Measurement and interpretation of \textit{Salmonella typhi} Vi IgG antibodies for the assessment of adaptive immunity

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ABSTRACT

Response to polysaccharide vaccination can be an invaluable tool for assessing functionality of the adaptive immune system. Measurement of antibodies raised in response to Pneumovax\textsuperscript{®}23 is the current gold standard test, but there are significant challenges and constraints in both the measurement and interpretation of the response. An alternative polysaccharide vaccine approach (\textit{Salmonella typhi} Vi capsule (ViCPS)) has been suggested. In the present article, we review current evidence for the measurement of ViCPS antibodies in the diagnosis of primary and secondary antibody deficiencies. In particular, we review emerging data suggesting their interpretation in combination with the response to Pneumovax\textsuperscript{®}23 and comment upon the utility of these vaccines to assess humoral immune responses while receiving immunoglobulin replacement therapy (IGRT).

1. Introduction

Primary immunodeficiencies (PIDs) are a group of over 300 diseases caused by inherited or genetic defects in one or more components of the immune system (Picard et al., 2015). Timely and appropriate diagnosis of PID is imperative to optimise patient treatment, reduce the risk of infections, comorbidities and avoid permanent tissue or organ damage (Chapel et al., 2014; Tang et al., 2017; Yazdani et al., 2017). Moreover, patient healthcare costs have been demonstrated to be substantially reduced after a diagnosis of PID (Modell et al., 2011). Secondary immunodeficiencies (SID) originate from external underlying factors that are not primary in origin but compromise the function of the immune system. Causes that may suppress the immune system include haematological malignancies, immunosuppressive drugs for example B cell ablation therapies like rituximab and pathogens such as the human immunodeficiency virus (HIV; reviewed in Dhalla and Misbah (2015))(Sánchez-Ramón et al., 2016).

Impairment of specific antibody responses is characteristic of a number of humoral immunodeficiencies, rendering individuals susceptible to recurrent and severe infections with encapsulated bacteria as well as rare opportunistic pathogens (McCusker and Warrington, 2011; de Vries, 2012; Sanges et al., 2017). The measurement of responses to T-cell dependent and T-cell independent antigens is essential for evaluation of the adaptive immune system (de Vries, 2008) and is recommended in peer-reviewed guidelines to aid in the clinical recognition and diagnosis of antibody deficiencies (de Vries, 2012; Orange et al., 2012; Bonilla et al., 2015). There are several primary antibody disorders in which assessment particularly rests on the determination of vaccine responses: transient hypogammaglobulinaemia of infancy (THI), IgG subclass 1, 2 and 3 deficiencies, IgA deficiency (IgAD) and specific polysaccharide antibody deficiency (SPAD). The growing category of undefined hypogammaglobulinaemia also requires careful assessment of vaccine responses. Likewise, utilising the antibody response to specific antigens has also been recommended in the diagnostic assessment and subsequent monitoring of IGRT in immunodeficient patients (Jolles et al., 2017).

The clinical measurement of T-cell independent immunity can be achieved by measuring the antibody response to several polysaccharide antigens such as isohaemagglutinins, meningococcal and pneumococcal polysaccharide vaccines (Menactra\textsuperscript{®} and Pneumovax\textsuperscript{®}23, respectively), as well as alternative vaccines such as \textit{Salmonella typhi} Vi polysaccharide (Typhim Vi\textsuperscript{®}). At present, the gold standard for assessment of T-cell independent responses is to determine the IgG response to Pneumovax\textsuperscript{®}23, which contains extracts of 23 pneumococcal capsular serotypes. Pneumovax\textsuperscript{®}23 provides protection against 80–90% of pneumococcal serotypes that cause invasive pneumococcal disease and...
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2. Vi capsular polysaccharide (ViCPS) vaccines

ViCPS is a linear homopolymer of poly-alpha(1 → 4)GalNAc-p in the organism *Salmonella typhi*, may depend on post translation modifications (Szu et al., 1991). Purified Vi antigen is a potent immunogen (Ahmadi et al., 2013) and hence a prime target for vaccine development against typhoid fever. Two main types of typhoid vaccines are currently available; ViCPS vaccines and a live-attenuated strain (Ty21a). Additionally, there is a limited availability of Vi-polysaccharide-protein conjugate (Vi-conjugate) vaccines, which are currently licensed for use in China and India only (Schadich et al., 2016) (Table 2).

ViCPS vaccines are licensed for use in adults and children over 2 years old and are used in areas where typhoid fever is endemic, as well as for travellers to those areas (Table 2, Schadich et al., 2016)). ViCPS vaccines have been shown to be effective in preventing

Table 1

<table>
<thead>
<tr>
<th>Challenges in the interpretation of response to Pneumovax®23</th>
<th>Solutions provided by measurement of response to ViCPS vaccines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of consensus analytical approach to measure response to Pneumovax®23 makes inter-laboratory comparisons difficult</td>
<td>ELISA based technology is consistently used to measure response to ViCPS</td>
</tr>
<tr>
<td>Analytical discrepancies are evident between techniques used to determine antibody concentrations to individual serotypes and when compared to the overall antibody response to all Pneumovax®23 containing serotypes (Janssen et al., 2014; Reynolds et al., 2015; Lopez et al., 2016; Dziadzio et al., 2017)</td>
<td>Consistency has been demonstrated by laboratory designed tests (LDT) and commercially available ELISAs (Bausch-Jurken et al., 2017)</td>
</tr>
<tr>
<td>Development of normal reference ranges for a healthy population has proven difficult in the background of prevalent pneumococcal disease (Hare et al., 2005; Orange et al., 2012; Bouilla et al., 2013)</td>
<td>Published normal reference ranges are available. Cut-offs to identify responders and non-responders have been identified (Ferry et al., 2004; Sánchez-Ramón et al., 2016; Bausch-Jurken et al., 2017; Kumarage et al., 2017).</td>
</tr>
<tr>
<td>High baseline pneumococcal antibody concentrations are prevalent and affects interpretation of response to Pneumovax®23 (Hare et al., 2009)</td>
<td>Low baseline concentrations of Typhi Vi IgG antibodies has been demonstrated in areas where disease prevalence is low and where it is endemic (Ferry et al., 2004; Sánchez-Ramón et al., 2016; Kumarage et al., 2017).</td>
</tr>
<tr>
<td>Use of pneumococcal conjugate vaccines in immunisation and re-immunisation schedules have made the interpretation of antibody response to Pneumovax®23 potentially challenging; misleadingly elevated responses to serotypes contained in conjugated pneumococcal vaccines are evident in individuals who have previously received the conjugated vaccine, masking a true polysaccharide response (Janssen et al., 2014; Schaballie et al., 2016)</td>
<td>Limited availability of conjugated vaccines against typhoid fever makes interpretation of antibody response to ViCPS less complicated (Szu, 2013; MacLennan et al., 2014; Mitra et al., 2016; Jin et al., 2017).</td>
</tr>
<tr>
<td>Lack of consensus on interpretation of response to Pneumovax®23</td>
<td>Conjugated vaccines are only licensed in India and China and their use will be more restricted than conjugated pneumococcal vaccines, especially in countries without endemic typhoid fever (Szu, 2013; MacLennan et al., 2014; Mitra et al., 2016; Jin et al., 2017).</td>
</tr>
<tr>
<td>Future availability of conjugated vaccines with increasing serotype coverage will render interpretation and use even more challenging</td>
<td>Cross reactivity lower as Typhim Vi does not possess multiple serotypes</td>
</tr>
<tr>
<td>High concentrations of pneumococcal IgG are present in antibody replacement products</td>
<td>Interpretation not complicated by antibodies to multiple serotypes</td>
</tr>
</tbody>
</table>

is recommended in all adults 65 years of age and older, as well as in individuals at-risk of infection who are > 2 years of age (Centers for Disease Control and Prevention, 2010; Daniels et al., 2016; Public Health England, 2017).

There have been a number of reports highlighting the utility of the antibody response to Pneumovax®23 in assessment of immune system functionality, however, despite being the most commonly used test and employed in many laboratories (Edgar et al., 2018), both measurement and interpretation remains challenging. Furthermore, widespread vaccination of children using conjugated pneumococcal vaccines, such as Prevenar 13®-three, adds to the complexity of result interpretation (summarised in Table 1). These factors have spurred the search for alternative polysaccharide antigens to assess T-cell independent humoral responses and consequently measurement of the antibodies induced in response to Typhim Vi® has gathered momentum in recent years. The focus of this review is to discuss the evidence in support of ViCPS anagenic potential in the organism.

The antibody response to ViCPS vaccination generates antigen-specific plasmablasts (Kantele et al., 1986; Kantele, 1990; Kantele and Makela, 1991; Sundstrom et al., 2008; Lundgren et al., 2009; Pukkanen et al., 2010) which aids distribution and localisation of the humoral response to effector sites using tissue specific homing receptors (Kantele et al., 2013). Post Typhim Vi® vaccination, the homing profile of ViCPS induced plasmablasts consisted of the following homing receptors: 84% peripheral lymph node, L-selectin, 56% intestinal, α4β7-integrin, and 4% cutaneous, cutaneous lymphocyte antigen (CLA). Ty21a plasmablasts had a different homing profile. Capsular polysaccharides are regarded as T cell independent Type II antigens and in mice the antibody response to the Vi antigen is governed by B1b cells (Marshall et al., 2012). In humans, the ViCPS antibody response involves splenic marginal zone B cells (Guinamard et al., 2000; Martin et al., 2001; Martin and Kearney, 2002; Weil et al., 2009) and a B1b analogue has been proposed in human common variable immunodeficiency (CVID) patients (Rakhmanov et al., 2009; Griffin et al., 2011). Two recent studies have investigated the presence of B cell populations in response to Typhim Vi® vaccination; Bausch-Jurken et al. report IgG responders to Typhim Vi® vaccination have a higher percentage of both B cells and switched memory B cells (Bausch-Jurken et al., 2017) and Evans et al. report the total number of B cells, but not percentage of B cells, to be higher in IgG responders than non-responders (Evans et al., 2018).
The assessment of the IgG response to ViCPS is recommended (Bonilla, 2018) but T cell independent type II antigens, such as ViCPS, produce a predominately IgM antibody response. The measurement of IgM response to polysaccharide vaccines has been suggested as additional tools to assess humoral immunity. Poor IgM responses to pneumococcal antigens in patients with antibody deficiencies has been associated with increased risk of infections and co-morbidities, and may have a particular utility in patients receiving IgG replacement therapy (Cavaliere et al., 2012; Schutz et al., 2012; Echeverria de Carlos et al., 2017). It can be envisaged that the IgM antibody response to polysaccharide ViCPS vaccinations may also have utility in assessing humoral immunity, but to date there are no publications exploring this avenue.

### 2.2. Baseline concentrations of Typhi Vi IgG antibodies

Independent studies have reported low baseline concentrations of Typhi Vi IgG antibodies in children and adult blood donors (median children 1.4 AU/mL, median adults 5.3 AU/mL, assay measuring range of 0–95 AU/mL and median adult 8.6 U/mL, assay measuring range 7.4–600 U/mL) (Ferry et al., 2004; Sánchez–Ramón et al., 2016). The percentage of individuals with baseline concentrations below assay measuring ranges has been reported to be 40–71% (Fig. 1) (Parker et al., 2014; Kumaranage et al., 2017; Evans et al., 2018) and age specific adult baseline concentrations suggest peaks in concentration between 32–41 years and 72–81 years (Parker et al., 2014). Median baseline Typhi Vi IgG concentrations are generally higher in adults compared to children (Kim et al., 1995; Ferry et al., 2004; Parker and Harding, 2016; Kumaranage et al., 2017) and the range of concentrations wider (7.4–300 U/mL vs. 7.4–39 U/mL assay measuring range 7.4–600 U/mL) (Kumarage et al., 2017), however, confirmation of this trend in larger cohorts is required (Fig. 1). Global baseline Typhi Vi IgG concentrations will likely remain relatively low since administration of the conjugate vaccine will only be directed towards countries with endemic typhoid fever and individuals travelling to them.

Some healthy individuals have been reported to have high pre-vaccination concentrations, significantly above the baseline concentrations ranges determined. Ferry et al. report 2/23 (Ferry et al., 2004), Kumaranage et al. report 2/24 (Kumaranage et al., 2017) and Evans et al. report 4/215 (Evans et al., 2018) healthy individuals with significantly higher baseline Typhi Vi concentrations, which may be indicative of prior pathogen contact, previous vaccination or cross reactivity with similar polysaccharide structures (Ferry et al., 2004).

Baseline concentrations have been suggested to be higher in areas of endemic Typhoid fever (19% to 58% levels, > 1 μg/mL) in the absence of vaccination (Klugman et al., 1996; Keddy et al., 1999; Panchanathan et al., 2001; Staats et al., 2010). A direct comparison between the baseline concentrations from an area with endemic typhoid fever to an area without the fever, using the same commercial assay, provides some support for this (median 8.6 (range 7.4–31.8) vs 10 (range 7.4–232)) (Sánchez–Ramón et al., 2016; Kumaranage et al., 2017). As with antibody responses to other vaccinations, high baseline concentrations of Typhi Vi IgG antibodies could affect the magnitude of response achieved (Evans et al., 2018), however Kumaranage et al. demonstrated that even in an area endemic of typhoid fever > 95% of a control group had a concentration below 100 U/mL suggesting that pre-vaccination concentrations may not be a limiting factor in the interpretation of response to Typhi Vi vaccination.

### 2.3. Response to ViCPS vaccines in a control population

Several studies have identified fold increase cut-offs in order to define an adequate response to ViCPS in healthy control populations. Using a one-sided 95% prediction interval, Schaballie and colleagues demonstrated a 2-fold increase and 11.2 U/mL post-vaccination concentrations were calculated. (A) Adult control group (n = 24) and (B) Children control group (n = 20).

Measuring range of the assay is 7.4–600 U/mL. Reprinted with permission (Kumarage et al., 2017).

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**Table 2**

<table>
<thead>
<tr>
<th>Vaccine type</th>
<th>Vaccine name</th>
<th>Developer</th>
<th>Licensed for use</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live attenuated Vi Polysaccharide (ViCPS)</td>
<td>Ty21a (Vivotif®)</td>
<td>Crucell</td>
<td>Adults and Children &gt; 5 years</td>
<td>(Ivanoff et al., 1994; Pakkanen et al., 2015)</td>
</tr>
<tr>
<td></td>
<td>Typhim Vi™</td>
<td>Sanofi Pasteur</td>
<td></td>
<td>(Acharya et al., 1987; Klugman et al., 1996; MacLennan et al., 2014; World Health Organization, 2014; Schadich et al., 2016)</td>
</tr>
<tr>
<td></td>
<td>Typhrix™</td>
<td>GSK</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Typhor™</td>
<td>Bharat Biotech</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Typho-Vi®</td>
<td>Biomed</td>
<td>Adults and Children ≥ 2 years</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vax-tVi®</td>
<td>Finlay Institute</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zeityph inj®</td>
<td>Boryung</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Typhevac inj®</td>
<td>Shanghai Institute of Biological Products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vi-TT conjugate</td>
<td>Peda-Typh™</td>
<td>Biomed</td>
<td>Licensed in India</td>
<td>(MacLennan et al., 2014) (Chinnasami et al., 2015) (Mitra et al., 2016)</td>
</tr>
<tr>
<td>Vi-rEPA conjugate</td>
<td>Typhar-TCV®</td>
<td>Bharat Biotech</td>
<td>Licensed in India</td>
<td>(MacLennan et al., 2014) (Jin et al., 2017)</td>
</tr>
<tr>
<td></td>
<td>Vi-rEPA®</td>
<td>Lanzhou Institute (China)</td>
<td>Licensed in China</td>
<td>(Szu, 2013; MacLennan et al., 2014)</td>
</tr>
</tbody>
</table>

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**Fig. 1.** Baseline concentrations of Typhi Vi IgG are low in both healthy children and adults.

Baseline Typhi Vi IgG concentrations in children and adult control groups. The Typhi Vi pre-vaccination concentrations were determined in the two control groups and separated into concentration ranges. The % individuals in the different concentration ranges were calculated. (A) Adult control group (n = 24) and (B) Children control group (n = 20).

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Table 3
Summary of post-vaccination ViCPS antibody concentrations and fold increases in healthy controls and primary antibody deficiency patients.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Units</th>
<th>Healthy controls</th>
<th>Antibody deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Post vaccination concentration (95% CI)</td>
<td>Fold increase in concentration (95% CI)</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Kim et al., 1995)</td>
<td>GMT</td>
<td>69.4</td>
<td>29†††</td>
</tr>
<tr>
<td>(Lim et al., 2007)</td>
<td>GMT</td>
<td>142.6</td>
<td>24†††</td>
</tr>
<tr>
<td>(Miyazu et al., 2015)</td>
<td>GMT</td>
<td>501.7 (305.3-824.5)</td>
<td>136.6 (82.5-222.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12-17 years</td>
<td>12-17 years</td>
</tr>
<tr>
<td>(Sánchez, 2016)</td>
<td>µg/ml</td>
<td>1.89 (0.4-8.9)</td>
<td>8</td>
</tr>
<tr>
<td>(Kumarage et al., 2017)</td>
<td>U/ml</td>
<td>679 (60-3029)</td>
<td>59 (7-236)</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Evans et al., 2018)</td>
<td>U/ml</td>
<td>107 (31-542)</td>
<td>12.3 (3.4-76.9)</td>
</tr>
<tr>
<td>(Sánchez-Ramón et al., 2016)</td>
<td>U/ml</td>
<td>171 (32-600)</td>
<td>12.3 (3.4-76.9)</td>
</tr>
<tr>
<td>(Kumarage et al., 2017)</td>
<td>U/ml</td>
<td>519 (80-2779)</td>
<td>32 (5-135)</td>
</tr>
<tr>
<td>(Kim et al., 1995)</td>
<td>GMT</td>
<td>79.1</td>
<td>12†††</td>
</tr>
<tr>
<td>(Lim et al., 2007)</td>
<td>GMT</td>
<td>58.7</td>
<td>10†††</td>
</tr>
<tr>
<td>(Acharya et al., 1987)</td>
<td>µg/ml</td>
<td>15–44 years</td>
<td>15–44 years</td>
</tr>
<tr>
<td>(Kumarage et al., 2017)</td>
<td>U/ml</td>
<td>3.2</td>
<td>16†††</td>
</tr>
<tr>
<td>(Klugman et al., 1987)</td>
<td>ELISA OD</td>
<td>0.2 (0.1–0.3)</td>
<td>4†††</td>
</tr>
<tr>
<td>(Bausch-Jurken et al., 2017)</td>
<td>U/ml</td>
<td>6.0 (2.1–16.8)</td>
<td>100**</td>
</tr>
<tr>
<td>(Moore et al., 2012)</td>
<td>ELISA U</td>
<td>7.2 (6.1–8.5)</td>
<td>13†††</td>
</tr>
<tr>
<td>(Miyazu et al., 2015)</td>
<td>GMT</td>
<td>148.6 (126.9–174.0)</td>
<td>22.6 (19.1–26.8)</td>
</tr>
<tr>
<td>(Kroon et al., 1999)†††</td>
<td>AU/L</td>
<td>105.6</td>
<td>20</td>
</tr>
<tr>
<td>Adults/children combined</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Schaballie et al., 2017)</td>
<td>U/ml</td>
<td>71.5</td>
<td>15</td>
</tr>
</tbody>
</table>

HYPOG: Hypogammaglobulinaemia.
CI: Confidence interval.
† † % ≥ 3 fold increase.
** % ≥ 2 fold increase.
§ Median concentration and range.
†† Post vaccination concentrations have been obtained from the same commercial assay for comparison.
††† Fold increase results determined using vaccination data taken 1 month post and pre vaccination.
†††† Study compared response in two cohorts; those with < 200 × 10⁶/L CD4+ cells and those with > 200 × 10⁶/L CD4+ cells.

concentration to define an adequate response to Typhim Vi® (Schaballie et al., 2017). Bausch-Jurken and colleagues used receiver operator characteristic (ROC) analysis to establish that a 1.95 fold-increase in ViCPS antibodies resulted in an area under the curve of 1.00 (p < 0.0001) and thus proposed that a 2-fold increase cut-off accurately discriminated between the control population and cohort of patients with known immunodeficiencies (Bausch-Jurken et al., 2017). Several studies have defined > 3-fold increases in antibody concentration post-vaccination as a normal response based on antibody concentration increases in > 94%, > 95% and 100% of control groups (Ferry et al., 2004; Sánchez–Ramón et al., 2016; Kumarage et al., 2017; Evans et al., 2018). Sanchez-Ramon and colleagues also used ROC analysis to demonstrate a 10 fold increase in antibody production to be 90.9% sensitive and 62.5% specific in the differentiation between healthy controls and patients with CVID (Sánchez–Ramón et al., 2016).

Many studies have demonstrated highly immunogenic IgG responses to ViCPS vaccines in healthy control populations (Table 3). A large clinical trial evaluating the immunogenicity of ViCPS vaccines in Nepal, an area of endemic typhoid fever, reported 76.9%, 79.1% and 62.5% of individuals (aged 5-14 yrs., 15-44 yrs. and 45–55 years old, respectively) achieved seroconversion, compared with a 95.5% seroconversion rate in French and US individuals (Acharya et al., 1987). More recently, two independent studies evaluated the response to ViCPS vaccines using the same commercial assay (The Binding Site, UK), allowing direct comparison between areas where the occurrence of typhoid fever is low with an area where it is endemic. A higher post vaccination concentration was reported where typhoid fever is endemic (median 519 U/mL [95% CI 80–2779] (Kumarage et al., 2017) vs. median 171 U/mL [range 32–600](Sánchez–Ramón et al., 2016)), which may be consequential to slightly higher baseline concentrations in areas where individuals may have prior exposure to the pathogen and thus forms part of a secondary response.

In a study to determine predictors of response to ViCPS vaccines, multivariate analysis revealed pre-vaccination concentration of ViCPS antibodies to be a significant predictor of antibody response (Moore et al., 2012). Klugman et al. (1987) also demonstrated a greater
increase in *Salmonella typhi* Vi antibody titres one month post vaccination in cohorts with lower pre-vaccination concentrations; a cohort with pre-vaccination ELISA readings of < 0.1 OD elicited a 4.11 fold increase, versus a 1.74 fold increase in those with a pre-vaccination ELISA reading of > 0.1 OD (Klugman et al., 1987). Despite these results, in the context of a low prevalence of typhoid fever in developed countries and the absence of routine use of conjugated vaccines, the impact of pre-vaccination concentrations is unlikely to have as much impact on the interpretation of response (Table 3).

Evaluation of immunotolerance to ViCPS vaccines is essential to ensure interpretation of response is not hampered by pre-exposure to ViCPS polysaccharide vaccines. Similar levels of antibody concentrations against ViCPS antigens have been shown to be elicited after primary and booster vaccinations (Keitel et al., 1994). In a study comparing response to ViCPS vaccines in cohorts who had received multiple vaccinations vs. a cohort who had not previously been vaccinated against typhoid fever, there was no evidence of immunotolerance (Roggelin et al., 2015). However, in a subsequent study a proportion of the population of the study failed to produce plasmablasts specific to ViCPS after a booster vaccination (Pakkanen et al., 2017) indicating this is an area requiring further investigation.

The comparison between children and adult responses in a healthy population has been demonstrated by Kumarage and colleagues; a greater response was elicited in the paediatric group compared with the adult group but it did not reach statistical significance (post vaccination: median concentration (95% CI) (U/mL) children: 679 (60–3029) versus adults: 519 (80–2779); median fold increase (95% CI, U/mL) children: 59 (7–236) versus adults: 32 (5–135) (Kumarage et al., 2017). Additionally, Kim and colleagues also report a higher seroconversion rate in children compared with adults, 98.4% versus 88.2% one month post vaccination (Kim et al., 1995). Data from paediatric populations is of particular interest given the widespread use of pneumococcal conjugate vaccines (PCV) in childhood vaccination schedules. Given that infants are unable to mount polysaccharide-specific responses before the age of 2 years, SPAD can only be assessed after this time point (Durandy et al., 2013). A diagnosis of a memory phenotype of SPAD has been described, which is an adequate initial response to a polysaccharide antigen that is not maintained at 6 months post vaccination (Orange et al., 2012). It is therefore essential that vaccines used in the diagnosis of antibody deficiencies are able to induce responses in healthy individuals that are relatively stable for at least a year post vaccination. Studies have assessed the longevity of response to ViCPS vaccines confirming a stable response at 12 months post-vaccination and beyond (Table 4).

### 3. Utility of ViCPS vaccines as diagnostic tools for antibody deficiency

#### 3.1. Primary immunodeficiency

Response to ViCPS vaccines has been recommended for the assessment of humoral immunity in individuals undergoing evaluation for antibody deficiencies (Anonymous, 1999; Ferry et al., 2004; Orange et al., 2012; De Silva et al., 2013) and could be of particular use in patients who have been vaccinated with a conjugated pneumococcal vaccine. Several studies have demonstrated the utility of measuring response to ViCPS vaccines as an indicator of humoral immunity in patients with primary antibody deficiencies, in differentiating responses in patients with hypogammaglobulinemia (HYPOG) and CVID from healthy controls as well as stratifying PID patients who are at increased risk of infection.

#### 3.1.1. Response to ViCPS vaccines is restricted in patients with primary antibody deficiencies

The IgG response to ViCPS vaccine may be defective in patients with primary antibody deficiencies (Sánchez–Ramón et al., 2016; Bausch-Jurken et al., 2017; Kumarage et al., 2017; Evans et al., 2018). Using a laboratory developed test (LDT) ELISA, antibody response to Typhim Vi® vaccination was determined in healthy controls, patients diagnosed with primary antibody deficiencies and patients with possible humoral immune deficiency (Bausch-Jurken et al., 2017). All patients diagnosed with an antibody deficiency had an abnormal antibody response to Typhim Vi®, as did seven patients with possible immunodeficiency that were subsequently determined to have primary or secondary immunodeficiencies (Fig. 2). Kumarage and colleagues used a commercially available ELISA assay to measure the antibody response to the polysaccharide Typhi Vi vaccine, Typbar®, in patients referred for immunological evaluation in Sri Lanka, an area where typhoid fever is endemic. In both paediatric and adult patients with primary antibody deficiencies (CVID and HYPOG), antibody response to Typbar® vaccination was lower than in the age-matched controls with 89% of paediatric PID patients and 45% of adult PID patients obtaining a three-fold increase in concentration post vaccination compared with 94% of the control groups (median fold-increase [95% CI]: adult control group 32 (5–135) versus adult PID group 2 (1–56) and children control group 59 (7–236) versus children PID group 18 (1–56) (Kumarage et al., 2017). Evans and colleagues determined response to Typhim Vi® in 20 newly diagnosed antibody deficient patients; 40% did not respond to Typhim Vi® (< 7.4 U/mL) and 30% achieved a > 3 fold increase (Evans et al., 2018). The median post-vaccination concentrations were statistically different between this cohort and healthy volunteers (p = 0.02).

#### 3.1.2. Differentiation between healthy individuals and patients with HYPOG and CVID

Two studies describe that the antibody response to ViCPS vaccination may differentiate between some CVID patients and HYPOG patients. Sánchez–Ramón and colleagues reported a multi-centre evaluation of the use of Typhim Vi® in previously diagnosed PID patients (Sánchez–Ramón et al., 2016). A ten-fold increase in Typhim Vi® antibodies provided good discrimination between healthy individuals and some patients with HYPOG and CVID (Fig. 3A) (Sánchez–Ramón et al., 2016). Kumarage and colleagues also demonstrated that a three-fold increase in Typhim Vi IgG differentiated between some CVID and HYPOG patients and their corresponding controls groups (Kumarage et al.,

### Table 4

<table>
<thead>
<tr>
<th>Reference Pre-vaccination</th>
<th>1 month</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
<th>36 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sero-conversion (90%CI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>(Kim et al., 1995)</td>
<td>88.2</td>
<td>96.2</td>
<td>75.3</td>
<td>56.5</td>
</tr>
<tr>
<td></td>
<td>(Lim et al., 2007)</td>
<td>89.1 (83.3–94.7)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Children</td>
<td>(Kim et al., 1995)</td>
<td>98.4</td>
<td>100</td>
<td>98.2</td>
<td>52.6</td>
</tr>
<tr>
<td></td>
<td>(Lim et al., 2007)</td>
<td>96.9 (93.4–100.4)</td>
<td></td>
<td></td>
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<tr>
<td>Geometric mean Titer (GMT) ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>(Kim et al., 1995)</td>
<td>79.1</td>
<td>120.3</td>
<td>41.7</td>
<td>28.3</td>
</tr>
<tr>
<td></td>
<td>(Lim et al., 2007)</td>
<td>64.0 ± 2.81</td>
<td>105.0 ± 2.29</td>
<td>84.1 ± 2.12</td>
<td>36.7</td>
</tr>
<tr>
<td>Children</td>
<td>(Kim et al., 1995)</td>
<td>69.4</td>
<td>49.2</td>
<td>36.7</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>(Lim et al., 2007)</td>
<td>151.3 ± 2.28</td>
<td>110.0 ± 1.91</td>
<td>100.2 ± 1.67</td>
<td></td>
</tr>
</tbody>
</table>
2017) (Fig. 3B). ROC curve analysis highlighted Typhim Vi® to be the more sensitive assay in distinguishing between CVID and healthy controls compared with Pneumovax® 23 (AUC 0.893 versus 0.538) (Sánchez–Ramón et al., 2016) (Fig. 3C). The failure to respond to ViCPS vaccination has been used to aid diagnosis of antibody deficiencies, particularly CVID (De Silva et al., 2013; Bausch-Jurken et al., 2017).

3.1.3. Stratification of patients at risk of infection

Ferry and colleagues demonstrated an approximate 10-fold increase between mean pre- and post-vaccination concentrations in 23 healthy adults (geometric mean and 95% CI pre-vaccination 3.9 (2.4–6.5) and post-Typhim Vi® vaccination 39.2 (24.8–61.3) (Ferry et al., 2004). Sánchez–Ramón and colleagues proposed that differentiation of CVID patients using a 10-fold increase cut-off may identify a subset of CVID patients with a more proficient humoral immunological response and thus may possess lower risk of infection and possibly CVID associated disorders. Supporting this, although not statistically significant, they reported two patients with a diagnosis of CVID with increased responses > 10-fold in concentration who had a lower number of respiratory infections and none of the inflammatory conditions linked with a diagnosis of CVID (Sánchez–Ramón et al., 2016).

3.2. Utility of measuring IgG antibodies in response to both Pneumovax® 23 and ViCPS vaccines

Individuals may respond very differently to similar types of antigen that vary in structure e.g. between different pneumococcal serotypes and between Pneumovax® 23 and Typhim Vi®. Guidelines have suggested measuring the response to two protein vaccines for the assessment of antibody deficiency (Chapel and Cunningham-Rundles, 2009). Currently, data analysing the response to two polysaccharides,
Fig. 3. Differentiation of HYPOG and CVID patients using measurement of Typhim Vi® antibodies.
A) Reprinted with permission (Sánchez–Ramón et al., 2016).
Box plots showing that the Ratio Typhim Vi was significantly higher in healthy control-group (HC) compared to CVID-group (p < 0.0001), but not with respect to HYPOG-group (p = 0.138). 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.
B) Reprinted with permission (Kumarage et al., 2017).
Responses to Typhi Vi vaccination in control groups, HYPOG and CVID patients. Typhi Vi responses were assessed in the children control group (n = 20), adult control group (n = 24), HYPOG (n = 8) and CVID (n = 8) groups. FI: fold increase.
C) Reprinted with permission (Sánchez–Ramón et al., 2016).
Receiver operating characteristic (ROC) curve analyses using Typhim Vi and PCP 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). PCP: pneumococcal capsular polysaccharides.

Pneumovax®23 and Typhim Vi®, are lacking.
Schaballie et al. identified 2/100 healthy individuals with abnormal response to Pneumovax®23 and Typhim Vi® vaccines. Upon subsequent investigation both had a history of repeated infections and the clinical characteristics of one patient were suggestive of SPAD diagnosis. A history indicative of immunodeficiency was not noted in any individual who had a poor response to just one vaccine (Schaballie et al., 2017) (Fig. 4).

In patients undergoing immunological evaluation, the responses to Typhi Vi® and Pneumovax®23 can divide the patients into 4 groups. Data from Sánchez-Ramón suggested that 13/45 (29%) HYPOG and CVID patients did not have a similar response to both Typhim Vi® and Pneumovax®23 (Fig. 5). Evans and colleagues demonstrated equivalent responses to Typhim Vi and Pneumovax in 8/9 antibody deficient patients (Evans et al., 2018). Further investigations will be of interest to determine if stratification of patients using both polysaccharide vaccines can identify patients at increased risk of infection. Moreover, Ochoa-Grullón and colleagues investigated the response to Typhim Vi® and Pneumovax®23 in 28 patients diagnosed with various haematological malignancies (Ochoa-Grullón et al., 2017). 11/28 (39%) of all patients did not have a similar response to both Typhim Vi® and Pneumovax®23 (Fig. 6). Given that there is a high incidence of infection among patients with haematological disorders and recipients of hematopoietic cell transplants (Blimark et al., 2015; Dhalla and Misbah, 2015) stratification using both polysaccharide vaccines may be of particular interest. Additional studies are needed to determine the utility of measuring response to Typhi Vi polysaccharide vaccines to aid identification of patients with increased risk of infection.

3.3. Secondary immunodeficiency
B-cell lineage lymphoproliferative diseases such as chronic lymphocytic leukaemia and multiple myeloma (Dhalla and Misbah, 2015) as well as treatment related immunosuppression such as with rituximab or other biologicals can prevent antibody production resulting in severe or prolonged HYPOG (Sánchez–Ramón et al., 2016).
Kroon and colleagues demonstrated that the response to Typhim Vi® is compromised in individuals infected with the human immunodeficiency virus (HIV) (Kroon et al., 1999). Thirty HIV positive individuals were vaccinated with Typhim Vi® and divided into 2 groups; those with greater than or < 200 × 10⁶/L CD4⁺ T lymphocytes. In the group with > 200 × 10⁶/L CD4⁺ T lymphocytes, 86% of individuals achieved a ≥ 4 fold increase in concentration (mean fold increase 29) compared to 89% in the healthy control group (mean fold increase 20). The response was significantly lower in those HIV patients with < 200 × 10⁶/L CD4⁺ T lymphocytes (25% achieving a ≥ 4 FI, mean 4.4).

Bausch-Jurken and colleagues reported the vaccination of 29 patients referred for immune system evaluation with the suspicion of PID.

Fig. 4. Failure to produce IgG antibodies in response to Pneumovax®23 and/or Typhim Vi® in healthy volunteers.
Venn diagram showing subjects (black dots) with abnormal antibody response for each detection method, among 100 healthy subjects (fifth percentile cut-off values were used). Subjects with normal test results are not shown. Arrows indicate the clinical history of two subjects with abnormal results for both tests. URTI: upper respiratory tract infections. Adapted from Schaballie et al. (2017).

Fig. 5. Combined measurement of IgG antibodies to Typhim Vi® and Pneumovax®23 identifies 4 groups of patients with immunodeficiency.
Specific antibody responses post-vaccination with Pneumovax®23 and Typhi Vi® in healthy controls and patients diagnosed with PID. Dashed line (50 mg/L) represents lower end of normal response to Pneumovax®23 in health individuals (Chua et al., 2011); dotted line (32 U/mL) represents lowest response to Typhim Vi® in healthy individuals (Sánchez–Ramón et al., 2016). Data from Sánchez–Ramón.
Specific antibody responses post-vaccination with Pneumovax and Typhim Vi in patients diagnosed with haematological malignancy and recurrent infection. Dashed line (50 mg/L) represents lower end of normal response to Pneumovax in healthy individuals (Chua et al., 2011); dotted line (32 U/mL) represents lowest response to Typhim Vi in healthy individuals (Sánchez–Ramón et al., 2016). CLL: chronic lymphocytic leukaemia; LF: lymphoma; FL: follicular lymphoma; HL: Hodgkin lymphoma; MM: multiple myeloma; MGUS: monoclonal gammopathy of undetermined significance.

Data adapted from Ochoa-Grullón et al. (2017).

(based largely on a history of recurrent upper respiratory tract infections) (Bausch-Jurken et al., 2017). Two patients were diagnosed with secondary antibody deficiency: one with homologous lymphohistocytosis secondary to Epstein-Barr virus infection who was treated with repeated doses of rituximab; and the other, HYPOG and T cell lymphopenia secondary to non-Hodgkin’s lymphoma treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). Both exhibited a < 2 fold increase in Typhim Vi responses.

Furthermore, the response to Typhim Vi* was investigated in 28 patients diagnosed with various haematological malignancies (Ochoa-Grullón et al., 2017). Only 5/28 achieved a post vaccination concentration of Typhim Vi antibodies > 32 U/mL and 5/28 achieved a more than 3 fold increase (Sánchez–Ramón et al., 2016). All patients with chronic lymphocytic leukaemia (n = 5) and follicular lymphoma (n = 7) were non-responders (Fig. 6).

4. Measurement of Typhim Vi* IgG antibodies as tool for monitoring patients with antibody deficiency

IGRT has been the mainstay for the management of primary and secondary immunodeficiencies, particularly in those with a more severe clinical phenotype (Orange et al., 2012; Julles et al., 2017). Monitoring of patients’ total IgG concentrations is performed on regular basis by measurement of the trough IgG levels and in particular situations, such as SPAD, assessment of specific antibody levels may also be undertaken. It has been proposed that for patients who are receiving IGRT, a potential way to assess underlying endogenous vaccine responses is to use immunisations not included in the standard vaccination schedules, as baseline levels of these antibodies in immunoglobulin preparations and endogenous levels would be expected to be low (Sobh and Bonilla, 2016; Julles et al., 2017). Although several studies have reported a lack of correlation between the measurement of total IgG and specific antibody production, others have reported an adequate correlation (Mikolajczyk et al., 2004; Chua et al., 2011; Adam and Church, 2015; Julles et al., 2017; Lee et al., 2017; Simao-Gurge et al., 2017).

The baseline concentration of Typhim Vi* antibodies in patients receiving IGRT has been shown to be low (Bausch-Jurken et al., 2017; Evans et al., 2018) and undetectable in four commercial preparations of gammaglobulin (Gammagard®, Gamunex®-C, Subgam®, and Hizentra®) (Bausch-Jurken et al., 2017) and so detection of ViCPS antibody response in these patients is feasible.

Furthermore, Bausch-Jurken and colleagues reported the assessment of the response to Typhim Vi* in 10 individuals with unknown PID receiving IGRT. 7/10 had a ≥ 2 fold-increase response and 3/10 a < 2 fold-increase (Fig. 2). Re-evaluation of these patients concluded that the 7 responders were immunologically normal and IGRT was discontinued (Bausch-Jurken et al., 2017). The suspected antibody deficiencies in these individuals were due to untreated atopic disease (e.g., allergic rhinitis, asthma). The 3/10 non-responders remained on IGRT and were diagnosed with SPAD. The response to Typhim Vi* was used as a key tool to aid the decision to cease treatment.

5. Conclusions

The response to ViCPS vaccines is of particular interest in the assessment and risk stratification of patients with suspected primary and secondary immunodeficiency. Although measurement of antibodies raised against Pneumovax®23 is the current gold standard test to assess adaptive immunity to unconjugated T-cell independent polysaccharide antigens, additional options, such as response to ViCPS vaccines, are urgently needed to allow assessment where the utility of Pneumovax®23 may be limited.

The use of ViCPS vaccine response in assessment of adaptive immunity has advantages (summarised in Table 1). The use of conjugate vaccines is limited (Breukels et al., 1999; Janssen et al., 2014; Schaballie et al., 2016) and generally baseline antibody concentrations of Typhi Vi IgG are low, minimising memory B cell reactivation. Furthermore, antibody concentrations have been reported in healthy control populations enabling assessment of response to ViCPS antibodies to be relatively simple.

Current data suggests that combined measurement of antibodies to Pneumovax®23 and Typhim Vi* may provide a wider window for exploration of the immune system (Sánchez–Ramón et al., 2016; Ochoa-Grullón et al., 2017; Schaballie et al., 2017). This will be particularly important for the growing group of patients with undefined hypogammaglobulinaemia who present with a broad range of disease severity. The four groups for classifying non-responsiveness to polysaccharide antigens are currently based solely on the response to Pneumovax®23 (Orange et al., 2012). An additional marker may provide further patient stratification and standardisation of definitions as well as aid in treatment decisions (Perez et al., 2017).

The utility of ViCPS has mainly been focused on diagnosis (Sánchez–Ramón et al., 2016; Bausch-Jurken et al., 2017; Kumarrage et al., 2017) but it may also be informative in individuals receiving IGRT (Bausch-Jurken et al., 2017).

In conclusion, the measurement of IgG antibodies raised in response to typhoid polysaccharide vaccines has utility for the investigation of adaptive immunity. This is likely to be an important addition to the diagnostic toolkit for immunologists, used alongside Pneumovax®23, in this complex and challenging area of immunological assessment.

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