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FASTTRACK CLINICAL RESEARCH
Thrombosis and antithrombotic treatment

Predictors of mortality in thrombotic thrombocytopenia after adenoviral COVID-19 vaccination: the FAPIC score

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Aims The clinical manifestation and outcomes of thrombosis with thrombocytopenia syndrome (TTS) after adenoviral COVID-19 vaccine administration are largely unknown due to the rare nature of the disease. We aimed to analyse the clinical presentation, treatment modalities, outcomes, and prognostic factors of adenoviral TTS, as well as identify predictors for mortality.

Methods and Results PubMed, Scopus, Embase, and Web of Science databases were searched and the resulting articles were reviewed. A total of 6 case series and 13 case reports (64 patients) of TTS after ChAdOx1 nCoV-19 vaccination were included. We performed a pooled analysis and developed a novel scoring system to predict mortality. The overall mortality of TTS after ChAdOx1 nCoV-19 vaccination was 35.9% (23/64). In our analysis, age ≤60 years, platelet count $<25 \times 10^3/\mu L$, fibrinogen <150 mg/dL, the presence of intracerebral haemorrhage (ICH), and the presence of cerebral venous thrombosis (CVT) were significantly associated with death and were selected as predictors for mortality (1 point each). We named this novel scoring system FAPIC (fibrinogen, age, platelet count, ICH, and CVT), and the C-statistic for the FAPIC score was 0.837 (95% CI 0.732–0.942). Expected mortality increased with each point increase in the FAPIC score, at 2.08, 6.66, 19.31, 44.54, 72.94, and 90.05% with FAPIC scores 0, 1, 2, 3, 4, and 5, respectively. The FAPIC scoring model was internally validated through cross-validation and bootstrapping, then externally validated on a panel of TTS patients after Ad26.COV2.S administration.

Conclusions Fibrinogen levels, age, platelet count, and the presence of ICH and CVT were significantly associated with mortality in patients with TTS, and the FAPIC score comprising these risk factors could predict mortality. The FAPIC score could be used in the clinical setting to recognize TTS patients at high risk of adverse outcomes and provide early intensive interventions including intravenous immunoglobulins and non-heparin anticoagulants.

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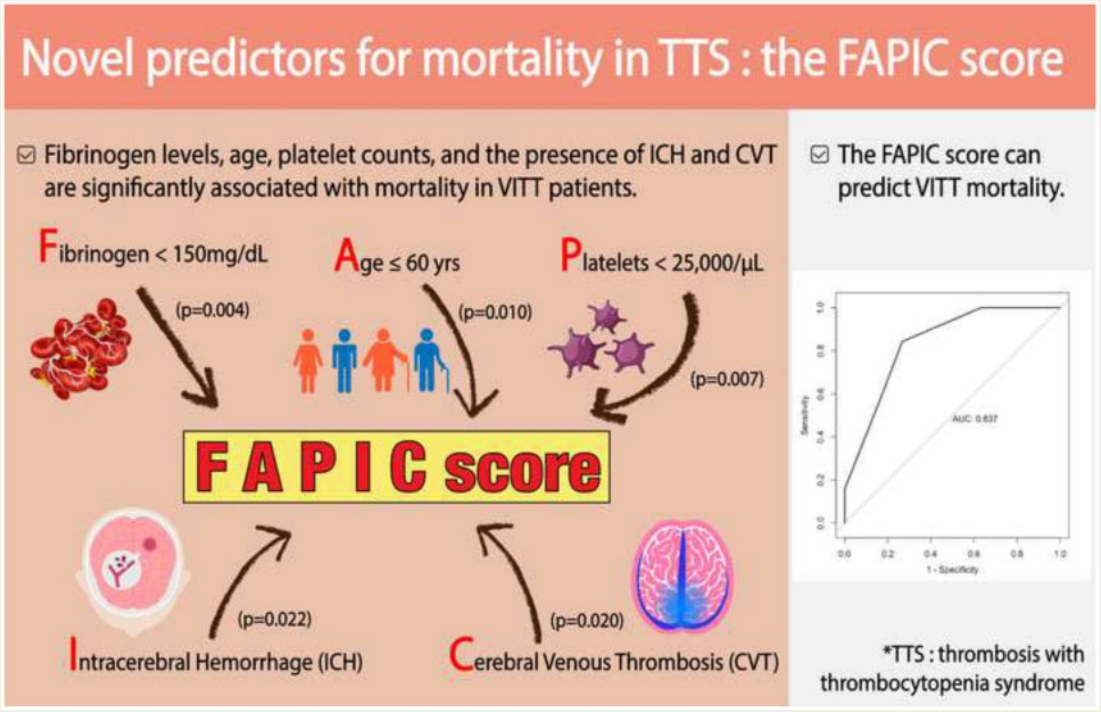
Graphical Abstract



The FAPIC scoring model, a summary score comprising fibrinogen, age, platelet count, intracerebral haemorrhage, and cerebral venous thrombosis, can be used to predict mortality in adenoviral vaccine-associated thrombosis with thrombocytopenia syndrome. AUC, area under the curve; VITT, vaccine-induced immune thrombotic thrombocytopenia.

Keywords Vaccine-induced thrombotic thrombocytopenia • Thrombotic thrombocytopenia syndrome • ChAdOx1 nCoV-19 • COVID-19 vaccine • Cerebral venous thrombosis

Introduction

Since its initial outbreak on 31 December 2019, coronavirus disease 2019 (COVID-19) has become a rampant pandemic with a total of 142 539 302 confirmed cases and 3 116 444 deaths as of 27 April 2021.[1] At such a pivotal time, rapid, worldwide vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to achieve herd immunity has become the most pressing issue for mitigating the global threat of the virus.[2,3] Currently, four vaccines have been approved either by the European Medicines Agency (EMA) or by the U.S. Food and Drug Administration (FDA), including two mRNA-based vaccines—BNT162b2 (Pfizer-BioNTech) and mRNA-173 (Moderna)—and two recombinant adenoviral vector vaccines—ChAdOx1 nCoV-19 (AstraZeneca) and Ad26.COV2.S (Johnson & Johnson/Janssen).[4,5] These vaccines have been developed and distributed at an unprecedented pace; as of 27 April 2021, a total of 570.63 million people worldwide have received at least one dose of the COVID-19 vaccine; 42.38% of the USA and 20.05% of Europe have been vaccinated at least once.[6] Although these vaccines are highly efficacious in protecting against SARS-CoV-2 infection by neutralizing antibodies,[7–9] there have been increasing reports of severe central vein thromboses after immunization with the ChAdOx1 nCoV-19 vaccine, some of which have been fatal.[10–16]

The UK Medicines and Healthcare Products Regulatory Agency (MHRA) and the EMA responded by reviewing the risk of thrombosis related to SARS-CoV-2 vaccine and confirmed that the risk of venous thrombo-embolism associated with the vaccines was not higher than that in the general population, but they acknowledged that the AstraZeneca vaccine may be related to a rare but serious adverse event associated with thrombosis, such as cerebral venous thrombosis (CVT) and thrombocytopenia,[17] although a causal association has not yet been confirmed.[18–20] The EMA compared the clinical picture with immune-mediated heparin-induced thrombocytopenia (HIT),[21] and two recently published case series have confirmed this similarity.[10,12] The patients in these case series had high levels of antibodies against antigenic complexes of platelet factor 4 (PF4), which are found in HIT, though none of the patients had previously received heparin.[10,12]

This evidence has resulted in conflicting reports and guidelines regarding the rollout of the ChAdOx1 nCoV-19 vaccine from different parts of the world, such as Canada, Germany, the EMA, and Thailand, but many countries have cautiously opted to continue

administration of the ChAdOx1 nCoV-19 vaccine.[22–24] With recent outbreaks in low- and middle-income countries such as India and Brazil, it is of urgent and critical importance to rapidly and comprehensively evaluate such vaccine-related adverse effects, especially as the ChAdOx1 nCoV-19 vaccine is both the major vaccine produced intrinsically in India and the largest component of the COVAX vaccine rollout plan.[25,26] Case reports and case series of rare, fatal thromboses associated with the ChAdOx1 nCoV-19 vaccine are accumulating; however, due to insufficient sample size, it is difficult to draw consistent, significant conclusions regarding the clinical presentation and treatment of these vaccine-associated thrombotic events, now called thrombosis with thrombocytopenia syndrome (TTS). Furthermore, no study to date has yet analysed risk factors for differential outcomes of TTS patients. Therefore, the present study aimed to perform a systematic review to investigate all published studies regarding TTS to analyse clinical and laboratory data, treatment modalities, and outcomes of patients and to discuss prognostic factors that may aid future therapeutic interventions.

Methods

Search strategy and selection criteria

This systematic review was performed in agreement with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P; Supplementary material online, *Table S1*).[27] As reports are being updated every day, a rapid review was conducted to summarize all published cases of TTS.

We initially carried out a search of PubMed ePubs, Scopus, Embase, and Web of Science databases to include all articles available regarding patients with COVID-19 vaccine-associated thrombosis after ChAdOx1 nCoV-19 vaccination published up to 28 April 2021, without limiting our search by language or date. Our initial search yielded 673 articles. After a review of individual abstracts and full texts, we identified seven studies (three case reports and four case series) that met the inclusion criteria for this systematic review.[10–16] In addition, we carried out an additional search in the same databases on 24 June 2021 and added 2 case series and 10 case reports.[28–39] The search terms used are described in detail in the Supplementary material online, *Table S2*. The detailed selection process is depicted in Supplementary material online, *Figure S1*; the characteristics of individual case studies are shown in Supplementary material online, *Table S3* and *S4*.

Cases were only included if they reported patients with a history of COVID-19 vaccination with the ChAdOx1 nCoV-19 vaccine prior to presentation, and if the patients had a haemorrhagic or thrombotic event documented by clinical and radiological findings. We excluded cases if they had received another type of COVID-19 vaccine. For the purposes of statistical analysis, we further excluded review articles, letters to the editors, abstracts, articles that did not contain sufficient information on the patients, and duplicate cases.

Three reviewers (J.I. Shin, S.H. Park, and S.B. Lee) independently examined the studies, and any disagreement among the authors was resolved by consensus. For each eligible case report and case series, we extracted data on the demographic, clinical, and laboratory findings at presentation, type of treatment, clinical course, and outcome.

Data collection

We identified 19 studies regarding TTS related to immunization with ChAdOx1 nCoV-19, and collected demographic and clinical characteristics including treatment and outcomes, comprising age, sex, onset of symptoms, ethnicity, pre-existing conditions, symptoms, laboratory results, immunological and platelet activation assays, number and location of thrombotic and/or haemorrhagic events, treatment modalities used, and mortality.

Statistical analysis

Statistical analyses were performed using the SPSS for Windows version 25.0 (SPSS Inc., IBM Corporation, Chicago, IL, USA) and R version 4.0.4 (R Core Team, Vienna, Austria). Basic demographic and clinical information was presented as the median and interquartile range (IQR) for continuous variables and the percentage for categorical variables. Continuous variables were compared with the Mann–Whitney U-test and categorical variables were compared using the Fisher's exact test. Spearman's correlation analysis was carried out to determine the relationships between continuous variables. Logistic regression analyses were also used to identify independent risk factors for mortality. In all statistical analyses, a two-tailed *P*-value of <0.05 was considered significant.

Briefly, the following steps were used to construct and validate the FAPIC score.

Step 1: construction of the FAPIC score

Demographic and clinical factors, laboratory measurements, and associated thromboses were considered as potential predictors. After univariable analyses of all parameters between survivors and non-survivors, binary variables that were significantly associated with mortality with a *P*-value of <0.05 were summed to create a FAPIC summary score for a logistic regression model. Only binary variables were considered in constructing the scoring model to achieve simplicity in application; cut-offs for continuous variables for dichotomization were pre-determined according to clinical relevance. Discriminative power of the FAPIC predictive model was assessed by drawing the receiver operating characteristic (ROC) curve and calculating the area under the curve (AUC) statistic (C-statistic). Model calibration was also assessed through Hosmer–Lemeshow goodness of fit analysis.

Step 2: internal validation

Internal validation of the prediction model was undertaken by two methods: the K-step cross-validation method and bootstrapping. First, K-step cross-validation was performed by taking 10% of the whole dataset for testing, and training the model on the remaining 90%, then repeating the procedure 20 times. Second, the predictive performance of the FAPIC scoring model was re-assessed via bootstrapping, sampling the whole dataset using 100 repetitions with replacement. For each method, the predictive model accuracy was assessed by computing the C-statistic.

Step 3: external validation

The external validation step was performed independently after the development of the model using different data. We independently collected all 16 published cases of TTS after Ad26.COV2.S vaccination to extract relevant clinical characteristics and mortality data,[40–44] and double-checked the data by reviewing the records in the Vaccine Adverse Event Reporting System (VAERS) of the U.S. Centers for Disease Control and Prevention (CDC). Baseline characteristics were compared between the original dataset and the validation set. Then, the performance of the FAPIC scoring model was assessed by computing the C-statistic.

Finally, a secondary analysis of steps 1–3 was performed after estimating missing values from the observed values using multiple imputation by chained equations (MICE). Twelve hundred rounds of imputation were performed, and the imputation algorithm was checked for convergence.

Table 1 Laboratory findings of patients with VITT after ChAdOx1 nCoV-19 vaccination according to outcome

Laboratory findings	Total patients ($n = 64$[a])	Survivors ($n = 40$)	Non-survivors (n = 23)	P-value
Platelet ($n = 62$)				
Platelet count (cells/mm^3)	35 000 (16 750, 70 250)	40 000 (26 000, 70 000)	19,000 (13,750, 75,750)	0.121
Platelet <25 × 10^3/μL	22/62 (35.5)	9/39 (23.1)	13/22 (60.9)	**0.007**
PT ($n = 41$)				
PT (s) ($n = 10$)	13.35 (12.95, 14.95)	14.10 (13.15, 20.40)	13.10 (12.80, 15.00)	0.366
PT INR ($n = 28$)	1.20 (1.10, 1.40)	1.20 (1.10, 1.30)	1.20 (1.10, 1.66)	0.488
PT, abnormal value[b]	31/41 (70.5)	18/27 (66.7)	13/17 (76.5)	0.735
aPTT ($n = 43$)				
aPTT (s) ($n = 23$)	29.90 (25.00, 39.43)	28.70 (24.00, 37.35)	32.70 (27.75, 43.85)	0.258
aPTT ratio ($n = 14$)	1.05 (0.98, 1.33)	1.05 (0.98, 1.33)	1.05 (0.91, 1.55)	0.962
aPTT, abnormal value[b]	16/43 (37.2)	10/26 (38.5)	6/17 (35.3)	1.000
Fibrinogen ($n = 50$)				
Fibrinogen (mg/dL)	140.00 (110.00, 262.50)	210.00 (120.00, 345.00)	120.00 (80.00, 140.00)	**0.003**
Fibrinogen <150 mg/dL	26/50 (52.0)	11/31 (35.5)	15/19 (78.9)	**0.004**
D-dimer ($n = 55$)				
D-dimer/upper limit of normal range	62.60 (20.80, 70.40)	45.80 (16.30, 70.40)	70.00 (32.22, 79.05)	0.143
D-dimer, abnormal value (>500 mg/L, FEU)	55/55 (100)	37/37 (100)	17/17 (100)	1.000
Anti-PF4/heparin antibody ELISA ($n = 47$)				
Anti-PF4/heparin antibody ELISA OD	2.16 (1.14, 2.92)	1.44 (0.64, 2.63)	2.26 (1.40, 3.13)	0.103
Anti-PF4/heparin antibody ELISA positive	46/47 (97.9)	26/27 (96.3)	19/19 (100.0)	1.000
Functional HIT Assay ($n = 21$)				
Platelet activation assay	19/21 (90.5)	9/10 (90.0)	9/10 (90.0)	1.000

Values are given as *n/N* (%) or median (IQR).
IQR, interquartile range; ELISA, enzyme-linked immunosorbent assay; HIT, heparin-induced thrombocytopenia; OD, optical density; FEU, fibrinogen equivalent unit.
[a]One patient had an unknown outcome.
[b]PT (prothrombin time)/PT (s) normal range: 10.0–12.0 s/PT INR normal range: 0.9–1.1.
[††]aPTT (activated partial thromboplastin time)/aPTT (s) normal range: 25.0–35.0 s/aPTT ratio normal range: 0.8–1.2.

Results

Demographics and clinical characteristics

The 64 patients with TTS were 21–71 years of age, with a median age of 45 years and an IQR of 22.75 years. Over two-thirds (68.5%) of patients were women. None of the patients reported a pre-existing prothrombotic condition.

Overall, patients presented to the hospital from a range of 5 to 24 days after vaccination, with a median time from vaccination of 10 days. Presenting symptoms from these cases are shown in Supplementary material online, *Table S5*. The most common symptoms for patients were neurological; notably, 22 out of 30 (73.3%) patients for whom symptom data were available presented with headache, followed by hemiparesis (30.0%), visual disturbance (26.7%), dysphasia (16.7%), dizziness (13.3%), and seizure (13.3%). Half (50.0%) of patients reported systemic symptoms, with fever in 23.3%, reduced consciousness in 16.7%, fatigue in 10.0%, and myalgia in 6.7% of patients. Gastrointestinal manifestations were present in seven patients (23.3%), including abdominal pain (13.3%) and vomiting (10.0%). Three patients (10.0%) reported bleeding tendency, including gum bleeds (6.7%), haematoma (6.7%), and petechial rash (3.3%). Other symptoms included dyspnoea (10.0%), chest pain (6.7%), back pain (6.7%), and arthralgia (3.3%).

Laboratory findings of TTS patients are delineated in *Table 1*. Most patients presented with thrombocytopenia, with a median platelet count (IQR) of 35 × 10^3/μL (16.75 × 10^3–70.25 × 10^3/μL). Thirty-one out of 41 (70.5%) patients had abnormal protrombin time (PT) and 16 (37.2%) had abnormal partial thromboplastin time (PTT), with median PT [international normalized ratio (INR)] of 1.20 and median activated partial thromboplastin time (aPTT; s) of 29.90. More than half (52.0%) had severe hypofibrinogenemia with fibrinogen levels <150 mg/dL. All 55 patients (100.0%) who were analysed had extremely elevated D-dimer levels, with an average of 62.60 times the upper limit of normal. Furthermore, the results of the correlation analysis indicated that platelet counts, fibrinogen levels, and D-dimer levels were associated (Supplementary material online, *Table S6*).

Forty-seven patients in our study underwent immunological testing for HIT antibodies; 46 (97.9%) had positive HIT antibody ELISA (enzyme-linked immunosorbent assay) tests with a median optical density (OD) of 2.16. Nineteen out of 21 (90.5%) patients who tested for functional PF4-dependent platelet activation assays had positive results.

Manifestations of thrombotic and haemorrhagic events

Sixty-one (95.3%) patients were identified with at least one thrombotic event (*Table 2*). More than one-third (35.9%) of these patients had two or more sites of thrombosis. The most common site of thrombosis was the brain (68.8%), with CVT in 59.4% of patients

Table 2 Thrombosis and haemorrhage of patients with VITT after ChAdOx1 nCoV-19 vaccination according to outcom

Thrombosis/haemorrhage	Total patients ($n = 64$[a])	Survivors ($n = 40$)	Non-survivors (n = 23)	P-value
Patients with thrombosis				
Presence of thrombosis	61/64 (95.3%)	38/40 (95.0%)	22/23 (95.7%)	1.000
Two or more sites of thrombosis	23/64 (35.9%)	13/40 (32.5%)	10/23 (43.5%)	0.424
Thrombosis sites				
Brain	44/64 (68.8%)	24/40 (60.0%)	19/23 (82.6%)	0.092
Cerebral venous thrombosis	38/64 (59.4%)	19/40 (47.5%)	18/23 (78.3%)	**0.020**
Acute middle cerebral artery thrombosis	5/64 (7.8%)	3/40 (7.5%)	2/23 (8.7%)	1.000
Arterial cerebral ischaemic attack	2/64 (3.1%)	2/40 (5.0%)	0/23 (0.0%)	0.529
Heart	3/64 (4.7%)	2/40 (5.0%)	1/23 (4.3%)	1.000
Myocardial infarction	1/64 (1.6%)	0/40 (0.0%)	1/23 (4.3%)	0.365
Intraventricular	2/64 (3.1%)	2/40 (5.0%)	0/23 (0.0%)	0.529
Pulmonary system	16/64 (25.0%)	13/40 (32.5%)	3/23 (13.0%)	0.133
Pulmonary embolism	13/64 (20.3%)	11/40 (27.5%)	2/23 (8.7%)	0.108
Pulmonary artery	1/64 (1.6%)	1/40 (2.5%)	0/23 (0.0%)	1.000
Not specified	2/64 (3.1%)	1/40 (2.5%)	1/23 (4.3$)	1.000
Gastrointestinal system	16/64 (25.0%)	9/40 (22.5%)	7/23 (30.4%)	0.554
Medium to large sized vessels	12/64 (18.8%)	10/40 (25.0%)	2/23 (8.7%)	0.183
Deep vein thrombosis	3/64 (4.7%)	3/40 (7.5%)	0/23 (0.0%)	0.293
Acute aortic thrombosis	2/64 (3.1%)	0/40 (0.0%)	2/23 (8.7%)	0.130
Aortoiliac	7/64 (10.9%)	6/40 (15.0%)	1/23 (4.3%)	0.407
Internal jugular vein thrombosis	3/64 (4.7%)	3/40 (7.5%)	0/23 (0.0%)	0.293
Inferior vena cava thrombosis	2/64 (3.1%)	2/40 (5.0%)	0/23 (0.0%)	0.529
Others	7/64 (10.9%)	5/40 (12.5%)	2/23 (8.7%)	1.000
Patients with haemorrhage				
Presence of haemorrhage	21/64 (32.8%)	9/40 (22.5%)	12/23 (52.2%)	**0.026**
Haemorrhage sites				
Intracerebral haemorrhage	12/64 (18.8%)	4/40 (10.0%)	8/23 (34.8%)	**0.022**
Subarachnoid haemorrhage	3/64 (4.7%)	1/40 (2.5%)	2/23 (8.7%)	0.548
Adrenal haemorrhage	3/64 (4.7%)	2/40 (5.0%)	1/23 (4.3%)	1.000
Not specified	3/64 (4.7%)	2/40 (5.0%)	1/23 (4.3%)	1.000

[a]One patient had an unknown outcome.

with thrombosis, the middle cerebral artery thrombosis in 7.8%, and other arterial cerebral ischaemic attack in 3.1%. Thirteen patients (20.3%) had pulmonary embolism, and one patient (1.6%) had pulmonary artery thrombosis. Gastrointestinal involvement was also common (25.0%). Other sites of thrombosis included deep vein (4.7%), internal jugular vein (4.7%), and inferior vena cava (3.1%) thrombosis.

Twenty-one patients (32.8%) presented with haemorrhage. Among patients with haemorrhage, 57.1% had intracerebral haemorrhage (ICH), followed by subarachnoid haemorrhage (SAH) and adrenal haemorrhage, each at 14.3%. In three cases, the location of haemorrhage was not specified.

Treatment approaches

The treatment modalities used in patients with TTS are shown in *Table 3*. Among the 39 patients for whom we had information about treatment, 26 (66.7%) received heparin products; unfractionated heparin (UFH) was administered in 25.6% and low molecular weight heparin (LMWH) was used in 28.2%. Steroids were used in 31.7% of patients and intravenous immunoglobulin (IVIG) was used in 43.9% of patients. Platelet transfusions were administered in 19.5% of cases, and red blood cell (RBC) transfusions were required in one patients (2.4%). Non-heparin anticoagulants—a direct oral anticoagulant (DOAC) or a direct thrombin inhibitor—were used in 14 (34.1%) patients, 6 (14.6%) of whom used DOACs, 7 (17.1%) of whom used direct thrombin inhibitors, and 1 of whom used an unspecified non-heparin anticoagulant. Twelve patients (29.3%) required surgery.

Characteristics in patients according to mortality

Overall, 23 (35.9%) patients died, 40 (62.5%) were alive and recovering, and 1 (1.6%) had an unknown outcome. A number of clinical and laboratory markers were significantly associated with mortality (*Table 4*). Severe thrombocytopenia of $<25 \times 10^3/\mu L$ ($P = 0.007$), hypofibrinogenaemia of <150 mg/dL ($P = 0.004$), the presence of CVT ($P = 0.020$), and the presence of ICH ($P = 0.022$) were significantly

Table 3 Treatment modalities in patients with VITT after ChAdOx1 nCoV-19 vaccination according to outcome

Treatment	Total patients ($n = 64$[a])	Survivors (n = 40)	Non-survivors (n = 23)	P-value
Treatment received				
Heparins	26/39 (66.7%)	17/23 (73.9%)	9/16 (56.3%)	0.312
Unfractionated heparin	10/39 (25.6%)	6/23 (26.1%)	4/16 (25.0%)	1.000
Low molecular weight heparin	11/39 (28.2%)	7/23 (30.4%)	4/16 (25.0%)	1.000
Fondaparinux	6/39 (15.4%)	5/23 (21.7%)	1/16 (6.3%)	0.370
Steroids	13/41 (31.7%)	9/24 (37.5%)	4/16 (25.0%)	0.503
Prednisolone	5/41 (12.2%)	4/24 (16.7%)	1/16 (6.3%)	0.631
Methylprednisolone	6/41 (14.6%)	4/24 (16.7%)	2/16 (12.5%)	1.000
Dexamethasone	4/41 (9.8%)	3/24 (12.5%)	1/16 (6.3%)	0.638
Transfusion				
Intravenous immunoglobulin	18/41 (43.9%)	13/24 (54.2%)	5/16 (31.3%)	0.203
Platelet	8/41 (19.5%)	2/24 (8.3%)	6/16 (37.5%)	**0.042**
Red blood cell	1/41 (2.4%)	0/24 (0.0%)	1/16 (6.3%)	0.400
Fibrinogen concentrate	1/41 (2.4%)	1/24 (4.2%)	0/16 (0.0%)	1.000
Plasmapheresis	1/41 (2.4%)	1/24 (4.2%)	0/16 (0.0%)	1.000
Surgery	12/41 (29.3%)	5/24 (20.8%)	7/16 (43.8%)	0.166
Neurosurgery	8/41 (19.5%)	1/24 (4.2%)	7/16 (43.8%)	**0.004**
Bowel resection	3/41 (7.3%)	3/24 (12.5%)	0/16 (0.0%)	0.262
Thrombectomy	2/41 (4.9%)	1/24 (4.2%)	1/16 (6.3%)	1.000
Tissue plasminogen activator	1/41 (2.4%)	1/24 (4.2%)	0/16 (0.0%)	1.000
Non-heparin anticoagulants	14/41 (34.1%)	13/24 (54.2%)	1/16 (6.3%)	**0.002**
Direct oral anticoagulant	6/41 (14.6%)	5/24 (20.8%)	1/16 (6.3%)	0.373
Direct thrombin inhibitor	7/41 (17.1%)	7/24 (29.2%)	0/16 (0.0%)	**0.029**
Eculizumab	2/41 (4.9%)	2/24 (8.3%)	0/16 (0.0%)	0.508

[a]One patient had an unknown outcome.

associated with adverse outcome. Furthermore, we found that age over 60 was negatively associated with mortality ($P = 0.010$). Patients at or under 60 years of age were more likely to have adverse clinical characteristics, such as thrombosis in the brain, CVT, and fibrinogen levels <150 mg/dL than those aged >60 years (Supplementary material online, *Figure S2*).

Regarding treatment, the administration of non-heparin anticoagulants was significantly associated with favourable outcome ($P = 0.002$). Specifically, all seven patients who received a direct thrombin inhibitor recovered and none died ($p = 0.029$), but the patients who received a direct thrombin inhibitor also had milder clinical profiles (Supplementary material online, *Table S7*). Platelet transfusion was also significantly associated with mortality (8.3% vs. 37.5%, $P = 0.042$), but in this case as well, patients who were administered platelets tended to have worse clinical profiles and risk factors such as lower platelet counts, and ICH. Seven out of eight (87.5%) patients who underwent neurosurgery died, while mortality was lower at 39.6% for those who did not receive surgery ($P = 0.004$).

Risk factors for mortality

According to logistic regression analyses, we found that platelet count $<25 \times 10^3/\mu L$ [odds ratio (OR) 4.815, 95% confidence interval (CI) 1.555–14.907, $P = 0.006$], fibrinogen levels <150 mg/dL (OR 6.818, 95% CI 1.811–25.672, $P = 0.005$), the presence of ICH (OR 4.800, 95% CI 1.253–18.384, $P = 0.022$), and the presence of CVT (OR 3.979, 95% CI 1.236–12.809, $P = 0.021$) were significantly associated with mortality (Supplementary material online, *Table S8*).

The FAPIC predictive scoring model for mortality

We designed a novel scoring system for mortality in TTS patients based on the predictive performance of our regression models. We included variables that were significantly associated with mortality in the univariate analyses and did not have missing values, which were age ≤60 years, platelet count $<25 \times 10^3/\mu L$, fibrinogen <150 mg/dL, the presence of ICH, and the presence of CVT. The model was a sum of scores consisting of one point for each of these five predictors. We named this scoring system FAPIC from the components of the model: fibrinogen, age, platelet count, ICH, and CVT. The predicted mortality increased with each point increase in the FAPIC score, with expected probability of death of 2.08% with FAPIC score 0, of 6.66% with FAPIC score 1, of 19.31% with FAPIC score 2, of 44.54% with FAPIC score 3, of 72.94% with FAPIC score 4, and of 90.05% with FAPIC score 5 (*Figure 1A*). The calculated C-statistic for the FAPIC score was 0.837 (95% CI 0.732–0.942) (*Figure 1B*). The Hosmer–Lemeshow goodness of fit test yielded a test statistic of 2.857 and a *P*-value of 0.582, signifying a good fit between the model and the observed data.

Table 4 Univariable analyses of demographic, clinical, laboratory findings, thrombosis, haemorrhage, and treatment in patients with VITT after ChAdOx1 nCoV-19 vaccination

Variables	Survivors ($n = 40$)	Non-survivors ($n = 23$)	*P*-value
Demographics			
Age	46.00 (34.25, 61.00)	37.50 (30.75, 52.50)	0.241
Age ≤60 years	30/40 (75.0)	23/23 (100.0)	**0.010**
Female sex	26/36 (72.2)	11/18 (61.1)	0.536
Time to presentation[a]	10.00 (7.00, 14.00)	10.00 (7.00, 10.25)	0.309
Clinical presentations			
Systemic	9/20 (45.0)	6/10 (60.0)	0.700
Neurological	16/20 (80.0)	10/10 (100.0)	0.272
Bleeding	1/20 (5.0)	2/10 (20.0)	0.251
Gastrointestinal	3/20 (15.0)	4/10 (40.0)	0.181
Cardiopulmonary	4/20 (20.0)	0/10 (0.0)	0.272
Laboratory findings			
Platelet count (cells/mm^3)	40 000 (26 000, 70 000)	19 000 (13 750, 75 750)	0.121
Platelet <25 × 10^3/μL	9/39 (23.1)	13/22 (60.9)	**0.007**
Fibrinogen (mg/dL)	210.00 (120.00, 345.00)	120.00 (80.00, 140.00)	**0.003**
Fibrinogen <150 mg/dL	11/31 (35.5)	15/19 (78.9)	**0.004**
D-dimer/upper limit of normal range	45.80 (16.30, 70.40)	70.00 (32.22, 79.05)	0.143
HIT ELISA (OD)	1.44 (0.64, 2.63)	2.26 (1.40, 3.13)	0.103
Platelet activation assay	9/10 (90.0)	9/10 (90.0)	1.000
Thrombosis and haemorrhage			
Presence of thrombosis	38/40 (95.0)	22/23 (95.7)	1.000
More than 2 sites of thrombosis	9/40 (22.5)	2/23 (8.7)	0.301
Cerebral venous thrombosis	19/40 (47.5)	18/23 (78.3)	**0.020**
Presence of haemorrhage	9/40 (22.5)	12/23 (52.2)	**0.026**
Intracerebral haemorrhage	4/40 (10.0)	8/23 (34.8)	**0.022**
Treatment			
Heparins	17/23 (73.9)	9/16 (56.3)	0.312
Steroids	9/24 (37.5)	4/16 (25.0)	0.503
Intravenous immunoglobulin	13/24 (54.2)	5/16 (31.3)	0.203
Platelet transfusion	2/24 (8.3)	6/16 (37.5)	**0.042**
Neurosurgery	1/24 (4.2)	7/16 (43.8)	**0.004**
Non-heparin anticoagulants	13/24 (54.2)	1/16 (6.3)	**0.002**
Direct thrombin inhibitor	7/24 (29.2)	0/16 (0.0)	**0.029**
FAPIC score	2.00 (1.00, 3.00)	4.00 (3.00, 4.00)	**< 0.001**

Values are give as median (interquartile range), or *n*/*N* (%).
HIT, heparin-induced thrombocytopenia; OD, optical density; VITT, vaccine-induced immune thrombotic thrombocytopenia.
[a]If time to admission after vaccination was not given, time to symptom onset after vaccination was used.

Internal and external validation of the FAPIC score

Internal validation of the FAPIC score demonstrated a good discrimination in both K-step cross-validation and bootstrapping methods. The calculated C-statistic for the FAPIC score was 0.786 (95% CI 0.757–0.814) and 0.807 (95% CI 0.787–0.827) in the K-step cross-validation and bootstrapping procedures, respectively (*Figure 2*).

Before externally validating the predictive performance of the FAPIC score in the Ad26.COV2.S dataset, we compared the clinical profiles of TTS after ChAdOx1 nCoV-19 and Ad26.COV2.S vaccines, which is shown in Supplementary material online, *Table S9*. For the validation dataset, the risk of death increased with each point increase in the FAPIC score, with an estimated mortality of 0% with FAPIC score 0–2, of 40.0% with FAPIC score 4, and of 50.0% with FAPIC score 5. There were no patients with TTS after Ad26.COV2.S who had a FAPIC score of 5. The ROC curve is shown in Supplementary material online, *Figure S3*; the C-statistic was 0.771 (95% CI 0.509–1.000).

With multiple imputation, 19 observations with missing variables in the FAPIC score were added. Good discriminatory performance of the FAPIC score was replicated on the complete dataset after multiple imputation (Supplementary material online, *Figure S4*).

Discussion

The incidence of CVT after COVID-19 vaccination has been reported as 2.5 cases per million in 4 months, higher than 1.3 cases

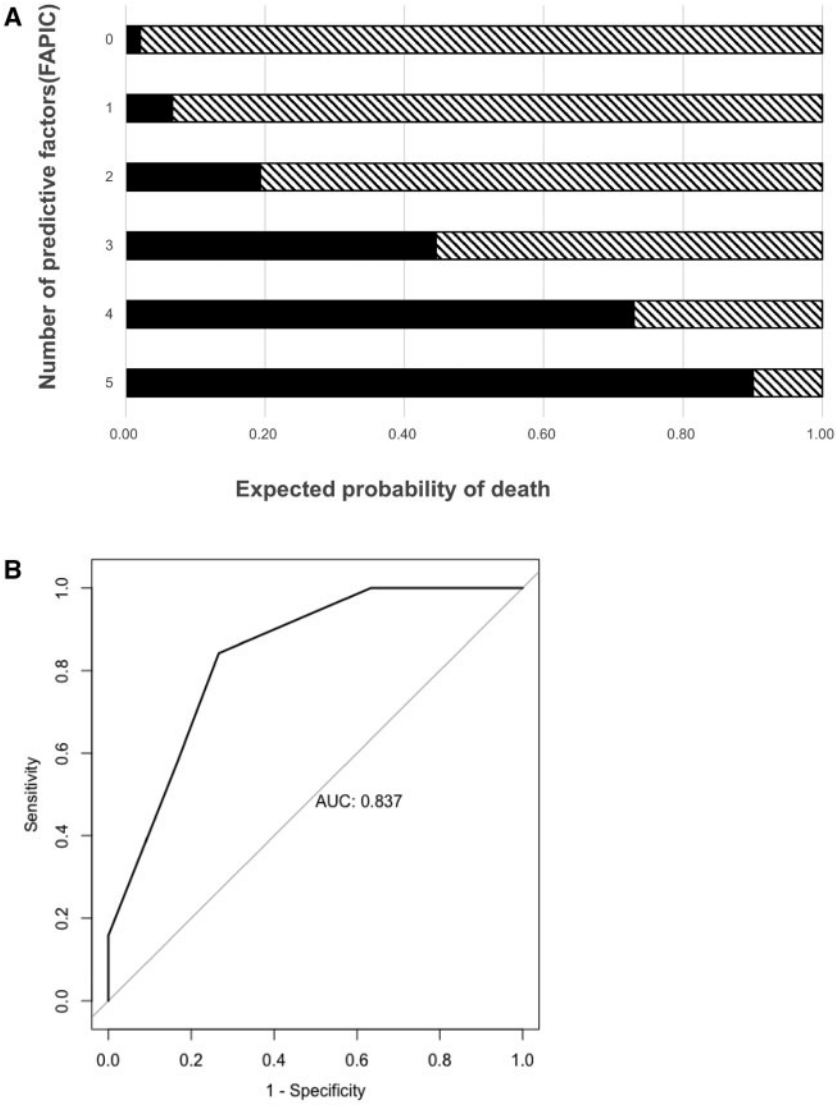


Figure 1 Estimated mortality in patients with TTS after ChAdOx1 nCoV-19 vaccination by FAPIC score (*A*) and the receiver operating characteristic (ROC) curve with the area under the curve (AUC) (*B*) of the FAPIC score. Variables included in the FAPIC score were: age ≤60 years, platelet count <25 × 10^3/μL, fibrinogen <150 mg/dL, the presence of intracerebral haemorrhage, and the presence of cerebral venous thrombosis.

per million in the initially reported incidence in the general population.[45] Balancing the risk of vaccine-associated adverse events and the benefits of population-wide prevention of COVID-19, many countries have opted to continue the rollout of ChAdOx1 nCoV-19 vaccinations cautiously, while some countries have halted distributions or implemented age restrictions.[18,19,22–24] As massive amounts of vaccinations including the ChAdOx1 nCoV-19 vaccine are continuing to be administered at the time of writing,[6] a rapid, systematic assessment of the clinical manifestations, treatment, and outcomes of TTS is crucial.

This systematic review summarizes 64 cases of TTS after ChAdOx1 nCoV-19 vaccination to analyse the clinical presentation, treatment modalities, outcomes, and prognostic factors associated with adverse outcomes (*Graphical Abstract*). Previously, the clinical picture of TTS has been compared with autoimmune HIT;[10,12] likewise, the patients in this systematic review had a similar clinical presentation to HIT without previous exposure to heparin products. Notably, in our study, 73.3% of patients whose symptoms were reported presented with a headache at initial presentation; other neurological symptoms such as hemiparesis, visual disturbance, and hemiplegia were also common. This is concordant with the hallmark presentation of typical CVT, as subacute headache is known to be present in 90% of CVT cases.[46,47] Patients could also present with a constellation of systemic, gastrointestinal, and bleeding symptoms. Furthermore, all patients had thrombocytopenia upon admission, with mean platelet count of 31 × 10^3/μL. Most patients (95.3%) had a thrombotic event, among which 59.4% had CVT; three patients did not present with thromboses, but with isolated haemorrhagic events. Haemorrhage was relatively common, occurring in 32.8% of patients and more than half being ICH; 8 out of 19 (80%) ICH cases were

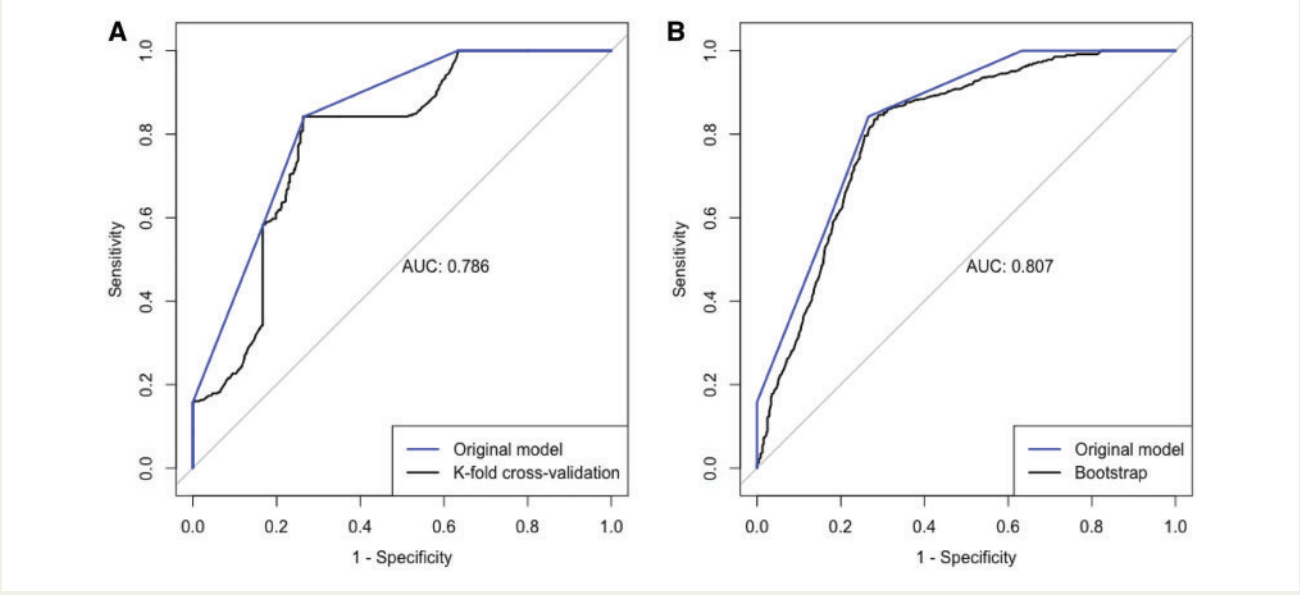


Figure 2 The receiver operating characteristic (ROC) curve and the area under the curve (AUC) of the FAPIC score on cross-validation (*A*) and bootstrapping (*B*).

associated with CVT. This spectrum of thrombotic and haemorrhagic events shows that TTS is not only limited to CVT, but can present with varying severity and locations.

In most cases, patients underwent immunological testing for anti-heparin/PF4 antibodies. In our study, 97.9% of patients with TTS after ChAdOx1 nCoV-19 vaccination tested positive for anti-PF4/heparin antibodies. Most had a very high OD for HIT ELISA without having had previous exposure to heparin, similar to what is seen in autoimmune HIT.[48] However, it should be noted that individual studies employed different methods for anti-heparin/PF4 antibodies, which have different diagnostic properties. The IgG ELISA tests for anti-heparin/PF4 antibodies typically have high sensitivity nearing 95–100%, but varying specificities.[49–51] For example, one study reported that the Asserachrom HPIA, used by Scully *et al.*,[11] had a 100% sensitivity and 77.8% specificity; and the LIFECODES PF4 IgG ELISA kit, used by Schultz *et al.*,[10] demonstrated 100% sensitivity and 31.6% specificity.[52] Both Asserachrom and LIFECODES anti-PF4 ELISA kits have been tested to successfully detect TTS antibodies.[53]

Furthermore, 19 out of 21 patients who underwent subsequent functional platelet activation assays yielded positive results as described by the original articles. Functional platelet activation assays provide more definitive, specific evidence that anti-heparin/PF4 antibodies contribute to the aberrant activation of platelets, which further support previous postulations that the mechanism of TTS may be similar to that of autoimmune HIT.[10,12] However, these results must be interpreted with caution, as functional platelet assays have significant heterogeneity in their specific methodology, and the results may be subject to error or misinterpretation (Supplementary material online, *Table S10–S11*). Furthermore, the four studies employed different methods in evaluating platelet aggregation, namely a modified heparin-induced platelet aggregation, the multiplate method, a flow cytometry-based method, and a serotonin release assay (SRA).[10–12,21,31,40,54,55] In the literature, the positive rate for functional platelet activation tests is reported to be far lower for patients with Ad26.COV.2.S-associated TTS.[40] However, as all 12 patients in this study were tested with the SRA, the different properties of confirmatory tests should be considered when interpreting the results of functional platelet activation assays.

This study was the first study to analyse risk factors for mortality in TTS. Notably, the overall mortality of TTS was high at 35.9%. This may have been partially because these patients were among the initial reported cases of TTS, and many of them received heparin products—LMWH or UFH—in the early stages of presentation. One of the most significant risk factors for mortality in our study was the presence of ICH. This is consistent with the literature, as risk factors suggestive of adverse outcomes in HIT include severity of thrombocytopenia,[56] and female gender has also been identified as a potential risk factor of thrombotic stroke as an outcome of HIT.[57] Cerebral haemorrhage has also been identified as an adverse prognostic factor for cerebral venous sinus thrombosis.[58]

In addition, patients who died were more likely to have lower platelet counts, lower fibrinogen levels, ICH, and CVT. The results of the correlation analysis also indicate that platelet counts are positively associated with fibrinogen, and negatively associated with D-dimer levels, pointing to a clinical picture similar to disseminated intravascular coagulation (DIC) with thrombocytopenia, hypofibrinogenaemia, and elevated D-dimer levels, which also predisposes patients to haemorrhage. This indicates a clinical picture in which severe TTS patients progress to a DIC-like state, predisposing them to haemorrhage and thus leading to an adverse outcome. Furthermore, age above 60 was a protective factor towards survival. Patients above 60 were also less likely to have an adverse clinical profile such as CVT

and low fibrinogen. This could be attributed to a less robust immune response post-vaccination due to immunosenescence,[59,60] resulting in a weaker autoimmune reaction and thus a less morbid clinical course.

From these associations, we developed a novel FAPIC score to predict mortality in patients with TTS. In our dataset, we found that risk of death increased with increasing FAPIC score, with a high C-statistic of 0.837 (95% CI 0.732–0.942). When the FAPIC score was internally validated through K-step cross-validation and bootstrapping, the model was found to have good discrimination, with a C-statistic of 0.786 (95% CI 0.757–0.814) and 0.807 (95% CI 0.787–0.827), respectively. Furthermore, its predictive power was replicated on a panel of TTS patients after Ad26.COV2.S administration, showing good discrimination (C-statistic = 0.771, 95% CI 0.560–1.000).

In our study, the use of non-heparin anticoagulants—direct thrombin inhibitors, such as argatroban, or DOACs, such as rivaroxaban and apixaban—was significantly associated with a favourable outcome. In fact, 13 out of the 14 patients who were administered non-heparin anticoagulants recovered. This is in accordance with the literature on HIT which recommends limiting heparin and initiating alternative anticoagulants such as DOACs or direct thrombin inhibitors.[21,61,62] The recent recommendations by the Expert Haematology Panel (EHP) and experts also suggest the use of these non-heparin-based anticoagulants in the setting of TTS.[63,64] In addition, as IVIG has been utilized as a treatment adjunct in autoimmune HIT,[65,66] there have been recommendations of the usage of IVIG and glucocorticoids in TTS to improve platelet counts and lower the risk of haemorrhagic transformation;[64,67] in our study, although survivors had a higher likelihood of having used IVIG of 54.2% compared with 31.3%, the difference was not statistically significant. However, our results regarding treatment must be interpreted cautiously due to the small sample size and potential confounding by indication.

Previously, there has been a comparison of the clinical profiles of CVT after ChAdOx1 nCoV-19 and Ad26.COV2.S, which reported that patients who received Ad26.COV2.S tend to present with CVT later, and have a more insidious clinical course despite a higher likelihood of ICH.[68] Patients with ChAdOx1 nCoV-19-associated TTS had a significantly shorter time to admission, higher rates of functional platelet assay positivity, and higher prevalence of ICH; they also tended to have higher D-dimer levels and higher prevalence of CVT with borderline significance. Other clinical characteristics were not significantly different.

A recent case series by See *et al.* reported all initial 12 cases of Ad26.COV2.S-associated TTS as Caucasian females aged 18–60, with additional risk factors such as obesity, hypothyroidism, and the use of combined oral contraceptives in 7 of them.[40] In our panel of 64 patients, TTS affected both males and females—although females accounted for 72.2%—at varying ages of 21–71; however, three patients who died were females aged 30–55 receiving oral contraceptives. More data regarding pre-existing conditions and medication use are required to evaluate the risk of developing TTS after vaccine administration.

There are some limitations to this study. As this study was a pooled analysis of published case reports and case series, we could not directly assess the electronic medical records of the 64 patients we reviewed. Our findings should be interpreted carefully considering that the representation of clinical information in the reports summarized may have been selective and incomplete *(Supplementary material online, Table S12–S13)*. The variables we analysed were limited to basic demographic, laboratory, and imaging findings, and the variables we included in our scoring system may reflect underlying disease progression rather than being root causes. Detailed, comprehensive review of pertinent clinical information such as comorbidities and medication history may result in more information on individuals at high risk for TTS incidence and adverse outcomes. Secondly, the sampling frame of our study was small and subject to publication bias. To mitigate this limitation, we aimed to perform an internal validation of the FAPIC score through cross-validation and bootstrapping methods, and an external validation on a distinct panel of TTS patients. More studies on national or international safety databases are also warranted to further verify the risk factors of mortality that were observed from this study. Furthermore, because of the extremely rare nature of TTS, the number of currently available cases was relatively small at 64 patients. Going forward, we expect higher statistical power and further insights from future accumulation of data. Further studies are needed to elucidate the exact pathophysiology of TTS and shed light on its clinical course; taking a step further, future investigations with more robust patient data are warranted to confirm whether the risk factors we identified play independently causal roles rather than simply being associated with mortality. Furthermore, exploration of predictors for the incidence of TTS from pre-vaccination profiles could aid clinical decision-making among available vaccines and potentially prevent the occurrence of TTS.

In conclusion, this study is the first to identify independent risk factors for mortality and propose a novel FAPIC score for predicting mortality in patients with TTS. We demonstrated that older age, severe thrombocytopenia, severe hypofibrinogenaemia, and the presence of CVT and ICH were significantly associated with adverse outcomes in TTS patients after ChAdOx1 nCoV-19 vaccination, and the sum of these factors could reliably predict mortality. Furthermore, we confirmed that the use of non-heparin anticoagulants was significantly associated with a favourable outcome, which further supports current recommendations that as soon as patients are suspected with TTS, heparin products should be halted and other forms of anticoagulation considered. The results of our study suggest that a combination of demographic, laboratory, and clinical markers may serve as predictors for mortality in TTS patients and aid identification of high-risk patients in the clinical setting.

In light of similar reports of TTS after vaccination with Ad26.COV2.S,[40] and reports of thrombotic thrombocytopenia in critically ill COVID-19 patients, the precise mechanism as to how ChAdOx1 nCoV-19 vaccination gives rise to thrombotic thrombocytopenia and production of anti-PF4/heparin antibodies and whether the vaccines share a common antigenic interaction or have independent pathophysiology still remain to be elucidated. Added to the clinical severity of TTS, the sheer rarity of the disease and the paucity of available information are adding to unnecessary fear and vaccine hesitancy.[69] This study has quantitatively analysed scattered evidence from clinical reports to assess risk factors and predict mortality with the largest statistical power available. We expect that our report and the FAPIC score could be utilized to evaluate TTS patients according to clinical severity, further consolidate evidence regarding better or worse outcomes, and thus ameliorate the uncertainty that still prevails regarding TTS. As evidence and experience

regarding TTS are being accumulated, we expect this report to guide future management of TTS in mitigating the extremely high mortality rate in these cases, as well as inform the medical and lay community to help combat vaccine hesitancy.

Author contributions

J.H., S.H.P., S.W.L., D.K.Y., and J.I.S. designed this study. M.H.L., S.H.P., S.B.L., and J.I.S. collected the data, and J.H., S.H.P., S.W.L., D.K.Y., and J.I.S. performed the statistical analysis. J.H., S.H.P., S.W.L., D.K.Y., and J.I.S. wrote the first draft of the manuscript. All authors had full access to all the study data. All authors reviewed, wrote, and approved the final version. The corresponding authors had final responsibility for the decision to submit for publication.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Data availability

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

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