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Multicenter study for the evaluation of the antibody response against salmonella typhi Vi vaccination (EMPATHY) for the diagnosis of *Anti*-polysaccharide antibody production deficiency in patients with primary immunodeficiency

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ABSTRACT

Evaluation of specific antibody (Ab) response to polysaccharide antigens is essential for diagnosis of primary immunodeficiencies. We assessed the specific Ab responses to the pneumococcal-polysaccharide (PPV) and to Salmonella typhi-polysaccharide (TyphimVi) vaccines in a prospective study (EMPATHY) in patients with common variable immunodeficiency (CVID-Group, $n = 22$), hypogammaglobulinemia (HYPOG-Group; $n = 27$) and healthy controls (HC-Group; $n = 16$). Specific Ab concentrations in response to PPV and to TyphimVi vaccines were measured by ELISA (The Binding Site, UK), defining 3-fold increase as normal response (Ratio:3×). The RatioTyphimVi:3× was significantly greater in HC than in CVID-Group ($p < 0.0001$), but not than HYPOG-Group ($p = 0.138$). However, the RatioPPV:3× showed no significant differences among the three groups. By ROC analysis, TyphimVi better differentiated HC from CVID (AUC:0.893, IC95%: 0.791–0.996, $p < 0.0001$) than PPV. Our results suggest that the use of specific Ab response to TyphimVi could represent a complementary assay for the diagnosis of *anti*-polysaccharide Ab production deficiency in patients with CVID.

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1. Introduction

Evaluation of specific antibody response to polysaccharide antigens is essential for diagnosis and management decisions of primary immunodeficiency diseases (PIDD) [1,2,3]. Indeed, deficiency of specific polysaccharide antibody responses renders individuals susceptible to

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recurrent and severe sinopulmonary infections with encapsulated bacteria, and an adequate diagnosis helps to define those who may benefit from immunoglobulin replacement therapy [4]. Measurement of the antibody response to pure nonconjugated Pneumovax 23® (PPV), a 23-valent pneumococcal polysaccharide vaccine, is the gold standard test to identify deficiencies in *anti*-polysaccharide antibody production.

A vaccine response is considered adequate when post-vaccination specific antibody concentration is >1.3 mg/L per serotype for serotype analysis, >50 mg/L if using a pneumococcal IgG ELISA [2,4–7] and more than a three to four-fold increase in *anti*-PPV antibody titers above the pre-vaccination concentration [8,9]. Measurement of fold-increase should always be interpreted in combination with the post-vaccination antibody concentration. However, the interpretation of pneumococcal antibody production using PPV can be challenging for the following reasons: the recent introduction of polysaccharide-protein conjugated vaccine in the vaccination schedule which could mask a pure PPV driven IgG2 polysaccharide response; cross-reacting and different immunogenicities among antibodies towards different serotypes, high PPV pre-immunization levels in general population, high prevalent natural infection by *S. Pneumoniae* [10,11]; and other issues related to inter-individual variability. It would therefore be of interest if an additional assay were available to assess the response to another polysaccharide antigen/vaccine in individuals with heterogeneous genetic disorders such as common variable immunodeficiency (CVID).

Recently, a novel vaccine response assay has become commercially available. The *Salmonella typhi Vi* IgG ELISA measures the response to the Typhim Vi polysaccharide vaccine which was licensed in 1988 for use in adults and children >18 months of age [12,13]. The main purpose of the current study was to analyse the specific production of antibodies raised in response to Typhim Vi using the *Salmonella typhi* Vi IgG ELISA Assay and compare to the production of antibodies raised to PPV and to evaluate its use as a complementary diagnostic tool for interpreting quantification of *anti*-polysaccharide responses in PIDD.

2. Patients and methods

2.1. Subjects

A prospective multicenter study (EMPATHY; including 18 Spanish centres) was conducted in 49 adult patients (aged 20 to 65 yrs) that presented with hypogammaglobulinemia, defined as serum IgG plus IgA ≤600 mg/dL, and were subsequently referred to the participating centers for evaluation of suspected immunodeficiency. All patients underwent clinical and immunological examination and were classified as either: Common Variable Immunodeficiency (CVID-Group; $n = 22$), according to the European Society of Immunodeficiencies (ESID) and the Pan American Group for Immune deficiency (PAGID) criteria [14], or Hypogammaglobulinemia (HYPOG-Group; $n = 27$). None of the patients had previously received intravenous gammaglobulin therapy. We sought to analyse the specific antibody response to purified Typhim Vi polysaccharide antigen from *S. typhi* in comparison to pneumococcal antigens in PPV for PIDD diagnosis. Sixteen asymptomatic volunteers of similar age and gender to the patient group that underwent Typhim Vi and PPV vaccination were selected to act as the healthy control group (HC-Group). The study was approved by the Ethical Committees of the participating centers and by the Agencia Española de Medicamentos y Productos Sanitarios (Spanish Agency of Medicines and Medical Devices).

After obtaining full informed and written consent, in all study subjects a morning blood sample was drawn for baseline pre-vaccination specific Ab levels, full blood count, total immunoglobulins (IgG, IgA and IgM) and IgG subclasses levels, baseline pneumococcal and *S. typhi* specific IgG antibodies. At the time of this study, vaccination with PPV (Pneumo23™, Sanofi Pasteur MSD Limited, Maidenhead, Berks, UK) and *Salmonella Typhi* vaccines (available in Spain as Typhim Vi™, Sanofi Pasteur MSD), were administered. After an interval of 28 days (±3 days) post PPV and Typhim Vi vaccination, blood was drawn from all subjects. Pre- and post-immunization serum samples were separated by centrifugation and then stored in aliquots at −40 °C until simultaneous performance of specific antibody tests.

2.2. Antibody testing

Total immunoglobulins were measured using commercial kits on an Immage (Beckman Coulter) nephelometer. IgG subclasses were measured using a Turbidimetry method (The Binding Site Group Ltd., Birmingham, UK) as previously described [15].

Specific antibodies to PPV and to Typhim Vi vaccines were measured using commercially available ELISA kits (VaccZyme™ *Anti*-PCP IgG EIA and VaccZyme™ Anti-*S. typhi* Vi human IgG EIA from The Binding Site Group Ltd., Birmingham, UK). Serum samples pre and post vaccinations for all patients and controls were tested in the same centralized laboratory at Hospital General Universitario Gregorio Marañón, which has accredited PVP ELISA test by the UNE-EN ISO 15189 quality standards in Spain. Samples were run in duplicate following manufacturer instructions. The results of specific antibody levels to Typhim Vi are expressed as U/mL (range, 7.4–600 U/mL). PPV antibody levels are given as mg/dL (range, 0.33–27 mg/L). The values of these responses are given as the ratio between pre- and post- immunization antibody levels. We used a three-fold increase between titres pre and post vaccination to define a normal antibody response according to prior studies [8,9].

2.3. Statistical evaluation

Descriptive data are presented as mean ± standard deviation (SD) or median values. Differences in antibody responses between groups were evaluated by Mann-Whitney *U* test. The Wilcoxon matched-pairs signed rank-sum test was used to assess the statistical significance between pre- and post-immunization antibody levels. Receiver operating characteristic (ROC) curves were used to select the optimal cut-off values of significant variables for diagnosis of CVID based on the optimum sensitivity and specificity. Data were analyzed with SPSS software (Chicago, Illinois). The criterion for significance was set at $P < 0.05$.

3. Results

3.1. Epidemiological characteristics of the study subjects

A total of 65 subjects were studied (Table 1): All three groups were aged matched and were not significantly different. Serum IgG, IgM and IgA were significantly lower for both the hypogammaglobulinemia and CVID groups compared to the HC group. In addition, IgG was also significantly lower between both hypogammaglobulinemia and CVID groups.

Serum IgG subclasses were significantly different between the groups (CVID vs HYPO vs HC) (Table 1). Pneumococcal and Typhim Vi vaccines were well tolerated and there was no moderate or severe adverse event in healthy control or patients groups. Pain at the injection site was the only adverse effect reported ($n = 7$) for both vaccines.

3.2. Polysaccharide antibody responses to PPV and Typhim Vi

The median pre-vaccination concentrations, post vaccination concentrations and fold increases in concentrations for both Typhi Vi and PPV antibodies are shown in Table 2 and Fig. 1A. Shapiro–Wilk test for Gaussian distribution demonstrated that Typhi Vi IgG and PCP IgG concentrations were not normally distributed ($p \leq 0.004$). The pre vaccination concentrations were not significantly different for Typhi Vi antibodies but were significantly different for the PCP IgG antibodies between the three groups. Post vaccination, significance was achieved between the PCP IgG concentrations from the three groups. The same was observed for Typhi Vi concentrations with the exception that the

Table 1
Epidemiological and clinical characteristics of patients included in the study.

	CVID	HYPOG	p^a	HC	p^b	p^b
Number of patients[a]	22	27	–	16	–	–
Gender F/M[a]	15/7	16/11	NS	9/6	NS	NS
Age, years[b]	45.8 ± 15.5	41.2 ± 18.9	NS	39.8 ± 12.8	NS	NS
Serum IgG	360.8 ± 161.5	524.1 ± 180.5	0.01	1055.8 ± 242.5	<0.0001	<0.0001
Serum IgA	50.7 ± 39.5	90.7 ± 72.3	0.07	161.4 ± 34.6	<0.0001	0.01
Serum IgM	55.1 ± 31.2	47.3 ± 25.4	NS	164.9 ± 56.7	<0.0001	<0.0001
Serum IgG1	279.4 ± 134.4	376.4 ± 100.3	0.02	548.4 ± 102.3	0.003	0.003
Serum IgG2	92.0 ± 61.6	142.4 ± 108.7	0.08	340.2 ± 174.7	0.005	0.006

[a] Comparison of CVID vs. HYPOG, MW test used for assessment of significance.
[b] Comparison of CVID vs. HC and HYPOG vs. HC, MW test used for assessment of significance.

median post vaccination concentration between the normal subjects and hypogammaglobulinemia group were not significantly different.

At baseline levels, 6/22 CVID patients (27%), 15/27 patients (55%) in the HYPOG-Group and 15/16 (94%) subjects in the HC-Group had *anti*-PPV titres (>50 mg/L).

Post vaccination, 11/22 CVID patients (50%), 25/27 patients (93%) in the HYPOG-Group and 16/16 (100%) individuals in the HC-Group had *anti*-PPV titres (>50 mg/L). Using the minimum post vaccination concentration for Typhi Vi in HC (32 U/mL) as a cut off, 9/22 (41%) and 17/27 (63%) of CVID and hypogammaglobulinemia patients achieved a post vaccination concentration > 32 U/mL.

Fig. 1A shows that the Typhim Vi Ratio was significantly higher in HC-Group compared to CVID group ($p < 0.0001$), but not with respect to HYPOG group ($p = 0.138$). By contrast, the Ratio PPV was not significantly different between the 3 groups (HC vs CVID ($p = 0.693$) and HC vs HYPOG ($p = 0.052$) (Fig. 1B). All subjects in the HC-Group were able to mount a ≥ 3-fold antibody response on Typhim Vi immunization versus only 40.3% of CVID-Group subjects ($p = 0.003$) and 64.4% of HYPOG-Group patients ($p = 0.006$), respectively. Using ROC analysis, the area under the curve (AUC) was higher using Typhim Vi vaccination [AUC Ratio Typhim Vi ≥3×: 0.893 (95%CI: 0.791–0.996, $p < 0.0001$)] (Fig. 2A) compared with *anti*-PPV vaccination [AUC Ratio PPV ≥3×: 0.538 (95%CI: 0.354–0.722), $p = 0.690$)] (Fig. 2A and B). The analysis revealed that Ratio Typhi was the most significant parameter that differentiated the Ab responses to PS vaccines between the CVID group and HC group. The statistically chosen cutoff value for the ratio of Typhim Vi in our HC population was 10 fold based in sensitivity for CVID diagnosis of 90.9%, and specificity of 62.5%, respectively. Using a cutoff of 10 fold increase, a greater number of subjects with <10 fold increase in Typhim Vi Ratio antibody response belonged to the CVID-Group (90.9% in the CVID-Group versus 51.9% in the HYPOG-Group versus 31.3% in the HC-Group, respectively) ($p = 0.0002$).

Discrepancies between the results of measurement of antibodies to polysaccharide antigens were observed mainly in the CVID group (59.7% no response to Vi-antigen vs. 72.2% no response to PPV-antigens). The global discrepancy between both methods was 34% (21/61), which could be influenced by baseline levels of *anti*-PPV antibodies (29.5% for global mean ≥ 0.08 g/L and 47.1% for controls mean ≥ 0.2 g/L).

3.3. Stratification of CVID patients

Using the response to Typhim Vi, two CVID patients had a ≥ 10 fold response (median value 24.8; range 12.2 to 37.5) and 20 had a ≤ 10 fold increase (median value 2.4; range 1 to 7.2,).

4. Discussion

Prior consensus on the use and interpretation of diagnostic vaccination in PIDD suggested the potential of Typhim Vi vaccine as an alternative diagnostic tool for assessing antibody production against polysaccharide antigen, although lack of available clinical data limited its routine recommendation [4,16]. Our results in this study indicate that the evaluation of the specific antibody response to Typhim Vi vaccine adds clinical value to the diagnosis of *anti*-polysaccharide antibody production deficiency in patients with CVID.

The CVID-Group elicited a similar lack of response to both Typhim Vi and PCP immunization (ratios of 1.8 and 1.3, respectively), which suggests the possibility to use Typhim Vi immunization in conjunction with classical Pneumo23 for diagnostic purposes and for clinical monitoring. Moreover, our results point to better power for discriminating between groups for *anti*-Typhim Vi antibodies production with respect to the gold-standard of PPV antibody production. Indeed, using statistical ROC analysis for our population to define the most optimal cutoff level for ratio Typhim Vi and PPV, only the response to Typhim Vi was significant. For Typhim Vi response a 10 fold increase was highly significant, with 90.9% sensitivity and 62.5% specificity, respectively, for CVID diagnosis.

In all three groups, relative to the post vaccination concentration, the pre vaccination concentration of pneumococcal antibodies was relatively high which resulted in lower median fold increases in concentration. The pre vaccination concentrations of Typhi Vi were not significantly different between the three groups and the median concentration was very low (≤9 U/mL). Thus, this could aid in the

Table 2
Responses to Typhim Vi and Pneumovax vaccination in healthy controls, hypogammaglobinemic and CVID patients.

	CVID	HYPOG	p^*	HC	p^a	p^a
Typhi Vi						
Pre-vaccination concentration (U/mL)	7.4 (7.4–69.8)	7.4 (7.4–75.2)	NS	8.6 (7.4–31.8)	NS	NS
Post-vaccination concentration (U/mL)	21 (7.4–277)	63.3 (7.4–600)	0.006	171 (32–600)	<0.0001	NS
Fold increase	1.8 (1 to 37.4)	8.6 (1 to 81.1)	0.02	12.3 (3.4–76.9)	<0.0001	NS
PPV						
Pre-vaccination concentration (mg/dL)	2.4 (0.33–27)	7.4 (0.6–27)	0.02	20.3 (2.9–27)	0.0003	0.007
Post-vaccination concentration (mg/dL)	5.8 (0.33–27)	22.4 (1.4–27)	0.006	27 (24.8–27)	<0.0001	0.002
Fold increase	1.3 (0.33–73)	2.1 (1–13.5)	NS	1.3 (1–8.6)	NS	NS

All values represent median (range min-max).
[a] Comparison of CVID vs. HC and HYPOG vs. HC, MW test used for assessment of significance.
[b] Comparison of CVID vs. HYPOG, MW test used for assessment of significance.

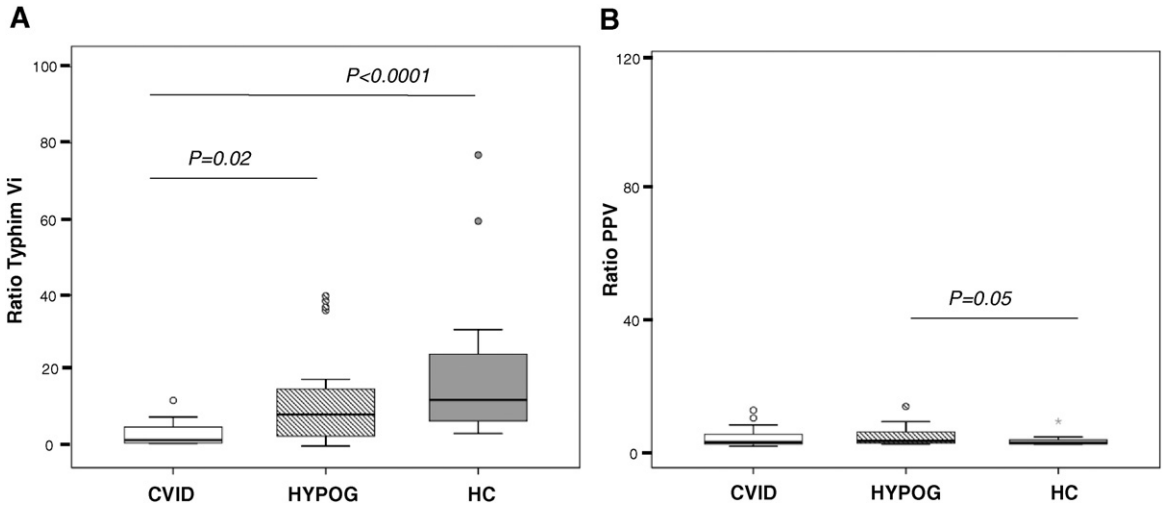
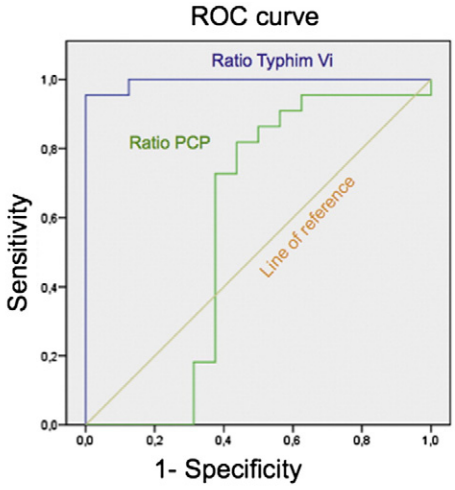


Fig. 1. Comparison between groups by increase in polysaccharide antibody responses. Fig. 1A: Box plots showing that the Ratio Typhim Vi was significantly higher in HC-group compared to CVID-group ($p < 0.0001$), but not with respect to HYPOG-group ($p = 0.138$). In contrast, the Ratio PPV did not show significant differences between the 3 groups (HC vs CVID, $p = 0.693$; and HC vs HYPOG, $p = 0.052$) (Fig. 1B). Each box plot represents the median (thick band) and the 25th and 75th centiles. The error bars represent the smallest and largest values that are not outliers.

differentiation between pre and post vaccination assessments and the diagnoses of hypogammaglobulinemia and CVID which was not possible with assessment of the antibody response to PPV.

Ferry and colleagues have proposed the use of a Typhim Vi as a suitable polysaccharide immunogen for PIDD investigation [8]. The pre- and post-vaccination fold increase was studied in a healthy population. The median value of for pre-vaccination titres was 3.9 AU/mL and for post-vaccination 39.2 AU/mL, indicating a 10 fold increase in titres. In agreement with the present study, we calculated that 10 fold increase in the HC group would give the best differentiation of a response between the HC group and CVID group [8]. Perhaps an intriguing observation from this study was the possible identification of two CVID groups of patients stratified according to their response to Typhim Vi. Unfortunately, the numbers of patients in the group that possess a ≥ 10 fold increase to vaccination were too small to examine further, but it is tempting to suggest that this group might have a more proficient immunological response and thus a lower predisposition to infection [17,18]. This is strengthened by the observation that the two patients with ≥10 fold increase to Typhim Vi had a lower number of respiratory infections and no CVID linked inflammatory conditions.



Variable	Area	P	95% CI	
			Lower limit	Upper limit
Ratio Typhim Vi	0.994	<0.0001	0.979	1.009
Ratio PCP	0.577	0.425	0.357	0.797

Fig. 2. Receiver operating characteristic (ROC) curve analyses using Typhim Vi and PPV ratio as predictor of diagnosis of CVID. The curves show the trade-off between sensitivity and specificity. An increase in sensitivity will be accompanied by a decrease in specificity. The accuracy of the prediction increases as the curve approaches the left-hand and top portions of the ROC space. The area under the curve (AUC) is the percentage of randomly drawn pairs for which the prediction is true. As observed, the ratio Typhim Vi better defined CVID patients ($p < 0.0001$).

With this in mind, Cavaliere et al. have recently demonstrated that PPV IgA and IgM vaccine response subgroups exist in CVID patients and that this stratification served as an indication to risk of pneumonia and bronchiectasis [19]. A larger study is required to understand the significance of this observation and to see whether there is any overlap with pneumococcal IgA and IgM response in the same cohort.

Variable pneumococcal polysaccharide vaccine responses have been reported in patients with CVID, with some patients showing some degree of responsiveness [20,21]. The high baseline level of pneumococcal antibodies from endemic infection ensures that interpretation of response between HC and CVID groups remains challenging. Discrepancies in antibody production between *anti*-Typhim Vi and *anti*-PPV tests were observed in this study.

Pneumovax 23 polysaccharide vaccine continues to be widely used in routine clinical immunology practice; even though systematic vaccination with the protein conjugated Prevenar vaccine may limit its use for assessing specific polysaccharide responses. Use of the *S. typhi* polysaccharide vaccine may provide further immunological insight to unravel subtle defects in the response to polysaccharide antigens.

In conclusion, this study strongly supports the diagnostic utility of *S. typhi* IgG ELISA for the diagnosis of the heterogeneous population of CVID patients. Further studies with larger number of subjects should be performed to confirm the utility of Typhim Vi to assess specific antibody responses in suspected antibody primary immunodeficiencies.

Conflicts of interest

ARP is employed by The Binding Site Group Ltd. All the other authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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