Clinical Immunology 215 (2020) 108416

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

Measurement of Typhim Vi® IgG antibodies in healthy donors as a tool for the diagnostic of patients with antibody deficiencies

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1. Introduction

Primary Immunodeficiencies (PIDs) are a group of disorders involving defects in one or more components of the immune system, and are characterized by an increased incidence of infections, autoimmunity, malignancies and diverse immune dysregulation-associated diseases. Although PIDs were previously considered rare conditions, the number of diagnosed cases is growing rapidly, and about 419 different forms of PIDs have already been recognized [1]. Humoral PIDs are the most frequent, accounting approximately for more than half of these diseases (ESID Online Database). Clinical manifestations of those entities are broad, ranging from patients with severe reduction of all immunoglobulins (Igs) and totally absent B cells to patients who have selective antibody deficiency with normal Igs in their serum. Early diagnosis and adequate therapy are the keys to survival and a better quality of life. Delays in diagnosis and/or inadequate management may lead to permanent organ damage (bronchiectasis), poor quality of life and/or death from overwhelming infections. Most patients with antibody deficiencies can live normal lives if an early diagnosis and dedicated therapies are implemented.

The main clinical use of vaccines is the prevention of infectious diseases. As knowledge of the immune system's role in host defense has evolved, it was observed that immune defects do not only affect the response to natural infection but also to immunization. Therefore, the clinical use of vaccines has also expanded to include a role in the diagnosis of immunodeficiencies [2]. There are several vaccines commonly recommended as a first-line diagnostic tool. Tetanus and diphtheria toxoids are used to determine the immune ability of patients with respect to protein antigens (Ags). Responses to protein Ags require the intact function of B and T cells and an altered response indicates that the functionality of B and T cells is abnormal, which is characteristic of the most severe forms of immunodeficiency. The pure polysaccharide pneumococcal vaccines (ie, the 23-valent pneumococcal polysaccharide vaccine [PPV-23] Pneumovax 23) are usually used to assess the response to polysaccharide Ags, which requires functional B cells only. The analysis of the immune response against the Pneumococca polysaccharide vaccine (PPSV) has been the standard method to assess the immune status in patients with a suspected PID. However, exposure to pneumococcal Ags may occur also from natural infection that may result in increased basal antibody titers and, therefore, possibly impair the diagnostic capacity of the immunization test. Moreover, the widespread dissemination of the conjugated vaccine Prevnar among pediatric population has complicated the immunological analysis, since the type of response obtained is T-dependent and it should be necessary to use individualized serotype analysis of the immune response to distinguish those patients that have the ability to generate a response to a polysaccharide Ag. These limitations led to exploring new possibilities in the evaluation of polysaccharide immune responses using an inactivated TyphimVi vaccine.

Salmonella enterica subspecies enterica serovar Typhi is a gram-negative bacterium that causes febrile typhoid fever. It is restricted to humans, causing 12 million cases of typhoid fever and approximately 129,000 deaths per year [3] in developing countries such as from Africa or South Asia. The S. typhi capsular polysaccharide, called Vi capsular polysaccharide (ViCPS) [4], plays an important role in bacterial virulence [5], and has been used in vaccine for a long time with success in controlling infections. S. typhi is not endemic in our country, so the ViCPS is considered a neoantigen in our population. The diagnostic protocol includes the analysis of the antibody production against these different Ags before and four weeks after the vaccination to measure the change produced by the immune boost with the appropriate Ag.

Regarding its diagnostic performance, in a recent study, the measurement of responses to the *Salmonella typhi* polysaccharide vaccine performed better than the aggregate (non-specific type) responses of 11

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https://doi.org/10.1016/j.clim.2020.108416
Received 22 January 2020; Received in revised form 5 March 2020; Accepted 7 April 2020
Available online 10 April 2020

pneumococcal antibodies for the discrimination of patients with common variable immunodeficiency (CVID) and hypogammaglobulinemia of healthy control subjects [6]. A 10-fold increase in IgG antibody against S typhi served to distinguish patients with CVID from control subjects with a sensitivity of 90.9% and a specificity of 62.5%. The lower performance of the pneumococcal vaccine in this study was largely due to the high level of pneumococcal IgG preimmunization in healthy subjects. Another study that used the same vaccine and the same commercial assay used previously found that a response ratio of only 2-fold increase (FI) had a sensitivity and specificity of 100% to distinguish control subjects from a group of patients previously diagnosed with antibody deficiency. In this study, the authors also showed that responses could be measured in patients receiving IgG therapy and that commercial IgG preparations did not contain measurable typhoid polysaccharide antibodies. These studies suggest that the measurement of the S typhi antibody could be useful for the diagnosis of PID [7]. However, different populations may have different immunization profiles given the differences, for example, in the vaccination schedule and infections that may explain the main changes in the basal levels of the immune status against different pathogens. To assess the immune response to this neoantigen in healthy individuals from our geographical area, we conducted the first prospective study at the "University Hospital of the Canary Islands" with the help of the "Border Health Center" of Santa Cruz de Tenerife, collecting 30 individuals who were vaccinated with the S. typhi polysaccharide vaccine and had their response measured. Twenty-seven adult patients attending the PID module of the Allergy Service of the Hospital were also included and their immune response to this neoantigen was evaluated.

2. Material and methods

2.1. Individuals

Healthy adults who were going to travel to countries where there is a potential risk of contracting typhoid fever were included prospectively in this study. They attended the Public Service of the Border Health Center in Santa Cruz de Tenerife between April 15 and 30, 2019, and were recommended, among others, to be vaccinated with the TyphimVi vaccine. All included subjects received full written and informed consent. The study was approved by the Ethical Committee of the University Hospital of the Canary Islands with the code 2017_82.

As part of the standard healthcare practice at our center [8], 27 adult patients attending the PID module and suspected to have an immunodeficiency were evaluated for vaccine response before any intravenous Ig (IVIG) treatment was prescribed. Data was collected retrospectively and anonymized.

2.2. Vaccination and sample collection

Vaccination consisted on intramuscular administration by deltoid puncture of a dose of 0.5 ml of sterile solution containing the cell surface Vi polysaccharide extracted from *S. enterica* serovar S. typhi (Sanofi Pasteur SA, Lyon, France). Five milliliter of blood samples were taken prior and 4 weeks after vaccination. After centrifugation at 3600 rpm for 10 min, all serum samples were identified with barcode and stored at −20 °C until analysis.

2.3. Immunoglobulin measurements

Total serum IgG, IgA and IgM were determined by immunoturbidimetry on the Roche/Hitachi cobas c system.

2.4. Specific anti-PCP and anti-S. typhi Vi IgG measurements

Specific antibodies for the TyphimVi vaccine were measured by the "VaccZyme™ Human Anti-Salmonella typhi Vi IgG Enzyme Immunoassay Kit" (Binding Site Group Ltd., Birmingham, United Kingdom) on an AP22 IF, a fully automated system for the ELISA and IFA methods. The VaccZyme assay measures the specific IgG against the virulence factor Vi of S. typhi, with a measurement range of 7.4–600 U/mL. For values lower than 7,4 U/mL, an empirical 7,4 U/mL value was attributed for fold increase calculations. Similarly, response to the vaccination against the pneumococcal conjugated vaccine, Prevnar13, was measured by the VaccZyme™ Anti-PCP IgG Kit EIA (Binding Site Group Ltd., Birmingham, United Kingdom), measuring range from 3,3–270 mg/L.

The statistical analysis was done on Microsoft Excel and GraphPad Prism8 for Windows.

2.5. Specific anti-S. typhi IgG in commercial IVIG preparations

Two different preparations of 50 mg/mL Plangamma™ Griffols were used to quantify the specific IgG against polysaccharide S. Typhi. Adequate dilution of those preparations to adjust the total IgG concentration to 900 mg/mL were done. Specific IgG was detected using the same procedure as conducted with serum samples and described before. Theoretical calculation was done to achieve the amount of specific IgG present in these commercial preparations used in the hospital.

3. Results

3.1. Basal levels of specific anti-S. typhi IgG

Anti-Typhim Vi IgG levels before vaccination were quantified in 30 healthy individuals. The specific anti-capsular polysaccharide S. Typhi Vi IgG was below the detection level of the assay in 25 of 30 individuals (83.3%), confirming its low prevalence in our population. The average of all values was 13.65 U/ml and the median < 7.40 U/mL. Twenty-seven individuals (90%) had specific anti-S. typhi Vi concentrations ≤32 U/mL, which corresponds to the lowest concentration post-vaccination found in a previous multicentric study with Spanish control population [6]. Two women, who had been previously vaccinated more than 10 years ago with the Typhim Vi vaccine, had 94,7 U/mL and 65.1 U/mL respectively Fig. 1.

3.2. Quantification of specific anti-S. typhi IgG levels after vaccination

Twenty-eight healthy individuals attended the second visit and had the specific anti-capsular polysaccharide S. typhi IgG levels measured in a post-vaccination blood sample collected 4 weeks after vaccination. 27 out of 28 (96%) showed increased values against S. typhi (Fig. 1,A) with respect to the basal levels. The mean of all values was 204.20 U/mL and the median was 161.10 U/mL Table 2: Interestingly, one individual did not show an increase in specific Typhi Vi IgG levels after vaccination (value < 7.4 U/mL pre- and post- ST vaccination). This individual presented also a slightly decreased total IgG (626 mg/L) and decreased total IgM levels (79 mg/L).

3.3. Fold increase between specific anti-S. typhi IgG levels pre- and post-vaccination in serum samples

A good immune response against polysaccharide vaccines has been defined as achieving a 2 fold increase (FI) of the specific IgG between before and after vaccination [9]. Other studies defined a 3× fold increase as a normal antibody response for Typhim Vi [6]. The median fold increase in our healthy individuals was 20,55 (mean FI 23,58) comparing pre- versus post- vaccine levels, and twenty-five out of 28 (89,2%) showed a FI above the three cut-off. One out of the 28 not achieving > 3 FI was the individual with a post-vaccination titer of anti-Typhim Vi IgG < 7,4 U/mL, and the other 2 individuals not able to generate a fold increase higher than 3 were the previously vaccinated

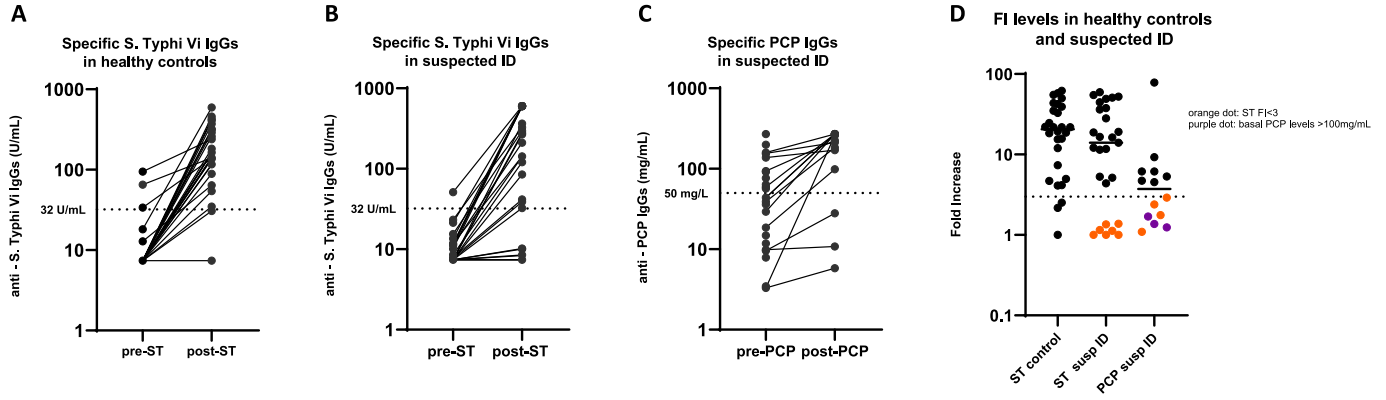

Fig. 1. Levels of specific immunoglobulins anti-S. Typhi Vi or anti-PCP in a control group of healthy individuals and in a cohort of patients with suspected ID. Levels were measured pre- and 4 weeks post-vaccination with Typhim Vi (ST, Sanofi) or Prevnar (PCP, Pfizer).

individuals presenting with higher basal levels. These two participants achieved fold increases of 2.5 and 2.1 times, respectively. Excluding these three individuals with poor response, the rest of the population that responded showed an increase of at least 4 times in specific levels of IgG against S. typhi (Fig. 1: D). No correlation was found between basal total IgG, IgA or IgM and specific anti-S. typhi IgG levels obtained post-vaccination.

3.4. Specific anti-S. typhi and anti-PCP IgG levels in patients with suspected PID

S. Typhim Vi immunization test has been included on our center for the evaluation of impaired polysaccharide antibody production. 27 patients were screened for suspected immunodeficiencies, undergoing S. Typhim Vi and PCP vaccine response evaluations. Only 1 out of 27 patients had anti-S. typhi IgG titers higher than 32 U/mL before vaccination, and 12 had levels below the limit of detection 7,4 U/mL. Post-vaccination, 3 patients had anti-S. typhi IgG < 7,4 U/mL and a total of 7 had post-vaccination levels < 32 U/mL Fig. 1:B. Moreover, those 7 individuals did not achieve a fold increase higher than > 3 in respect to their basal level, and therefore are considered to have a poor vaccine response. The diagnosis of these patients includes 1 Good Syndrome, 1 Bruton disease, 4 CVID (1 with granuloma and lymphoma), 1 lymphoma Table 1:.

The anti-PCP titers were also evaluated in this cohort of patients with suspected immunodeficiencies. Before vaccination, 12 patients had anti-PCP IgG levels higher than 50 mg/mL, of which 6 were above 100 mg/mL Fig. 1:C. Only 1 out of the 5 with post-immunization results had a fold increase > 3, while all but 1 had a good response by S. typhi, achieving a fold increase of at least 3. Post-vaccination with Prevnar13, the anti-PCP levels were measured in 14 patients, with 7 (50%) obtaining fold increase levels < 3, with 4 patients in agreement with a bad response to S. Typhim Vi and 3 patients with a particularly good response to S.Typhim Vi but high basal anti-PCP levels.

Table 1
Adult patients attending the PID module.

PID group	Age	Gender	S. Typhi response	Presenting complaint	Clinical diagnosis	IVIG/Atb
1	35	M	R	Recurrent pneumonia	A.H.	NO
2	72	F	R	Non-CF bronchiectasias	A.H:	NO
3	68	F	R	Chronic rhinosinusitis	hypogammaglobulinemia	**atb**
4	60	M	R	Hypogammaglobulinemia	A.H:	NO
5	49	F	R	Chronic rhinosinusitis	hypogammaglobulinemia	**atb**
6	56	M	R	Pneumonia	A.H:	NO
7	60	F	**NR**	Non-CF bronchiectasias	CVID + GRANULOMAS + LYMPHOMA	**IVIG**
8	57	F	R	Recurrent bronchitis	hypogammaglobulinemia	atb
9	65	F	**NR**	Recurrent bronchitis	SAD + LYMPHOMA	**IVIG**
10	28	M	R	Hypogammaglobulinemia	A.H:	NO
11	53	F	R	Hypogammaglobulinemia	A.H.	NO
12	48	M	R	Severe pneumonia	A.H:	NO
13	26	M	R	Hypogammaglobulinemia	Asymptomatic SAD	NO
14	24	M	R	Hypogammaglobulinemia	A.H:	NO
15	21	M	R	Hypogammaglobulinemia	A.H:	NO
16	59	F	**NR**	Chronic rhinosinusitis	CVID	**IVIG**
17	64	M	R	Chronic rhinosinusitis	SAD	**atb**
18	58	F	R	Hypogammaglobulinemia	A.H:	NO
19	70	F	**NR**	Recurrent bronchitis	Sd. GOOD	**IVIG**
20	38	F	**NR**	Acute otitis media	CVID	**IVIG**
21	39	F	**NR**	Chronic rhinosinusitis	CVID	**IVIG**
22	70	M	R	Hypogammaglobulinemia	A.H:	NO
23	49	M	R	Severe pneumonia	A.H:	NO
24	80	F	R	Non-CF bronchiectasias	MYELOMA	NO
25	53	F	R	Recurrent pneumonia	A.H:	NO
26	40	M	R	Non-CF bronchiectasias	hypogammaglobulinemia	**atb**
27	33	M	**NR**	Hypogammaglobulinemia	BRUTON	NO

M: Male**; F**: Female; **R**: Responder; **NR**: non responder. **AH**: asymptomatic hypogammaglobulinemia; **CVID**: Common Variable Immunodeficiency **IVIG**: intravenous immunoglobulin. **SAD**: Specific Antibody deficiency Atb: profilaxis with antibiotics

Table 2
Descriptive statistics of healthy individuals and patients with suspected immunodeficiency.

Descriptive stats	CONTROL							PID suspected					
	AGE	IgG (mg/dL)	IgA (mg/dL)	IgM (mg/dL)	pre-ST	post-ST	FI ST	pre-ST	post-ST	FI ST	pre-PCP	post-PCP	FI PCP
Number of values	29	30	30	30	30	28	28	27	27	27	27	14	14
Minimum	20	626	140	49	7,4	7,4	1	7,4	7,4	1	3,3	5,81	1095
Median	37	1009	249,5	104	7,4	161,1	20,55	7,54	142,5	14,03	43,8	218,3	3727
Maximum	58	1506	394	306	94,73	595,6	61,95	51,18	600	59,35	270	270	78,39
95% CI of median													
Lower confidence limit	25	983	207	90	7,4	135,9	12	7,4	32,38	4376	14,8	28	1,37
Upper confidence limit	40	1170	289	143	7,4	259,1	32,91	11,49	363,9	36,11	91,6	270	6164
Mean	35,79	1053	254,3	124,3	13,65	204,2	23,58	11,56	244,5	20,59	67	181,6	9072
Std. Deviation	12,05	209,6	75,86	57,12	19,14	145,4	18,27	8962	239,4	19,88	69,57	103,2	20,1
Lower 95% CI of mean	31,21	975	225,9	103	6506	147,8	16,49	8013	149,8	12,72	39,48	122	-2532
Upper 95% CI of mean	40,38	1132	282,6	145,7	20,8	260,6	30,66	15,1	339,2	28,45	94,52	241,2	20,68

CI- confidence interval; ST – S. Typhim Vi IgG levels (in U/mL); FI – fold increase; PCP – pneumococcus capsular polysaccharide.

3.5. Specific anti-S. typhi IgG in commercial Ivig preparations

Two different preparations of 50 mg/mL PlangammaTM Griffols were used to quantify the specific IgG against polysaccharide S. Typhi. After dilution of these two preparations to theoretical 900 mg/dL of total IgG present in healthy individuals, calculated results were below detection limit of 7 U/mL of specific S. typhi IgG in both samples.

4. Discussion

The evaluation of the immune response to vaccination with polysaccharides is a first-step diagnostic tool to assess the functionality of the adaptive immune system [2]. The measurement of antibodies in response to PCP is the gold standard, but its interpretation presents many challenges due to the high basal levels that are frequently found in healthy populations and with PID [10]. The use of the Typhim Vi pure polysaccharide vaccine has appeared as a suitable alternative candidate for the PCP vaccine to assess the immune response of patients with suspected PIDs. The advantages of the Typhim Vi vaccine are several: simplified interpretation due to the lack of multiple serotypic variants, there is no conjugated polysaccharide vaccine currently in routine use and, most importantly, the low anti IgG-S. typhi levels before vaccination in most populations, since the vaccine recommendation in our media is only for travelers who go to countries with high risk of infection [11]. In this study, we have addressed the question of whether this vaccine would be suitable for use in our geographic area with a diagnostic purpose to assess the immune status of people receiving this immunization. For this reason, we contacted the Border Health Service and designed the study that consisted of recruiting people who were going to travel to high-risk countries, who had an indication for the vaccine and who also agreed to participate in the study of serological analysis. The individuals within the study were a young population, with 14 individuals (46.7%) in a range of 20 to 30 years. As described elsewhere [12], the basal results obtained before vaccination were below the detection level using the Anti- S. typhi immunoassay in most of the samples (83,3%) obtained from non-immunized individuals. These data will allow us to establish a reference diagnostic cut-off for our population. Two individuals had been previously immunized in 2001 and 2007 and, as described by others, showed higher basal level of specific anti-Typhim Vi CPS IgG (65.1 and 94.7 U/mL), highlighting the importance of knowing the vaccine status of the individuals at the time of the immune evaluation.

Twenty-eight out of the 30 included individuals attended the second visit to preform blood analysis for their specific immune response to the S. typhim Vi vaccine. Unfortunately, one individual who presented with a low level of total IgM was one of the non-attendees, which prevented speculation of a consequence of this. The other individual with a low level of total IgG, ST16, was the only one who did not respond to the S. typhim Vi vaccine. This result is very consistent with the possibility that this individual has a defect in some of the humoral immune response pathways that may be responsible for this quantitative and qualitative defect. We do not have clinical data to corroborate this hypothesis.

The immune response FI obtained in this study with healthy individuals was considered ideal for polysaccharide immune vaccines [6] and verified in 89.3% of the total population. These data are in line with those obtained in other studies (92% in the Japanese population [12] and 93% in the US population [13].

In suspected immunodeficient patients, the assay performed good in identifying patients with impaired antibody production in response to polysaccharides. This is fundamental in the evaluation of humoral immunodeficiencies, and the S. typhim Vi is particularly relevant in our center because it is the only immunization test that can actually measure polysaccharide vaccine responses since the introduction of the conjugated vaccine Prevnar in the vaccine plan for children and for adults at risk.

Finally, the specific IgG anti-S. typhi was also measured in two different Ivig preparations available at the University Hospital of the Canary Islands. In both preparations, the calculated anti-S. typhi specific IgG was below the detection limit of the ELISA method (< 7 U/mL), which shows that the plasma donors that are present in our geographic area are representative of the results obtained with the healthy individuals evaluated in this study. This undetectable level of specific anti-S. typhi IgG opens the possibility of using this vaccine as an appropriate method to investigate immune responses also in patients with PID and/or secondary immunodeficiencies taking these Ig preparations. Possible discontinuation on Ivig courses could be addressed using the immune response to this neoantigen.

To our knowledge, this is the first report that shows the basal levels and the immune response to the Typhim Vi CPS vaccine performed in healthy individuals in the population of the Canary Islands. This study showed that the Typhim Vi polysaccharide vaccine has a good immunogenicity in our population. The administration of the vaccine as a single dose was highly immunogenic with a high proportion (89.3%) of individuals who achieved a 4-fold increase in antibody titers against S. typhi. Although it has the usual limitations associated with any prospective study design (limited number of people participating during the time of recruitment, some absences on the second visit), the results obtained are very valuable to establish the use of this vaccine in patients with PID in our geographical area. In addition, given the absence of specific anti-*S.typhi* IgG antibodies in IVIG preparations used in our clinical setting, the use of this vaccine can be spread to assess these deficits in the population treated with Ig.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgements

The authors want to thank the laboratory technicians from the Immunology Section Hospital Universitario de Canarias (MJ Placer, G Camacho, M Romera, M Padilla); registered nurses (S García-Rodríguez, A Pérez-Granados, L Pérez) for their help with the procedures and dedication.

GEDIP group: Pneumology (I. Suarez-Toste); Microbiology (T. Delgado-Melián), Preventive Medicine (J. Duque-Arimany), Pediatrics (C. Martínez-Faci, V. Velasco); Internal Medicine (M.C. Durán, A. Martínez-Riera), Ear-Nose-Throat (G. de Lucas, A. Perez-Orribo); Haematology (P. Machado, T. Martín-Santos), Immunology (Y Barrios; A Franco); Allergy (V Matheu, I Sanchez-Machin, P Poza-Guedes, R Gonzalez, C Alava, E Mederos).

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