Clinical and biological features of cerebral venous sinus thrombosis following ChAdOx1 nCoV-19 vaccination

Vaccines for COVID-19 were developed with unprecedented speed and since January 2021, the AstraZeneca/Oxford University ChAdOx1 nCoV-19 vaccine has been administered to over 400 million people globally. In April 2021, the Medicines and Healthcare products Regulatory Agency (MHRA) and the European Medicines Agency reported a possible association between ChAdOx1 nCoV-19 and a rare syndrome of unusual site thrombosis combined with thrombocytopenia, termed vaccine-induced immune thrombotic thrombocytopenia (VITT). Frequency of VITT varies across age groups. Overall, 411 cases of VITT have been reported to the MHRA by 21 July 2021 with fatality rate of 17.76% (73/411).

We report our experience of four VITT cases from a single tertiary referral centre in London, UK, who suffered cerebral venous sinus thrombosis (CVST) with or without thrombosis elsewhere. Baseline clinical and laboratory features are shown in table 1. Informed written consent was obtained from all patients before publication. All patients fulfilled the proposed diagnostic criteria for VITT. Each case was reported via the MHRA Yellow card scheme and other national VITT-CVST surveillance projects.

All four patients were women aged 41–46 years and diagnosed with VITT 7–28 days post ChAdOx1 nCoV-19 vaccination. Each presented with head-ache and varying degrees of neurological deficit. Detailed case histories are provided in the online supplemental material 1. Comparative baseline characteristics and laboratory markers of four patients presenting with cerebral venous sinus thrombosis following AstraZeneca/Oxford University ChAdOx1 nCoV-19 vaccine are shown in table 1.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline characteristics and laboratory markers of four patients presenting with cerebral venous sinus thrombosis following AstraZeneca/Oxford University ChAdOx1 nCoV-19 vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Patient 2</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>Female</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>46</td>
</tr>
<tr>
<td><strong>Medical history</strong></td>
<td>Migraine</td>
</tr>
<tr>
<td><strong>Clinical presentation</strong></td>
<td>Headache, collapse, hemiparesis</td>
</tr>
<tr>
<td><strong>No. of days post vaccine</strong></td>
<td>14</td>
</tr>
<tr>
<td><strong>Thrombosis</strong></td>
<td>CVST</td>
</tr>
<tr>
<td><strong>Admission bloods</strong></td>
<td></td>
</tr>
<tr>
<td>Hemosit, Acid-Steel HIT-1g2+ (&lt;1 U/mL)</td>
<td>0.05 (neg)</td>
</tr>
<tr>
<td>Immucor ELISA PF4 HIT-1g2+ (&lt;0.4 Optical Density (OD))</td>
<td>2.48</td>
</tr>
<tr>
<td>HYPHEN Biomed ELISA PF4 HIT-1g2+ (&lt;0.4 OD)</td>
<td>1.67</td>
</tr>
<tr>
<td>Platelets (150–400×109)</td>
<td>39</td>
</tr>
<tr>
<td>D-dimer (&lt;500ng/mL, EAU)</td>
<td>&gt;20000</td>
</tr>
<tr>
<td>Fibrinogen (1.9–4.3g/L)</td>
<td>0.7</td>
</tr>
<tr>
<td>Troponin (ng/L) (&lt;19.8)</td>
<td>4.3</td>
</tr>
<tr>
<td>PT (12.8–17.4 s)</td>
<td>14.7</td>
</tr>
<tr>
<td>APTT (25.0–35.0 s)</td>
<td>43.2</td>
</tr>
<tr>
<td>DRVVT ratio</td>
<td>Negative</td>
</tr>
<tr>
<td>Antithrombin (70–130 IU/dL)</td>
<td>107</td>
</tr>
<tr>
<td>Protein C activity (70–130 IU/dL)</td>
<td>72</td>
</tr>
<tr>
<td>Free protein S antigen (70–130 IU/dL)</td>
<td>68.8</td>
</tr>
<tr>
<td>Factor VIII (50–150 IU/dL)</td>
<td>64.3</td>
</tr>
<tr>
<td>VWF-Ag (50–150 IU/dL)</td>
<td>220</td>
</tr>
<tr>
<td>VWF-Rco (50–150 IU/dL)</td>
<td>235</td>
</tr>
<tr>
<td>Plasminogen activity (70–130 IU/dL)</td>
<td>72</td>
</tr>
<tr>
<td>PAI-1 (ng/mL)**†</td>
<td>10.1</td>
</tr>
<tr>
<td>E-selectin**†</td>
<td>6.9</td>
</tr>
<tr>
<td>ICAM-1**†</td>
<td>78.8</td>
</tr>
<tr>
<td>VCAM-1**†</td>
<td>1192.8</td>
</tr>
<tr>
<td>Thrombomodulin**†</td>
<td>4.9</td>
</tr>
<tr>
<td>P-selectin**†</td>
<td>33.1</td>
</tr>
<tr>
<td>C3 (0.79–1.52 mg/dL)</td>
<td>1.25</td>
</tr>
<tr>
<td>C4 (0.16–0.30 mg/dL)</td>
<td>0.10</td>
</tr>
<tr>
<td>Clq (ng/mL)**†</td>
<td>289.4</td>
</tr>
<tr>
<td>CSB-9 (ng/mL)**†</td>
<td>184.8</td>
</tr>
</tbody>
</table>

**Mean (range) levels in eight control plasma samples tested in parallel. PAI-1: 2–4.1 (2.9–6.7); E-selectin: 7.5 (2.8–13.3); ICAM-1: 98.8 (71.0–148.0); VCAM-1: 671.9 (190.7–1076); thrombomodulin: 3.1 (4.8–5.8) and P-selectin: 26.5 (0–34.7)ng/mL.

†Performed in postadmission bloods.

Mean (range) in control plasma as defined by manufacturer: Clq: 129.6 (33.9–268.1) and CSB-9: 147 (75–219) ng/mL. Values in bold are abnormal.

APTT, activated partial thromboplastin time; CSB-1, cerebral venous sinus thrombosis; DRVVT, dilute Russell’s viper venom time; ICAM-1, intercellular adhesion molecule-1; PAI-1, plasminogen activator inhibitor-1; PE, pulmonary embolus; PF4, platelet factor 4; PT, prothrombin time; VCAM-1, vascular cell adhesion molecule-1; VWF-Ag, Von Willebrand antigen; VWF-Rco, Von Willebrand factor ristocetin factor.
Figure 1  Cranial images from four patients with cerebral venous sinus thrombosis vaccine-induced immune thrombotic thrombocytopenia. (A) Initial non-contrast CT scan of the head performed on admission of patient 1. A left parietal cortical vein (red arrow) is hyperdense and expanded, as is the anterior aspect of the superior sagittal sinus (blue arrow). Diffuse high attenuation is seen within the sulcal spaces of the right parietal lobe in keeping with subarachnoid haemorrhage (green arrow). (B) Midline sagittal slice from CT venogram (CTV) performed at admission of patient 1. There is an extensive filling defect throughout the entirety of the imaged superior sagittal sinus (red arrows). Contrast can be seen anterior to the thrombus. (C,D): MR susceptibility-weighted imaging sequence performed 2 weeks following admission of patient 1. (C) A filling defect is still present within the left cortical parietal vein (red arrow). Foci of susceptibility are present within the sulcal and cortical superior parietal lobule in keeping with subarachnoid haemorrhage with haemosiderin staining (green arrows). (D) Multiple dilated deep medullary veins (yellow arrow) within the left cerebral hemisphere which have developed as a result of the venous obstruction. (E) Initial CTV head performed on admission for patient 2. A large thrombus is seen within the mid-superior sagittal sinus where it is expanded (red arrow). It extends anteriorly with no contrast opacification anteriorly. (F) CTV performed at 2 weeks for patient 2 shows interval improvement with a reduction in size of the thrombus (red arrow) at the mid-superior sagittal sinus and contrast visible anteriorly. (G) Patient 3 initial unenhanced CT scan performed at the time of admission. There is subarachnoid haemorrhage (green arrow) within right post-central sulcus with cortical oedema posteriorly. Hyperdensity within the posterior superior sagittal sinus in keeping with acute sinus venous thrombosis (red arrow). (H) Reconstructed 3D Maximum Intensity Projection (MIP) projection from a contrast-enhanced MR venogram for patient 4 performed on day 3 of admission. Complete lack of contrast opacification within the left transverse or sigmoid sinus due to extensive venous thrombosis is seen.

material. Neuroimaging for patient 1 demonstrated extensive thrombosis involving both the dural venous sinuses and superficial cortical veins as well as associated subarachnoid haemorrhage in the parietal sulci bilaterally (figure 1A–D) but no thrombus detected in imaging of the abdomen. Patient 2 initially presented with superior sagittal sinus thrombosis associated with right-sided neurological deficit (figure 1E), branch intracranial portal venous thrombus and non-occlusive segmental pulmonary arteries filling defects consistent with pulmonary emboli (CT pulmonary angiogram (CTPA)). CT venogram (CTV) performed 2 weeks later showed improvement with a reduction in thrombus size (figure 1F). In patient 3, CTV demonstrated extensive dural venous sinus thrombosis affecting the superior sagittal, left transverse and sigmoid sinuses (online supplemental figure S1) and CTPA revealed a large saddle embolus with extensive thrombus extending into all lobar branches bilaterally with features of right heart strain (online supplemental figure S2). MRI further delineated multiple sites of thrombosed cortical veins and subarachnoid haemorrhage (figure 1G). For patient 4, CTV demonstrated extensive CVST with secondary area of infarct/oedema in the left posterior temporal lobe (figure 1H). CT scan of the abdomen demonstrated portal (online supplemental figure S3A) and hepatic vein thrombus (online supplemental figure S3B).

Of the typical abnormal blood parameters reported in the literature for VITT, thrombocytopenia and hypofibrinogenemia were evident in three out of four and two out of four patients, respectively, and all four exhibited grossly raised D-dimer. We confirm the importance of selecting appropriate anti-platelet factor 4 (PF4) antibody tests as all patients tested negative in the AcuStar HIT-IgG (PF4-H) chemiluminescent assay but strongly positive in two anti-PF4 ELISAs (Immucor, Hyphen BioMed) (table 1). Additional autoantibody tests revealed low levels of antinuclear antibodies in patients 2 and 4 (23–30 units at 1:40 serum dilution, assay cut-off=20 units), while antiphospholipid (aPL) antibodies were undetectable in nine different aPL assays employed (IgG, IgM, IgA anticardiolipin and anti-ß2GPI; IgG anti-domain I of ß2GPI; IgG, IgM anti-phosphatidylserine/prothrombin). Thus, our results reinforce the conclusion that anti-PF4 is the key pathogenic antibody in VITT.

A uniform management approach was taken with urgent plasma exchange (PLEX) initiated in combination with Intravenous Immunoglobulin (IVig) (1 g/kg in two divided doses over 2 days timed appropriately around PLEX) to minimise loss of IVig, high-dose steroids (1 g intravenous methylprednisolone followed by 20 mg dexamethasone intravenous or oral for 4 days with tapering dose over the next few days) and non-heparin-based anticoagulants (initially argatroban) with rituximab
(375 mg/m²) in two patients. All four patients survived with complete resolution of symptoms and laboratory markers suggesting that patients are not developing disseminated intravascular coagulation. This is slightly surprising given the very high D-dimer and reduced fibrinogen, which must therefore reflect localised fibrin formation and breakdown.

Further serological analysis (table 1) in our four patients may point towards additional approaches for VITT management. Results from our functional platelet aggregation assay suggest that although it is generally recommended to avoid heparin anticoagulant in VITT, it may not aggravate progressive thrombosis (online supplemental figure S4). Platelet aggregation induced by serum from three patients in the absence of heparin was reduced with both low-dose and high-dose unfractionated Heparin compared with control serum (in contrast to classical HIT serum). Serum from patient 3 who was not thrombocytopenic at any time had no effect on donor platelet aggregation (online supplemental figure S4).

Compelment inhibition with ecizumab was also shown to benefit VITT and indeed complement activation was evident in our patients as demonstrated by low levels of C3 (patient 2) and C4 (patients 1, 3 and 4) coupled with raised C3a (patients 1 and 3) and C5b-9 (patients 3 and 4) (table 1). It is worth noting that thrombin, FXa and plasmin generated during the fibrinolytic process are all capable of inducing complement activation and C5b-9 terminal complex assembly. We propose measurement of both total complement levels and activation products may support stratified patient management with anticomplement biologics.

To our knowledge, this is the first study to interrogate immune, coagulant/haemostatic, platelet and endothelial disturbances combined with imaging in VITT. Our clinical and laboratory findings are remarkably uniform, consistent with a genuine syndrome and the good outcomes reported here suggest that rapid aggressive therapy directed at pathogenesis could be beneficial. As the number of VITT cases rises globally, it is of utmost importance to understand the biological mechanisms that drive or further complicate VITT.

Christina Crosette-Thambiah,1,2 Charis Pericleous,3 Namir Asmar,4 Joshua Bomstyk,2 Anita Ranger,2 Abdul Shelab,2 Sai Priya Ramji,3 Soma Banerjee,3 Mike Laffan,1,2 Deepa J Arachchilage1,2

1Centre for Haematology, Department of Immunology and Inflammation, Imperial College London, London, UK
2Department of Haematology, Imperial College Healthcare NHS Trust, London, UK
3National Heart and Lung Institute, Imperial College London, London, UK
4Department of Neurology, Imperial College Healthcare NHS Trust, London, UK
5Neurology, Imperial College Healthcare NHS Trust, London, UK
6Department of Stroke & Neurosciences, Imperial College Healthcare NHS Trust, London, UK

Correspondence to Dr Deepa J Arachchilage, Centre for Haematology, Department of Immunology and Inflammation, Imperial College London, London, UK; d.arachchilage@imperial.ac.uk

Twitter Deepa J Arachchilage @DeepaArachhil1

Acknowledgements The authors would like to thank Dr Nina Solajo, Dr Fatake Chowdhury, Dr Kamala Gurung and Dr Isaac Obisanya, all clinical teams at intensive care and hyperacute stroke unit, nursing staff of the Apheresis Team and laboratory staff involved in care of the patients at Imperial College Healthcare NHS Trust. The authors would like to specially thank the haematology and biochemistry laboratory staff (Steve Fox, Francine Leutche-Djoufie, Harsha Hirani and Nelesh Morjaria) at Royal Brompton Hospital for performing most of the additional laboratory assays and Dr Marta Peverelli at National Heart and Lung Institute, Imperial College London.

Contributors DJA conceived the study, involved in data collection, data verification, data analysis, figures and data interpretation, wrote the original draft and reviewed and edited the manuscript. CC-T contributed to data collection, data verification, data analysis, figures and writing and editing the original manuscript. TF performed some of the laboratory assays and involved in data analysis, data interpretation and writing and editing of the manuscript. ML interpreted the data and wrote and revised the manuscript. JB and AR contributed to data collection. NA and SR provided radiology images and edited the manuscript. AS and SB contributed to writing the manuscript. All authors reviewed and approved the final version of the manuscript.

Funding This study was partly funded by Imperial College COVID19 Research Fund (PB8531 received by DJA and G26266 received by CP and DJA). CP was funded by Versus Arthritis (21223).

Competing interests DJA received research funding from Bayer PLC and Leo Pharma outside of this research project. ML received consultation and speaker fees from AstraZeneca, Sob, Leo Pharma, Takeda and Pfizer but it was not related to this research project. Others have no conflict of interests to declare.

Patient consent for publication Obtained.

Ethics approval This study was approved by research ethics committee approval (17NW0161).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Raw data can be made available via direct contact with DJA (d.arachchilage@ imperial.ac.uk).

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the information. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible
Letter

To cite Crossette-Thambiah C, Pericleous C, Asmar N, et al. J Neurol Neurosurg Psychiatry Epub ahead of print: [please include Day Month Year]. doi:10.1136/jnnp-2021-327340

Received 11 June 2021
Accepted 26 August 2021

J Neurol Neurosurg Psychiatry 2021;0:1–4.
doi:10.1136/jnnp-2021-327340

ORCID iD
Deeja J Arachchillage http://orcid.org/0000-0001-5993-4850

REFERENCES
1 WCC-DGWHO. Available: https://covid19.who.int/
Supplementary Material: Clinical and biological features of cerebral venous sinus thrombosis following ChAdOx1 nCov-19 Vaccination.

Table of Contents
Supplementary Material: Clinical and biological features of cerebral venous sinus thrombosis following ChAdOx1 nCov-19 Vaccination.......1
Clinical case descriptions ................................................................................... 1
Laboratory Methods ........................................................................................... 4
Anti-PF4 antibodies ......................................................................................... 4
Antiphospholipid (aPL) and anti-nuclear antibodies (ANA) ...................... 4
Complement levels and activation markers.................................................. 4
Coagulation, platelet and endothelial activation markers..........................4
Functional activity of VITT patient serum in aggregating donor platelets with or without heparin .................................................................5
Supplementary Tables & Figures ...................................................................... 5
Table S1: Changes of laboratory markers over time in Patient 1 ..........5
Figure S1: Patient 3 - CT Venogram ............................................................. 6
Figure S2: Patient 3 - CT Pulmonary Angiogram .......................................6
Figure S3: Patient 4 - CT abdomen and pelvis with portovenous phase contrast ........................................................................................................6
Figure S4: Functional activity of serum of patients with VITT in aggregating donor platelets with or without heparin ...............................8
Figure S5: Changes in D-dimer, platelet count, fibrinogen, and anti-PF4 antibodies in response to treatment .................................................................9

Clinical case descriptions

Patient 1 is a 46-year-old female with history of migraine with no other significant medical history. Initial presentation was to on call general practitioner with a severe headache 8 days following her first dose of ChAdOx1 nCoV-19 when she was prescribed rizatriptan (5HT1-receptor agonist). Twenty-four hours later whilst alone at home, she developed nausea, right sided weakness of the face, arm and legs and was found on the floor ~96 hours later by police. On initial assessment she was agitated with GCS 10/15 (E4V1M5), no verbal output and no ability to follow commands or visual cues. MRC muscle power score was 1 (range 0-5) throughout the right side and seizure activity was noted. Blood tests revealed a platelet count...
of 39x10^9/L, fibrinogen of 0.7g/L and grossly elevated D-dimer at 31,770 ng/ml (Fibrinogen equivalent unit [FEU]) (Table 1). The blood film reflected a true thrombocytopenia with no evidence of schistocytes. ELISA for IgG anti-PF4 was positive (OD 2.54). Neuroimaging demonstrated extensive thrombosis involving both the dural venous sinuses and superficial cortical veins as well as associated subarachnoid haemorrhage in the parietal sulci bilaterally (Figure 1a-b). She was transferred to our intensive care unit with combined hyperacute stroke team and neurosurgical input.

Treatment with IVIg (0.5mg/kg), methylprednisolone 1g, PLEX with Octaplas and rituximab (375mg/m^2) were started urgently. Anticoagulation was instigated with argatroban aiming for an APTT of 1.5-2.0 for the first 48hrs increased to 2.0-2.5 in the absence of further bleeding or deterioration on the subarachnoid haemorrhage on repeat imaging. Levetiracetam was commenced for seizure control. Following 5 days of PLEX and weaning dose of steroids, the patient was mobilising independently with significant improvements in her expressive and receptive dysphasia. Platelet count stabilised at 350x10^9/L (Figure S5). Table S1 summarises changes of additional haemostatic markers over time since admission. No new or significant interval changes were noted on repeat neuroimaging (Figure 1c-d). Argatroban was changed to Fondaparinux and eventually to Apixaban 5mg bd on discharge.

**Patient 2** is a 41 year old female with history of migraine who presented with severe headache to emergency service 7 days following the first dose of ChAdOx1 nCoV-19. She was treated for migraine and discharged. Two days later she represented with abdominal pain and nausea. Her platelet count fell from 165 to 76 x10^9/L in 24 hours and D-dimer was >20,000ng/ml (Table 1). Imaging confirmed superior sagittal sinus thrombosis (Figure 1e) and branch intrahepatic portal venous thrombus. Urgent PLEX, IVIg, steroids and Argatroban were commenced and the patient was transferred to ICU. Diagnosis of VITT was confirmed with anti-PF4 ELISA (OD 2.2). Platelet count normalised by Day 3 of 5 of PLEX. Soon after admission, a new right sided neurological deficit developed although no increase of clot burden or haemorrhage was noted on CT or MRI. Rituximab (375mg/m^2) was commenced in view of the fluctuating neurology. On Day 10 of admission and despite ongoing improvements in laboratory markers, the patient developed chest pain and CTPA demonstrated nonocclusive filling defects within segmental lower lobe pulmonary arteries consistent with pulmonary emboli. PLEX was therefore recommenced until anti-PF4 antibodies were retested. Reassuringly, anti-PF4 had fallen (OD 1.21), PLEX was discontinued and Argatroban switched to Fondaparinux. The patient continued to improve with a resolution in neurological symptoms,
headache, and chest pain. CT venogram performed 2 weeks later showed improvement with a
reduction in size of the thrombus (Figure 1f). Platelet count remained stable, D-dimer improved
to 66ng/ml, anti-PF4 fell further (OD0.37) (Figure S5b) and discharged home with apixaban
5mg bd on Day 22.

Patient 3 is a 43-year-old female with a history of hypertension on Ramipril and provoked
DVT post-operatively as a teenager. Twenty-eight days post ChAdOx1 nCoV-19, she
developed shortness of breath, severe holo-cephalic headache, left sided upper limb
paraesthesia with bilateral specks in vision and presented to the emergency department
following an episode of collapse associated with loss of consciousness. Initial concerns were
for pulmonary embolus and CTPA revealed a large saddle embolus with extensive thrombus
extending into all lobar branches bilaterally with features of right heart strain (Figure S2). A
treatment dose of LMWH was given. Laboratory bloods showed a platelet count of 161,
fibrinogen of 2.75, D-dimer of >20,000ng/mL and positive anti-PF4 (OD 2.35) (Table 1).
LMWH was switched to Fondaparinux combined with IVIg. CT venogram (CTV) was advised,
which indeed demonstrated extensive dural venous sinus thrombosis affecting the superior
sagittal, left transverse and sigmoid sinuses (Figure S1) and MRI further delineated multiple
sites of thrombosed cortical veins and subarachnoid haemorrhage (Figure 1g). As such the
patient was moved to ITU with neurosurgical presence, commened on PLEX and
dexamethasone IV and fondaparinux was switched to argatroban. Following 5 days of PLEX
and IV dexamethasone the platelet count improved from 150x10^9/L to 196x10^9/L and D-dimer
fell from >20,000 to 3709ng/mL. Symptoms significantly improved and anti-PF4 dropped (OD
0.38) by Day 6 and was discharged home with apixaban 5mg bd. She was not
thrombocytopenic at any time (Figure S5c).

Patient 4 is a 46-year-old female with history of migraine who developed an occipital headache
with nausea and vomiting 9 days following her first dose of ChAdOx1 nCoV-19. Two days
after initial onset the patient presented to the emergency department of a district general
hospital after having no relief with sumitriptan and was discharged with safety-net advice. Two
days later, she re-presented with headache of worsening severity and CTV demonstrated
extensive cerebral venous sinus thrombosis with secondary area of infarct/oedema in the left
posterior temporal lobe (Figure 1h). Although no neurological deficit was apparent on history
or examination, platelet count was 57x10^9/L, D-dimer 80,000ng/mL, fibrinogen 1.4g/L and
positive anti-PF4 (OD 2.18) (Table 1). CT Abdomen demonstrated portal (Figure S3a) and
hepatic vein thrombus (Figure S3b). The patient was transferred to ITU with neurosurgery present on site for emergency PLEX, Dexamethasone and Argatroban were commenced and IVIg was initiated but held due to an anaphylactoid type reaction. Platelet count normalised by Day 4 of PLEX, and D-dimer fell to 7000ng/mL. Post PLEX, anti-PF4 fell (OD 0.23) and headache had improved (Figure S5d).

**Laboratory Methods**

**Anti-PF4 antibodies**
Testing for anti-PF4 antibodies was performed by ELISA in two UK reference laboratories using LIFECODES (Immucor) and HYPHEN BioMed. HIT testing with Acustar HIT-IgG assay (Werfen) was performed for comparison. The positive thresholds were based on the normal ranges <0.4 OD for ELISA and < 1.00 for Acustar HIT IgG assay.

**Antiphospholipid (aPL) and anti-nuclear antibodies (ANA)**
Serum IgG, IgM and IgA anticardiolipin and anti-β2GPI, IgG and IgM anti-phosphatidylserine/prothrombin and ANA were measured by ELISA as per manufacturer’s instructions (Werfen, UK). Antibodies against IgG anti-Domain I of β2GPI were measured using an established in-house assay 6.

**Complement levels and activation markers**
C3 and C4 plasma levels were measured on the Beckman Coulter AU680 analyser using photometric analysis. C3a-desArg and SC5b-9 were measured by ELISA following manufacturer’s instructions (Quidel Corp, UK).

**Coagulation, platelet and endothelial activation markers**
Lupus anticoagulant (using Dilute Russell’s viper venom time), antithrombin, Factor VIII, von Willebrand factor antigen (VWF:Ag), VWF Ristocetin cofactor (VWF:RCoF) and plasminogen activity and were performed on the ACL TOP 500. Plasma levels of plasminogen activator inhibitor-1 (PAI-1), thrombomodulin and cell adhesion molecules [E-selectin, intercellular (IC)AM-1, vascular cell (VC)AM-1 and P-selectin] were measured by in-house ELISA using validated antibody pairs and research-level standardised protocols (DuoSet ELISA, R&D, UK).
**Functional activity of VITT patient serum in aggregating donor platelets with or without heparin**

Functional activity of serum (antibodies) from patients with VITT in aggregating donor platelet with or without heparin (low [0.43IU/mL] or high dose [100IU/mL]) was assessed using Multiplate analyzer (Roche Diagnostics, UK) which can rapidly detect and quantify abnormalities of platelet function in whole blood. The increase in impedance by the attachment of platelets onto the Multiplate sensors is transformed to arbitrary aggregation units (AU) and plotted against time. The most important parameter in assessing the platelet function is the area under the aggregation curve (AUC).

**Supplementary Tables & Figures**

**Table S1: Changes of laboratory markers over time in Patient 1**

<table>
<thead>
<tr>
<th>Days since admission</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antithrombin (70-130 IU/dL)</td>
<td>107</td>
<td>108</td>
<td>111</td>
<td>109</td>
<td>119</td>
</tr>
<tr>
<td>Factor VIII (%) (50-150 IU/dL)</td>
<td>64.3</td>
<td>66</td>
<td>125</td>
<td>139</td>
<td><strong>172</strong></td>
</tr>
<tr>
<td>VWF:Ag (50-150 IU/dL)</td>
<td><strong>220</strong></td>
<td><strong>229.9</strong></td>
<td><strong>198.5</strong></td>
<td><strong>199</strong></td>
<td><strong>177.1</strong></td>
</tr>
<tr>
<td>VWF:Rco (50-150 IU/dL)</td>
<td>235</td>
<td><strong>151.5</strong></td>
<td><strong>155.6</strong></td>
<td><strong>161</strong></td>
<td><strong>144</strong></td>
</tr>
<tr>
<td>Plasminogen activity (70-130 IU/dL)</td>
<td>72</td>
<td>82</td>
<td>90</td>
<td>85</td>
<td>97</td>
</tr>
<tr>
<td>C3 (0.79–1.52mg/dL)</td>
<td>1.25</td>
<td>0.86</td>
<td>0.73</td>
<td>0.67</td>
<td>1.24</td>
</tr>
<tr>
<td>C4(0.16-0.38 mg/dL)</td>
<td><strong>0.10</strong></td>
<td><strong>0.10</strong></td>
<td><strong>0.11</strong></td>
<td><strong>0.09</strong></td>
<td>0.24</td>
</tr>
<tr>
<td>Troponin (ng/L) (&lt;19.8)</td>
<td>4.3</td>
<td>4.0</td>
<td>2.1</td>
<td>1.2</td>
<td>1.8</td>
</tr>
</tbody>
</table>

VWF:Ag : Von Willebrand Antigen

VWF:Rco : Von Willebrand factor ristocetin cofactor activity

Values in bold are abnormal (above or below the normal reference range)
**Figure S1: Patient 3 - CT Venogram**

Sagittal section from CT venogram performed 2 days following admission. There is an extensive filling defect throughout the entirety of the superior sagittal sinus and contrast can be seen anterior to it (red arrows).

**Figure S2: Patient 3 - CT Pulmonary Angiogram**

Axial CT at the level of the main pulmonary artery. A filling defect (red arrow) can be seen extending from the main pulmonary artery into both lobar pulmonary arteries in keeping with a large saddle embolus. The left pulmonary artery is expanded due to the significant thrombus.

**Figure S3: Patient 4 - CT abdomen and pelvis with portovenous phase contrast**
a) Extensive filling defect extending from the portal confluence into the main portal vein (red arrows) which is expanded. This continued into the left and right portal veins (not shown). A small amount of contrast can be seen within the posterior aspect of the main portal vein.

b) Filling defect within the right hepatic vein (blue arrow). The middle and left hepatic veins can both be seen filled with contrast.
Figure S4: Functional activity of serum of patients with VITT in aggregating donor platelets with or without heparin

Aggregation of donor platelets after incubation with serum from the patients was measured by whole-blood impedance aggregometry. The measurements were performed in the presence of unfractionated heparin (UFH) low (0.43IU/mL) or high (100IU/mL) concentrations and in the absence of added heparin (saline). Serum from a healthy donor and serum from a patient with confirmed classical heparin-induced thrombocytopenia (HIT) were also tested. The red and
blue lines represent duplicate measurements. AU denotes arbitrary units, and AUC the area under the curve. Serum from patient with classical HIT showed marked platelet aggregation with low dose UFH. Patient 3 had normal platelet count all throughout the admission. Serum from all patients showed higher platelet aggregation in the absence of heparin compared to patient with classical heparin induced thrombocytopenia and healthy donors. When patient serum was incubated with low or high dose heparin, there was variable response in platelet aggregation.

Figure S5: Changes in D-dimer, platelet count, fibrinogen, and anti-PF4 antibodies in response to treatment
(a) Patient 1

![Graph showing changes in D-dimer, platelet count, fibrinogen, and anti-PF4 antibodies in response to treatment](image-url)
(b) Patient 2

![Graph showing changes in D Dimer, Platelets, and Fibrinogen over time for Patient 2. OD: 2.19 at admission, 1.20 on Day 3, and 0.37 on Day 7. Treatment with Argatroban and Fondaparinux is indicated.]
(c) Patient 3

![Graph showing D Dimer, Platelets, Fibrinogen, and Anti-PF4 levels over the days of admission. The graph illustrates the changes in these parameters with time.](image-url)
Panels a-d show these laboratory measurements for Patients 1-4 respectively. From top to bottom of each panel: plasma exchange (PLEX), intravenous immunoglobulin (IVIg), steroids (methylprednisolone, dexamethasone) and rituximab are indicated by arrows; top graph: D-dimer; middle graph: platelet count; bottom graph: fibrinogen; enumerated anti-PF4 OD; and non-heparin anticoagulants.