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Third-dose SARS-CoV-2 mRNA vaccine increases Omicron variant neutralisation in patients with chronic myeloid disorders

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Abstract:

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To the Editor:

Concerns remain over the response to vaccination against SARS-CoV-2 in patients with haematological malignancy. We and others have reported high serological and T cell response rates to vaccination in patients with chronic myeloid neoplasms.(1-3) However, some patient groups were identified with a suboptimal response necessitating further evaluation of the effects of additional vaccine doses.(4) Moreover, the degree of immune response offered by current vaccines against variants of concern also requires further evaluation. To address these concerns, we performed a comprehensive immunological evaluation of the response to SARS-CoV-2 vaccination in patients with chronic myeloid neoplasms (MPN) after three doses of vaccine, including neutralising titres against the Omicron variant and T cell responses.

Antibody response was assessed using anti-Spike (anti-S) IgG with anti-nucleocapsid (anti-N) IgG used to determine previous infection, as described previously.(2) Neutralising antibody analysis was performed by assessing inhibitory effect of plasma on entry of HIV-1 particles pseudotyped with Wuhan or Omicron BA.1 Spike proteins into cells expressing the ACE2 receptor.(2) T cell response was evaluated using a fluorospot assay assessing IFN γ /IL-2 secretion upon re-exposure to S peptides (Mabtech, Supp. Methods). Plates were analysed using the IRIS reader providing both spot-forming unit (SFU) frequency and relative spot volume (RSV).

To date samples have been collected from 104 patients with MPN or post allo-SCT in total. Testing was performed in 61 after 2 doses and 33 patients after 3 doses. Generalized mixed linear model, regular t-test, Fisher exact test and Pearson correlation coefficients were used as appropriate for hypothesis testing. Samples were collected at a median of 49 days after a second dose of vaccine (range 22-88) and 43 days after a third dose (27-72). This study received ethical approval from the Edgebaston Research and Ethics Committee, IRAS identification 285396 - 20/WM/0187/AM04 and was conducted in accordance with the Declaration of Helsinki.

Serological analysis was performed after a third dose of mRNA vaccine in 33 patients including chronic myeloid leukaemia (CML, n=13), essential thrombocythaemia (ET, n=4), polycythaemia vera (PV, n=6) and myelofibrosis (MF, n=10). Of the MPN patient cohort, treatments included ruxolitinib (n=9), hydroxycarbamide (n=4), pegylated interferon-alpha (n=3) and active surveillance (n=4). Of the CML cohort, TKI therapy included nilotinib (n=5), dasatinib (n=2), bosutinib (n=1) and ponatinib (n=5). Patient characteristics of those analysed after a third dose are summarised in Supplemental Table 1.

In 24 paired samples and excluding those with elevated anti-N optical density (n=4), a statistically significant increase was seen after 3 doses in mean anti-S IgG EC50 (4094 vs 1265 after two doses; p= 0.006). Similarly, mean neutralising antibodies against Wuhan Spike pseudotypes were 231 after 2 doses and 1461 after 3 doses in paired samples (n=19, p=0.05).

Mean neutralising antibodies titres against the Omicron pseudotype from 19 paired samples increased from 82 after two doses to 365 after a third dose (p=0.026, Figure 1a). After three doses of vaccine patients from the initial cohort were more likely to have detectable neutralising antibodies against Omicron with 95.7% having detectable response (ID₅₀ > 25) compared with 70% after two doses (18/19 vs 13/19, p=0.062, Figure 1b). However, the mean level of Wuhan Spike neutralising antibodies was significantly higher than the mean level of Omicron neutralising antibodies after 2 doses of vaccine (241 vs 116, p= 0.026, Figure 1c). Similarly, after three doses mean neutralising antibody titres were 1168 against Wuhan spike compared with 357 against Omicron (p=0.075, Figure 1d).

T cell fluorospot analysis was performed in 30 patients. A positive response was observed in 90% (27/30) after a third dose, with similar response rate as observed after two doses (88.3%, 53/60). Significantly higher mean RSV was observed for IFN γ in polyfunctional cells compared with monofunctional (21885 vs 9184, $p < 0.001$). After a third vaccine dose IFN γ SFUs and RSV were, however, significantly higher at 242 and 9184 vs 72 and 5031 respectively after the second dose ($p < 0.001 / < 0.001$, Figure 2 a,b). Of note, we observed an association in the time between a third dose of vaccine and sampling and reduction in T cell reactivity, as indicated by SFUs for IL-2 ($r = -0.5$, $p = 0.026$; Supplementary Figure 1). There was no significant trend observed for other indicators of vaccine response and time between vaccine dose and sampling.

We next assessed the impact of therapy on T cell responses. After 3 doses patients on ruxolitinib (a JAK1/2 inhibitor) had lower SFU and RSV of IFN γ than other patients at 79 and 7690 vs 302 and 9727 ($p = 0.004 / 0.015$, Figure 2 c,d). Patients taking ruxolitinib also had reduced polyfunctional IFN γ and IL-2 RSV compared to other patients with 9425 vs 22437 IFN γ RSV and 1858 vs 4771 IL-2 RSV respectively ($p = 0.047 / p = 0.022$). Finally, patients on ruxolitinib were also more likely to have a negative T cell response after 3 doses than other patients (4/8 vs 2/22, $p = 0.029$). Patients with MF had lower SFU for IFN γ than patients with CML, PV and ET at 93 vs 294, 224 and 446, $p = 0.075 / 0.349 / 0.024$, Figure 2e) Similarly patients with MF had lower RSV than those with CML, PV and ET at 7532 vs 9528, 10499 and 10630, $p = 0.035 / 0.017 / 0.021$ (Figure 2f). We observed significant correlation between humoral and T cell response, for both anti-S IgG and Wuhan spike neutralising antibody titres, and SFU for IL-2 secreting T cells ($r = 0.4$, $p = 0.03$ and $r = 0.6$, $p = 0.003$ respectively, Supplementary Figure 2 i-ii). We also observed a significant correlation between neutralising titres for Omicron pseudotype and SFU for IFN γ secreting T cells ($r = 0.4$, $p = 0.049$; Supplementary Figure 2 iii).

Of 29 assessed patients, 7 (24.1%) reported confirmed SARS-CoV-2 breakthrough infection after a third dose with two requiring hospitalisation and high dependency unit admission, both of whom were treated with ruxolitinib. We previously reported that, after two doses of vaccine, only one out of 55 patients completing a post-vaccination survey developed confirmed Covid-19 infection, although this was prior to the emergence of the Omicron variant and the related surge in cases.(4) After a third dose, multivariate analysis did not identify neutralising antibody levels towards Omicron, or T cell response as significantly associated with SARS-CoV-2 infection, however there was a trend towards association between breakthrough infection and ruxolitinib treatment (12.31 95% CI 0.54, 770.87, $p = 0.1$).

Patients with haematological malignancy have been identified as a particularly vulnerable group with reduced response to vaccination when compared with other cancer patients.(5) This is increasingly relevant due to the poor outcomes observed with SARS-CoV-2 infection including in patients with chronic myeloid malignancy. However, most studies evaluating immunological vaccine response have reported only on serology, which underestimates the frequency of responders when compared with studies also evaluating cellular response.(6) Moreover, patients with haematological malignancy have been shown to frequently have discordant serological and cellular responses, although we do show moderate correlation between immune responses in our analysis.(7-9)

The Omicron variant is capable of immune escape and impaired efficacy of current vaccines, particularly after two doses of vaccine. A T cell response, however, can be induced by a wide range of epitopes, and vaccine induced memory T cells retain activity against variants in comparison with neutralizing antibodies.(10) As such, the increase in T cell reactivity after 3 doses observed in our analysis is of particular significance in view of the reduced Omicron neutralisation. Our data however demonstrates that cellular response to vaccination continues to be impaired in patients taking ruxolitinib, which is known to have pleiotropic

effects upon the immune system. This may also be reflective of reduced immune function in patients with more advanced MF, typically associated with inflammation, who frequently require JAKi therapy for symptomatic control. Taken together these findings highlight the need for additional approaches for these patients.(11) Finally further longitudinal studies with larger cohorts are required to elucidate long-term efficacy of vaccination against SARS-CoV-2 in this population.

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Contributions:

PH and HdL designed and analysed data and wrote the manuscript. PH performed the research. AK, TL, MM and KJD performed the research and reviewed the manuscript. MD performed statistical analysis and reviewed the manuscript. JS, CS, RD, CW, SA, NCG, JOS, SK, DR DM and CH assisted with patient recruitment and reviewed the manuscript.

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Figure Captions:

Figure 1. Humoral Response to third dose of SARS CoV-2 mRNA Vaccination in patients with Chronic Myeloid Disorders

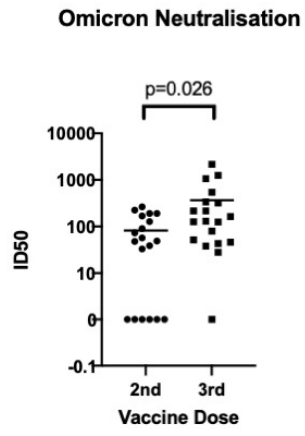
- a) Increased neutralisation of Omicron variant pseudotypes after third vaccine dose compared with after second dose.
- b) Reduced frequency of patients with detectable Omicron neutralising antibody response after second vaccine dose compared with after third dose.
- c) Neutralising antibody response against Wuhan and Omicron pseudotype after two doses of vaccine showing reduced response against Omicron.
- d) Neutralising antibody response against Wuhan and Omicron pseudotype after three doses of vaccine showing reduced

Figure 2: T cell Response to second and third dose of SARS CoV-2 mRNA Vaccination in patients with Chronic Myeloid Disorders

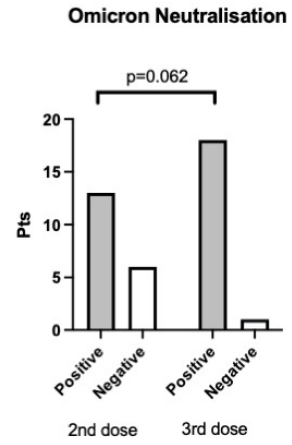
- a) Frequency of IFN γ spot forming units (SFUs) after two and three doses of vaccine showing increased T cell response after third dose.
- b) Increased relative spot volume (RSV) of IFN γ SFUs after three doses of vaccine compared with after two doses.
- c) Reduced frequency of SFUs for IFN γ in patients taking ruxolitinib after three doses of vaccine.
- d) Reduced RSV of IFN γ SFUs in patients taking ruxolitinib after a third dose of vaccine.
- e) Reduced frequency of SFUs for IFN γ in patients with diagnosis of myelofibrosis (MF) when compared with chronic myeloid leukaemia (CML) and essential thrombocythaemia (ET) diagnosis.
- f) Reduced RSV of SFUs for IFN γ in patients with diagnosis of myelofibrosis (MF) when compared with CML, polycythaemia vera (PV) and ET diagnosis.

1

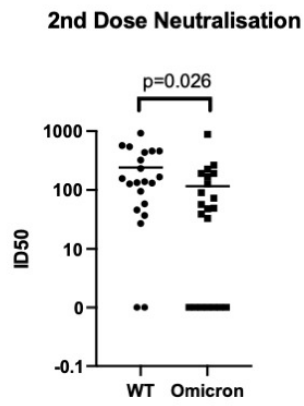
a.



b.



c.



d.

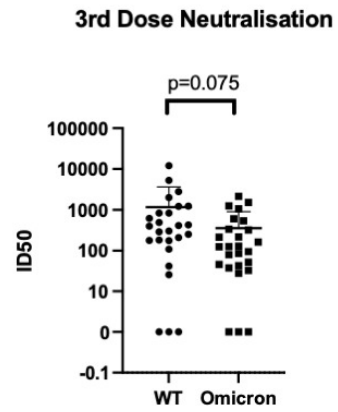


Figure 2

