Cerebral Venous Thrombosis post BNT162b2 mRNA SARS-CoV-2 vaccination: A Black Swan Event

Bingwen Eugene FAN[1,2], Jia Yi SHEN[3], Xin Rong LIM[4], Tian Ming TU[3], Cheng Chieh Ray CHANG[1], Hnin Su Wai KHIN[3], Jasmine Shimin KOH[3], Jai Prashanth RAO[5], Soon Lee LAU[6], Guat Bee TAN[6], Yew Woon CHIA[7], Kay Yaw TAY[3], Shahul HAMEED[3], Thirugnanam UMAPATHI[3], Kiat Hoe ONG[1,2], Banumukala Madhava Rao Vishnu PRASAD[2]

[1]Department of Haematology, Tan Tock Seng Hospital, Singapore
[2]Department of Laboratory Medicine, Khoo Teck Puat Hospital, Singapore
[3]Department of Neurology, National Neuroscience Institute, Singapore
[4]Department of Rheumatology, Allergy and Immunology, Tan Tock Seng Hospital, Singapore.
[5]Department of Neurosurgery, National Neuroscience Institute, Singapore.
[6]Department of Laboratory Medicine, Tan Tock Seng Hospital, Singapore
[7]Department of Cardiology, Tan Tock Seng Hospital, Singapore

Correspondence:
Bingwen Eugene Fan
Department of Haematology
Tan Tock Seng Hospital, Singapore
Bingwen_Eugene_Fan@ttsh.com.sg

Manuscript Type: Correspondence

Key words: COVID-19, Cerebral Venous Thrombosis, Coagulopathy, SARS-CoV-2 vaccine

To the editor:

COVID-19 vaccine associated cerebral venous thrombosis (CVT) are rare adverse events and can be considered as *black swan events*. "Black swan events", originally described by Nassim N. Taleb in economic theory, are rare, unexpected events whose potential to occur only becomes apparent

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/ajh.26272

after they have happened, where they are neither predicted nor anticipated, and only on retrospect, a clear basis is found for their occurrence that justifies their apparent randomness.

The modified Adenovirus vector COVID-19 vaccines (ChAdOx1 nCoV-19 (Oxford–AstraZeneca) and Ad26.COV2.S (Johnson & Johnson/Janssen)) and mRNA based COVID-19 vaccines (BNT162b2 mRNA (Pfizer–BioNTech) and mRNA-1273 (Moderna)) have shown both safety and efficacy against COVID-19 in phase 3 clinical trials and are now being utilized in global vaccination programmes. However, as CVT is a rare neurovascular thrombotic event with an estimated incidence of 1.6 cases per 100,000 per year [1], clinical trials were underpowered to detect the incidence of CVT. As such, rare cases of post-vaccine associated CVT from the use of ChAdOx1 nCoV-19 and Ad26.COV2.S COVID-19 vaccines, including the mechanism of vaccine-induced thrombotic thrombocytopenia (VITT), have emerged in real-world vaccination with post-marketing surveillance, triggering a wave of temporal suspension of use in several European countries.

The incidence and pathogenesis of CVT after mRNA COVID-19 vaccines remains unknown. Mass vaccination in Singapore commenced on 30th Dec 2020 with majority of people receiving the BNT162b2 mRNA vaccine, with mRNA-1273 vaccination initiated later on 12th March 2021. We present 3 cases of CVT post BNT162b2 mRNA SARS-CoV-2 vaccination (Table 1), review existing data on the incidence and discuss mechanisms of CVT in BNT162b2 mRNA vaccination.

Patient 1

A healthy 54-year-old Chinese male with well controlled hyperlipidaemia, developed severe headache and vomiting 24 hours after his 2nd dose of BNT162b2 mRNA vaccine, and acute left hemiparesis 2 days later. CT Brain revealed a large right temporo-parietal lobe intraparenchymal haemorrhage with associated midline shift and uncal herniation, necessitating a decompressive craniectomy. CT angiogram excluded underlying vascular malformations and CT venogram confirmed dural venous sinus thrombosis (transverse and sigmoid sinus). Full blood count, coagulation profile and thrombophilia screen were unremarkable. Tests for anti-platelet factor 4 (anti-PF4) antibodies

and heparin induced platelet aggregation (HIPA) were negative. Bilateral lower limb deep vein thrombosis ultrasound scans were negative. Whole body CT did not reveal any other thrombosis or malignancy. He was anticoagulated on unfractionated heparin (UFH) and subsequently converted to low molecular weight heparin (LMWH) and is currently undergoing rehabilitation.

Patient 2

A healthy 62-year-old Chinese female with a history of well-controlled hypertension presented with headache and vomiting 9 days after her 2nd dose of BNT162b2 mRNA COVID-19 vaccine. Her full blood count and coagulation panel were unremarkable. CT Brain and CT venogram on admission confirmed acute right cerebral bleed involving occipital and temporal lobes associated with subarachnoid haemorrhage due to thrombosed right transverse and sigmoid sinus veins. UFH was started with close therapeutic monitoring. On day 4 of admission there was a drop in her Glasgow Coma Scale from 14 to 9, with repeat CT showed increasing size of haemorrhagic right cerebral venous infarcts with worsening of mass effect and development of early hydrocephalus, requiring decompressive craniectomy. This was later complicated by intracranial empyema requiring drainage. Post operatively, UFH infusion was resumed and later converted to LMWH. Thrombophilia workup was negative. Bilateral lower limb deep vein thrombosis scan was negative for thrombosis. Whole body CT performed 3 weeks into admission was negative for malignancy, however, right upper lobe segmental artery pulmonary embolism, left internal iliac artery and right common iliac vein thrombi were detected, likely from immobility in ICU and perioperative discontinuation of anticoagulation. Incidentally, a left iliopsoas haematoma was detected. Despite her haematoma, UFH was resumed due the increased thrombotic burden and later converted to LMWH, with bridging to warfarin a week later.

Patient 3

A 60-year-old Chinese female with family history of thrombosis (her son had unprovoked pulmonary embolism) and medical history of diabetes mellitus, hypertension and hyperlipidaemia, presented 8

days after her 2nd dose of BNT162b2 mRNA COVID-19 vaccine for right ataxic hemiparesis. CT brain and venogram confirmed extensive dural venous thrombosis and venous infarct in bilateral periolandic gyri. This was complicated by acute right occipital lobe intraparenchymal hematoma and bilateral subarachnoid haemorrhage. Her CVT was treated with LMWH, followed by bridging to warfarin. Thrombophilia workup apart from a low anti thrombin III (AT) level of 55% (reference range 80-130%), was unremarkable. She had no previous history of deep vein thrombosis. Her low levels of AT III during acute illness could possibly be attributed to acute and extensive thrombosis which can lower levels transiently, hence plans were made to recheck AT III levels when the patient is asymptomatic. Whole body CT scan performed did not reveal any malignancy. She made an uneventful recovery and was discharged.

Platelet flow cytometry (Becton Dickinson FACSCanto TM Flow analyzer) performed on blood specimens from patient 1 and 2 both demonstrated increased expression of CD62P (platelet surface P-selectin) and PAC1 (activated GP IIb/IIIa) in the presence of an ADP agonist), with normal expression of CD36, CD42a, CD42b, CD61, Cd63, GPVI and CD235a as compared to healthy controls.

Emerging data from both cohort studies and pharmacovigilance databases suggest an increased incidence of CVT post BNT162b2 mRNA vaccination. A preprint by Taquet et al [2] evaluating data from the United States, proposes that COVID-19 increases the risk of CVT (incidence of 39.0 per million people) more than people who have received a COVID-19 mRNA vaccine (incidence of 4.1 per million people, n= 366,869 in each cohort, 95% CI 1.1–14.9, adjusted RR=6.36, P<.001), however vaccination still posed a higher risk for developing CVT than the background incidence of CVT (0.41 per million people).

Smadja et al [3] evaluated COVID-19 vaccination thrombotic risk reported to the World Health Organisation (WHO) Global Database for Individual Case Safety Reports (VigiBase), a global pharmacovigilance database monitoring safety of ChAdOx1 nCoV-19, BNT162b2 mRNA and mRNA-1273 vaccines. For the 3 vaccines, 2161 thrombotic events (795 venous and 1374 arterial thrombotic

events) were reported from 12th December 2020 to 16th March 2021, with a combined reporting rate of 0.21 [95% CI: 0.19–0.22] cases of thrombotic events per 1 million person vaccinated-days. The reporting rate of unexpected CVT post COVID-19 vaccine for mRNA-1273 (Moderna) was 0.9%, with BNT162b2 mRNA (Pfizer–BioNTech) was 0.4%, and for ChAdOx1 nCoV-19 was 1.1%. Overall median time to venous thrombotic event was 4 days. A caveat for this dataset is that incidence or prevalence are not appropriate since there is no information about precise denominator for each separate vaccine and about the extent of underreporting.

In the Yellow Card report [4] covering the period of 9th Dec 2020 to 26th May 2021, the Medicines and Healthcare products Regulatory Agency in the United Kingdom noted 33 cerebrovascular venous and sinus thrombosis (24 cerebral venous sinus thrombosis, 3 CVT and 3 superior sagittal sinus thrombosis, 1 Transverse sinus thrombosis) out of a total of 10.6 million second doses of BNT162b2 mRNA vaccine given, or 33/10,600,00 (0.00311%).

There has only been a single published case series of 2 female patients with extensive CVT post BNT162b2 mRNA vaccination by Dias et al [5]. Both patients had normal platelet counts and negative tests for anti-PF4 antibodies, excluding VITT. The first patient had borderline low protein S, possibly depressed due to extensive thrombosis. The relationship of CVT to BNT162b2 remains uncertain given the patients' pre-existing risk factors of oral contraceptives use and renal cell carcinoma.

As of 31st May 2021, 4,047,651 million doses of mRNA vaccines have been administered in Singapore, with 3 cases of probable COVID-19 vaccine associated CVT, deriving an incidence of 3/4,047,651 (0.000074%) per dose, or 3/1,766,497 (0.00017%) for people who have completed 2 vaccination doses, as compared to a higher incidence of 2/44,479 (0.0045%) of CVT associated with COVID-19 infection in a local review [6]. In our 3 cases described, patients affected were predominantly older, with development of CVT on the 1st to 9th day after the 2nd dose of BNT162b2 mRNA vaccination. All had extensive CVT, with the first and second patient developing life threatening intraparenchymal haemorrhage requiring decompression craniectomy. All had normal

platelet counts, with 2 patients having negative HIPA, suggestive that the mechanism of CVT was unrelated to VITT described with the modified Adenovirus vector COVID-19 vaccines. Therefore, anticoagulation with LMWH or UFH is appropriate and should not be avoided in mRNA COVID-19 vaccine associated CVT.

COVID-19 has strong association with immunothrombosis, resulting in stroke and CVT [6]. CVT occurring post COVID-19 mRNA vaccination could be a possible recapitulation of the thrombotic complications seen in acute COVID-19, where the WHO has cautioned that "COVID-19 vaccination itself may be associated with an increased risk of developing COVID-19-like disease or its complications [7]". While the SARS-CoV-2 virion has a higher thrombogenic potential, the spike glycoprotein also carries significant thrombogenicity. We therefore postulate that the pathogenesis of BNT162b2 mRNA vaccine induced CVT may possibly be related to spike glycoprotein interaction causing immunothrombosis from 1) Disruption of endothelial homeostasis and the blood-brain barrier 2) SARS-CoV-2 spike glycoprotein directly causing platelet activation 3) Aberrant activation of the alternative pathway of complement.

Firstly, evidence for endothelial dysfunction is demonstrated in a 3D microfluidic model of the human blood-brain barrier (BBB) [8], where spike glycoprotein, upon binding to the angiotensin converting enzyme 2 receptor, induces loss of the BBB integrity, activation of endothelial cells with upregulation of cell adhesion molecules (ICAM-1 and VCAM-1), leukocyte chemotaxis factors and proinflammatory cytokines (IL-1 β and IL-6), triggering a proinflammatory response and upregulation of matrix metalloproteinases in human brain endothelial cells. We postulate that this mechanism may contribute to a significant breech in brain endothelial barrier integrity. A combination of endothelial dysfunction, exposed tissue factors, immune-vascular cross talk and procoagulant cytokines from vessels can promote thrombogenesis and cause catastrophic CVT.

Secondly, Zhang et al [9] demonstrate that the spike glycoprotein potentiates platelet aggregation and dense granule secretion in response to various platelet agonists, including ADP, with platelets

demonstrating accelerated spreading and clot retraction. Flow cytometry demonstrated an enhancement of both platelet activation markers of integrin αIIbβ3 activation (CD62P) and P-selectin expression (PAC-1) in the presence of agonist, *similar* to the flow cytometry findings in our 2 patients, which can be attributed to possible prior spike glycoprotein sensitization from mRNA COVID-19 vaccination. Despite clear evidence that vaccine mRNA is degraded within short days [10] [11], the duration of persistence of the spike glycoprotein in-vivo for the BNT162b2 mRNA vaccine is still unknown, and expert opinion from the Infectious Diseases Society of America vaccines information FAQ postulates that the spike glycoprotein may last for weeks. While platelets can be activated in the immediate post thrombotic state or after acute bleed, post-vaccination persistence of spike glycoprotein in tissues or in the circulation could be a plausible cause for platelet activation, as seen in the unusual persistence of increased expression of platelet activation markers CD62P and PAC1 in the flow cytometry of patient 2, performed 4 weeks post neurosurgery.

Thirdly, in vitro studies [12] have demonstrated that spike proteins can activate the alternative pathway. Carvelli et al [13] report activation of C5a and sC5b-9 as well as deposition of activated complement activation products in injured tissues and organs in severe COVID-19 infection, through activation of any of three initiating pathways (classical, lectin, or alternative pathway) by the spike protein or immune-antigen complexes, with concurrent monocyte activation and neutrophil NETosis. Spike protein dysregulation of the complement system can perpetuate a vicious cycle of inflammatory tissue damage and cause significant endothelial dysfunction, playing a pivotal role in immune-mediated thrombogenesis.

We propose that a combination of one or more conditions— a sufficiently high enough titre of spike glycoprotein in the circulation, coupled with a high number of activated platelets and aberrant complement activation by the spike glycoprotein— need to be present to cross the thrombotic threshold and trigger CVT. This may explain the rarity of this clinical observation.

In conclusion, robust pharmacovigilance for COVID-19 vaccines is key in advancing global COVID-19 vaccination efforts with both speed and safety. However rare they may be, the reporting of adverse and *black swan events* such as COVID-19 vaccine-associated CVT is necessary to minimise uncertainty in the medical fraternity and the public, to increase awareness of regulatory authorities and clinicians to such events, so that these can be evaluated and managed promptly. Compared to most medications where adverse reactions that occurs in fewer than 1 in 10,000 are considered as "very rare", COVID-19 vaccines appear safe, given that risk of COVID-19 vaccine-associated CVT is by far much rarer than developing CVT in COVID-19 infection.

Acknowledgements

The authors greatly appreciate the efforts of our fellow healthcare workers and the support of their families during this pandemic.

Author Contribution

BE Fan, JY Shen, BMRV Prasad conceived the study. All authors were involved in patient care, and contributed substantially in the acquisition, analysis and interpretation of data, critical revision of manuscript for important intellectual content.

Disclosure

The authors declare that they have no conflict of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics approval

The study was approved by the Singapore Health Services institutional review board (CIRB 2020/2410). Waiver of consent was granted by the Singapore Health Services institutional review board.

Data Availability Statement

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

References

1. Devasagayam S, Wyatt B, Leyden J, Kleinig T. Cerebral venous sinus thrombosis incidence is higher than previously thought: a retrospective population-based study. Stroke. 2016;47(9):2180–2182.
2. Taquet M, Husain M, Geddes JR et al. Cerebral venous thrombosis: a retrospective cohort study of 513 284 confirmed COVID-19 cases and a comparison with 489 871 people receiving a COVID-19 mRNA vaccine. https://osf.io/a9jdq/
3. Smadja D, Yue QY, Chocron, R, et al. Vaccination against COVID-19: Insight from arterial and venous thrombosis occurrence using data from VigiBase. Eur Respir J 2021: 2100956. doi:10.1183/13993003.00956-2021
4. Coronavirus vaccine - weekly summary of Yellow Card reporting https://www.gov.uk/government/publications/coronavirus-covid-19-vaccine-adverse-reactions/coronavirus-vaccine-summary-of-yellow-card-reporting https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/990930/COVID-19_Pfizer_Vaccine_Analysis_Print_26.05.2021.pdf
5. Dias L, Soares-dos-Reis R, Meira J, et al. Cerebral Venous Thrombosis after BNT162b2 mRNA SARS-CoV-2 vaccine, Journal of Stroke and Cerebrovascular Diseases, 2021. doi:10.1016/j.jstrokecerebrovasdis.2021.105906.

6. Tu TM, Goh C, Tan YK, et al. Cerebral Venous Thrombosis in Patients with COVID-19 Infection: a Case Series and Systematic Review. J Stroke Cerebrovasc Dis. 2020;29(12):105379. doi:10.1016/j.jstrokecerebrovasdis.2020.105379

7. COVID-19 Vaccines: Safety Surveillance Manual Module: Responding to adverse events following COVID-19 immunization (AEFIs) https://www.who.int/vaccine_safety/committee/Module_AEFI.pdf?ua=1

8. Buzhdygan TP, DeOre BJ, Baldwin-Leclair A, et al. The SARS-CoV-2 spike protein alters barrier function in 2D static and 3D microfluidic in-vitro models of the human blood-brain barrier. Neurobiol Dis. 2020;146:105131. doi:10.1016/j.nbd.2020.105131

9. Zhang, S., Liu, Y., Wang, X. et al. SARS-CoV-2 binds platelet ACE2 to enhance thrombosis in COVID-19. J Hematol Oncol 13, 120 (2020). https://doi.org/10.1186/s13045-020-00954-7

10. Summary of the Public Assessment Report for Pfizer/BioNTech COVID-19 vaccine Updated 4 June 2021. https://www.gov.uk/government/publications/regulatory-approval-of-pfizer-biontech-vaccine-for-covid-19/summary-public-assessment-report-for-pfizerbiontech-covid-19-vaccine

11. Probst J, Weide B, Scheel B, et al. Spontaneous cellular uptake of exogenous messenger RNA in vivo is nucleic acid-specific, saturable and ion dependent [published correction appears in Gene Ther. 2009 May;16(5):706]. Gene Ther. 2007;14(15):1175-1180. doi:10.1038/sj.gt.3302964

12. Yu, J. et al. (2020) 'Direct activation of the alternative complement pathway by SARS-CoV-2 spike proteins is blocked by factor D inhibition', Blood, 136(18), pp. 2080–2089. doi:10.1182/blood.2020008248.

13. Carvelli J, Demaria O, Vély F, et al. Association of COVID-19 inflammation with activation of the C5a-C5aR1 axis. Nature. 2020;588(7836):146-150. doi:10.1038/s41586-020-2600-6

Table 1: Clinical characteristics, neuroimaging, laboratory tests and outcomes of the 3 patients*

Patient number	1	2	3
Clinical Characteristics			
Age (years)	54	62	60
Gender	Male	Female	Female
Past Medical History	Hyperlipidemia	Hypertension	Diabetes mellitus Hypertension Hyperlipidemia
Number of vaccine doses	2	2	2
Onset of symptoms post vaccination (days)	1	9	8
Presenting symptoms	Headache followed by left sided weakness	Headache, vomiting, behavioural changes	Giddiness, vomiting and right sided weakness and numbness
Neuroimaging			
Site of Thrombosis	Right transverse sinus Right sigmoid sinus Right internal jugular veins	Right transverse sinus Right sigmoid sinus Right internal jugular veins	Right transverse sinus Right sigmoid sinus Right internal jugular veins Bilateral cortical veins
Haemorrhage	Right temporo-parietal intraparenchymal haemorrhage	Right temporal-occipital haemorrhage and subarachnoid haemorrhage (SAH)	Right occipital hematoma, bilateral SAH and bilateral peri-rolandic gyri infarcts
Laboratory Tests			
White Blood Count ($x10^9$/L)	12.9 (4.0-9.6)	18.79 (3.82-9.91)	7.34 (4.0-10.0)
Haemoglobin (g/dL)	13.5 (13.6-16.6)	12.8 (11.2-14.9)	13.5 (12-16)
Platelets ($x10^9$/L)	300 (150-360)	383 (173-414)	346 (140-440)
Prothrombin Time (sec)	13.9 (11.7-14.0)	10.4 (9.7-11.6)	10.3 (9.9-11.4)
Activated Partial Thromboplastin Time (sec)	28.7 (27.0-37.0)	22.6 (25.5-35.8)	28.2 (25.7-32.9)
INR	1.0	0.96	0.98
ESR (mm/hr)	35 (1-10)	50 (1-20)	-
ANA (Titre)	<80 (<80)	0.07 (<1.0)	-
Anti-dsDNA (Titre)	<25 (0-25)	57.1 (<100)	-
Anti-Cardiolipin IgM	Negative	Negative	Negative
Anti-Cardiolipin IgG	Negative	Negative	Negative
Lupus anticoagulant	Absent	Absent	-
Anti B2 glycoprotein 1	Negative	Negative	-
Factor V levels (%)	-	134 (64-161%)	-
Factor VIII levels (%)	-	>200 (53-196%)	-
Anti-thrombin III (%)	94 (80-130)	87 (93-125)	55 (80-120)
Protein C (%)	87 (70-150)	146 (83-144)	177 (70-140)

Protein S (%)	76 (65-130)	99 (65-115)	69 (64-123)
Factor V Leiden Gene	-	Negative	-
Anti-PF4 antibody	Negative	-	-
Heparin induced platelet aggregation	Negative	Negative	-
Platelet flow cytometry	Increased CD62P and PAC1 expression	Increased CD62P and PAC1 expression	Not performed
Treatment and outcome			
Anticoagulation	UFH followed by LMWH then warfarin	UFH followed by LMWH then warfarin	LMWH then warfarin
Clinical Outcome	Left hemiparesis, on rehabilitation	Left hemiparesis, on rehabilitation	Full recovery

*Reference ranges differ as patients were admitted to 3 different hospitals.