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Evaluation of Polysaccharide Typhim Vi Antibody Response as a predictor of Humoral Immunodeficiency in Haematological Malignancies

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ABSTRACT

An increasing healthcare challenge in the management of haematological malignancy (HM) is secondary immunodeficiency. From January 2019, the EMA included the evaluation of specific antibody (Ab) responses to better select patients for immunoglobulin replacement therapy (IgRT). We evaluated Ab responses to pneumococcal and *Salmonella typhi* pure polysaccharide immunization in a cohort of 42 HM patients and 24 healthy-controls. Pre-post specific Ab concentrations were measured by ELISA at 4 weeks. Globally, significantly lower Typhim Vi (TV) seroprevalence (9%) compared to 23-valent pneumococcal polysaccharide vaccine (PPV) (76%) ($p < 0.001$) was observed. TV non responders (88%) were higher than PPV non responders (62%) ($p < 0.0001$) and correlated better to infectious history. By ROC analysis, pre-post 5-fold TV increase was the best cut-off to discriminate HM with recurrent infections and controls (sensitivity 91%, specificity 100%). Despite the small sample cohort, our results suggest that specific anti-*S typhi* Ab response is a useful complementary assay in the diagnosis and management decision of SID to HM.

1. Introduction

Haematologic B-cell malignancies (HM) comprise a large and heterogeneous group of lymphoproliferative disorders that range from slow-growing, indolent lymphomas, to more aggressive forms of non-Hodgkin's lymphoma (NHL) [1,2].

Exciting novel and diverse chemoimmunotherapies used to treat HM-Group have greatly improved overall survival (OS), progression-free survival (PFS) and complete response rate (CRR), while expanding the secondary immunodeficiency (SID) spectrum to many lymphoproliferative disorders beyond chronic lymphocytic leukaemia (CLL), multiple myeloma (MM) and that associated to hematopoietic stem cell transplantation (HSCT) [3]. Hence, the SID profile has evolved to incorporate a younger age, new infectious agents, reactivation of latent viral infections and even opportunistic infections [3,4]. The impact of new molecules targeting B-cell differentiation, signalling pathways (B-cell receptor inhibitor (BCR), B cell apoptosis agonist (Bcl-2); and chimeric antigen receptor (CAR) T cells) in terms of infectious complications has not been established.

Recurrent or severe infections are the most common cause of morbidity and mortality mainly in patients with CLL (between 30 and 50% of deaths) [3,5,6] and MM (22% of deaths within the first year after

Abbreviations: SID, Secondary immunodeficiency; HM, Haematological malignancy; HC, Healthy control; IgRT, Immunoglobulin Replacement Therapy; PPV, pure pneumococcal polysaccharide vaccine; TV, *Salmonella typhi Vi vaccine*

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diagnosis) [7]. Hypogammaglobulinemia is highly prevalent in CLL (25% at diagnosis and 85% during disease course) and has been used to decide immunoglobulin replacement therapy (IgRT) when associated with recurrent infections [8]. However, the predictive value of hypogammaglobulinemia for recurrent or severe infections has been questioned [9,10]. Moreover, quantification of IgG levels in the most common IgG monoclonal gammopathy of undetermined significance (MGUS) and MM is not informative. Poor specific antibody (Ab) responses to pneumococcus was suggested as a good predictor of infections at a pivotal clinical trial of MM in 1994 [11]. Extrapolated from primary immunodeficiency (PID) experience, specific Ab response after immunization seems to be a better predictor of humoral immunodeficiency and for IgRT initiation [3,4,12].

Humoral immune responses to T-cell dependent antigens, such as tetanus-toxoid, and T-cell independent antigens such as pneumococcal, *Salmonella typhi* and meningococcal polysaccharide vaccines can be assessed in patients with HM and recurrent infections [13]. From January 2019, a new indication of the European Medicines Agency (EMA) has included the defect of specific Ab response as indication for IgRT in SID with recurrent infections [14].

The gold-standard test for assessment of T-cell independent responses is to determine IgG response to 23-valent polysaccharide vaccine (PPV). A vaccine response is considered adequate when post vaccination specific Ab concentration is 3- to 4-fold increase in anti-PPV Ab titres above the pre-vaccination concentration. Measurement of fold-increase should always be interpreted in combination with the pre-vaccination Ab concentration. Since the assessment of production of polysaccharide antibodies requires PPV, the interpretation of response is hindered by the inclusion of pneumococcal conjugate vaccine (PCV), which is recommended by the Centres for Disease Control and Prevention (CDC) in HM-Group among other risk groups since 2014 [15].

Recent studies in PID indicate that the response to Typhim Vi (TV), a pure polysaccharide *S. typhi* vaccine, might act as a neoantigen in several populations and non-endemic areas [16], which could reliably be measured at diagnosis and in patients receiving IgRT. In commercial IgRT preparations, IgG TV is undetectable [17].

In the present study, we sought to determine the specific Ab response to TV and PPV in a retrospective observational study in a cohort of HM-Group. Additionally, we have highlighted the utility of measuring IgG response to TV in patients undergoing assessment for IgRT.

2. Material and methods

2.1. Subjects

A retrospective observational study was conducted at the Hospital Clínico San Carlos of Madrid, Spain, between 2013 and 2015. Forty-two patients diagnosed with HM based on the WHO Classification of Tumours of Hematopoietic and Lymphoid Tissues [18] were subsequently referred from the Hematology Dept. to the Clinical Immunology Dept. for evaluation of suspected immunodeficiency (HM-Group). Twenty-four asymptomatic adult volunteers were studied as healthy control group (HC-Group). Baseline assessment included blood count, serum immunoglobulins (IgG, IgA, and IgM), IgG subclasses levels (IgG1, IgG2), pre-post polysaccharide and protein IgG specific Ab responses, lymphocytes subpopulations (T $CD4^+$, T $CD8^+$, CD4/CD8 ratio, $CD19^+$ B cells, NK cells), C3 and C4 concentrations, biochemistry and total proteins. Visits were documented every 6 months, as per routine and the follow-up period was at least 1.5 years per patient. All the procedures were approved by the Ethical Committee of the centre.

2.2. Serum collection and Ab testing

In order to compare different polysaccharide vaccines, all subjects received the gold-standard 23-valent pneumococcal polysaccharide vaccine (PPV) (Pneumo23®, Sanofi Pasteur MSD Limited, Maidenhead, Berks, UK), and TyphimVi polysaccharide vaccine (TV) (available in Spain as Typhim Vi™, Sanofi Pasteur MSD). Eight patients (19%) had received conjugated polysaccharide vaccines (PCV) 2 years prior study initiation. Patients were also vaccinated with tetanus-toxoid (TT) (Diftavax®, Sanofi Pasteur) vaccine, into the right or left deltoid muscle. Blood samples were obtained *via* venepuncture on day 0 prior to vaccination and on day 30 (± 3) after vaccination. Serum was collected and stored at −40 °C until simultaneous performance of specific Ab tests.

Specific Ab to PPV and TV vaccines were measured using commercially available ELISA kits (VaccZyme™ anti-pneumococcal capsular polysaccharide IgG ELISA and VaccZyme™ anti-*S. typhi* Vi human IgG ELISA, The Binding Site Group Ltd). The anti-PCP IgG assay is composed of a mixture of the following capsular polysaccharide serotypes: 1–5, 6B, 7F, 9 N, 9 V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33F. Serum samples pre- and post-vaccination were collected for all patients and controls. Samples were tested in duplicate according to the manufacturer instructions at the Hospital Clínico San Carlos. Specific Ab concentrations were reported for PPV IgG as mg/dL (range, 0.33–27 mg/dL) and TV IgG as U/mL (range, 7.4–600 U/mL). Responders were defined as individuals obtaining a fold increase (FI) > 3 for PPV IgG and TV IgG according to published data in PID [4,19]. However, no data are currently reported in patients with SID. Protective Ab concentrations were defined as ≥4.4 mg/dL or PPV IgG and ≥ 0.15 IU/mL for TT IgG [20]. No protective Ab concentrations have been defined for TV vaccination at present.

Commercial immunoglobulin preparations (Gammagard®, Hizentra® and HyQvia®) were tested for the presence of TV IgG Ab. The immunoglobulin preparations were tested at multiple dilutions (1:1; 1:10).

2.3. Statistical analysis

Descriptive data and continuous variables are presented as mean ± standard deviation (SD) or median values with interquantile range (IQR). We compared categorical variables between groups by the Pearson's chi-square or the non-parametric Mann-Whitney *U* test when appropriate. Correlations were assessed using Pearson correlation coefficients. Titer ratios using ROC curves to select optimal Ab cut-off values for both polysaccharide antigens. Statistics were analysed with SPSS (Chicago, Illinois) and GraphPad Prism software (GraphPad Software, La Jolla, CA, USA version 5), $p < 0.05$ was considered statistically significant.

3. Results

3.1. Epidemiological and immunological characteristics of the study subjects

A total of 66 subjects were studied. Their epidemiological and immunological characteristics are summarized in Table 1. Age and gender were not significantly different between HM-Group and the HC-Group. Serum immunoglobulin (Ig) levels were significantly lower for HM group compared to HC-Group. All subjects underwent clinical and immunological evaluation. Median baseline IgG level at first documentation was 3.5 g/L (excluding paraprotein). Patients were classified as: non-Hodgkin lymphoma (NHL) (n = 17, 40%), monoclonal gammopathy of undetermined significance (MGUS) (n = 13, 31%), chronic lymphocytic leukaemia (CLL) (n = 10, 24%), Hodgkin lymphoma (HL) (n = 1, 2%) and multiple myeloma (MM) (n = 1, 2%). Aside from minor injection site reactions, no moderate or severe adverse events were reported post vaccination. None of the patients had received IgRT at least 12-months before study inclusion or under chemotherapy or progression of underlying disease at evaluation.

Table 1
Epidemiological and immunological characteristics of study subjects.

	HM (No.:40)			HC (No.:24)
Subgroup	NHL (No.:17)	MGUS (No.:13)	CLL (No.:10)	
Gender F/M (No.)	12/5	8/5	6/4	20/4
Age at baseline [α]	69 ± 10	61 ± 11	67 ± 11	60 ± 18
	69 (16)	62 (16)	67 (19)	64 (31)
Disease duration since diagnosis [α]	12 ± 6	6 ± 3	11 ± 3	
	11 (9)	7 (7)	7 (6)	
IgG	424 ± 303	1360 ± 793	553 ± 358	1121 ± 231
	356 (530) ***	1131 (1106)	344 (608) ***	1160 (321) ***
IgA	48 ± 61	141 ± 92	96 ± 155	263 ± 95
	18 (81) ***	111 (146) §	22 (68) ***	244 (114) ***
IgM	42 ± 45	252 ± 385	29 ± 31	116 ± 55
	25 (67) ***	95 (169)	12 (37) ***	95 (40) ***

Data are presented as mean (Standard Deviation; SD); median (Interquantilic Range; IQR). α years.
*** $p < 0.001$ between HM-group at baseline and HC-group; Mann-Whitney *U* test.
§ $p < 0.05$ between MGUS-group at baseline and HC-group; Mann-Whitney *U* test.

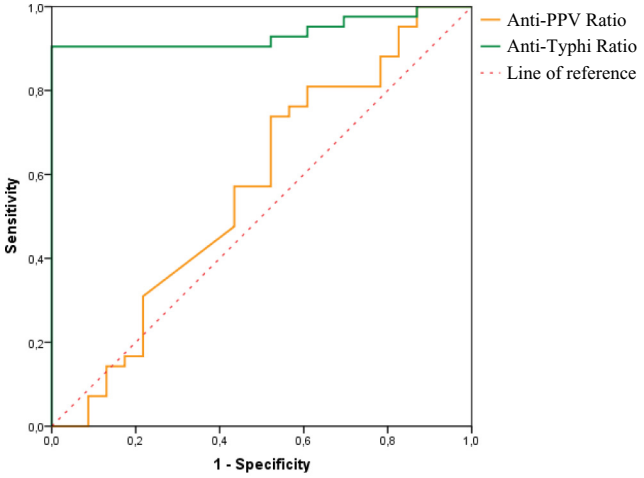
3.2. Baseline concentrations and polysaccharide Ab response

Three of 42 (7%) HM patients had baseline concentrations of TV IgG antibodies above the cut-off of 7.4 U/mL *versus* 32 of 42 (76%) above 4.4 mg/dL for PPV($p < 0.001$). In the HC-Group, 7 of 24 (30%) for TV *versus* 18 out of 24 (75%) for PPV, showing low seroprevalence of *S. Typhi* in both groups, compatible with previous reports [21] (Table 2). Only 3 out of 8 (37%) patients who had received PCV vaccine previous to PPV had baseline titers against pneumococcus (mean 4.1 ± 2.2 SD; median 4.0 (interquartile range3.0)).

The median post vaccination concentration of TV IgG in the HC-Group was 88 U/mL (range 34–600 U/mL). Using the minimum post vaccination concentration for TV IgG in HC (34 U/mL) as a cut off, 5 of 42 HM-Group achieved a post vaccination > 34 U/mL.

Analysis of specific anti-polysaccharide Ab responses was based on baseline concentrations and response ratio to contextualize individual response. There is currently no published data on the pre-to post ratio cut-off for SID, therefore response ratios in HM and HC were calculated as seen in PID [19,21]. Eight of 42 (19%) of the HM-Group achieved a 3-fold increase for TV IgG post-vaccination *versus* 24 of 24 (100%) in the HC-Group.

Using ROC analysis to determine the optimal threshold for a normal vaccination response in SID, the performance of TV ratio was higher to discriminate HM-Group and HC-Group [AUC: 0.93 (95%CI:0.87–0.99), $p < .0001$] compared with PPV ratio [AUC: 0.56 (95%CI:0.41–0.72), p:NS (Fig. 1)]. The statistically chosen cut-off value was a 5-FI for TV IgG with sensitivity diagnosis of 91%, specificity of 100%, and positive predictive value of 97%.



			95% CI	
Variable	Area	P	Lower limit	Upper limit
Ratio TV	0.93	<0.0001	0.87	0.99
Ratio PPV	0.56	0.36	0.41	0.72

Fig. 1. ROC curve.

Table 2
Immunological Ab assessment.

Subgroup	NHL	MGUS	CLL	HC
No. of pts	17	13	10	24
Baseline TV	5.7 ± 1.3	5.6 ± 1.9	6.1 ± 1.9	7.6 ± 2.6
IgG	5.9 (4.2–7.0)	5.9(4.2–6.9)	5.6 (4.8–7.0)	7.4(5.8–8.6)
Ratio TV	4.0 ± 10.4	23.8 ± 61.7	1.6 ± 1.0	20.9 ± 21.7
	1.12 (0.9–2.3)	2.4(1.4–13.9)	1.1 (1.0–1.9)	11.8(8.9–23.5)
Baseline PPV	7.12 ± 8.7	14.8 ± 11.6	5.9 ± 6.5	14.5 ± 11.2
IgG	3.0(1.2–12.7)	13.8(3.2–27.0)	4.5 (1.8–7.3)	8.8(4.6–27.0)
Ratio PPV	1.9 ± 2.0	3.2 ± 4.3	4.7 ± 7.0	8.9 ± 19.7
	1.0(0.8–2.0)	1.0(2.9)	2.0(1.0–5.3)	1.3(1.0–5.0)
Baseline TT	0.4 ± 0.8	1.8 ± 2.5	0.5 ± 0.7	-
IgG	0.1(0.3–0.5)	1.1(0.1–2.2)	0.2(0.4–1.1)	
Ratio TT	3.2 ± 7.2	18.0 ± 49.7	10.7 ± 21.9	-
	1.0(0.8–1.5)	2.1(0.4–8.9)	1.2(1.2–12.8)	

Data are presented as mean (Standard Deviation; SD); median (Interquantilic Range; IQR).
Detectable basal levels for TV IgG: 7.4 U/mL, PPV IgG 4.4 mg/dl and TT IgG 0.1 U/mL
For the purpose of this manuscript MM and HL subgroup are not presented in the table due to low number of patients.

When using our proposed cut-off of 5-fold increase of Typhim Vi IgG, only 5 of 42 (12%) HM-Group presented an adequate Ab response, two of them without infectious history. As expected due to high baseline PPV concentrations, the ratio PPV:3x showed no significant differences among the two groups, which suggests that TV was superior at predicting humoral response than PPV ($p < 0.0001$) in our cohort of HM-Group.

A Venn diagram shows patients (HM-Group) with abnormal Ab response (Fig. 2). Of the total number of patients, only two (2/42; 5%) presented with an adequate response to both polysaccharide and protein antigens, corresponding to the subgroup of MGUS patients with recent diagnosis (2015).

In the present study, we assessed the pre- and post-vaccination ratio in a HC-Group. The median pre- vaccination titres for TV IgG was

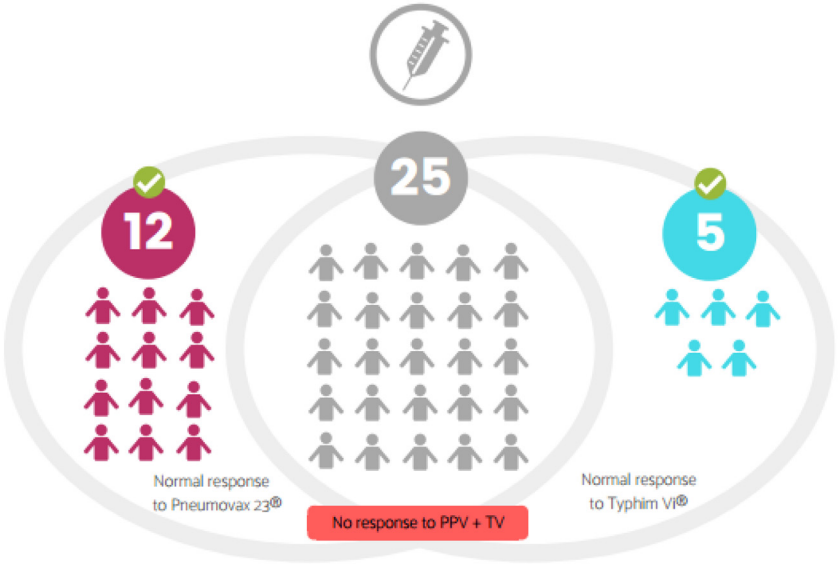


Fig. 2. Venn diagram showing failure ratio response to PPV® and/or TV in HM-Group.

7.4 U/mL and for post vaccination 88 U/mL (mean 160 ± 140 SD; (interquartile range 162)) yielding > 10-fold increase. Post vaccination, approximately 96% of HC-Group had fold increases in concentration ≥ 3 and 74% ≥10. None of the HC-Group had pre-vaccination TV IgG concentrations > 100 U/ml.

The patients were categorized into two groups based on their response to TV: HM responders (R, $n = 5$), and non-responders (NR, $n = 28$). The median FI for TV IgG was significantly lower in NR compared to R ($p < .0001$). However, no difference was found for PPV IgG FI between R and NR (Table 3).

3.3. Combined interpretation of IgG antibodies to Tetanus-Toxoid (TT) Protein and Typhim Vi (TV) polysaccharide vaccines in HM patients

Fig. 3 shows the quadrant distribution of TT and TV responses by fold-increase and the associated infectious history. For a complete interpretation, PPV responses were added in each quadrant. Thirteen out of 42 (31%) patients of the HM-Group presented a 3-FI Ab response against TT (Q2 and Q4). Eight out of these 13 (62%) patients overlapped TT and PPV responses, either due to high baseline PPV levels (75%) or 3-FI after PPV vaccination (50%) (Q2 and Q4). The remaining 5 TT-responder patients are highly suspicious of an underlying PID (Q2 and Q4) due to frequency of infections from childhood. Of interest, in 1 patient the lymphoma was the clinical onset of CVID (Q3), whole exome sequencing (WES) analysis revealed heterozygous mutations in two genes associated with PID (TACI and LRBA) and a homozygous mutation in PLCG2 (somatic mutation described in cancer). Two additional HM patients (Q3, Fig. 3) are also highly suspicious of being PID, currently awaiting confirmation by genetic results. The diagnostic suspicion of PID was based on the ESID diagnostic criteria: increased susceptibility to infections prior to the development of malignancy (6 patients) or family history of PID or parental consanguinity (1 patient). To confirm this suspicion, whole-exome sequencing (WES) is being carried out on the patients' genomic DNA (gDNA). We will search for rare variants (Minor Allele Frequency < 0.01) of known PID-causing genes and then, all mutations will be confirmed by Sanger sequencing. Molecular and cellular characterization also will be tested.

3.4. TV and clinical association

No significant association was found between TV IgG ratio and IgG levels (< 400 mg/dL) ($p = 0.88$). Interestingly, 3 patients (#18, #30, #40) with normal serum IgG levels presented with polysaccharide Ab deficiency. IgRT was implemented for two (#18, #40) of them due to history of recurrent infections, despite preserved IgG levels. The third patient (#30) remains infection-free. Fourteen out of 31 (45%) patients, excluding paraprotein subgroup, presented panhypogammaglobulinemia and 12 out of 14 (86%) TV Ab deficiency.

Two individuals of the HM-Group (patient #33 and #42) with MGUS diagnosis had normal responses to both TV and PPV without clear history of infections.

Severe bacterial infections requiring hospitalization and/or

Table 3
Responders and Non-responders according to TV IgG response.

No.	HM-TV Responders	HM-TV Non-Responders	p*	HC	p* HM-TV R and HC	p* HM-TV NR and HC
	5	37		24		
Baseline IgG PPV	14.0 ± 11.1 6.6 (4.0–27.0)	8.2 ± 9.3 3.2 (1.3–14.8)	0.37	16.1 ± 12.0 8.4 (4.6–27.0)	NS	0.31
FI IgG PPV	1.7 ± 1.5 1.0 (0.5–3.3)	3.2 ± 4.7 1.0 (0.9–3.6)	NS	9.2 ± 20.0 2.3 (0.9–5.2)	NS	NS
Baseline IgG TV	4.7 ± 1.9 5.2(3.2–6.1)	6.1 ± 1.7 6.0 (4.7–7.0)	NS	7.8 ± 2.5 7.4 (6.2–8.7)	0.004	0.003
FI IgG TV	62.8 ± 87.8 23.2(13.9–131.9)	1.7 ± 1.0 1.0 (1.0–2.2)	< 0.0001	21.2 ± 22.1 11.9 (8.4–25.4)	0.014	< 0.0001

Data are presented as mean (Standard Deviation; SD); median (Interquantilic Range; IQR)
Significance between R and NR by Mann Whitney-U test.

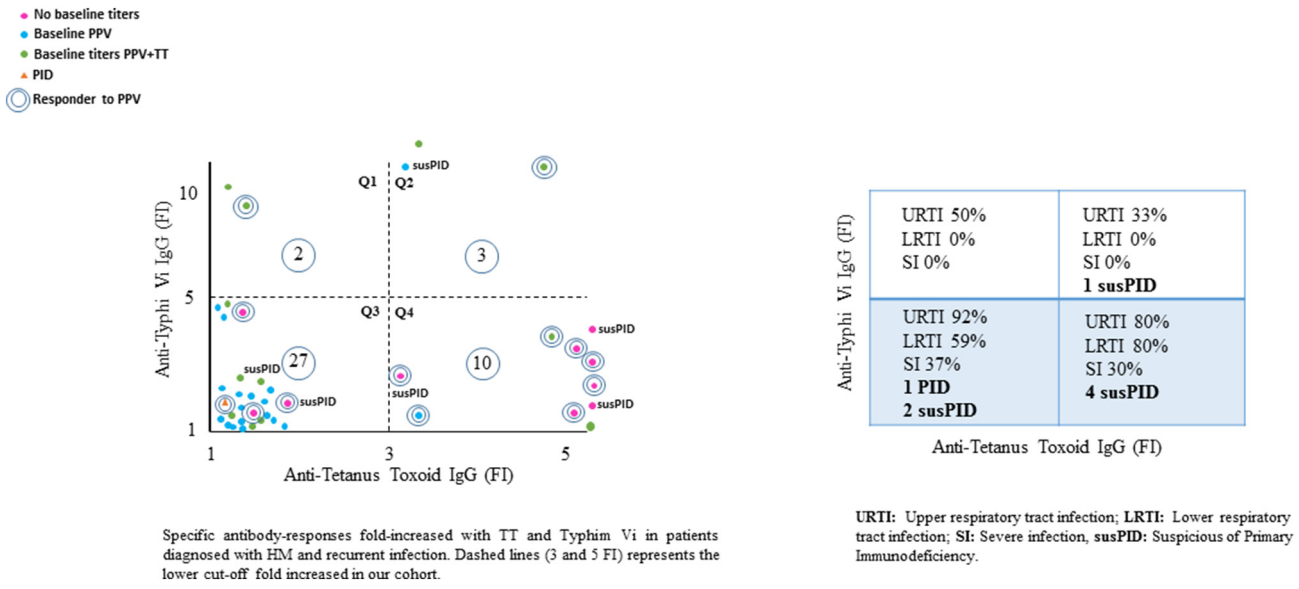


Fig. 3. Combined interpretation of IgG antibodies to proteic tetanus-toxoid (TT) and polysaccharide Typhim Vi (TV) vaccines in HM patients.

Table 4
History of infections according to HM sub-classification.

Clinical	NHL (n = 17)	MGUS (n = 13)	CLL (n = 10)	HL (n = 1)	MM (n = 1)
URTI	11 (65)	4 (30)	6 (60)	1 (100)	0 (0)
LRTI	11 (65)	2 (15)	7 (70)	1 (100)	1 (100)
SBI	6 (35)	1 (8)	3 (30)	1 (100)	1 (100)
VZV	4 (24)	2 (15)	2 (20)	0 (0)	0 (0)
LSD	5 (29)	3 (23)	2 (20)	1 (100)	0 (0)

Data are presented as No. (%). URTI: Upper respiratory tract infection; LRTI: Lower respiratory tract infection (*e.g.* Pneumonia, Bronchitis); SBI: Severe Bacterial Infection; VZV: Varicella zoster virus; LSD: Lung structural damage (*e.g.* bronchiectasis).

administration of intravenous antibiotics were observed in 17 out of 42 (40%) HM-Group, 94% (16/17) associated with TV Ab deficiency. The 2 patients that presented with an adequate response to TV were clinically asymptomatic without requiring IgRT. Eight out of 42 (19%) HM-Group presented with at least one episode of herpes zoster infection in the last year of which 63% (5/8) of these patients were associated with TV Ab deficiency (Table 4).

Bronchiectasis were observed in 5 of 42 (12%) of the HM-Group, three of them with preserved IgG levels, however all of them with antibody failure to TV.

3.5. TV booster response to cessation of IgRT

According to current guidelines [3] we re-assessed TV Ab production in 5 patients of the HM-Group (3 CLL and 2 NHL) after a period of 12 months on IgRT. Interestingly, one patient was able to mount a 3-FI IgG response to TV (32 U/mL) after 12-months, indicating humoral reconstitution. During the follow-up period of 1.2 years, the patient remained stable and infection free after withdrawing IgRT.

3.6. Haematological status

None of the patients were receiving chemotherapy during the immunological evaluation period. Excluding the paraprotein subgroup, 21 patients (72%) had received one cycle chemotherapy (4–6 weeks), 6 patients (21%) at least two cycles and 2 patients (7%) were in a "watch and wait" phase. Mean follow-up duration since study inclusion was 26 ± 15 months (median 24 months). During the follow-up period, the HM remained stable in 40 patients (95%) and worsened in 2 patients (5%). Two patients (5%) died, one patient due to pneumonia and other patient due to HM progression. Six patients (14%) of the NHL group had received stem cell transplant and two patients (5%) of the CLL group were splenectomised secondary to refractory autoimmune haemolytic anaemia (AHAI).

4. Discussion

Despite the new EMA IgRT indication, there are no standardized specific Ab responses in SID and data from PID may not be of use, given the usual lack of secondary responses in the latter. Initial assessment of specific Ab responses in HM was proposed by Helen Chapel in the 80's [22] as a more reliable predictor of infections to start IgRT. Interpretation of adequate polysaccharide Ab responses in PID remains controversial due to diverse reasons [23], being one of the major factors the inclusion of Prevenar 13® (PCV) into the routine vaccination schedule. In our cohort of HM patients, low baseline concentrations of TV IgG were found in all subjects, showing a very low seroprevalence of TV IgG, in contrast to PPV IgG baseline concentrations. Pioneering works by Chapel and Griffits evaluated the value of pneumococcal vaccination responses in the pre-rituximab era in HM patients for IgRT decision taking. However, in almost all published studies IgRT initiation in SID to HM was guided by recurrent or severe infections in addition to hypogammaglobulinemia [8,22,24].

To date, there is no published data on the evaluation of TV IgG response in HM patients. Although we are aware of the small sample size of our HM cohort, the preliminary data presented on the evaluation of polysaccharide Ab response in HM patients comparing PPV and TV are original and may set indicative cut-off levels until standardization is achieved with larger prospective cohorts. The study included TV IgG response compared to pure polysaccharide immunization, before PCV was recommended by CDC, and includes a cohort of patients, such as MGUS and non-Hodgkin lymphoma, which enter in new EMA indications. Our results suggest that TV ratio was the most significant parameter to differentiate infectious risk between HM and HC subjects, which underlines its clinical utility in SID.

Baseline concentration of PPV Ab in our cohort was relatively high, in agreement with previous publications [21], which resulted in lower median fold increases in concentration. Of note, the ratio PPV:3× showed no significant differences among the two groups. Using statistical ROC analysis to define the most optimal cut-off level for ratio TV

and PPV, only the response to Typhim Vi was significant. In our HM cohort, a cut-off level of 5 FI in TV Ab concentration was the most accurate for selection of SID patients that may benefit from IgRT. Interestingly, TV IgG responses may help to discriminate primary from secondary responses in previously immunocompetent patients, a feature that is particularly useful in SID patients.

Three patients with normal Ig concentrations presented with recurrent infections associated with an inadequate Ab response to TV. This represents a differential feature of SID, which may lack primary responses while eliciting secondary responses to PPV or TT antigens. The overall specific Ab production evaluation suggests that SID standards of immunological assessment differ from PID. These findings might further support the relevance of patient's ability to mount a specific Ab response together with Igs quantification.

Although no statistical significance was observed between the subgroups and their Ab response, 36% of MGUS patients had an adequate fold increase in TV IgG, which may indicate specific Ab responses at earlier diagnosis stages as a biomarker of humoral immunodeficiency and underline the convenience of early vaccination in these patients.

Hematopoietic malignancies are a relatively common complication in patients with immunodeficiencies and can be the first or even the only manifestation of an underlying PID [25]. However, there is no data on the real incidence of patients with PID within the population of HM. About 12% of haematological cancers are estimated to be driven by oncogenic pathogens, which may be more pronounced or accelerated in PID patients [26,27]. Other intrinsic and extrinsic mechanisms of oncogenesis make PID patients especially vulnerable to HM as well as to other malignancies. In our cohort of HM patients derived from Haematology Dept., 1 patient was diagnosed of PID with lymphoma as its first clinical manifestation; another patient had PID and MGUS; and 6 (14%) additional patients were highly suspicious of PID and await genetic testing that might eventually confirm diagnosis. This finding is clinically relevant, since an underlying PID may impact infectious and toxicity risk of cancer therapies, and actionable PID mutations may be susceptible to selected targeted therapies. It may also suggest that implementation of an immunological work-up, based on serum immunoglobulins and immunization response, to stratify HM patients prior to immune insult or even at diagnosis of HM [4] could help to optimize HM management.

Baseline concentrations of TV IgG in patients receiving IgRT has been shown to be undetectable [28,29]. Therefore, the detection of TV IgG response in these patients is feasible. The utility of TV has been mainly focused on diagnosis [17,21,28,29], but it may also be informative of humoral reconstitution in individuals receiving IgRT, which is other distinctive feature of SID, mainly in a proportion of lymphomas and after progenitor cells transplantation [12,28]. This could be particularly relevant, as our very preliminary results show the value of booster TV Ab response as a marker of humoral reconstitution that help to decide IgRT cessation, whereas currently, discontinuation of IgRT is based on clinical data. Indeed, after 1-year follow-up on IgRT, 1 of 5 HM patients was able to mount a 3-FI in TV IgG and remains asymptomatic after IgRT withdrawal. It would be also interesting in further studies to measure TV IgA or IgM responses in patients who have already initiated IgRT.

The response to TT was limited to the HM-Group in the setting of routine clinical practice. Nevertheless, our data indicates that combined Ab responses measurement may provide a wider window for exploration of the immune system.

5. Conclusions

Our results suggest that TV IgG responses may be susceptible of standardization in non-endemic countries, as a useful tool for the diagnosis of SID and to facilitate a more rigorous selection of patients to start IgRT, allowing for better therapeutic decisions. Also, TV booster after 12-months on IgRT may be used as a biomarker to withdraw IgRT, which could be particularly useful in patients with lymphoma that undergo immune reconstitution or after progenitor cells transplantation. We expect that our study may support new scientific approaches within SID population, as closer interdisciplinary collaborations grows.

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Data availability

The datasets generated for this study are available on request to the corresponding author.

Author contributions

JOG and SSR contributed conception and design of the study. AR, RP have contributed to the immunological study of PID. CO, ER and KGH contributed to the database. LF and IS performed the statistical analysis. JOG wrote the first draft of the manuscript. SSR and LW wrote sections of the manuscript. SSR and LW critically reviewed the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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