LETTER

CHRONIC MYELOPROLIFERATIVE NEOPLASMS

Antibody and T-cell responses to SARS-CoV-2 vaccination in myeloproliferative neoplasm patients

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TO THE EDITOR:

Safe and effective vaccines to prevent]. severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections are critical to halt coronavirus disease of 2019 (COVID-19). BNT162b2 (Pfizer/BioNTech) and mRNA-1273 (Moderna) are SARS-CoV-2 mRNA vaccines that are delivered in lipid nanoparticles to express the SARS-CoV-2 spike protein. Completion of the vaccine series results in near 100% development of neutralizing antibodies in healthy adults, as well as elicitation of strong T-cell responses [1]. Hematologic malignancy patients however are known to have decreased immunologic responses to SARS-CoV-2 vaccination [2]. Myeloproliferative neoplasms (MPNs), including chronic myeloid leukemia, essential thrombocythemia, polycythemia vera, and myelofibrosis (MF) remain a vulnerable patient population and are immunocompromised due to impaired innate and adaptive immunity, heightened inflammation, and effects of ongoing treatment [3]. As a result, patients are at high risk for complications related to SARS-CoV-2 infection [4, 5].

We investigated humoral and T-cell responses to the BNT162b2 and mRNA-1273 vaccine series in MPN patients and compared to healthy controls. Although most reports of SARS-CoV-2 immunogenicity have focused on quantifying anti-spike antibodies in peripheral blood, T-cell responses are an essential component of the anti-viral immune response against SARS-CoV-19 [6]. In particular, neutralizing antibody activity is significantly reduced against SARS-CoV-2 variants after vaccination, although T-cell responses still appear to be intact [7, 8]. T-cell responses are therefore particularly important to prevent severe COVID-19 caused by emerging variants such as Omicron. In addition to serologic measurements against the SARS-CoV-2 spike protein, we utilized two IFNγ-release assays, the ELISpot and whole blood assay previously developed in convalescent and vaccinated healthy individuals, to assess T-cell responses in MPN patients 1 month after vaccination [9].

Patients with a diagnosis of MPN by World Health Organization 2016 criteria presenting at Massachusetts General Hospital and eligible for SARS-CoV-2 vaccination were recruited [10]. All patients gave informed consent, and the protocol was approved by the Institutional Review Board. A total of 26 MPN patients were enrolled and had baseline blood samples collected, of whom 21 patients completed vaccination and had 1-month post-vaccination blood samples collected. An additional 7 MPN patients were enrolled who had post-vaccination samples collected only. Table 1 lists baseline characteristics. Data from 26 vaccinated participants with no history of active malignancy were used as healthy controls. Healthy participants were significantly younger compared to MPN patients (median age 36 vs. 62, $p < 0.001$), with no other differences between groups.

To measure serologic responses, qualitative ELISA for human IgG/A/M against SARS-CoV-2 spike protein using donor serum was performed per manufacturer instructions (The Binding Site; Birmingham, UK; sensitivity and specificity 98.4 and 94.7%) [11]. In total, 4/21 MPN patients had positive serology for SARS-CoV-2 at baseline; 3/4 patients had known history of SARS-CoV-2 infection. 27/28 (96%) MPN patients had evidence of positive serology 1-month post-vaccination, compared to 25/26 (96%) healthy controls (Fig. 1A). The one MPN patient who lacked a serologic response was a MF patient on ruxolitinib. We also measured quantitative anti-spike IgG concentrations in MPN and healthy donors per manufacturer instructions (Euroimmun; Lubeck, Germany). There was a trend toward lower median post-vaccination IgG binding antibody units (BAU) [12] in MPN patients compared to healthy controls (1723 vs. 3482 BAU/ml, $p = 0.10$). Median anti-spike concentrations significantly increased from pre- to post-vaccination timepoints in MPN patients (4 vs. 1723 BAU/ml, $p < 0.001$). There were otherwise no significant differences in post-vaccination concentrations based on MPN subtype, age, gender, treatment, or number of days post-vaccine, although there was a trend toward higher concentrations in patients who received the mRNA-1273 vaccine ($p = 0.069$). In the 4 MPN patients who had positive baseline serology, post-vaccination concentrations appeared similar to that of MPN

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Table 1. Cohort characteristics.

	MPN	Control	*p* value
Number of patients vaccinated	28	26	
Age (years; median, range)	62 (25–90)	36 (23–81)	<0.001
Gender (% male)	13 (46%)	14 (51.6%)	0.59
Vaccine type			0.15
BNT162b2	14 (50%)	11 (35.5%)	
mRNA-1273	14 (40%)	19 (61.3%)	
Days post-vaccine (median, range)	40 (13–128)	34 (10–176)	0.36
History of COVID-19	3 (11%)	0	
Diagnosis			
CML	7 (25%)		
PV	4 (14.2%)		
ET	5 (17.9%)		
MF	8 (28.5%)		
Other MPN	4 (14.3%)		
Treatments			
None	6 (20.6%)		
Ruxolitinib	5 (17.2%)		
Hydroxyurea	6 (20.7%)		
Interferon	1 (3.4%)		
TKI	9 (31.0%)		
Other	2 (6.9%)		

CML chronic myeloid leukemia, *ET* essential thrombocythemia, *PV* polycythemia vera, *MF* myelofibrosis, *MPN* myeloproliferative neoplasm, *TKI* tyrosine kinase inhibitor.

patients without SARS-CoV-2 exposure (2611 vs. 1489 BAU/ml, $p = 0.68$).

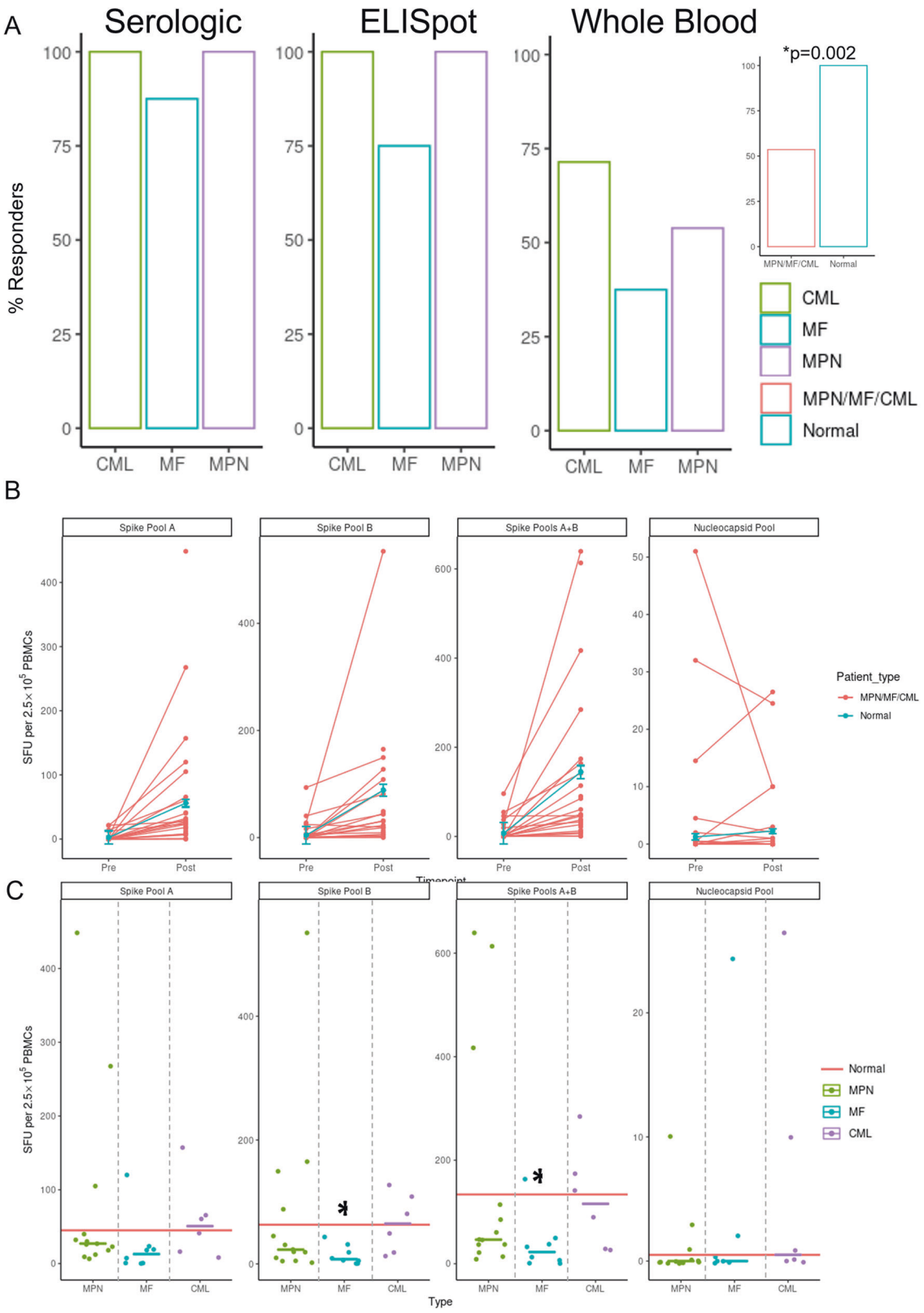
IFNγ ELISpot testing in participants is described elsewhere (Supplementary Fig.) [9]. Briefly, peripheral blood mononuclear cells (PBMCs) were stimulated with commercially available overlapping 15mer peptide pools spanning the SARS-CoV-2 spike (Spike Pool A: AA1-643; Spike Pool B: AA633-1273) and nucleocapsid proteins. PBMCs were incubated with peptide pools (final concentration 2 μg/ml) from Spike Pool A, Spike Pool B, and Nucleocapsid Pool in duplicate wells of the Human IFNγ single-color ELISpot plate (ImmunoSpot; Shaker Heights, OH). A positive threshold was considered if there were a mean of ≥6 spot-forming units (SFUs) per 2.5×10^5 PBMCs to either Spike Pool A or B after subtraction of background, based on prior receiver operator curve (ROC) analysis of ELISpot responses in convalescent donors (sensitivity 90% specificity 92%) [9].

Six MPN patients had a positive ELISpot on baseline testing, including three MPN patients with documented prior infection and one with likely exposure. Due to technical issues, ELISpot testing could not be performed on one post-vaccination sample. In total, 25/27 vaccinated MPN patients (93%) were ELISpot responders on post-vaccination testing, compared to 26/26 (100%) healthy controls ($p = 0.99$) (Fig. 1A). The two MPN patients who lacked an ELISpot response included an MF patient on clinical trial with ruxolitinib and a bromodomain and extra-terminal motif inhibitor, and the MF patient on ruxolitinib who also lacked a serologic response. Median ELISpot SFU's to Spike Pool A, Spike Pool B, and total spike response (Spike A + Spike B) increased across timepoints in MPN patients (Spike A 0–24, $p < 0.001$; Spike B 0–20, $p < 0.001$; total spike 0–46, $p < 0.001$) (Fig. 1B). MPN patients had significantly lower median SFU's to Spike Pool B (20 vs. 63, $p = 0.012$) and trended toward lower total spike response (46 vs. 134, $p = 0.058$) on post-vaccination ELISpot compared to healthy controls (Fig. 1C). MF patients also had significantly lower SFUs to Spike Pool B (8 vs. 63, $p < 0.01$) and total spike responses (22 vs. 134, $p < 0.01$) compared to healthy controls. Post-vaccination ELISpot SFU's to Spike Pool A and total spike response were similar between the 4 MPN patients with evidence of prior SARS-CoV-2 exposure and MPN patients with no history of exposure, although sample sizes are overall limited (Spike Pool A: 44 vs. 19, $p = 0.21$; Spike Pool B: 32 vs. 19, $p = 0.76$; Total Spike: 94 vs. 38, $p = 0.43$). There were no significant increases in ELISpot SFU's to the Nucleocapsid Pool across timepoints. We found that older age was significantly associated with lower post-vaccination SFUs to Spike Pool B ($p < 0.01$) and total spike response ($p = 0.03$) in all cohorts, with no other effects of gender, vaccine type, timing of blood draw relative to vaccination, treatment, or absolute lymphocyte count (ALC). In multivariable linear modeling with both diagnosis and age included, MPN diagnosis was no longer significantly associated with lower SFUs to Spike Pool B and total spike responses, although age remained significant ($p = 0.02$; $p = 0.03$).

In an effort to develop a T-cell response assay that required less intensive processing than the ELISpot assay, a whole blood assay based on the in vitro diagnostic QuantiFERON TB Gold Plus assay was also used to assess T-cell response (Supplementary Fig.) [9]. Heparinized whole blood from donors was stimulated with S1 and S2 protein subdomains from the SARS-CoV-2 spike protein (final concentration 5 ug/ml), with measurement of IFNγ released into plasma on quantitative ELISA (QuantiFERON, Qiagen; Hilden, Germany). IFNγ release of ≥0.3 IU/ml was considered a positive threshold, based on prior ROC analysis of convalescent and SARS-CoV2 naïve donors (sensitivity and specificity 100%) [9]. MPN patients were significantly less likely to have T-cell responses by whole blood assay compared to healthy controls (54% vs. 100%, $p = 0.002$) (Fig. 1A). MF patients had the lowest whole blood response rates, although this was not significant. Older age was significantly associated with decreased likelihood of having a whole blood response on univariate analysis (OR 0.95, CI 0.90–0.98). However, on multivariable logistic modeling, age was no longer significantly associated with positive whole blood responses when adjusting for MPN diagnosis. As 100% of healthy controls had a whole blood response, we were unable to model the effect of MPN diagnosis on whole blood response likelihood when accounting for older age. We found no other differences in whole blood response rates based on gender, treatment, vaccine type, ALC, or number of days post-vaccine.

For the first time, we report antibody and T-cell responses to completed series of the BNT162b2 and mRNA-1273 vaccines in MPN patients. Our results are encouraging, with >90% serologic and ELISpot responder rates seen. This is in agreement with recent studies that have found high seroconversion rates among MPN patients [13]. We however found a trend toward lower anti-Spike IgG concentrations in MPN patients after vaccination compared to healthy donors, which may indicate a less robust serologic response. Memory T-cell responses have also been reported in 80% of MPN patients after a single dose of the BNT162b2 vaccine [14]. We did find a signal for lower immunologic responses in T-cell assays in MF patients, although our sample size may have been too limited for statistical significance. Consistent with prior studies, we found significant effects of older age on T-cell response [15], with no other effects by other variables.

Despite these high response rates, our data demonstrated subtle deficiencies in cellular immunity in MPN patients. We found a lower magnitude of T-cell responses on ELISpot testing after vaccination in MPNs compared to healthy controls, although this finding was partially confounded by the older age of MPN patients. It is unknown if lower SFUs on ELISpot testing, which would indicate a lower number of activated T-cells after vaccination, translates into less clinical protection against COVID-19, even if above the cut-off threshold. In addition, we discovered



discordance between ELISpot and whole blood assays in MPN patients only. While healthy controls had 100% responses across both T-cell assays, MPN patients only had a 54% response on whole blood testing, despite >90% responses on ELISpot testing. The whole blood IFNγ assay was designed as a simpler assay more suitable to high-throughput clinical testing. As a result, the whole blood assay also requires processing of the spike protein by antigen presenting cells followed by presentation on the cell surface major histocompatibility complex to cognate T-cells. It is possible that the decreased whole blood response rate therefore

Fig. 1 Serologic and T-cell Responses in Myeloproliferative Neoplasm Patients. A Seroconversion rates and percentage of positive T-cell responders on ELISpot and whole blood IFNγ release assays within myeloproliferative neoplasm (MPN) patients and in healthy donors. Comparisons between MPN (MPN/myelofibrosis [MF]/chronic myeloid leukemia [CML]) patients and normal controls were made using chi-square tests for response rate evaluations. Magnitude of T-cell responses on IFNγ release ELISpot assay as measured by mean spot-forming units (SFUs) per 2.5×10^5 peripheral blood mononuclear cells (PBMCs) against Spike Pool A, Spike Pool B, total spike responses (Spike A + B), and nucleocapsid peptide pools (**B**) from baseline to post-vaccination timepoints in MPN patients and normal controls (mean, error bars indicate standard error) and (**C**) across MPN subtypes in post-vaccination samples. Red line indicates median SFUs in normal controls, and asterisk indicates significant difference in SFUs between MF patients and healthy controls. Comparisons of pre and post-vaccination measurements were conducted using Wilcoxon signed-rank tests. Comparisons between all MPN patients and normal controls, and within MPN (MPN/MF/CML) patients were made using Wilcoxon rank-sum tests or Kruskal–Wallis tests.

reflects deficiencies in cellular immunity required for T-cell activation in MPN patients. It is unknown whether this results in clinically significant differences in future SARS-CoV-2 infections or if it is a limitation of this particular assay for T-cell response testing. However, given the importance of T-cell responses especially in protecting against severe disease caused by SARS-CoV-2 variants, further investigation into these subtle abnormalities is warranted.

In summary we report high serologic and T-cell responses to BNT162b and mRNA-1273 vaccination in MPN patients. Prospective studies with endpoints of clinical infection would be necessary to understand how immune responses to vaccines translate to reduction of COVID-19 cases.

DATA AVAILABILITY

For original data, please contact jhow@bwh.harvard.edu or ghobbs@partners.org.

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AUTHOR CONTRIBUTIONS

JH collected, analyzed, and interpreted the data and wrote the manuscript; KMEG, ELE, and KK collected, analyzed, and interpreted the data; YL and DN analyzed and interpreted the data; RCL provided important insight into the manuscript; MBL provided figures and provided important insight into the manuscript; MVM provided important insight into the manuscript and sponsored the study; JH and GSH designed the study and sponsored the study; all authors edited, read, and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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