has been performed to assess the protective capacity of the anti-pneumococcal titer in IVIG.^{8,12} This is the first prospective study to measure both total IgG and trough and peak levels of specific antipneumococcal capsular polysaccharide antibodies (PnPsAbs) against 16 serotypes in IVIG-treated children with PID (7 successive IVIG infusions). Objectives were (1) to estimate the proportion of subjects achieving the effectiveness thresholds recommended for pneumococcal vaccines and (2) to compare trough PnPsAb levels with those measured in both healthy adults and IVIGs.

METHODS

Study Cohort

Twenty-three pediatric patients (aged <18 years, intent to treat) with PID (classification of Notarangelo et al)¹⁶ were enrolled in an academic, multicenter, 1-arm open-label noninterventional study, without modification of the doctor-patient relationship, in accordance with the Declaration of Helsinki. Patients with known hypersensitivity to plasma products were excluded. Informed consent was obtained from parents or legal guardians. The study protocol was approved centrally and locally by independent ethics committees as required by Belgian law. Before inclusion, all patients were on regular IVIG substitution therapy (various IVIG brands). At inclusion, the mean time since diagnosis was 4.84 years (95%

confidence interval [CI] 2.51–7.17 years). To reduce potential variability due to local pneumococcal seroepidemiology factors, a single IVIG product containing antiendemic-pathogen antibodies was used for all patients. Patients were first stabilized with ≥ 5 consecutive infusions with the same IVIG product (Multigam, Brussels, Belgium; no sampling), followed by 7 infusions after inclusion. Paired plasma samples were taken before infusion (trough) and 20 minutes after its end (peak). At the seventh infusion, only the preinfusion sample was collected. All samples were investigated in a single laboratory.

Adult Plasmas and IVIGs

Healthy adult plasma samples (from 8 large pools of plasma donations prepared as in Laub et al)¹⁷ and 17 IVIG batches were tested in parallel for PnPsAbs. The IVIG preparations were produced from large plasma pools containing >12 000 donations collected

TABLE 1 Demographic and Baseline Characteristics

Parameter	Total (%) ^a
Enrolled patients	23
Intent to treat	22
Gender	
Female	10 (45.5)
Male	12 (54.5)
Age (y)	
Mean	9.2
1st quartile	4.4
Median	8.9
3rd quartile	14.3
Minimum, maximum	0.7–17.2
Time since first clinical diagnosis (y)	
Mean	4.8
95% CI	2.5–7.2
Diagnosis	
Common variable immunodeficiency	11 (50)
Specific antibody deficiency	5 (23)
X-linked agammaglobulinemia	2 (9)
IgG subclasses deficiency	2 (9)
Other agammaglobulinemia	2 (9)
Other	6 (27)
Severe bacterial infection (episodes)	
Pneumonia	7
Proven IPD	10

^a n (%) except as noted.

from healthy donors aged 18 to 65 years living in Belgium. It was fractionated at a single site (Central Department for Fractionation of the Red Cross, Brussels, Belgium). The multistep purification process notably included an octanoic acid precipitation step, inactivating viruses and thrombolytic agents. The variation coefficient for the 16 PnPSAbs measured in the different IVIG lots ranged from 2% to 11% (serotypes 3, 5, 9V, range 15%–17%).

Nephelometry

Total IgG and subclasses were measured by nephelometry with a BN Prospec apparatus according to the supplier's recommendations (Dade Behring, Marburg, Germany).

Enzyme-Linked Immunosorbent Assays

With an automated instrument, PnPSAbs were determined by enzyme-linked immunosorbent assay (ELISA) according to the protocol recommended by WHO (www.vaccine.uab.edu). Briefly, plates were coated with pneumococcal polysaccharide (serotype 1, 3, 4, 6B, 7F, 9N, 9V, 12F, 14, 18C, 19A, 19F, or 23F; ATCC, Merck, Manassas, VA). Plasma samples and the human antipneumococcal reference standard, kindly provided by the US Food and Drug Administration, were diluted appropriately with buffer-casein absorbant containing 10 µg/mL C-polysaccharide and 10 µg/mL 22F capsular PnPs (adult and patient samples; Staten Serum Institute, Copenhagen, Denmark) to neutralize polysaccharides contaminating the coating PnPs antigens. After a 1 hour of incubation at 37°C and several washings, horseradish-conjugated rabbit anti-human IgG antibody (Sigma-Aldrich, MO) was added and binding to the polysaccharide-coated plates was revealed with 3,3'-5,5'-tetramethyl benzidine. Color development was stopped with H₂SO₄, measured at 405 nm, and concentrations

were calculated by interpolation from the standard curve. The test was fully validated with the WHO QC-sera panel (National Institute for Biological Standards and Controls, South Mimms, United Kingdom), and its sensitivity was 0.7 to 2.82 ng/mL, depending on the serotype. Results were the same whether the PnPSAbs were measured in serum or plasma.

Statistical Analyses

All statistical tests were 2-sided at the 5% level of significance with SAS version 9.2 (SAS Institute, Cary, NC). No adjustment for multiplicity was done. Arithmetic means and 95% CIs were calculated for immunoglobulin. Each result was expressed as a mean for all patients per visit. Weighted GMCs and 95% CI were calculated for the PnPSAbs. The weighted GMC corresponding to each serotype was first calculated per visit for all patients, and then the results were aggregated for all visits. Individual PnPSAb values were evaluated as follows: for each patient at each visit, the plasma PnPSAb content was compared

with the cut-offs ≥ 0.2 , ≥ 1.0 , and ≥ 1.3 µg/mL and scored. Percentage, SD, and 95% CI (not shown) were calculated. Similarly, PnPSAb values were also compared with the levels (-2 SD) measured in healthy adult plasma pools. Spearman's correlation was used when appropriate.

RESULTS

Study Population, Treatment, and Infection

Twenty-three patients with confirmed PID were recruited. One patient dropped out for reasons unrelated to any adverse event. The clinical characteristics of the patients are summarized in Table 1. Twenty-two intent-to-treat patients were treated with a mean IVIG dose of 0.40 g/kg (Table 2), the median interval between infusions being 28 days (mean 37 days; range 26–50 days). During the study, there were no cases of IPD, and five serious bacterial infections (0.36 episode/patient-year) occurred. Other infections are listed Table 2. Antibiotics were administered to 15 patients (68%). By the end of the

TABLE 2 Efficacy End Points

Study Duration (d)	
Mean	227
Median	191
Treatment duration (d)	
Mean (95% CI)	179 (135–224)
Median	147
IVIG dose (g/kg), mean; 95% CI	0.40 (0.36–0.45)
Total relative weekly dose ^a (g/kg)	
Mean (95% CI)	0.10 (0.08–0.12)
No. and types of infections in intent-to-treat group	
Serious infectious diseases	
Proven invasive pneumococcal disease	0
Pneumonia	2
Sepsis ^b	3
Episode/patient/y	0.36
Other infectious diseases	
Bronchitis	4
Sinusitis	5
Other URTI	22
Gastrointestinal	6
Urinary tract infection	1
Episode/patient/y	2.77

URT, upper respiratory tract infection.

^a Weekly dose is defined as sum doses / period between visit 1 and the last infusion in days / 7.

^b Not related to antibody deficiency.

study, the antibiotic consumption was greatly reduced in 8 (57%) of the antibiotics-treated patients and slightly in 2 (14%). IVIG tolerance was described as good (13%) to very good (78.3%) at the final evaluation, and fever was reported in 2 patients (8.7%).

Trough and Peak IgG Levels

Tables 3 and 4 show total trough and peak IgG levels at each assessment time for all patients. The mean trough value (close to the median) for all visits was 7.77 ± 0.29 (range 3.22–14.1 g/L), and the mean peak value was 13.92 ± 0.35 (range 8.03–33.90 g/L), demonstrating an ~1.8-fold increase from pre- to postinfusion. Most mean trough IgG levels were maintained in the range 7.56 to 7.98 g/L (for 95% CI, see Table 3), that is, within the normal range for healthy individuals.¹⁸ At each assessment time, $35.1\% \pm 7.6\%$ of patients were between 5 and 7 g/L, and $22.1\% \pm 8.8\%$ were between 7 and 8 g/L (Table 4). A good correlation was found between total peak IgG and the relative weekly IVIG dose at the same visit (Spearman correlation coefficient 0.802; $P = .0001$) and a weaker correlation between trough level and relative weekly IVIG dose infused at the previous visit (correlation coefficient = 0.599; $P = .0032$).

Distribution of Trough PnPsAb Levels in the Patient Population

To allow inspection of data showing different orders of magnitude, we used the reverse cumulative distribution plot (recommended by Reed et al)¹⁹ for analyzing antibody levels after immunization) in which the horizontal axis shows the antibody level, and the vertical axis represents the percentage of subjects having at least the indicated antibody level. An example is shown for the trough PnPsAb values of visit 2 (Fig 1). The reverse cumulative distribution plots differed markedly according

TABLE 3 Total IgG (g/L) in All Subjects at Each Assessment Time

Visits	Mean	95% CI (LL–UL)	SD	Min	Q1	Median	Q3	Max
Visit 1								
Before	7.85	7.12–8.57	1.64	5.68	6.65	7.21	9.49	11.80
After	14.55	12.29–16.81	5.10	9.05	11.20	13.70	15.30	33.90
Visit 2								
Before	7.50	6.67–8.32	1.86	3.22	6.18	7.19	8.63	11.10
After	13.72	12.29–15.14	3.13	9.18	11.70	12.60	15.60	22.20
Visit 3								
Before	7.40	6.87–7.92	1.13	5.94	6.47	7.25	7.97	9.94
After	13.65	12.44–14.85	2.51	9.59	12.00	12.60	16.20	18.00
Visit 4								
Before	7.56	6.56–8.56	2.13	4.22	5.90	7.17	9.32	11.50
After	14.10	12.75–15.45	2.97	8.55	11.90	13.50	16.70	19.50
Visit 5								
Before	8.14	7.00–9.28	2.44	3.47	6.58	7.65	10.03	14.10
After	13.79	12.53–15.05	2.61	10.50	11.90	13.20	15.50	19.30
Visit 6								
Before	7.99	7.03–8.95	2.11	4.58	6.56	7.39	9.15	12.20
After	13.69	12.13–15.26	3.25	8.03	12.10	12.80	15.70	20.60
Visit 7								
Before	7.97	7.07–8.86	1.92	4.43	6.41	7.87	9.06	12.10
Mean all visits								
Before	7.77	7.56–7.98	0.29					
After	13.92	13.64–14.20	0.35					

Total IgG mean, 95% CI, and SD are shown before and after infusion, per visit and for all visits. LL, lower limit. Max, maximum; Min, minimum; Q1, first quartile; Q3, third quartile; UL, upper limit.

to the serotype and showed wide dispersion (range 0.046–8.8 $\mu\text{g}/\text{mL}$). On the basis of the trough levels present or exceeded in 85% of the patients, anti-serotype 14 antibodies appeared particularly abundant (the

corresponding curve is skewed to the right), whereas levels of anti-serotype 4 and 12F antibodies were >10 times lower. The highest trough values (>1 $\mu\text{g}/\text{mL}$) were found for 6B, 14, 19A, and 19F in most patients.

TABLE 4 IgG Distribution in All Subjects at Each Assessment Time: Percent of Subjects Achieving the Defined IgG Level Before and After Infusion, per Visit, and for All Visits

Visits	<5 g/L	≥ 5 and <7 g/L	≥ 7 and <8 g/L	≥ 8 and <10 g/L	≥ 10 g/L
Visit 1					
Before	0.0	40.9	27.3	18.2	13.6
After	0.0	0.0	0.0	4.5	95.5
Visit 2					
Before	4.5	45.5	9.1	31.8	9.1
After	0.0	0.0	0.0	4.8	95.2
Visit 3					
Before	0.0	40.0	35.0	25.0	0.0
After	0.0	0.0	0.0	5.3	94.7
Visit 4					
Before	10.0	35.0	20.0	15.0	20.0
After	0.0	0.0	0.0	4.8	95.2
Visit 5					
Before	10.0	30.0	15.0	20.0	25.0
After	0.0	0.0	0.0	0.0	100.0
Visit 6					
Before	4.8	28.0	28.6	19.0	19.0
After	0.0	0.0	0.0	15.8	84.2
Visit 7					
Before	5.0	25.0	20.0	35.0	15.0
Mean all Visits					
Before	4.9 (4.1) ^a	35.1 (7.6) ^a	22.1 (8.8) ^a	23.4 (7.5) ^a	14.5 (8.2) ^a
After	0.0	0.0	0.0	5.9 (5.3)	94.1 (5.2)

^a Number in parentheses is standard deviation.

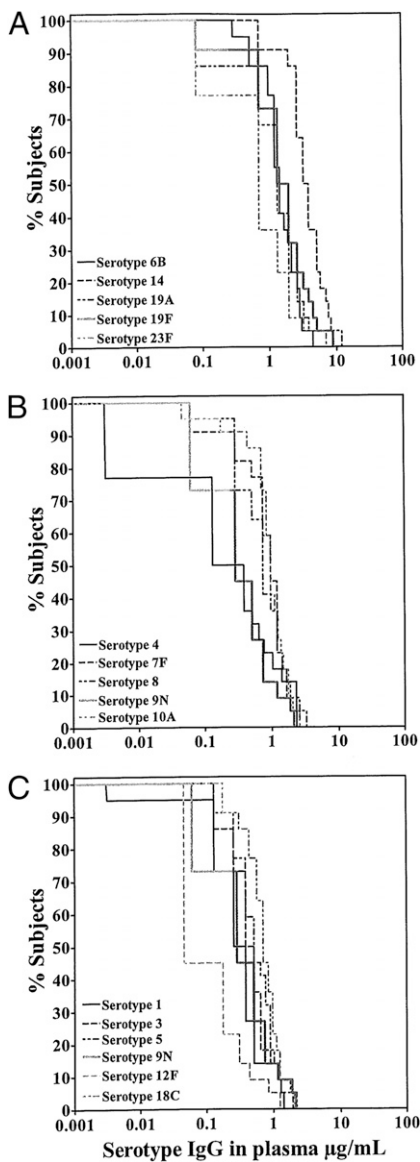


FIGURE 1
Reverse cumulative distribution curves of trough levels for 16 pneumococcal serotypes (visit 2). Reverse cumulative distribution curves showing concentrations of anti-PnPsAb against 16 serotypes ($\mu\text{g/mL}$; x axis) in plasmas of IVIG recipients (% subjects; y axis) before the second infusion (trough level). A, Serotypes 6B, 14, 19A, 19F, and 23F; B, serotypes 4, 7F, 8, 9N, and 10A; C, serotypes 1, 3, 5, 9N, 12F, and 18C.

Weighted GMCs and Estimated Protective PnPsAb Concentrations in Patients Before and After Infusion

Table 5 shows the trough (7 infusions) and peak (6 infusions) weighted GMCs of IgG against each pneumococcal serotype. Results of the 7 infusions are

aggregated. Again, the trough and peak weighted GMCs varied strongly according to the serotype. Trough GMCs ranged from 0.172 (serotype 12F) to 3.544 (serotype 14) $\mu\text{g/mL}$. Peak GMCs ranged from 0.379 (serotype 12F) to 7.956 (serotype 14) $\mu\text{g/mL}$ (this represents an ~ 20 -fold difference). The peak/trough ratio ranged from 2.1 to 2.7. We divided the peak GMC range arbitrarily into 3 levels: low ($\leq 1 \mu\text{g/mL}$; serotypes 1, 4, 12F), medium ($1\text{--}1.8 \mu\text{g/mL}$; serotypes 3, 5, 7F, 8, 9N, 9V, and 18C), and high ($2.1\text{--}8 \mu\text{g/mL}$; serotypes 6B, 10A, 14, 19A, 19F, and 23F).

To estimate the protection induced by passive immunization, we tentatively used the thresholds recommended by WHO for predicting, for all serotypes, vaccine effectiveness against IPD. The threshold $\geq 0.2 \mu\text{g/mL}$ (22F ELISA) is recommended as protection correlate for conjugate vaccines. This threshold was achieved at trough level in 89% to 100% of the patients (Table 5) for all serotypes except 4, 9V, and 12F and at peak level in 93% to 100% of the patients. For 4, 14, and 19F, a threshold of $\geq 1 \mu\text{g/mL}$ is reported to give a better correspondence between the serological response rate and vaccine effectiveness.²⁰ In this study, for antibodies against 19A, 19F, 6B, and 14, a trough level $\geq 1 \mu\text{g/mL}$ was found in 80% to 98% of the patients. For antibodies against the other serotypes, only 1% to 53% of the patients achieved this threshold. The trough threshold of $\geq 1.3 \mu\text{g}$, required for the polysaccharide vaccine, was reached for serotype 14 in 93% of the patients and for the other serotypes in 0% to 76% of the patients. For 9 serotypes, peak antibody levels reached a level $\geq 1 \mu\text{g/mL}$ in 83% to 100% of the patients. For 6 serotypes, peak values reached a level $\geq 1.3 \mu\text{g/mL}$ in 87% to 100% of the patients. For antibodies against the other serotypes, the $1.3 \mu\text{g/mL}$

threshold was achieved in 2% to 77% of the patients.

Comparing Patient Trough and Peak PnPsAb Levels With Those Found in Adults and IVIG Preparations

Figure 2A shows the serospecific PnPsAb (-2 SD) contents measured in adult donor plasmas. Figure 2B shows the percentage of PID patients with PnPsAb levels equal to or higher than those measured in adult plasmas (-2 SD). For all serotypes, peak levels in patients were similar to the levels measured in adult plasma pools, as were the trough values for serotypes 1, 3, 4, 7F, 9N, and 23F. Antibodies against the other serotypes reached levels similar to those found for adults (-2 SD) in 46% to 76% of the patient subjects. When the paired mean weighted PnPsAb GMCs of the patients were compared for each serotype with the levels measured in IVIG products, good regression fits were observed ($R^2 = 0.912\text{--}0.914$; $P < .0001$) for both trough and peak values (Fig 2C).

DISCUSSION

Patients with antibody deficiency are susceptible to recurrent and/or severe infection by encapsulated bacteria and to a range of gastrointestinal and malignant complications.²¹ Immunoglobulin replacement therapy reduces recurrent infections and subsequent complications and may decrease antibiotic use and hospitalization.⁸ The relationship between trough IgG and reduction of infections has been addressed in several studies. On the basis of a multicenter double-blind randomized crossover study including 43 PID patients, Eijkhout et al⁹ concluded that doubling the dose ($0.6\text{--}0.8 \text{ g/kg}$) significantly reduced the number and duration of infections. In a large meta-analysis including 17 studies totaling 676 patients and 2127 patient-years of

TABLE 5 Trough and Peak PnPsAb Concentrations (GMCs) and Proportion of Subjects Achieving Various Reference Thresholds

Serotype	Trough Weighted GMC	95% CI	Trough % Subjects (SD), $\mu\text{g/mL}$			Peak Weighted GMC	95% CI	Peak % Subjects (SD), $\mu\text{g/mL}$			Peak/Trough Ratio
	$\mu\text{g/mL}$	LL–UL	≥ 0.2	≥ 1	≥ 1.3	$\mu\text{g/mL}$	LL–UL	≥ 0.2	≥ 1	≥ 1.3	
1	0.416	0.388–0.447	89 (7)	15 (3)	6 (2)	0.875	0.801–0.953	100	32 (3)	22 (8)	2.1
3	0.515	0.477–0.558	92 (7)	13 (4)	9 (4)	1.099	1.030–1.179	99 (2)	54 (7)	36 (8)	2.1
4	0.326	0.278–0.382	60 (11)	19 (6)	14 (6)	0.577	0.545–0.612	95 (3)	22 (7)	16 (2)	2.1
5	0.608	0.545–0.549	97 (3)	20 (11)	5 (5)	1.300	1.166–1.442	100	70 (15)	50 (8)	2.1
6B	1.551	1.448–1.653	100	83 (7)	65 (8)	3.727	3.523–3.935	100	100	99 (2)	2.4
7F	0.836	0.736–0.949	93 (6)	47 (8)	31 (4)	1.779	1.660–1.906	100	87 (5)	77 (3)	2.1
8	0.812	0.765–0.858	99 (2)	41 (5)	26 (8)	1.593	1.496–1.692	100	83 (3)	68 (7)	2.1
9N	0.428	0.378–0.481	89 (5)	13 (2)	11 (4)	1.046	0.955–1.139	99 (2)	50 (17)	31 (8)	2.4
9V	0.456	0.400–0.517	64 (5)	35 (8)	25 (3)	1.000	0.882–1.125	96 (5)	63 (7)	54 (10)	2.2
10A	0.963	0.903–1.021	98 (3)	53 (8)	30 (8)	2.101	2.023–2.180	100	96 (5)	91 (5)	2.2
12F	0.172	0.155–0.189	35 (5)	1 (2)	0	0.379	0.349–0.411	93 (7)	8 (3)	2 (3)	2.2
14	3.544	3.235–3.861	100	98 (3)	93 (4)	7.956	7.658–8.245	100	100	100	2.2
18C	0.695	0.608–0.791	94 (4)	33 (2)	15 (5)	1.683	1.497–1.891	99 (2)	88 (5)	77 (6)	2.4
19A	1.754	1.629–1.881	100	80 (6)	76 (3)	4.760	4.597–4.929	100	100	100	2.7
19F	1.787	1.671–1.901	100	80 (6)	69 (8)	3.859	3.676–4.030	100	100	99 (2)	2.2
23F	1.096	0.982–1.212	99 (2)	52 (15)	36 (11)	2.307	2.131–2.483	100	98 (3)	87 (7)	2.1

The percentage of subjects achieving threshold antibody concentrations 0.2, 1.0, and 1.3 $\mu\text{g/mL}$ are shown separately for trough and peak levels. The peak/trough GMC ratio is shown and is highly significant for all serotypes ($P < .0001$). LL, lower limit; UL, upper limit.

follow-up with diverse diagnoses and IVIG products, Orange et al¹⁰ showed the pneumonia incidence to decline by 27% with each gram-per-liter increment in trough level. The pneumonia incidence was 5 times higher with a 5 g/L than with a 10 g/L trough level. A patient-individualized approach to IgG replacement therapy is supported by clinical experience and justified by a prospective analysis in patients with common variable immunodeficiency disorders over a follow-up period of 22 years.^{21,22} Protective IgG trough levels were achieved with a wide range of replacement doses (0.2–1.2 g/kg/mo), whereas patients with bronchiectasis required twice as much IgG replacement therapy.^{21,23}

In most of our patients, the mean trough total IgG level was maintained in the range 7.56 to 7.98 g/L. At each assessment time, 90% to 95% of the patients showed a trough level above the lowest threshold (5 g/L) deemed acceptable for adults according to the European Medicine Agency (5–6 g/L).²⁴ The main goal of this study was to investigate PnPsAb levels and their correlates of protection in IVIG-treated PID patients. PnPsAbs in IVIG products have proved

active in an opsonophagocytosis test thought to predict the functional potential of these antibodies.^{11,20} We have used WHO 22F-ELISAs to measure trough and peak PnPsAbs specific to 16 serotypes. The reverse cumulative curves show a wide dispersion of levels and marked differences between serotypes (with 12F and 14 at the 2 extremes of the spectrum). For serotype 14, the trough GMC was 20 times as high and the peak GMC 22 times as high as for serotype 12F. Different criteria can be used as surrogates to evaluate the protection conferred by peak and trough PnPsAb levels. The WHO working group for evaluating new pediatric pneumococcal vaccines concluded that the percentage of subjects reaching the WHO reference value of 0.2 $\mu\text{g/mL}$ by 22F-ELISA could provide the best estimate of protection against IPD.^{3,4} Our results show that 93% to 99% of the subjects had peak levels $\geq 0.2 \mu\text{g/mL}$ for all serotypes and 89% to 90% had trough levels $\geq 0.2 \mu\text{g/mL}$ for 13 serotypes (not 4, 9V, and 12F). Trough levels of antibodies against serotypes 19A, 19F, 6B, and 14 reached the minimum protective level of 1 $\mu\text{g/mL}$, found to correspond better with

vaccine effectiveness against IPD due to serotypes 4, 14, and 19F in a conjugate vaccine trial conducted in the American Indian population and also found to protect normal children against pneumonia.^{3,20,25,26} Only PnPsAbs against serotype 14 showed trough levels $\geq 1.3 \mu\text{g/mL}$ (93% of subjects), considered protective or adequate in all age groups after immunization with polysaccharide vaccine. Pieretti and Cunningham-Rundles²⁷ report that for antibodies against ≥ 7 of 14 serotypes, 11 of 20 immunodeficient patients had trough levels $\geq 1.3 \mu\text{g/mL}$. On the basis of trough level $\geq 0.2 \mu\text{g/mL}$, our data indicate that PID patients treated with 0.4 g/kg IVIG are protected against IPD caused by most serotypes. Higher PnPsAb serum levels might be desirable to prevent non-IPDs such as pneumonia.^{25,28}

To better understand this wide variability according to serotype, trough and peak levels of the different PnPsAbs were compared to the levels present in adult plasma pools (the starting material for IVIG production). Peak levels were comparable to adult levels, but in only 6 serotypes did trough levels reach the adult plasma levels (87%–96% of

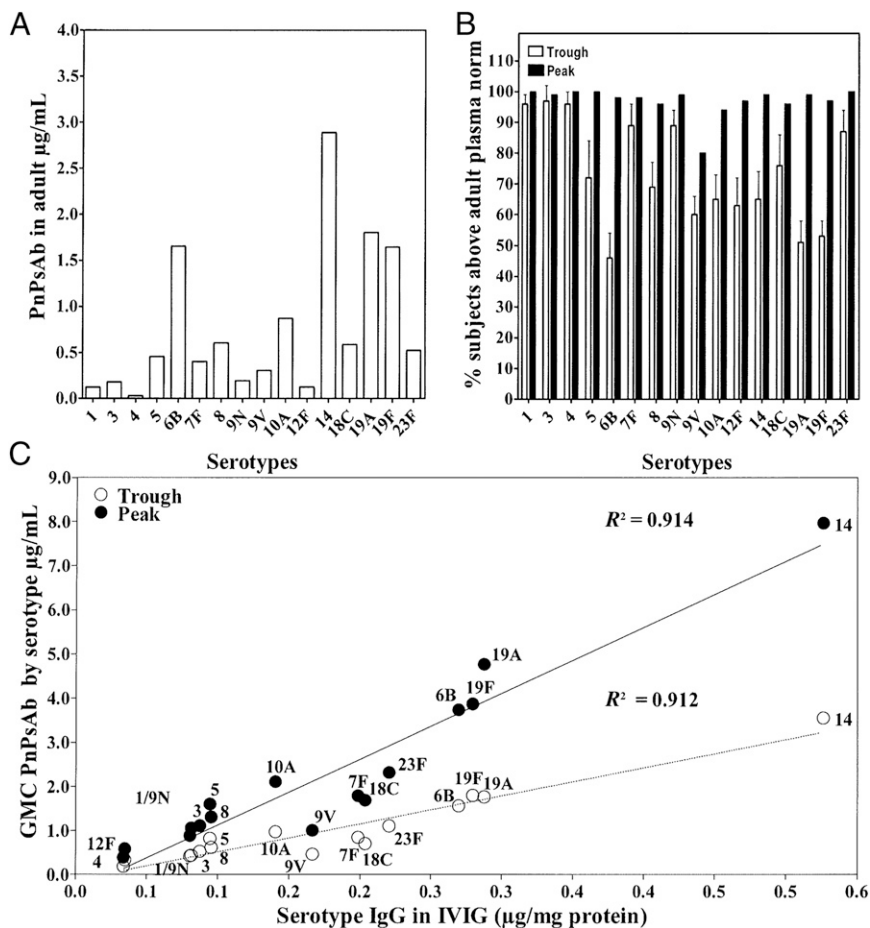


FIGURE 2 IVIG-treated subjects (%) achieving PnPsAb concentrations measured in adults and correlation with IVIG products. A, Concentrations (-2 SD) in $\mu\text{g/mL}$ (y axis) of IgG against 16 serotypes (x axis) in adult plasma pools. B, Percentage of subjects (y axis) achieving the PnPsAb level found in adult plasma pools (trough and peak). C, Trough and peak GMCs in patients ($\mu\text{g/mL}$; y axis) are shown for each serotype as a function of the levels ($\mu\text{g/mg/protein}$) in IVIG product.

subjects). The peak-to-trough level ratio in patients ranged from 1.8 (total IgG) to 2.1 to 2.7 for PnPsAbs according to the serotype. Differential catabolism or IgG consumption can be suspected because in a pharmacokinetic study of 63 PID children and adults,¹⁵ different half-lives were found for total IgG (30 ± 11 days) and IgGs against serotypes 14 (40.77 days), 19A (60.04 days), and 23F (29.98 days). We found no correlation between dose and trough level for any serotype ($P > .05$). Chua et al¹⁴ likewise observed only a weak correlation ($R^2 = 0.5615$) between total IgG level and trough total PnPsAbs against 23 serotypes (in-house ELISA).

Seventeen batches of IVIGs were analyzed for their PnPsAb contents. Between-lot variability of specific antibody levels was lower than in Lejtenyi and Mazer,¹² probably because, as these authors claimed, the plasma donations used here for IVIG production were collected in a geographically restricted region. Mikolajczyk et al,¹¹ studying 7 IVIG preparations, also revealed differences among products. We show good correlations between both the peak and trough weighted GMCs for antibodies against each serotype and the mean levels of these antibodies in the infused IVIG ($R^2 = 0.912-0.914$). This demonstrates a tight relationship between the serospecific PnPsAbs present

in the IVIG preparation and those circulating in patients. It is worth stressing the impact of the pneumococcal serotypes present in the environment of donors and patients on specific PnPsAb levels, notably those present in donations for producing therapeutic IVIGs. Globally, the most common serotypes causing IPD in young children before introduction of the seven-valent pneumococcal conjugate vaccine were 14, 6B, 1, 23F, 5, and 19F.²⁹ Of these, serotypes 14, 6B, 23F, and 19F have the highest GMC values in our patients, whereas serotypes 1 and 5, viewed as noncarried serotypes, have the lowest values. Vaccination with conjugate vaccines considerably affects pneumococcal disease incidence, serotype distribution, and carriage in both vaccinated and unvaccinated subjects.³⁰⁻³³ How the new pneumococcal conjugate vaccines will influence the dynamic epidemiology of nonvaccine serotypes and specific PnPsAb levels in adult plasma donors (and thus in the derived IVIG products) is of concern.⁸

CONCLUSIONS

Our study shows a linear relation between serotype-specific antipneumococcal antibodies in pediatric PID patients and the administered IVIG product, as well as a good correlation with adult plasma levels. Our findings highlight the need to monitor serospecific antibody levels continuously in both IVIG batches and PID patients.

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Pneumococcal Antibody Levels in Children With PID Receiving Immunoglobulin

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