Concentrations of Pneumococcal IgA and IgM are compromised in some individuals with antibody deficiencies

Ainara Echeverría de Carlos, Ricardo Gómez de la Torre, Enrique García Carus, Luis Caminal Montero, Jose Bernardino Díaz López, Hector Suárez Casado, Luis Molinos Matín, Lourdes Tricas Aizpún, Stephen Harding, and Antony R. Parker

ABSTRACT
The response to pneumococcal vaccination is assessed by measurement of antigen specific IgG only and is compromised in a number of antibody deficiencies. We measured the concentrations of Pneumococcal IgA and IgM in individuals with both normal and abnormal pneumococcal capsular polysaccharide (PCP) IgG concentrations. A higher number of individuals had abnormal pre-vaccination IgA and IgM concentrations below the lower limit of the normal range compared to the control group. Post vaccination a lower number of individuals had IgA and IgM concentrations below the upper limit of the normal range compared to the control group. Non responders had a higher percentage of individuals with a prior history of infection. In addition, individuals with a history of prior infection had lower pre- and post-vaccination concentrations of PCP IgG, IgA, and IgM. Post-vaccination IgA and IgM concentrations identified four groups of responses which correlated with prior history of infection. A higher percentage of individuals with abnormal PCP IgA and IgM concentrations had a history of prior infection compared to the percentage of individuals with normal concentrations. In individuals with an antibody deficiency, measurement of Pneumococcal IgA and IgM correlates with the number of individuals with prior history of infection.

KEYWORDS
Pneumococcal; IgA; IgM; antibody deficiency; pneumovax

Introduction
The response to the polysaccharide vaccine Pneumovax® involves production of antigen specific IgG, IgA, and IgM antibodies. IgG is the most abundant immunoglobulin and maybe produced by memory B cells. Circulating serum IgA is considered a second line of defence, mediating elimination of pathogens that have breached the mucosal surface. IgM is the first antibody to...
appear in response to initial exposure to an antigen. Post vaccination, IgG is the most stable response with both IgA and IgM concentrations declining over a 12-month period.\[2\]

Currently, the response to pneumococcal vaccination is assessed by measurement of IgG immunoglobulin only and is compromised in a number of antibody deficiencies.\[3-5\] The IgA and IgM responses to pneumococcal vaccination have been reported to be compromised in some pediatric patients with antibody deficiencies\[6\] and correlates with common variable immunodeficiency (CVID) patients and their history of infection.\[7\]

Using reported unknown vaccination reference ranges\[8\] we measured the concentrations of Pneumococcal IgA and IgM responses to Pneumovax in individuals diagnosed with antibody deficiencies who had normal and abnormal pneumococcal capsular polysaccharide (PCP) IgG concentrations.

**Experimental**

Serum samples were obtained from 30 blood donors (15 males and 15 females; median age 34 years, range 19–81) which served as the Control group. Only subjects who were free of recurrent infections or inflammation (assessed by questionnaire) were included in the analysis. Samples were stored at −80°C. The samples were collected in donor centres by Biomex Solutions (Heidelberg, Germany) and purchased from Quest Biomedical (Solihull, UK). Sample collection was approved by the Institution Ethics Review Board (#05142), with all donors providing written informed consent. The vaccination status of this population was unknown.

Pre- and post-Pneumovax vaccination samples were available from 32 adults diagnosed with primary antibody deficiencies (PAD, median age 53 years, range 19–75 years, 15:17 M:F) according to ESID guidelines (Hospital Universitario Central de Asturias, Oviedo, Spain): 16 common variable immunodeficiency (CVID, median IgG 2.7 g/L, range 0.58–3.8; IgA 0.07 g/L, range 0.07–0.61; IgM 0.45 g/L, range 0.14–2.6), 5 hypogammaglobulinemia (HYPO median IgG 5 g/L, range 4–5.3; IgA 0.78 g/L, range 0.6–0.9; IgM 0.34 g/L, range 0.14–0.43), 4 IgA deficiency (IgAD median IgG 8.6 g/L, range 6.4–11; IgA 0.07 g/L, range 0.07–0.09; IgM 0.56 g/L, range 0.28–0.88) and 7 IgG deficiency (IgGD median IgG 4.6 g/L, range 1.6–5.65; IgA 1.86 g/L, range 1.1–2.8; IgM 0.8 g/L, range 0.55–1.3).

Prior history of respiratory tract infections for the 32 patients was documented by consultant immunologists. Patients were grouped according to whether the individual had a history of recurrent infection or not. Ethics were approved by El Comite de Ethica de la Investigacion del Principado de Asturias ha evaluado del Estudo no 153/15.

Total immunoglobulins were measured using a IMMAGE 800 nephelometric analyzer (Beckman Coulter, Inc). Serum specific IgG, IgA, and IgM
antibodies were measured using commercial ELISA kits (VaccZyme Pneumococcal capsular polysaccharide (PCP) IgG, IgA, and IgM ELISAs, The Binding Site Group Ltd., Birmingham, UK).

Adult reference ranges for individuals with unknown vaccination status were used ([8], 95% CI): PCP IgG, 11–265 mg/L; PCP IgA, 7–125 U/mL; and PCP IgM, 17–140 U/mL. Lower and upper limit of the normal reference ranges were used as cut offs (LLNR and ULNR). A responder was defined as an individual with a concentration of antibodies above the ULNR post vaccination. A Non Responder was defined as an individual with a concentration of antibodies lower than the ULNR post vaccination.

Correlation between total immunoglobulin concentrations and PCP IgG, IgA, and IgM pre- and post-vaccination concentrations were assessed by linear regression analysis. Fishers exact test and Mann Whitney U test were used to compare concentrations above or below LLNR and ULNR to prior history of infections. One-way ANOVA was used to compare antibody concentrations between different PAD classifications. A \( p \)-value <0.05 were considered to be statistically significant.

Results and discussion

A number of PAD patients had pre-vaccination PCP IgG, IgA, and IgM concentrations below the LLNR. With the exception of PCP IgM, this was significantly higher than the number of individuals below LLNR in the control group (Table 1, Figure 1). In addition, a number of PAD patients had post vaccination PCP IgG, IgA, and IgM concentrations below the ULNR. This was lower than the number of individuals with concentrations below the ULNR in the control group. 4/32 (13%) had concentrations of all 3 PCP isotypes within their respective reference ranges. PAD patients with a history of prior infection had lower pre and post vaccination concentrations of PCP IgG, IgA, and IgM than those without history of infection (Table 2). PAD patients without a history of prior infection had significantly lower pre-vaccination concentrations of PCP IgG and IgA than those individuals in the

| Table 1. Number and percentage of individuals with PCP IgG, IgA, and IgM pre vaccination concentrations below the lower limit of the reference range (LLNR) and post-vaccination concentrations below the upper limit of the reference ranges (ULNR). PCP IgG, IgA, and IgM were measured in the Control group (n = 30) and PAD patients (n = 32). Reference ranges were used to establish concentrations below LLNR and ULNR. [8] |
|---------------------------------|---------------------------------|---------------------------------|
|                                   | Control group                  | PAD group                       |
|---------------------------------|---------------------------------|---------------------------------|
| Pre vaccination (below LLNR)     | Post vaccination (below ULNR, % Non Responders) |
| PCP IgG (mg/L)                  | 0/30 (0%)                      | 16/32 (50%)                     |
| PCP IgA (U/mL)                  | 2/30 (7%)                      | 14/28 (50%)                     |
| PCP IgM (U/mL)                  | 2/30 (7%)                      | 7/32 (22%)                      |
|                                 | Control group                  | PAD group                       |
|                                 | 30/30 (100%)                   | 22/32 (69%)                     |
|                                 | 30/30 (100%)                   | 19/28 (68%)                     |
|                                 | 30/30 (100%)                   | 17/32 (53%)                     |

*PCP IgA was not measured in 4 individuals with IgAD (total n = 28 PAD patients).
Figure 1. The percent of individuals with pre-vaccination concentrations below LLNR or percentage non responders post vaccination for PCP IgG, IgA, and IgM. PCP IgG, IgA and IgM concentrations were measured in 32 patients with PAD and reference ranges were used to establish concentrations below LLNR or Non Responder status. Pre-and post-vaccination status for each PAD is shown.
Control group. Non responders had a higher percentage of individuals with a history of prior infection than the responder group (Table 3).

There was not significant association between the concentration of total immunoglobulins and the pre- and post-vaccination concentrations for any of the pneumococcal immunoglobulins (p = 0.3–1.0 and p = 0.1–1.0, respectively).

With the exception of individuals with IgAD, all PADs had individuals with pre-vaccination PCP IgG, IgA, and IgM concentrations below the LLNR (Figure 1). All PADs had PCP IgG, IgA and IgM Non Responders. The difference between the pre vaccination concentrations of the PAD groups was not significant (p = 0.2–0.4). There were no differences in PCP IgG and IgM post vaccination concentrations between the different PAD groups (p = 0.1–0.2) but there was a significant difference between the post vaccination PCP IgA concentrations (p = 0.01).

The post vaccination PCP IgA and IgM concentrations identified four groups of responses (IgA/IgM non responders, IgA only responders, IgM only responders and IgA/IgM responders; Figure 2). The percentage of

### Table 2.

|                     | Control group | PAD without history of infection | p value† | PAD with history of infection | p value†† |
|---------------------|---------------|---------------------------------|----------|-------------------------------|-----------|
| Number of individuals | 30            | 15                              | NA       | 17                            | NA        |
| Pre vaccination     |               |                                 |          |                               |           |
| PCP IgG (mg/L)      | 32 (20–260)   | 22 (3.3–113)                    | <0.0001  | 8 (3.3–74)                    | 0.034     |
| PCP IgA* (U/mL)     | 20 (6–115)    | 7 (0–128)                       | 0.006    | 6 (0–56)                      | 0.73      |
| PCP IgM (U/mL)      | 40 (13–129)   | 43 (0–270)                      | 0.41     | 21 (0–180)                    | 0.049     |
| Post vaccination    |               |                                 |          |                               |           |
| PCP IgG (mg/L)      | NA            | 309 (4–780)                     | NA       | 29 (3.3–565)                  | 0.04      |
| PCP IgA* (U/mL)     | NA            | 23 (0–149)                      | NA       | 5 (0–384)                     | 0.02      |
| PCP IgM (U/mL)      | NA            | 171 (2–292)                     | NA       | 75 (0–392)                    | 0.7       |

*PCP IgA was not measured in 4 individuals with IgAD (total n = 28 PAD patients).
†Control group vs PAD without history of infection.
††PAD without history of infection vs. PAD with history of infection.
NA=not applicable

### Table 3.

|                     | PCP IgG | PCP IgA* | PCP IgM |
|---------------------|---------|----------|---------|
| Non Responder       | 22      | 20       | 40      |
| Responder           | 10      | 19       | 17      |
| Percentage of individuals with a prior history of infection | 55 | 68 | 65 |

*PCP IgA was not measured in 4 individuals with IgAD (total n = 28PAD patients).
individuals with a history of prior infection was higher in those with no PCP IgA and IgM responses (10/14, 71%) > IgM only responders (3/5, 60%) > IgA and IgM Responders (3/7, 43%). IgA only responders were not included as this consisted of only 2 patients.

The post vaccination concentrations of PCP IgA and IgM in individuals with abnormal and normal PCP IgG concentrations are shown in Table 4. With one exception, in both PCP IgG populations, a higher percentage of individuals with PCP IgA and IgM concentrations below ULNR had a history of prior infection. In PAD patients with normal pre vaccination concentration of PCP IgG (n = 16, Table 1), 6 patients had IgA and IgM pre vaccination concentrations below LLNR (3 IgA and 3 IgM), 4 were non responders for either PCP IgA and/or IgM. In PAD patients with normal PCP IgG post vaccination (n = 10), 8 patients were IgA and IgM non responders (4 IgA and 4 IgM), with 6 non responders in either PCP IgA and/or IgM. Since 2 of these patients would have been identified at pre vaccination, 4 further PCP IgA and/or IgM non responders would have been identified. 8/26 (31%) patients with normal PCP IgG at pre and post vaccination would have been identified as PCP IgA and/or IgM non responders.

Assessment of the adaptive immune response currently relies on measurement of the IgG response to vaccination. Measurement of additional immunoglobulins, IgA and IgM, may provide complete identification of an abnormal antibody response. We hypothesised that the pneumococcal IgA and IgM concentrations and responses may be compromised in individuals with antibody deficiencies. In addition, that this may correlate with a history of prior infection.
The number of PAD patients with PCP IgG, IgA and IgM pre-vaccination concentrations below the LLNR was higher than in the Control group. The pre-vaccination concentration of PCP IgG and IgA in PAD patients without infection were significantly lower than that in the Control group.

There was no significant association between the concentration of total immunoglobulins and the pre and post vaccination concentrations of any of the pneumococcal immunoglobulins. A large number of individuals had pre- and post-vaccination PCP IgG, IgA, and IgM concentrations below the lower and upper limit of the normal reference ranges. In individuals with both normal and abnormal PCP IgG concentrations between 40–83% individuals had PCP IgA, PCP IgM, or both IgA and IgM lower than their respective normal reference ranges. Only 4/32 (13%) had the concentrations of all 3 pneumococcal antibodies within the reference ranges post vaccination suggesting that the majority of antibody deficient patients do not have pneumococcal responses to all 3 immunoglobulins.

Individuals with a history of prior infection had lower pre- and post-vaccination concentrations of PCP IgG, IgA, and IgM. In individuals with both normal and abnormal PCP IgG concentrations between 40–83% individuals had PCP IgA, PCP IgM, or both IgA and IgM lower than their respective normal reference ranges. Only 4/32 (13%) had the concentrations of all 3 pneumococcal antibodies within the reference ranges post vaccination suggesting that the majority of antibody deficient patients do not have pneumococcal responses to all 3 immunoglobulins.

The IgA and IgM responses to pneumococcal vaccination may differentiate between transient and permanent immunodeficiencies[6] and has been shown to correlate to the number of individuals with a history of infection in CVID patients receiving IVIG.[7]

In this study, we have extended the utility of the observation in Cavaliere[7] to more than CVID patients with the inclusion of HYPO, IgAD, and IgGD

**Table 4.** PCP IgA and/or IgM concentrations above or below ULNR in individuals with normal and abnormal PCP IgG post vaccination concentrations. PCP IgA and IgM were measured in individuals with abnormal PCP IgG (n = 22) and normal PCP IgG (n = 10) post vaccination. PCP IgA and IgM reference ranges were used to establish concentrations below or above the ULNR.[8]

|                          | Number of individuals with a concentration <ULNR (%) | Number of individuals with a concentration >ULNR (%) | Number of individuals with a history of infection (%) |
|--------------------------|------------------------------------------------------|------------------------------------------------------|------------------------------------------------------|
| Abnormal PCP IgG (<265 U/mL) | PCP IgM Only                                    | 13/22 (59)                                           | 9/22 (41)                                            | 3 (33)                                               |
|                          | PCP IgA Only*                                    | 15/18 (83)                                           | 11/18 (61)                                           | 1 (33)                                               |
|                          | PCP M and PCP IgA                                | 12/18 (67)                                           | 9/18 (50)                                            | 3 (50)                                               |
| Normal PCP IgG (>265 U/mL) | PCP IgM Only                                    | 4/10 (40)                                            | 2/10 (20)                                            | 2 (33)                                               |
|                          | PCP IgA Only*                                    | 4/10 (40)                                            | 3/10 (30)                                            | 2 (33)                                               |
|                          | PCP M and PCP IgA*                               | 2/10 (20)                                            | 2/10 (20)                                            | 3 (38)                                               |

*PCP IgA was not measured in the IgAD patients.
patients. We show that the PCP IgA and IgM responses are compromised in other PADs in addition to CVID. In addition, using patients samples obtained prior to treatment, we also show that the IgA and IgM responses correlate with a prior history of infection which complements the observations reported in Cavaliere et al.[7] Measurement of PCP IgA and IgM before treatment maybe important to support initial treatment decisions that a clinician may consider or indeed define any watch and wait strategy. Once receiving treatment, measurement of PCP IgA and IgM may risk stratify the patients further to aid the decision of whether to continue, change or cease certain treatments. A further strength of the study is that the PCP IgA and IgM responses have been interpreted using reference ranges developed in the laboratory with volunteer serum samples. Cavaliere developed PCP IgA and IgM concentration cutoffs based on the CVID population.[7] This will support interpretation in the clinical laboratory.

Conclusions

31% of individuals with PCP IgG concentrations within the reference range, had abnormal concentrations of PCP IgA and IgM. Abnormal concentrations correlate to a higher percentage of individuals with a history of infection.

References

1. Woof, J.M.; Kerr, M.A. The Function of immunoglobulin A in Immunity. *J. Pathol.* 2006, 208, 270–82.
2. Schutz, K.; Hughes, R.G.; Parker, A.; Quinti, I.; Thon, V.; Cavaliere, M.; Wurfel, M.; Herzog, W.; Gessner, J.E.; Baumann, U. Kinetics of IgM and IgA Antibody Response to 23-Valent Pneumococcal Polysaccharide Vaccination in Healthy Subjects. *J. Clin. Immunol.* 2012, 33, 288–298.
3. Bonilla, F.A.; Bernstein, I.L.; Khan, D.A.; Ballas, Z.K.; Chinen, J.; Frank, M.M.; Kobryninski, L.J.; Levinson, A.I.; Mazer, B.; Nelson, R.P., Jr.; Orange, J.S.; Routes, J.M.; Shearer, W.T.; Sorensen, R.U. Practice Parameter for the Diagnosis and Management of Primary Immunodeficiency. *Ann. Allergy Asthma Immunol.* 2005, 94, S1–63.
4. Bonilla, F.A.; Khan, D.A.; Ballas, Z.K.; Chinen, J.; Frank, M.M.; Hsu, J.T.; Keller, M.; Kobryninski, L.J.; Komarow, H.D.; Mazer, B.; Nelson, R.P., Jr.; Orange, J.S.; Routes, J.M.; Shearer, W.T.; Sorensen, R.U.; Verbsky, J.W.; Bernstein, D.I.; Blessing-Moore, J.; Lang, D.; Nicklas, R.A.; Oppenheimer, J.; Portnoy, J.M.; Randolph, C.R.; Schuller, D.; Spector, S.L.; Tilles, S.; Wallace, D.; Bonilla, F.A.; Khan, D.A.; Bernstein, D.I.; Blessing-Moore, J.; Khan, D.; Lang, D.; Nicklas, R.A.; Oppenheimer, J.; Portnoy, J.M.; Randolph, C.R.; Schuller, D.; Spector, S.L.; Tilles, S.; Wallace, D.; Bonilla, F.A.; Ballas, Z.K.; Chinen, J.; Frank, M.M.; Hsu, J.T.; Keller, M.; Kobryninski, L.J.; Komarow, H.D.; Mazer, B.; Nelson, R.P., Jr.; Orange, J.S.; Routes, J.M.; Shearer, W.T.; Sorensen, R.U.; Verbsky, J.W. Practice Parameter for the Diagnosis and Management of Primary Immunodeficiency. *J. Allergy Clin. Immunol.* 2015, 136, 1186–205.
5. Orange J.S.; Ballow M.; Stiehm E.R.; Ballas Z.K.; Chinen J.; De La Morena M.; Kumararatne D.; Harville T.O.; Hesterberg P.; Koleilat M.; McGhee S.; Perez E.E.;
Raasch J.; Scherzer R.; Schroeder H.; Seroogy C.; Huissoon A.; Sorensen R.U.; Katial R. Use and interpretation of diagnostic vaccination in primary immunodeficiency: A working group report of the Basic and Clinical Immunology Interest Section of the American Academy of Allergy, Asthma & Immunology. *J. Allergy Clin. Immunol.* 2012, 130, S1–24.

6. Moschese, V.; Cavaliere, F.M.; Graziani, S.; Bilotta, C.; Milito, C.; Chini, L.; Quinti, I. Decreased IgM, IgA, and IgG Response to Pneumococcal Vaccine in Children with Transient Hypogammaglobulinemia of Infancy. *J Allergy Clin Immunol* 2016, 137, 617–619.

7. Cavaliere, F.M.; Milito, C.; Martini, H.; Schlesier, M.; Drager, R.; Schutz, K.; Brunetti, G.; Pesce, A.M.; Thon, V.; Warnatz, K.; Quinti, I. Quantification of IgM and IgA Anti-Pneumococcal Capsular Polysaccharides by a New ELISA Assay: a Valuable Diagnostic and Prognostic Tool for Common Variable Immunodeficiency. *J. Clin. Immunol.* 2012, 33, 838–846.

8. Parker, A.R.; Allen A.; Harding S. Concentration of Anti-pneumococcal Capsular Polysaccharide IgM, IgG, IgA Specific Antibodies in Adult Blood Donors. *Pract. Lab. Med.* 2016, 1–5.