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COVID-19 vaccination antibody responses in patients with Aplastic Anaemia and Paroxysmal Nocturnal Haemoglobinuria

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Paroxysmal Nocturnal Haemoglobinuria (PNH) and Aplastic Anaemia (AA) represent part of a spectrum of rare, potentially life-threatening, bone marrow failure disorders that are thought to result from an autoimmune attack targeting normal bone marrow haematopoietic stem cells. There is concern that patients with these disorders may be less able to mount an effective immune response due to their underlying disease and/or treatment-related immunosuppression, and are at risk of more severe SARS-CoV-2 infections. In the Leeds PNH service we have seen suboptimal meningococcal vaccine responses in up to 42% of patients on anti-complement therapy (unpublished data).

We performed a post implementation real-world prospective observational study to investigate the antibody response to SARS-CoV-2 vaccination in adult patients with AA and/or PNH. All patients under the care of the UK PNH National Service in Leeds were eligible, except for prior allogeneic bone marrow transplantation (detailed methods, appendix p1). Blood samples were obtained at timepoints pre-vaccination and 4-6 weeks post each vaccination. Serum spike-specific composite IgA, IgG and IgM antibodies were tested at the Leeds University laboratory using an enzyme-linked immunosorbent assay (ELISA, The Binding Site Group™). Responses from all patients and healthy controls were compared. Post-hoc subgroup analyses comprised the variables: diagnosis (classical PNH, AA/PNH overlap and AA), age, calcineurin inhibitor (CNI) therapy, vaccine type and, in patients with PNH on complement inhibitory treatment, prior history of a suboptimal meningococcal vaccination response.

SARS-CoV-2 antibody responses were measured in 175 patients and 45 healthy volunteers (demographics, appendix p5). Four patients were excluded from final analysis due to concurrent immunosuppression for other indications. After one vaccination, patients had a markedly reduced seroconversion rate of 63·4% (n=83/131) compared with 95·1% (n=39/41) of healthy volunteers which was similar across patient subgroups (63·2% in classical PNH, 69·7% in AA/PNH overlap and 50% in AA) (Figure 1). Overall, patients showed a 2·4-fold lower antibody response compared to healthy volunteers (median result 1·2 [95% CI 1·1-1·4]; p<0·0001 versus 2·9 [95% CI 2·0-3·7]). The absolute antibody levels were significantly lower in all patient subgroups (classical PNH median antibody result: 1·1 [95% CI 2·0-3·7]; AA/PNH: 1·4 [95% CI 1·0-1·8]; AA: 1·2 [95% CI 0·7-1·8]; healthy volunteers: 2·9 [95% CI 2·0-3·7]; p<0·0001). After second vaccination, seropositivity and magnitude of antibody responses improved, equivalent to healthy volunteers. Overall patient seroconversion rates

were 99·4% (n=155/156) compared with 97·6% (n=40/41) healthy volunteers, with no difference in antibody levels (patient median antibody result: 3·3 [95% CI 3·0-3·6]; healthy volunteers: 4·0 [95% CI 3·6-4·5]; p=0·0968). Healthy volunteers showed a 38·6% increase in antibody response between first and second vaccinations (p=0·0002) and marked improvements were seen across all patient subgroups (classical PNH: 175·2% increase, p<0·0001; AA/PNH: 184·2% increase, p<0·0001; AA: 147·1% increase, p=0·0034) (appendix p7).

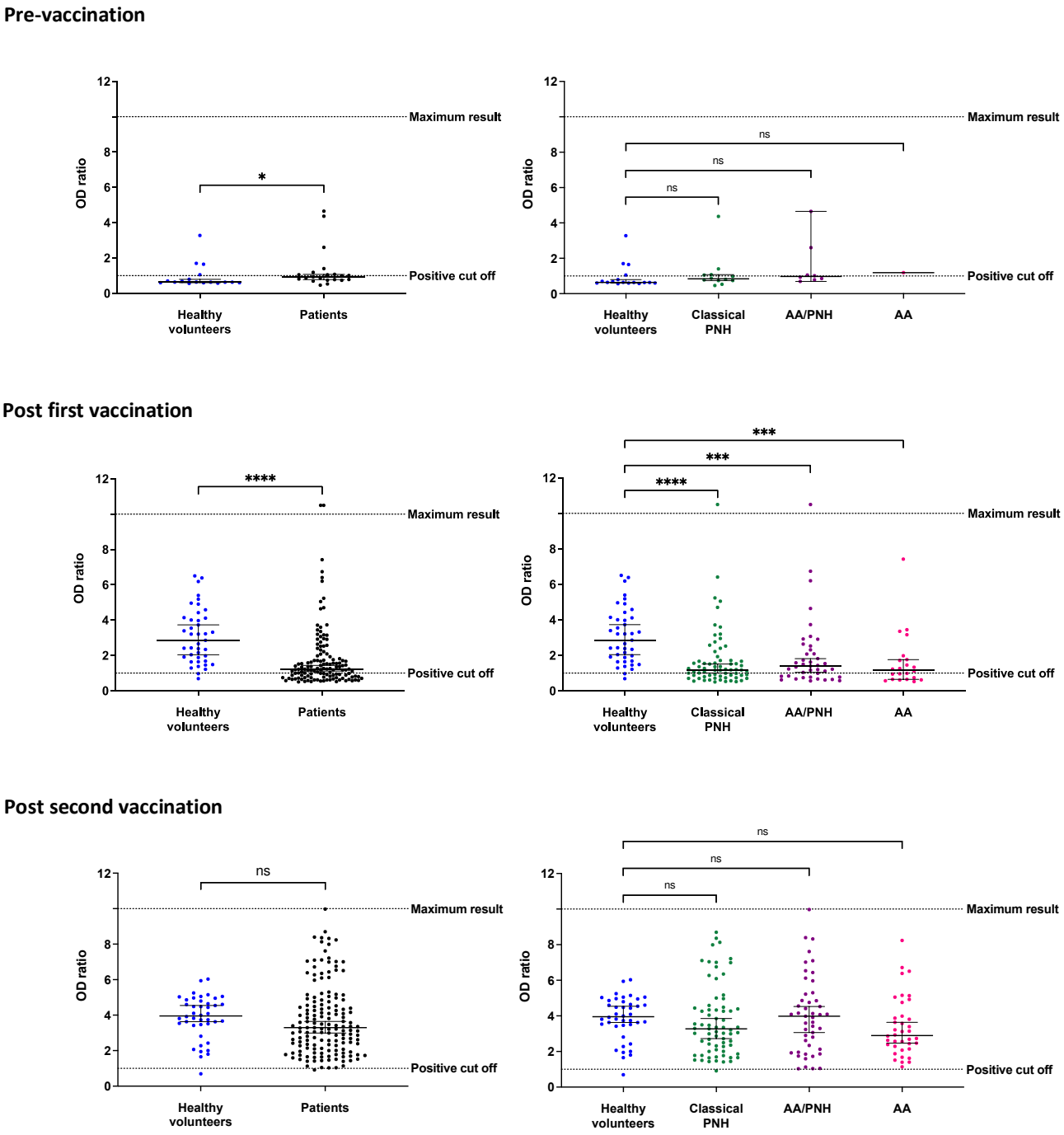

Figure 1: Anti SARS-CoV-2 spike-specific IgA/G/M antibody responses pre and post first and second vaccination in healthy volunteers and patients. The OD ratio is a calibrated optical density result calculated as per manufacturer's guidelines with a cut-off for positivity of 1, indicated by the dotted line. Dot plots show antibody results for individuals, each dot represents the mean of experimental duplicates. Error bars show median and 95% confidence intervals. Results that were above the maximum optical density reading were assigned a number above the maximum result. Statistical comparisons between groups not otherwise shown were not significant. n, number; ns, not significant (>0·05); *p<0·05; **p<0·01; ***p<0·001; ****p<0·0001.

In univariate analysis, current CNI therapy did not show a significant difference in antibody response (appendix p8). Advancing age was found to be moderately inversely correlated (r(39)=-0·61, p<0·0001) with antibody results in healthy volunteers after first vaccination and weakly correlated after second vaccination (r(40)=-0·35, p=0·0246). However, no significant correlation was seen in any patient groups after either vaccination (appendix p9). Increased antibody levels were seen after two vaccinations in patients who received BNT162b2 (median result: 4·52, [95% CI 3·58-5·3] versus ChAdOx1-S: 2·81, [95% CI 2·42-3·29]; p<0·0001). A similar pattern was seen in the healthy volunteers (appendix p11). A prior history of suboptimal response to meningococcal vaccination was not associated with a reduced antibody response following two SARS-CoV-2 vaccinations (appendix p12).

Multivariate analysis was conducted to see if the patient group association seen in univariate models could be explained by potential confounding factors. Patient group versus healthy volunteers was significantly associated with non-response to first vaccination even after controlling for other variables (OR: 9·097, 95% CI [1·493 – 55·429]; $p = 0·017$). Female sex versus male (OR: 2·635, 95% CI [1·241 – 5·594]; $p = 0·012$) and receiving ChAdOx1-S (OR: 2·520, 95% CI [1·095 – 5·799]; $p = 0·03$) also had significant associations with non-response. Other variables were not significant (appendix p13). No significant association was seen for any variable and non-response after second vaccination (data not shown).

Real-world post-implementation data is vital for understanding the effectiveness of vaccination programmes in rare diseases that are minimally represented in clinical trials. Scheinberg et al have shown impaired humoral immune responses in other haematological diseases, autoimmune disorders and patients on immunosuppressants.[1] This is the largest study to our knowledge providing outcome data in patients with PNH and AA.

Protective SARS-CoV-2 antibody levels are currently unknown. Before vaccines were available, we reported adverse clinical outcomes of COVID-19 in our patients[2] and 5 patients known to the National PNH Service in Leeds died due to SARS-CoV-2 infection (unpublished data). No vaccinated patient enrolled in the present study has died within 28 days of a positive SARS-CoV-2 test which supports that the achieved antibody responses are clinically significant. Breakthrough COVID-19 infections post second vaccination have occurred in 16 patients in the study at the time of writing. 15/16 were on complement

inhibitor therapy. All patients had minor illness except one who required hospital admission but recovered without intensive care or ventilatory support.

The reason for the difference in response after one but not two vaccines is unclear. Whilst our healthy volunteer and patient cohorts were not perfectly age and sex matched which limits the direct comparability of the groups, the seroconversion rates seen are in line with other studies.[3] Limitations of our data include the small sample size, as is the challenge in evaluating rare diseases, and, due to the inception of the study at the time of UK vaccination programme rollout, very few samples could be obtained pre-vaccination. Overall, when compared with studies in the general UK population,[3] lower responses after first vaccine were noticed in our patient cohorts, in line with previous data on other diseases treated with immunosuppression.[1]

Individuals receiving B-cell depleting therapies have shown reduced humoral responses to SARS-CoV-2 vaccination in other studies,[4] and we know that patients with AA and PNH have markedly reduced absolute B cell numbers.[5] However, the responses seen after second vaccination were considerably better than those seen in other disorders such as haematological malignancies.[1] Another interesting question raised is whether complement inhibition itself may impair vaccine responses. Complement components can enhance antibody mediated viral neutralisation and have demonstrated importance in determining responses to other vaccinations,[6] although there is limited understanding in the role in SARS-CoV-2 viral mediated immunity which merits further study.

It is reassuring to note current CNI therapy did not impact responses. CNI therapy in studies of other diseases including renal disorders report variable impacts on humoral response.[7] A large number of our patients had prior ATG therapy, and were able to mount a good response to vaccination. However, the study did not recruit any who received ATG therapy within 12 months of vaccination, when the effect on immune responses is likely to be most profound. Interestingly, patients with a prior history of suboptimal response to meningococcal conjugate vaccination still mounted a response to two SARS-CoV-2 vaccinations (appendix p12). This prompts the question as to whether the improved response observed is due to more efficacious novel mRNA and viral vector vaccine technologies or whether polysaccharide conjugate vaccines are poorly immunogenic in our patients.

Vaccination side effects (appendix p14) were comparable with published studies.[8] There were a small number of adverse events, including one superficial femoral vein thrombosis, one case of Vaccine Induced Thrombotic Thrombocytopenia, four cases of breakthrough haemolysis, and two cases of transient drops in haematological parameters in the aplastic anaemia cohort. It is reassuring that the majority of patients on complement inhibitors were vaccinated without significant symptomatic breakthrough haemolysis. There have been case reports of de-novo AA post vaccination,[9] but COVID-19 infection itself currently appears to pose the greater risk for this occurring.[10] It is conceivable that these events and reported side effects are simply temporally related rather than directly attributable to vaccination. Longer follow up of larger cohorts is required to further characterise rare adverse events. However, benefits of vaccination continue to significantly outweigh any potential risk.

Our data stress the importance of at least two vaccinations in patients with PNH and/or a history of AA in order to achieve a good SARS-CoV-2 antibody response and it is expected that they will benefit from booster programmes. Whilst we have shown robust antibody levels following two vaccinations, further studies are required to determine longevity of response, degree of effective IgG responses, T-cell responses and long-term infection outcomes.

Declaration of interests

None declared

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Author contributions

AP, DN, PM, SR, DP, MG, RK drafted and edited the manuscript. DN, PH, AP obtained funding. DN, PH, AP, SR, CM and DP designed the study. AP, CM, BF, EC, RS, ES, TZ, PM, PH, MG, RK, TM, SR, LA, AT, NH, NY, CB identified and recruited patients and collected clinical data. AP, CM, MH, DC carried out the laboratory work. AP, DN, JD completed the data analysis. All authors had full access to all of the data and reviewed and approved the final manuscript.

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References

1. Kearns P, Siebert S, Willicombe M, et al. Examining the Immunological Effects of COVID-19 Vaccination in Patients with Conditions Potentially Leading to Diminished Immune Response Capacity – The OCTAVE Trial. 2022.
2. Pike A, Muus P, Munir T, et al. COVID-19 infection in patients on anti-complement therapy: The Leeds National Paroxysmal Nocturnal Haemoglobinuria service experience - Pike - 2020 - British Journal of Haematology - Wiley Online Library. 2021.
3. Wei J, Stoesser N, Matthews P, et al. Antibody responses to SARS-CoV-2 vaccines in 45,965 adults from the general population of the United Kingdom. *Nature microbiology* 2021; **6**(9).
4. Herishanu Y, Avivi I, Aharon A, et al. Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. *Blood* 2021; **137**(23).
5. Richards S, Dickinson A, Cullen M, et al. Presentation clinical, haematological and immunophenotypic features of 1081 patients with GPI-deficient (paroxysmal nocturnal haemoglobinuria) cells detected by flow cytometry. *British journal of haematology* 2020; **189**(5).
6. Kurtovic L, Beeson J. Complement Factors in COVID-19 Therapeutics and Vaccines. *Trends in immunology* 2021; **42**(2).
7. Stumpf J, Siepmann T, Lindner T, et al. Humoral and cellular immunity to SARS-CoV-2 vaccination in renal transplant versus dialysis patients: A prospective, multicenter observational study using mRNA-1273 or BNT162b2 mRNA vaccine. *The Lancet regional health Europe* 2021; **9**.
8. Menni C, Klaser K, May A, et al. Vaccine side-effects and SARS-CoV-2 infection after vaccination in users of the COVID Symptom Study app in the UK: a prospective observational study. *The Lancet Infectious diseases* 2021; **21**(7).
9. Tabata S, Hosoi H, Murata S, Takeda S, Mushino T, Sonoki T. Severe aplastic anemia after COVID-19 mRNA vaccination: Causality or coincidence? *Journal of autoimmunity* 2022; **126**.
10. Avenoso D, Marsh J, Potter V, et al. SARS-CoV-2 infection in aplastic anaemia. *Haematologica* 2021.