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BNT162b2 mRNA SARS-CoV-2 vaccination does not cause upregulation of endothelial activation markers or hypercoagulability: A prospective, single-arm, longitudinal study

To the Editor:

Reports of thrombosis post coronavirus disease 2019 (COVID-19) mRNA vaccination have sparked concerns about the safety of these immunizations. As of October 31, 2021, the Health Sciences Authority of Singapore reported 13 suspected cases of cerebral venous thrombosis (CVT) with the Pfizer-BioNTech/Comirnaty (BNT162b2) and Moderna/Spikevax (mRNA-1273) COVID-19 vaccines out of 9 953 673 total number of doses administered.[1]

We previously reported on three patients who developed CVT post BNT162b2 vaccination, occurring 1–9 days after the second dose.[2] Compared to vaccine-induced thrombotic thrombocytopenia (VITT) associated with the use of adenovirus vector ChAdOx1 nCoV-19 and Ad26.COV2.S COVID-19 vaccines, these patients with CVT post BNT162b2 vaccine were negative for antiplatelet factor 4 (PF4) antibodies. In a study of 30 healthcare workers who received the BNT162b2 vaccine, no hypercoagulable state was found.[3] Their coagulation parameters remained unchanged postvaccination except for a slight increase in platelet levels 14 days after the second dose of BNT162b2 vaccine. Similarly, no differences were detected in thromboelastometry, thrombin generation, thrombin receptor activating peptide, adenosine diphosphate, and arachidonic acid-induced platelet aggregation tests after first dose of the BNT162b2 or the ChAdOx1 vaccines in healthy volunteers by Campello et al.[4]

No study to date has evaluated markers for endothelial activation post mRNA vaccination. *Virchow's triad* of venous stasis, hypercoagulable state, and endothelial dysfunction summarizes the pathophysiologic mechanisms leading to thrombosis. SARS-CoV-2 infection is characterized by COVID-19 associated coagulopathy, evidenced by elevated D-dimer, Von Willebrand factor (vWF), Factor VIII levels, hyperfibrinogenemia in critically ill patients.[5,6] Endothelial cell adhesion molecules, including serum levels of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (V-CAM1) were elevated in severe COVID-19 infection.[7] We hypothesized that post BNT162b2 vaccination, markers of endothelial activation as well as parameters of coagulation may be elevated. A prospective, observational, pilot study was performed to evaluate the endothelial and coagulation profile in a series of healthy participants who had received two doses of the BNT162b2 mRNA vaccine, with the aim to determine if the BNT162b2 vaccination results in endothelial activation or hypercoagulability by studying the endothelial adhesion molecules and coagulation parameters pre and post mRNA vaccination.

Eighteen participants who received the BNT162b2 vaccine were enrolled in this study. Participants completed a questionnaire on their cardiovascular and thrombotic risk factors, including the chronic medications they were taking prior to vaccination. All participants had three blood samples planned: prevaccination, after first dose of BNT162b2 vaccine, and after second dose of BNT162b2 vaccine. The median age of the participants was 35 years (interquartile range [IQR 31–44]), and 14 (78%) were female. Fifteen participants did not have any cardiovascular or thrombotic risk factors. Two reported a medical history of hypertension and one had a history of stroke; and they were on antihypertensive medications and antiplatelet therapy, respectively, during the period of vaccination and blood taking. All 18 participants completed two doses of BNT162b2 vaccination, with the second dose of BNT162b2 administered a median of 21 (IQR 21–22) days after the first dose. All tolerated the vaccination with no serious adverse reaction and no thrombotic events. Blood samples were collected at three-time points: prevaccination (on day of vaccination), a median of 17 (IQR 16–18) days after the first dose of BNT162b2 vaccine, and a median of 9 (IQR 7.5–14.5) days after the second dose of BNT162b2 vaccine. Only one participant defaulted the blood sampling after the first BNT162b2 vaccine dose but completed prespecified blood sampling prevaccination and post-second dose of vaccine.

The following biomarkers were assayed by enzyme-linked immunosorbent assay: *ICAM-1*, *VCAM-1*, and P-selectin (all R&D Systems, Abrington, UK). Coagulation tests were performed using the STA R Max Series coagulation analyzer (Diagnostica Stago, France) and Sysmex CN-6000 automated coagulation analyzer (Sysmex Corporation, Kobe, Japan). Prothrombin time (PT) was measured with Innovin (Siemens Healthcare, Marburg, Germany), activated partial thromboplastin time (aPTT) with Dade Actin FSL (Siemens Healthcare), fibrinogen (modified Clauss) with STA Liquid FIB, D-dimer with STA Liatest D-Dimer. Clotting factor levels (Factor VIII) were measured with STA Deficient VIII. vWF antigen was assayed with an immunoturbidimetric method using STA Liastest vWF: Ag kit. Clot waveform analysis (CWA) was performed with Sysmex CN-6000 automated coagulation analyzer (Sysmex Corporation) with parameters obtained from aPTT and for PT, as per International Society of Hemostasis and Thrombosis Scientific and Standardization Committee recommendation. Four quantitative parameters were recorded—"Min1" (maximum velocity), "Min2" (maximum acceleration), "Max2" (maximum deceleration), and "Delta change" (difference between initial maximum and final maximum values of light transmittance). For continuous data, median and

TABLE 1 Descriptive statistics of endothelial activation markers and coagulation parameters pre and post BNT162b2 vaccine

					ANOVA repeated measures	
		Prevaccination	Post first dose vaccination	Post second dose vaccination	Mauchly's test of sphericity	Test of differences over time[b]
Laboratory tests	Reference range	Median[a] (IQR)	Median[a] (IQR)	Median[a] (IQR)	*p*-value	*p*-value
ICAM-1 (ng/mL)	<95.0	63.5 (50.6–69.9)	66.1 (56.5–79.7)	69.5 (59.8–78.1)	.13	.04
VCAM-1 (ng/mL)	<187.0	132.4 (119.9–144.5)	133.6 (128.4–146.1)	135.4 (121.8–154.7)	<.001	.72
P-selectin (ng/mL)	<103.0	66.2 (29.9–108.8)	41.7^ (11.4–72.6)	32.7^ (10.2–88.4)	.96[c]	.27[c]
Fibrinogen (g/L)	1.8–4.5	3.2 (2.53–3.68)	3.16 (2.72–3.53)	3.31 (2.57–3.69)	.13	.56
D-dimer (μg/mL)	<0.50	0.32 (0.22–0.38)	0.21^ (0.14–0.35)	0.29 (0.24–0.34)	.35	.53
F VIII (%)	60.0–150.0	103.5 (74.7–132.3)	108 (84.5–117)	111 (86.3–138)	.008	.47
vWF Ag (%)	56.0–160.0	107.5 (71.5–132.3)	106 (73–122.5)	109.5 (78–137)	.20	.48
PT (sec)	10.0–11.7	10.8 (10.38–11.13)	10.8^ (10.5–10.9)	10.5 (10.2–10.9)	.80	.005
Min1 (PT) (%/s)	1.95–5.67	3.59 (3.01–4.48)	3.68 (3.08–4.08)	3.82 (3.18–4.62)	.048	.096
Min2 (PT) (%/s^2)	0.97–2.93	1.84 (1.51–2.32)	1.87 (1.9–2.08)	1.96 (1.62–2.39)	.04	.10
Max2 (PT) (%/s^2)	0.75–2.35	1.48 (1.19–1.92)	1.52 (1.26–1.70)	1.59 (1.32–1.93)	.07	.06
Delta change (PT) (%)	6.52–17.28	10.8 (9.5–13.15)	11.3 (9.6–12.1)	11.7 (9.98–13.83)	.06	.11
aPTT (sec)	23.9–34.1	30.1 (28.8–32.8)	29.6 (28.1–32.7)	29.5 (27.1–31.5)	.33	.03
Min1 (aPTT) (%/s)	2.86–6.78	4.47 (4.05–5.40)	4.76 (4.03–5.51)	4.86 (4.24–5.72)	.69	.10
Min2 (aPTT) (%/s^2)	0.46–1.10	0.73 (0.64–0.83)	0.74 (0.65–0.87)	0.76 (0.69–0.91)	.12	.03
Max2 (aPTT) (%/s^2)	0.37–0.93	0.62 (0.54–0.70)	0.61 (0.55–0.72)	0.64 (0.59–0.75)	.07	.04
Delta change (aPTT) (%)	25.21–63.09	42.4 (36.33–53.03)	44.2 (40.05–50.53)	46.1 (37.85–52.03)	.12	.23

Note: F VIII, Factor VIII; vWF Ag, von Willebrand factor antigen; PT, prothrombin time; APTT, activated partial thromboplastin time. Reference intervals for clot waveform parameters were established in the TTSH Hematology laboratory locally based on 124 healthy controls in accordance with the Clinical and Laboratory Standards Institute guidelines. ICAM-1, intercellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; P-selectin; reference data from 81 normal controls, TTSH Immunology Research laboratory.

[a]Median (range) was presented to compare with the reference ranges. All data were normally distributed (checked via Shapiro–Wilk's test) except for those indicated by ^.

[b]*p*-value based on Greenhouse–Geisser would be reported if the p-value based on the Mauchly's test was <.05.

[c]Tests were computed based on log-transformed variable.

IQR were presented due to its skewed distribution. Tests of association between coagulation and endothelial parameters were performed. Normality of the data was assessed using histogram and Shapiro–Wilk's test. The data were transformed if it was shown to be skewed. Linear mixed models were used to estimate the difference in blood markers between two-time points, treating participants as a random effect. As the study population comprised only 18 participants, no adjustment was made in the model. The repeated measures analysis of variance was also used to analyze the same data, as part of sensitivity analyses. The Mauchly's test of sphericity was assessed as part of the analysis; if the *p*-value based on the Mauchly's test was <.05, the *p*-value based on Greenhouse–Geisser would be reported in the test of differences of blood markers over time. Analyses were performed using STATA 17 (StataCorp 2021. Stata Statistical Software: Release 17, College Station, TX: StataCorp LLC.). Statistical significance was declared if a 2-sided *p*-value < .05. Bonferroni correction was used in instances of multiple comparisons.

Our results show no evidence of endothelial activation (ICAM, VCAM-1, and P-selectin) or hypercoagulability, with the median values of endothelial cell adhesion molecules and coagulation parameters remaining within normal limits pre and postvaccination (Table 1). There was a statistically significant increase in median ICAM levels post first and second dose of vaccination, although this remained below the normal limit of ICAM levels. A statistically significant decrease in PT and aPTT was observed postvaccination, with a corresponding increase in aPTT CWA for maximum acceleration (max2) and maximum deceleration (max2) when comparing the differences in median values post first and second dose of vaccination with prevaccination levels. However, these parameters remain within the reference ranges for PT, aPTT, and CWA. Even though we did not capture data on the reactogenicity of mRNA vaccination in our participants, which encompasses manifestations of the inflammatory response to vaccination such as injection-site pain, redness, or induration, as well as systemic symptoms such as fever, we postulate that these mild variations in endothelial markers and coagulation parameters, though statistically significant, may be related to a local inflammatory immune response to vaccination. While patients with moderate to severe COVID-19 have increased hypercoagulability and endothelial activation, with an increased incidence of thrombosis, the localized expression of the spike protein with mRNA vaccination does not result in severe inflammation that can cause a hypercoagulable state in healthy subjects, as demonstrated by our study data from our study and those of the two previous studies mentioned,[3,4] or excessive endothelial activation as shown by our study. One participant who did not report any cardiovascular risk factors demonstrated raised vWF Ag and Factor VIII levels of 181% and 198%, respectively, prior to vaccination. Her levels of vWF Ag dropped to 163% 16 days after the first dose of BNT162b2 vaccine and 179% 9 days after the second dose of BNT162b2 vaccine while her levels of Factor VIII were 184% and 183% after the first and second dose of BNT162b2 vaccine, respectively. Another participant with a history of stroke did not demonstrate significant baseline levels or increase in her coagulation parameters nor upregulation of endothelial adhesion molecules.

There are several limitations of our study. First, the small sample size may not be representative of the larger population. Second, our study focused on only the BNT162b2 vaccine and hence the results are not generalizable to all COVID-19 vaccines as VITT is reported to be more strongly associated with adenovirus vector vaccines than mRNA vaccines.[8] Third, majority of our study participants were younger, healthy individuals without any cardiovascular risk factors, and it is unclear if the results will be similar in a population with such risk factors. Lastly, we did not evaluate markers of inflammation such as C-reactive protein, interleukin-6, or tumor necrosis factor, which would be important in establishing any postvaccination increase in local or systemic inflammation, or tests of platelet function to detect postvaccination platelet activation.

In conclusion, our findings provide reassuring preliminary data that BNT162b2 vaccination does not result in endothelial activation or a hypercoagulable state. While the definition and mechanism of VITT are more clearly defined and linked to the presence of PF4 antibodies, the cause of rare non-VITT thrombosis remains elusive.[9,10] Additional studies will be required to identify the population at risk of vaccine-associated thrombosis and how to monitor for this rare but serious complication.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Bingwen Eugene Fan and Xin Rong Lim conceived the study. All authors contributed substantially to the acquisition, analysis, and interpretation of data, critical revision of the manuscript for important intellectual content.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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A population-based study of acute panmyelosis with myelofibrosis in the United States: 2004–2015

To The Editor:

Acute panmyelosis with myelofibrosis (APMF) is a rare subtype of acute myeloid leukemia (AML) characterized by acute panmyeloid proliferation with increased blasts, cytopenias, bone marrow fibrosis, and absence of splenomegaly. APMF is estimated to account for <1% of AML.[1] There is controversy regarding how to differentiate APMF from other myeloid malignancies such as AML with fibrosis, acute megakaryoblastic leukemia, or myelodysplastic syndrome with fibrosis.[2]

There is a paucity of studies describing the clinical features and outcomes of APMF. The prognosis of APMF is very poor, with a reported median survival of 1–9 months.[1,3] No consensus in treatment exists. No study has yet utilized the National Cancer Database (NCDB) or Surveillance, Epidemiology, and End Results (SEER) database to report risk factors, treatments received, and additional clinical features. Our study utilizes SEER and the NCDB to better describe the outcomes and survival trends of patients diagnosed with APMF from 2004 to 2015.[4,5]

We queried the United States SEER database and NCDB using the ICD-O-3 code 9931/3. The NCDB is a joint project of the Commission on Cancer of American College of Surgeons and the American Cancer Society that is a nationwide oncology outcomes database for >1500 cancer programs in the US and Puerto Rico, capturing 70% of all newly diagnosed cases of cancer in the US.[4] SEER is a program of the National Cancer Institute that collects and publishes cancer incidence and survival data covering about 28% of the US population.[5]

Due to the challenges of diagnosing APMF, we only included patients in our cohort if the diagnostic confirmation included those with recorded positive histology or positive histology plus positive immunophenotyping and/or positive genetic studies. The SEER 17 registries (2004–2015) were used to find data on incidence, overall survival (OS), and relative survival (RS); the Alaska Native Tumor registry was excluded from this analysis. Incidence was age-adjusted to the U.S. 2000 standard population. Incidence ratios (IRs) were calculated with SEER*Stat software (version 8.3.6; NCI, Bethesda, MD).

Abbreviations: AAPMF, acute panmyelosis with myelofibrosis; CDS, Charlson Deyo Score; CI, confidence interval; HR, hazard ratio; HCT, hematopoietic cell transplantation; IRs, incidence ratios; IQR, interquartile range; NCDB, NCDB, National Cancer Database; NHAPI, non-Hispanic Asian Pacific Islander; NHB, non-Hispanic Black; NHW, non-Hispanic White; OS, overall survival; RS, relative survival; SEER, Surveillance, Epidemiology and End Results.