Supplementary Material: Clinical and biological features of cerebral venous sinus thrombosis following ChAdOx1 nCov-19 Vaccination.

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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 APTTTR 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 7days 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 (OD1.21), 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-ß2GPI, IgG and IgM antiphosphatidylserine/prothrombin and ANA were measured by ELISA as per manufacturer’s instructions (Werfen, UK). Antibodies against IgG anti-Domain I of ß2GPI were measured using an established in-house assay.

**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>172</td>
</tr>
<tr>
<td>VWF:Ag (50-150 IU/dL)</td>
<td>220</td>
<td>229.9</td>
<td>198.5</td>
<td>199</td>
<td>177.1</td>
</tr>
<tr>
<td>VWF:Rco (50-150 IU/dL)</td>
<td>235</td>
<td>151.5</td>
<td>155.6</td>
<td>161</td>
<td>144</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 5S: Changes in D-dimer, platelet count, fibrinogen, and anti-PF4 antibodies in response to treatment
(a) Patient 1
(b) Patient 2

![Graph showing D Dimer, Platelets, and Fibrinogen levels over time.](image)

- **D Dimer (mg/ml)**
  - Initial: >30,000 mg/ml
  - Days 1-15: Stable at ~5,000 mg/ml

- **Platelets (x10^9/L)**
  - Days 1-15: Fluctuates from 250 to 450 x10^9/L

- **Fibrinogen (g/L)**
  - Days 1-15: Fluctuates from 1.5 to 3.5 g/L

**Anti-PF4 OD:**
- Day 1: 2.19
- Day 3: 1.20
- Day 15: 0.37

**Therapy:**
- **Argatroban**
- **Fondaparinux**
(c) Patient 3

![Graph showing Dimer, Platelets, and Fibrinogen over Days of Admission]

- **D Dimer (ng/ml)**
  - Initial value: >20,000
  - Decreases over days:
    - Day 1: ~15,000
    - Day 2: ~10,000
    - Day 3: ~5,000
    - Day 4: ~1,000
    - Day 5: ~250

- **Platelets (×10^9/L)**
  - Initial value: 150
  - Increases over days:
    - Day 1: 150
    - Day 2: 160
    - Day 3: 170
    - Day 4: 180
    - Day 5: 190

- **Fibrinogen (g/L)**
  - Initial value: 3.5
  - Decreases over days:
    - Day 1: 3.0
    - Day 2: 2.5
    - Day 3: 2.0
    - Day 4: 1.5
    - Day 5: 1.0

**Anti-PF4 OD**
- **Argatroban**
  - OD: 2.19
- **Apixaban**
  - OD: 0.38
(d) Patient 4

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.