

Reconstitution of IgG Subclasses Following Immunoglobulin Administration in Adult Patients with Common Variable Immune Deficiency

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Keywords

Intravenous Ig · Subcutaneous Ig · Enzyme-facilitated Ig · IgG subclasses · Common variable immune deficiency

Abstract

Background: Immunoglobulin (Ig) therapy reduces the frequency and severity of infection among patients with antibody deficiency disorders. However, a subset of patients lacks adequate clinical response. **Objective:** The purpose of this study was to determine in adult common variable immune deficiency (CVID) patients (A) if lack of clinical response to Ig therapy correlates with lack of reconstitution of IgG subclass (es), (B) correlation between Ig dosing and/or IgG trough levels and IgG subclass reconstitution, (C) and most common impaired *Streptococcus pneumoniae* (*S. pneumoniae*) serotype antibody response. **Methods:** Single-institution, retrospective chart review for CVID patients at immunology clinics from 2015 to 2019. Patients were monitored every 3–6 months for IgG dosage, IgG trough levels, IgG subclass reconstitution, infectious episodes (chronic sinusitis, bronchitis, upper respiratory, and lower respiratory tract infections), urinary tract infections, and antibiotic use. Follow-up was calculated in patient years. **Results:** Twenty-five of 41 patients achieved complete reconstitution of all IgG sub-

classes, and 16/41 demonstrated intermittent or lack of reconstitution. There were significantly less ($p < 0.001$) infections among fully reconstituted patients (0.66 ± 0.19 infections per patient year) as compared to those with intermittent or lack of reconstitution (1.26 ± 0.13 infections per patient year). There was a significant correlation between IgG trough levels and IgG subclass reconstitution. Most common impaired *S. pneumoniae* serotype included 3, 4, 9n, 10a, 11a, 12f, and 15b. **Conclusions:** Incomplete IgG subclass reconstitution was associated with increased frequency of infections. IgG trough levels correlate with IgG subclass reconstitution. A limited number of *S. pneumoniae* serotype antibodies are commonly impaired in CVID.

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Introduction

Common variable immune deficiency (CVID) is a heterogeneous and the most common antibody deficiency disorder among adults, with an estimated prevalence of 1/20,000–1/50,000 [1]. It is characterized by low IgG, low

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IgA, and/or IgM, impaired response to vaccination and increased susceptibility to infections [2–4]. Monogenic defects contribute to approximately 10–20% of CVID patients [5–7]; therefore, the genetic basis for the majority of CVID cases remains unclear. The most common, identified defects are observed in B cell compartments including decreased switched memory B cells, increased CD21_{low} B cells, and failure to differentiate B cells into plasma cells [8]. Immunoglobulin replacement is an established standard therapy for antibody deficiency disorders. Immunoglobulin is administered via intravenous (IVIG), subcutaneous (SCIG), and enzyme-facilitated subcutaneous (fSCIG) routes (9–12). Regardless of route of administration, immunoglobulin therapy has been effective in reducing the frequency and severity of infections [9–13].

Although the majority of patients respond to Ig therapy in reducing the frequency and severity of infections, a subset of patients continues to suffer from recurrent infections. To explain such a phenomenon, Bonagura et al. [14] proposed the concept of “biological trough level.” He reported that different IgG trough levels, in 2 different patients with common variable immunodeficiency, were required to keep them free from recurrent infections. We proposed that the failure to reconstitute IgG subclass (es) may be one of the factors responsible for continued recurrent infections. A role of IgG subclass reconstitution in CVID has not been reported. Furthermore, specific antibody responses against individual *Sterptococcus pneumoniae* (*S. pneumoniae*) serotypes have not been reported. Therefore, in this study, we examined IgG subclass (es) reconstitution following Ig therapy and its relationship with recurrent infections in adult CVID patients. Furthermore, we studied the relationship between Ig dosing and IgG trough levels with IgG subclass reconstitution. Finally, we examined most frequently impaired *S. pneumoniae* serotype antibody responses and contribution of IgG subclasses for low total IgG and in adult patients with CVID.

Our data show that lack of IgG subclass reconstitution was associated with an increased frequency of infections as compared to those patients who had complete reconstitution of IgG subclasses. IgG trough levels correlated with IgG subclass reconstitution; however, Ig dosing has no correlation with IgG subclass reconstitution or IgG trough levels. The most commonly impaired specific antibody responses following Pneumovax-23 vaccination in CVID included serotypes 3, 4, 9n, 10a, 11a, 12f, and 15b. Isolated or in combination with other IgG subclasses, low IgG1 subclass contributed to low total IgG levels in majority of CVID patients.

Materials and Methods

This is a retrospective chart review of adult patients with CVID that were diagnosed and are followed at primary immunodeficiency clinics at University of California at Irvine. Charts were reviewed for the 2015–2019 period. We identified 41 patients with CVID who met ESID/PAGID criteria [2]. We reviewed patient charts for demographic data including age, gender, presenting manifestations, comorbidities, IgG, and IgG subclass levels at the time of diagnosis. Since 2016, we monitored patients every 3–6 months for IgG and IgG subclass levels, infectious episodes, and antibiotic use. Although most patients had very tight follow-up, we did not exclude patients from analysis if they had periods greater than 6 months’ duration without follow-up. Date calculator was used to determine the days between the last date in 2015 (or the earliest date of established care beyond that time) and the date of the patient’s last follow-up visit. Each patient’s time in follow-up was then converted into years by dividing by 365.25 (to account for leap year). Patients were categorized into 3 groups, fully reconstituted, intermittently reconstituted, and not reconstituted based upon IgG subclass reconstitution during entire period of follow-up. Intermittent reconstitution was categorized for the lack of IgG subclass (es) reconstitution on at least 2 occasions during follow-up.

*The University of California Institutional Review Board
Approved This Study*

Statistical analysis was performed with unpaired *t* test and unpaired *t* test with Welch’s correction, and Mann-Whitney test using GraphPad Prism version 8.4.3 for Windows, GraphPad Software, San Diego, CA, USA. *p* value of <0.05 is considered significant.

Results

Demographic Data

Demographic data for all 41 patients are included in Table 1. Diagnosis was established according to ESID/PAGID criteria [2] characterized by low IgG and low IgA and/or low IgM and impaired response to Pneumovax-23. Impaired responses were defined as unprotected *S. pneumoniae* antibody titers (<1.3 µg/mL) and/or <2-fold increase over low titers at baseline against more than 70% serotypes [15]. There was a female predominance with a female-to-male ratio of approximately 3.6:1. The average age was approximately 58.9 years. The majority of patients presented with upper and lower respiratory infections. Upper respiratory tract infections included sinusitis, otitis media, pharyngitis, and bronchitis, and lower respiratory tract infections included pneumonia. Eighteen of the 41 patients (48%) had associated autoimmune diseases. Allergic manifestations included 15 patients (36.6%) with chronic allergic rhinitis and/or rhinosinusitis and 6 patients (14.6%) with allergic asthma. Allergic

Table 1. Demographics and clinical presentation data

ID	Age, years	Gender	IgG1, mg/dL	IgG2, mg/dL	IgG3, mg/dL	IgG4, mg/dL	Associated autoimmune disease	Associated allergic disease	Presenting infectious manifestations
1	71	Male	339	56*	12*	5.6*		Asthma	Bronchitis
2	70	Female	284*	129*	34	2*	Celiac disease	Asthma	Upper respiratory infections; bronchitis
3	60	Female	249*	<97*	349	<7*			Recurrent otitis media, chronic sinusitis
4	38	Female	169*	131*	20*	10.5		Asthma	Chronic sinusitis; pneumonias
5	61	Female	288*	65*	11*	1*			Chronic sinusitis
6	49	Female	<150*	<97*	104*	<7*		Asthma	Pneumonias
7	75	Male	<150*	202*	35*	<7*	Psoriasis		Pneumonias
8	68	Female	411	117*	24.6	9.3	SLE Temporal arteritis Dermatomyositis Celiac disease		Pneumonias; urinary tract infections
9	49	Female	115*	11*	39	<0.3*			Pharyngitis; pneumonias
10	73	Female	214*	392	24.3	10.6		Bradykinin-mediated angioedema	None
11	50	Female	300*	94.5*	35	3.2*	SLE APAS		Upper respiratory infections
12	34	Female	<15*	2*	1*	<0.3*		Allergic rhinitis	Chronic sinusitis; pneumonias
13	27	Female	105*	59.2*	12.3*	8	SLE	Allergic rhinitis	None
14	64	Female	189*	171	52.5	1.2*	Rheumatoid arthritis		Recurrent urinary tract infections
15	66	Female	277*	100*	43.1	4.9*	Multiple sclerosis	Allergic rhinitis	Chronic sinusitis; bronchitis
16	46	Female	312*	185	36.6	16.4			Chronic sinusitis; bronchitis
17	63	Male	242	210	89.7	2.8*		Allergic rhinosinusitis	Chronic sinusitis
18	47	Female	316*	108*	18*	25.3	Hypothyroidism	CSU	Pneumonia
19	62	Female	295*	171*	21*	1*	Addison's disease	Allergic rhinosinusitis	Chronic sinusitis
20	75	Female	251*	197	108	20.3	Hypothyroidism		Recurrent urinary tract infections
21	69	Female	309*	101*	34.4	4.6*			Upper respiratory infections Recurrent urinary tract infections
22	71	Male	97*	39*	19*	5.5	Ulcerative colitis		Chronic sinusitis; pharyngitis; pneumonias
23	17	Male	343	149	10.6*	102		Allergic rhinitis Asthma	Recurrent otitis media
24	72	Female	285*	200	22.6	13.3		Allergic rhinosinusitis	None
25	43	Female	347*	231*	47	36.9		Allergic rhinitis Asthma	Bacterial sinusitis
26	68	Female	237*	159	27*	5		Allergic rhinitis	Bacterial sinusitis; pharyngitis; pneumonias
27	67	Male	256	174	66.7	7.2*			Upper respiratory infections; chronic sinusitis, bronchitis
28	60	Female	305*	165	18.2*	22			Upper respiratory infections; bronchitis

Table 1 (continued)

ID	Age, years	Gender	IgG1, mg/dL	IgG2, mg/dL	IgG3, mg/dL	IgG4, mg/dL	Associated autoimmune disease	Associated allergic disease	Presenting infectious manifestations
29	66	Female	81.3*	119*	19.6*	<7*	Rheumatoid arthritis		Urinary tract infections
30	75	Male	309	102*	5.8*	1.8*	Myasthenia gravis ITP Pernicious anemia		Upper respiratory infections
31	52	Female	309*	102*	10.5*	<7.3*			Upper respiratory infections
32	47	Female	308*	208	54.7	3.4*	Hashimoto's thyroiditis		Upper respiratory infections
33	77	Female	295*	129*	28.6	14.2			None
34	56	Female	300*	127*	7.4*	5.7*		Allergic rhinoconjunctivitis	Upper respiratory infections; bronchitis
35	66	Female	382	104*	43.4	25.8	Raynaud's syndrome	Allergic rhinitis	Recurrent urinary tract infections
36	78	Male	504	85.4*	49	10.3	ITP		Bronchitis
37	54	Female	245*	120*	32.3	13.6	SLE		Bronchitis; recurrent urinary tract infections
38	61	Male	349*	146*	31	21			Upper respiratory infection; pneumonia
39	71	Female	355*	114*	23	13.5		Allergic rhinitis	Bacterial sinusitis; bronchitis; recurrent urinary tract infections
40	44	Female	105*	61.3*	17.1*	1.2*	ITP Hypothyroidism		Recurrent urinary tract infections
41	52	Female	443	195*	22	<0.3*		Allergic rhinitis	Bacterial sinusitis

APAS, anti-phospholipid antibody syndrome; ITP, idiopathic thrombocytopenia; SLE, systemic lupus erythematosus; CSU, cold stimulated urticaria; Ig, immunoglobulin. * Low IgG subclass value.

manifestations were in 35% of fully reconstituted group and 42% of incomplete (intermittent and no) reconstitution group. Autoimmune manifestations were in 48% of fully reconstituted and 45% in incomplete reconstituted group. None of the patients had bronchiectasis, and no patient was on prophylactic antibiotic or on any immunosuppressive or biological agents. All patients received uninterrupted immunoglobulin replacement therapy.

The mean IgG levels at the time of diagnosis were 455.8 mg/dL (control range, 694–1,618 mg/dL). The mean serum IgA levels in 20 patients with low IgG and low IgA were 29.7 mg/dL (control range, 68–378 mg/dL), and 28 patients with low IgG and low IgM had mean IgM levels of 31.1 mg/dL (control range, 65–263 mg/dL). Three patients had IgA < 7 mg/dL, 2 in fully reconstituted and 1 in intermittently reconstituted groups.

Reconstitution of IgG Subclasses by Ig Replacement

Patients received Ig replacement therapy via IVIG, SCIG, or fSCIG routes (Table 2). IgG subclasses were fully reconstituted in 25 patients (61%), 12 patients (29%)

achieved intermittent reconstitution, and 4 patients (10%) without any reconstitution. Among the 25 fully reconstituted patients, 19 were on IVIG, 3 on SCIG, and 3 on fSCIG. Among the 12 patients with intermittent reconstitution, 10 were on IVIG, and 1 each on SCIG and fSCIG. Among the 4 patients without any reconstitution, 2 were on IVIG, and 1 each on SCIG and fSCIG. The mean dose of Ig on IVIG was 453.4 mg/kg/4 weeks, on SCIG 445.7 mg/kg/4 weeks, and on fSCIG 441.4 mg/kg/4 weeks.

Infections per Patient Year according to IgG Subclass Reconstitution

A surrogate for infections included use of antibiotics, as prescribed by a physician or nurse practitioner for sinusitis, upper respiratory tract infections, and bronchitis, was determined using clinical data obtained at each follow-up appointment for those that had been on consistent Ig therapy for at least 3 months. Infections were tabulated among those that achieved complete reconstitution of their IgG subclasses and those with intermittent

Table 2. Reconstitution of IgG subclasses by Ig therapy

Route of administration	Dose of Ig (N), mean, mg/kg/4 weeks	Fully reconstituted (N)	Intermittent reconstitution (N)	No reconstitution (N), (mean, mg/kg/4 weeks)
IVIG (31)	453.4	19	10	2
SCIG (5)	445.7	3	1	1
fSCIG (5)	441.4	3	1	1
Total		25	12	4

N, number of patients; Ig, immunoglobulin; IVIG, intravenous immunoglobulin; SCIG, subcutaneous immunoglobulin; fSCIG, facilitated subcutaneous immunoglobulin.

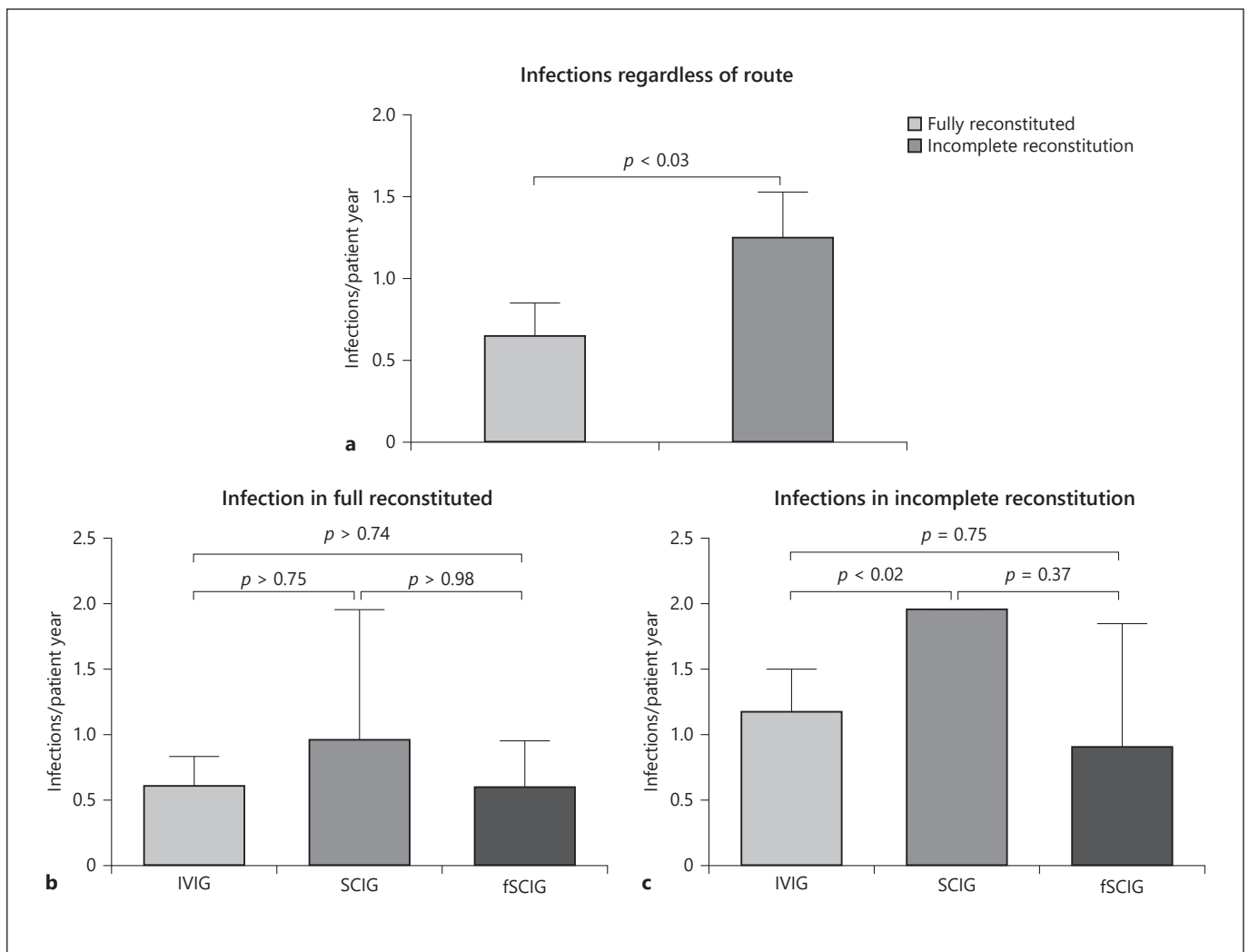


Fig. 1. Infections according to IgG subclass reconstitution and route of Ig administration. **a** Infections per patient year in patients with full reconstitution and incomplete reconstitution (intermittent plus lack or reconstitution) of IgG subclasses for all 3 routes of Ig administration. Infections according to different routes of administration in patients with complete reconstitution (**b**) and

incomplete IgG subclass reconstitution (**c**). Collective time in years is 45.5 in incomplete reconstitution and 71.4 in fully reconstituted group. Ig, immunoglobulin; IVIG, intravenous immunoglobulin; SCIG, subcutaneous immunoglobulin; fSCIG, facilitated subcutaneous immunoglobulin.

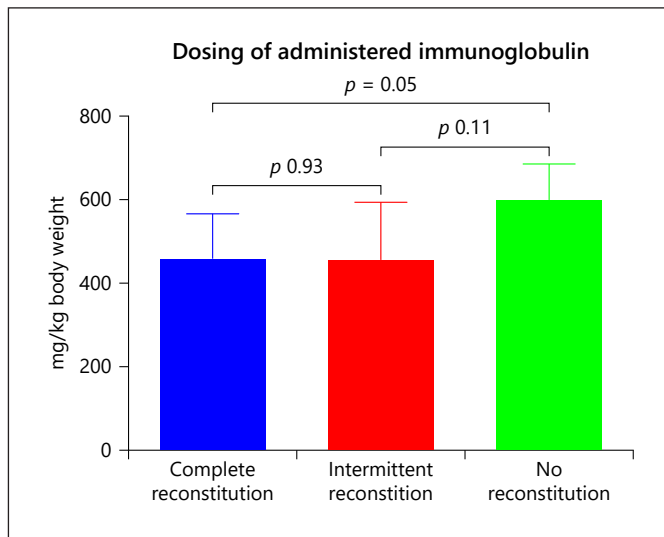


Fig. 2. Dosing of Ig and IgG subclass reconstitution. Data are expressed in mg/kg body weight/4-week dose of Ig as mean $\pm$ SEM for all 3 groups. Number of subjects in complete reconstitution group ($n = 25$), intermittently reconstituted group ($n = 12$), and lack of reconstitution of IgG subclasses ($n = 4$). Ig, immunoglobulin; SEM, standard error of measurement.

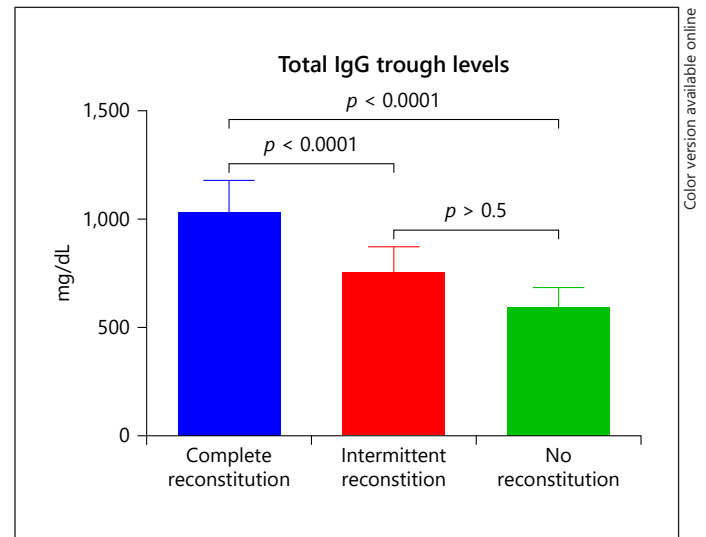


Fig. 3. IgG subclass reconstitution and IgG trough levels. Data are shown for IgG trough levels at mean $\pm$ SEM significantly higher levels of IgG trough levels were observed in fully reconstituted group as compared to intermittently reconstituted and lack of reconstituted group. Ig, immunoglobulin; SEM, standard error of measurement.

and lack of reconstitution. As there were different amounts of time spent in our care while on >3 months of Ig therapy, infections were then averaged per patient year to allow for comparison. The cumulative time of follow-up (in years) for all patients was 71.38 years for fully reconstituted group and 45.55 years for incomplete (intermittent and no) reconstitution group. Figure 1a shows that the infection rate per patient year for all patients, regardless of routes of Ig administration, was significantly ($p \leq 0.035$) higher among those with incomplete (intermittent plus lack of reconstitution) IgG subclass reconstitution (mean $\pm$ SEM, 1.26 ± 0.13) as compared to those with complete reconstitution (mean $\pm$ SEM, 0.66 ± 0.19). When data were analyzed according to route of Ig administration, no significant difference was observed among various route of administration in fully reconstituted group (Fig. 1b); however, in incomplete reconstituted group (Fig. 1c), IVIG route ($n = 31$) had significantly ($p < 0.02$) lower infections than SCIG route ($n = 5$) but not significantly lower than fSCIG route ($n = 5$).

Relationship of Ig Dosing and IgG Trough Levels with IgG Subclass Reconstitution

Dosing of IgG varies among various centers around the world. Orange et al. [19] reported correlation between Ig dosing and clinical outcome in patients treated

with SCIG, as well as a correlation between IgG trough levels and infections [20]. Therefore, we examined the relationship between Ig dosing and IgG trough levels and the reconstitution of IgG subclasses. Dosing of IgG ranged from 336–651 mg/kg/4 weeks with a mean $\pm$ SEM value of 457 ± 21.9 mg/kg/4 weeks in the group with complete reconstitution of IgG subclasses. Among the group with intermittent reconstitution of IgG subclasses, dosing ranged from 394–830 mg/kg/4 weeks with a mean $\pm$ SEM value of 454 ± 37.3 mg/kg/4 weeks, and dosing ranged from 513–685 mg/kg/4 weeks with a mean $\pm$ SEM value of 598 ± 49.6 mg/kg/4 weeks in patients who did not reconstitute their IgG subclasses (Fig. 2). There was no significant difference ($p = 0.93$) in the dosing between completely reconstituted ($n = 25$) and intermittently reconstituted ($n = 12$) group; however, a significant difference ($p = 0.035$) was observed in dosing between completely reconstituted group and lack of reconstitution ($n = 4$) group.

Both correlation [20, 21] and lack of correlation [22, 23] have been reported between high and low IgG trough levels with bacterial infections in CVID. Since reconstitution of IgG subclasses correlated with infections, we analyzed IgG trough levels in all 3 groups of IgG subclass reconstitution (Fig. 3). IgG trough levels (mean $\pm$ SEM) in fully IgG subclass reconstituted group ($1,035 \pm 28.7$

Table 3. Antibody response to individual *S. pneumoniae* serotypes

Pneumococcal serotype	Patients with unprotected titers and/or impaired response* (%)
1	20/38 (52.6)
2	10/18 (55.6)
3	31/38 (81.8)
4	33/38 (86.8)
5	15/38 (39.5)
6b	28/38 (73.7)
7f	23/38 (60.5)
8	21/38 (55.3)
9n	29/38 (76.3)
9v	27/38 (71.1)
10a	14/18 (77.8)
11a	14/18 (77.8)
12f	30/38 (79.0)
14	24/38 (63.2)
15b	13/18 (83.3)
17f	10/18 (55.6)
18c	20/38 (52.6)
19a	6/18 (33.3)
19f	24/38 (63.2)
20	9/18 (50.0)
22f	13/18 (34.2)
23f	28/38 (73.7)
33f	10/18 (55.6)

S. pneumoniae, *Streptococcus pneumoniae*; PPSV, pneumococcal polysaccharides vaccine. * Unprotected titers = <1.3 µg/mL and impaired response = <2-fold increase in titers over low baseline values 4 weeks following immunization with PPSV-23.

mg/dL) were significantly higher ($p < 0.0001$) than in the intermittently reconstituted group (755.6 ± 31.6) and significantly higher ($p < 0.0001$) than group without reconstitution (598.3 ± 49.7). No significant ($p > 0.5$) difference was observed in trough levels between intermittent reconstituted and lack of reconstituted group.

Specific Antibody Response to S. pneumoniae Individual Serotypes

Antibody response to individual serotypes of *S. pneumoniae* in CVID has not been reported. In each case of newly diagnosed CVID, we confirmed the presence of specific antibody deficiency as per established criteria with unprotected pneumococcal titers (<1.3 µg/mL) and/or impaired response to Pneumovax-23 (<2-fold increase) in 70% of tested serotypes [15]. Some of the testing predated the availability of 23 serotype testing, and consequently, 14 serotypes were tested. Table 3 shows data for serotypes most frequently associated with impaired response and/or unprotected levels. Commonly impaired

S. pneumoniae serotype antibody responses in the majority of patients (>75% of patients) included serotype 3, 4, 9n, 10a, 11a, 12f, and 15b.

Distribution of IgG Subclass Deficiency Contributing to Low Total IgG in CVID Patients

At the time of diagnosis, IgG subclass (es) contributing to low total IgG were stratified into 2 groups: those who had only isolated low IgG subclass and those with 2 or more low IgG subclasses. Ten patients had isolated IgG subclass deficiency (Fig. 4a) that included IgG1 ($n = 4$), IgG2 ($n = 3$), IgG3 ($n = 1$), and IgG4 ($n = 2$). Majority of patients had combination of low IgG subclasses (Fig. 4b): IgG1, IgG2, and IgG3 ($n = 5$); IgG1, IgG2, and IgG4 ($n = 6$); IgG2, IgG3, and IgG4 ($n = 2$); IgG1, IgG2, IgG3, and IgG4 ($n = 8$); IgG1 and IgG2 ($n = 5$); IgG1 and IgG3 ($n = 2$); IgG1 and IgG4 ($n = 2$); and IgG2 and IgG4 ($n = 1$). Therefore, isolated and in combination, IgG1 was low in 35 of 41 (85%) patients.

Discussion

Patients with antibody deficiencies require lifelong replacement therapy with Ig to prevent recurrent and often severe infections and irreversible complications. Regardless of route of administration, IVIG, SCIG, or fSCIG, Ig is effective in reducing frequency and severity of infections (9–13). However, a subset of patients continued to have recurrent infections. Orange et al. [20], in a meta-analysis, reported a relationship between IgG trough levels and frequency of pneumonia. We hypothesized that breakthrough infections on Ig therapy may be also due to lack of reconstitution of IgG subclasses. There are no published data on the reconstitution of IgG subclasses following immunoglobulin therapy in CVID and relationship with infections. We observed complete stable IgG subclass reconstitution in 61% of patients, intermittent reconstitution in 29%, and 10% of patients never achieved full reconstitution. The reasons are unclear. There was no significant difference in dosing of Ig; however, there was significant difference in IgG trough levels. Although Ig distributors claim physiological levels of IgG subclasses in their products IgG subclass concentrations may be different. Lamari et al. [24] reported different IgG subclass levels among 2 preparations of IVIG. Because of small number of subjects, we could not analyze data according to Ig products. In our study, we observed a significant increase ($p < 0.035$) in infection/patient year in patients who had had intermittent or persistent lack of

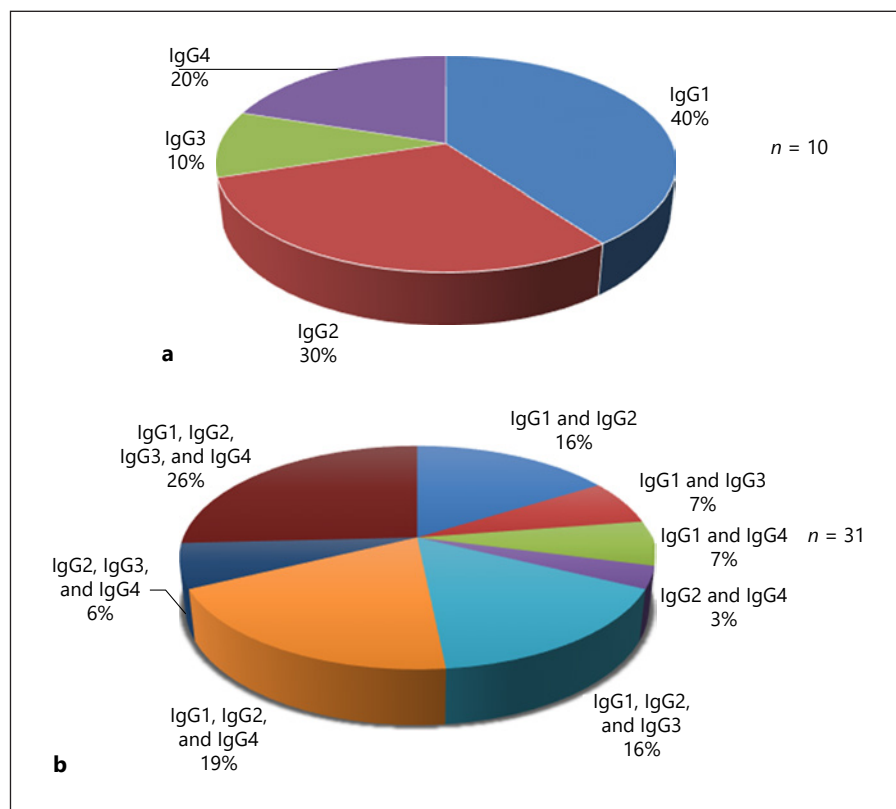


Fig. 4. a Distribution of low isolated IgG subclasses in CVID patients ($n = 10$). Low IgG subclasses were observed for all 4 subclasses; however, low IgG1 subclass was most common. Data are expressed as % of cases. **b** Distribution of multiple IgG subclass deficiency in CVID ($n = 31$). Majority of patients had combined IgG subclass deficiency involving IgG1 ($n = 25$). Data are expressed as % of cases. Ig, immunoglobulin; CVID, common variable immune deficiency.

IgG subclass reconstitution as compared to those with complete reconstitution of IgG subclasses. No significant difference was observed between routes of Ig administration among IgG subclass fully constituted group. However, we observed significantly lower infection rate in IVIG group ($n = 21$) as compared to SCIG group, though the number of subjects in the later group is too small ($n = 5$) to draw a conclusion. No significant difference was observed between patients receiving SCIG ($n = 5$) and fSCIG ($n = 5$). In an earlier publication in patients with primary hypogammaglobulinemia, no significant difference was observed in infection rate between those who were fully reconstituted versus those without IgG subclass reconstitution [17]. Furthermore, full reconstitution was observed with fSCIG [17]. The discrepancy between 2 studies appears to be because, in our previous study [17], we collected data only at 1 time point during 1 year of observation as compared to current study where we followed data at 3–4-month intervals over 5 years period. Therefore, in prior study fully reconstituted group could have included intermittently reconstituted patients. Furthermore, patients on fSCIG in both studies were few to draw a definitive conclusion. In current study, none of the patients from previous study on primary hypogammaglob-

ulinemia (17) were included. Salehzadeh et al. [18] reported that some patients with CVID following IVIG therapy did not reconstitute their IgG subclasses; however, authors did not give any details about number of patients or IgG subclasses that were not reconstituted. Furthermore, they did not report their data in relation to infections. Finally, 50% of their patients had normal total IgG levels, therefore, would not qualify for a diagnosis of CVID.

In different studies, different dosing and different trough levels have been reported. In the majority of studies, IgG trough levels have been reported in relation to severe bacterial infections (SBIs). Quinti et al. [21] compared SBIs between IgG trough levels less than 400 mg/dL and greater than 400 mg/dL and reported less frequency of pneumonia in patients with trough levels of <400 mg/dL. Orange et al. [19] reported, in a meta-analysis, a decreased frequency of pneumonia with higher trough levels in patients with primary immunodeficiencies. Gathmann et al. [1] also reported association of higher trough level with decreased frequency of SBIs in primary hypogammaglobulinemia; however, this association was restricted to SBIs and not to moderate infections. In contrast, Eijkhout et al. [22] observed no difference in SBIs

between low- and high-dose regimens; however, they observed a significant reduction in overall infection. In our study, we observe no significant difference in reduction in upper and lower respiratory tract infections, including pneumonia and urinary tract infections among various routes of Ig administration in both fully reconstituted and incomplete reconstituted group. We had only 9 patients with history of pneumonia and were equally distributed among IgG subclass fully reconstituted and incompletely reconstituted groups.

Bonagura et al. [14] proposed the concept of biological IgG trough levels, by demonstrating different IgG trough levels required in 2 patients with CVID to keep those patients free from getting recurrent sinopulmonary infections. Suri et al. [16] from India reported much lower “biological trough levels” in patients with x-linked agammaglobulinemia treated with IVIG as compared to those reported by Bonagura et al. [14]. The reason for such discrepancy in biological trough levels is unclear.

Dosing of immunoglobulin varies from center to center and country to country. Gathmann et al. [1] compiled data from 28 medical centers in Europe, noting that initial dosing ranged from 129–750 mg/kg/month. In general, the start dose is 400 mg/kg; however, some centers started with 1,000 mg/kg and reduce to 400 mg/kg/month. Some of the centers in their study determine Ig dosing based upon target trough levels ranging from 500–800 mg/dL. Orange et al. [19] reported a correlation between dosing and trough levels in that each 100 mg/kg increase in SCIG dosing resulted in 120 mg/dL increases in trough levels. Lee et al. [24] observed that each 100 mg/kg increase in IVIG resulted in 73 mg/dL increases in trough levels. However, we did not observe significant correlation between Ig dosing and IgG trough levels. All 3 groups of patients had similar mean dose, yet trough levels in IVIG group were significantly higher. These observations suggest that there may be factors other than weight-based Ig dosing that contribute to changes in IgG trough levels, including age, tissue distribution, and/or Ig catabolism.

None of these studies has compared IgG trough levels in relation to IgG subclass reconstitution. We observed significantly higher IgG trough levels among patients in whom IgG subclasses were fully reconstituted ($p < 0.0001$) as compared to those in whom IgG subclasses were either intermittently reconstituted or did not reconstitute.

The criteria for interpretation of *S. pneumoniae*-specific antibody response continued to change based upon “expert opinion” [15, 25, 26] rather than on solid scientific data. Interpretation is based upon (A) concentration of antibodies after immunization, (B) fold increase in an-

tibody levels over pre-immunization levels, and (C) percent of serotype to which the patient has responded. The latter has changed from 50 to 70%. The concentration of antibodies has varied from 1:00 to 0.3 µg/mL; some laboratories have used even 0.5 and 2.0 µg/mL. Furthermore, fold increase in antibody levels has also varied from 2- to 4-fold increase. Hoffman et al. [27] demonstrated significant variation in the response to various serotypes. They observed antibody titers of >1.3 mg/dL against serotype 19A in 84% of adults with recurrent respiratory tract infections, whereas only 43% of patients had antibody levels of >1.3 against serotype 12F. Borgers et al. [28] examined pneumococcal polysaccharide immune responses against 14 serotypes in healthy subjects ranging from children to aged subjects. They demonstrated that percent of subjects responded to vaccination varied with age of subjects, and criteria for response used, that is, 2- versus 4-fold increase and type of serotypes. There are no data available for all 23 serotypes in healthy subjects and in primary immunodeficiency regarding response to different serotypes. In our study, we analyzed our data based upon antibody levels, fold increase, and serotypes. Our data show that 7 *S. pneumoniae* serotypes including 3, 4, 9n, 10a, 11a, 12f, and 15b were impaired in majority of CVID patients. We have previously reported an impairment of serotypes 3, 4, 9n, 9f, 12f, and 23f in patients with selective IgM deficiency [29], and impaired serotypes 3, 4, 6b, and 12f in majority of patients with primary hypogammaglobulinemia [17].

We analyzed distribution of IgG subclasses that contributed to low total IgG in patients with CVID. Our findings revealed low IgG1 in 78.1%, IgG2 deficiency in 73.2%, IgG3 in 43.9%, and IgG4 in 51.2% patients with CVID. Previously, we reported hypogammaglobulinemia low IgG1 in 60.6%, IgG2 in 49.3%, subnormal IgG3 in 28.2%, and subnormal IgG4 in 22.5% [17]. Although IgG4 accounts for >10% of total IgG, isolated IgG4 deficiency is associated with respiratory tract infections and asthma [30–32]. Barton et al. [33] found that low IgG1 levels in 97%, low IgG3 in 58.8%, and low IgG2 in 35.3% of CVID patients with CVID. Salehzadeh et al. [18] reported isolated or combined low IgG1 levels in 75%, low IgG2 in 25%, and IgG3 in 50% of CVID patients; however, almost 25% of these patients had normal total IgG, therefore, would not qualify for a diagnosis of CVID.

In summary, lack of IgG subclass (es) reconstitution is associated with increased infections, and lack of IgG subclass reconstitution correlate with total IgG trough levels but not with the dosing of Ig. Therefore, there may be a value of determining IgG subclasses for subclass deficien-

cy, especially in patients with breakthrough infections. Because of possible differences in IgG subclass concentrations among various Ig products, a change in IgG product might be appropriate. A limited number of pneumococcal serotype antibodies are impaired in majority of CVID that might be considered in defining impaired specific antibody response to pneumococcal vaccine. Finally, as expected, low IgG1 levels contribute to low total IgG in CVID; however, in 15% of cases other IgG subclasses deficiency contributes to low IgG. Further studies in a large cohort of patients are required to determine the effect of route of administration, preferably with the same Ig product, in the reconstitution of IgG subclasses and relation with infections.

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Statement of Ethics

Human Subject Review Committee of Institutional Review Board, University of California at Irvine approved the protocol. Since it is a retrospective chart review study, no consent was obtained from subjects.

Conflict of Interest Statement

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Author Contributions

David Reeder retrieved all data information from EMR and patient's chart, compiled, and wrote a draft of the paper. S.G. developed the concept, supervised David Reeder, and guided him throughout the projects, performed statistical analysis, and wrote final version of the manuscript.

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