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

Christina Crossette-Thambiah[1,2*], Charis Pericleous[3*], Namir Asmar[4], Joshua Bomsztyk[2], Amita Ranger[2], Abdul Shlebak[2], Saipriya Ramji[4], Soma Banerjee[5,6], Mike Laffan[1,2], Deepa RJ Arachchillage[1,2]

[*]Equally contributed.

[1] Centre for Haematology, Imperial College London, London, UK.

[2] Department of Haematology, Imperial College Healthcare NHS Trust Imperial College London, London, UK.

3. National Heart and Lung Institute, Imperial College London, London, UK.

4. Department of Neuroradiology, Imperial College Healthcare NHS Trust Imperial College London, London, UK.

5. Department of Stroke Medicine, Imperial College Healthcare NHS Trust Imperial College London, London, UK.

6. Department of Brain Sciences, Imperial College London, London, UK.

Address for correspondence:

Dr Deepa RJ Arachchillage, Department of Haematology, Imperial College Healthcare NHS Trust and Imperial College London, Hammersmith Hospital, 4th Floor, Commonwealth Building, Du Cane Road, London W12 ONN

Tel: +44 (0) 20 7351 8400, FAX: +44 (0) 2073518402

E-mail: d.arachchillage@imperial.ac.uk

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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[1]. In April 2021, the Medicines and Healthcare products Regulatory Agency (MHRA) and the European Medicines Agency (EMA) 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 Medicine & Healthcare prodcuts Regulatory Agency (MHRA) by 21st of July 2021 with fatality rate of 17.76% (73/411)[2].

We report our experience of four VITT cases from a single tertiary referral centre in London, United Kingdom 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[3 4] and reported via the MHRA Yellow card and other national VITT-CVST surveillance projects.

All four patients were women aged 41-46 years old and diagnosed with VITT 7-28 days post ChAdOx1 nCov-19 vaccination. Each presented with headache and varying degrees of neurological deficit. Detailed case histories are provided in the Supplemental 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 (Figure1a-d) but no thrombosis detected in imaging of the abdomen. Patient 2 initially presented with superior sagittal sinus thrombosis (Figure 1e) and branch intrahepatic portal venous thrombus, and later developed a new right sided neurological deficit and nonocclusive segmental pulmonary arteries filling defects consistent with pulmonary emboli [CT pulmonary angiogram (CTPA)]. CT venogram performed 2 weeks later showed improvement with a reduction in thrombus size (Figure 1f). In Patient 3, CT venogram (CTV) demonstrated extensive dural venous sinus thrombosis affecting the superior sagittal, left transverse and sigmoid sinuses (Figure S1) and CTPA revealed a large saddle embolus with extensive thrombus extending into all lobar branches bilaterally with features of right heart strain (Figure S2). MRI further delineated multiple sites of thrombosed cortical veins and subarachnoid haemorrhage (Figure 1g). For Patient 4, CTV demonstrated extensive cerebral venous sinus thrombosis with secondary area of infarct/oedema in the left posterior temporal

lobe (Figure 1h). CT Abdomen demonstrated portal (Figure S3a) and hepatic vein thrombus (Figure S3b).

Of the typical abnormal blood parameters reported in the literature for VITT, thrombocytopenia and hypofirninogenemia were evident in three and two patients respectively, and all exhibited grossly raised D-dimer. We confirm the importance of selecting appropriate anti-platelet factor 4 (PF4) antibody tests[5] 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 anti-nuclear antibodies (ANA) in Patient 2 and 4 (23-30 units at 1:40 serum dilution, assay cut off = 20 units), while antiphospholipid antibodies (aPL) 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 are the key pathogenic population in VITT.

A uniform management approach was taken with urgent plasma exchange (PLEX) initiated in combination with IVIg (1g/Kg into divided doses in two days with the timing of PLEX to minimise loss of IVIg), high dose steroids (1g IV methylprednisolone followed by 20mg dexamethasone IV or oral for four days with tapering dose over the next few days) and non-heparin-based anticoagulants (initially argatroban) with rituximab (375mg/m^2) in two patients. All four patients survived with complete resolution of symptoms and laboratory markers supporting this therapeutic approach in a syndrome currently estimated as having a 70% mortality rate especially in those presenting with evidence of bleeding[3 6]. Transformation of cerebral infarction to haemorrhage is a well recognised complication of CVST and may have been exacerbated by severe thrombocytopenia and hypofibrinogenemia (both of which were apparent in our first case) as well as the need for anticoagulation.

Overall management of the four cases presented here represents an aggressive approach to VITT and we believe this played a central role in the favourable outcome of our patients. Our first case presentation was dramatic and was critically unwell with a GCS of 10 on arrival. Her laboratory markers also reflected severe disease. Given the high mortality rates in such presentation a decision was made to start PLEX early and resulted in a rapid improvement in clinical status. Whilst IVIg and PLEX deal with VITT antibodies already formed, a short course of high dose steroid was given to reduce further antibody formation and reduce the cerebral oedema associated with the extensive CVST and cerebral haemorrhage in the first patient. We

adopted the same strategy for the subsequent three patients although their presentation was less dramatic. With respect to anticoagulation, argatroban was chosen as its short half life of 45 minutes permits monitoring and rapid adjustment. Once clinically stabilised and platelet recovery was observed, anticoagulation was switched to once-daily fondaparinux and then to the oral anticoagulant apixaban on discharge. Table S1 (patient 1) and Figure S5 summarise changes in laboratory markers in response to treatment.

Intriguingly, all four patients had notably raised levels of von Willebrand Factor antigen and activity (VWF:Ag, VWF:RCo respectively) as well as plasminogen activator inhibitor-1 (PAI-1) compared to control plasma tested in parallel. Circulating factor VIII, thrombomodulin, E-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell (VC)AM-1 and P-selectin were also elevated at variable degrees (Table 1; methods summarised in Supplemental Material page 4). Taken together, these findings suggest ongoing fibrinolysis and are indicative of an inflammatory platelet and endothelial response. In contrast, prothrombin time (PT), activated partial thromboplastin time (APTT), antithrombin, protein S and protein C measurements were unremarkable 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 a functional platelet aggregation assay suggest that although it is generally recommended to avoid heparin anticoagulant in VITT, it may not aggravate progressive thrombosis (Figure S4). Platelet aggregation induced by serum from three patients in the absence of heparin was reduced with both low and high dose UFH compared to control serum (in contrast to classical HIT serum). Serum from Patient 3 who was not thrombocytopenic at any time had no difference in donor platelet aggregation (Figure S4).

Complement inhibition with eculizumab was also shown to benefit VITT[7] and indeed complement activation was evident in our patients as demonstrated by low levels of C3 (Patient 2) and C4 (Patient 1, 3 and 4) coupled with raised C3a (Patient 1 and 3) and C5b-9 (Patient 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 anti-complement 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[3 4] and the good outcomes reported here suggest that rapid agressive 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.

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Declaration of interests: All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare as follows: DJA received research funding from Bayer plc and Leo Pharma outside of this research project. ML received consultation and speaker fees from Astra-Zeneca, Sobi, Leo-Pharma, Takeda and Pfizer but not related to this research project. Others have no conflict of interests to declare. As the corresponding author, DJA was responsible for the study design, had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Contributors: DJA conceived the study, involved in data collection, data verification, data analysis, figures, data interpretation, writing the original draft reviewing and editing the manuscript. CCT contributed to data collection, data verification, data analysis, figures, writing the original manuscript and editing it. CP performed some of the laboratory assays, involved in data analysis, data interpretation, writing and editing the manuscript. ML interpreted the data 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. ML interpreted the data, wrote, and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

Ethical approval: This study was approved by Research Ethics Committee (REC) approval (17/WA/0161) and informed written consent was obtained from all patients for collection of research bloods and use of their clinical data for publication.

Data sharing: Raw data can be made available via direct contact with the corresponding author (d. arachchillage@imperial.ac.uk).

The lead author (DJA) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no pertinent aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

References

1. (https://covid19.who.int/). WCC-DGWHO.
2. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1005194/Coronavirus_vaccine-_summary_of_Yellow_Card_reporting_14.07.21_clean.pdf).
3. Scully M, Singh D, Lown R, et al. Pathologic Antibodies to Platelet Factor 4 after ChAdOx1 nCoV-19 Vaccination. *N Engl J Med* 2021;384(23):2202-11. doi: 10.1056/NEJMoa2105385 [published Online First: 2021/04/17]
4. https://b-s-h.org.uk/about-us/news/guidance-produced-by-the-expert-haematology-panel-ehp-focussed-on-vaccine-induced-thrombosis-and-thrombocytopenia-vitt/.
5. Platton S, Bartlett A, MacCallum P, et al. Evaluation of laboratory assays for anti-Platelet Factor 4 antibodies after ChAdOx1 nCOV-19 vaccination. *J Thromb Haemost* 2021 doi: 10.1111/jth.15362 [published Online First: 2021/05/12]
6. Greinacher A, Thiele T, Warkentin TE, et al. Thrombotic Thrombocytopenia after ChAdOx1 nCov-19 Vaccination. *N Engl J Med* 2021 doi: 10.1056/NEJMoa2104840 [published Online First: 2021/04/10]
7. Tiede A, Sachs UJ, Czwalinna A, et al. Prothrombotic immune thrombocytopenia after COVID-19 vaccine. *Blood* 2021 doi: 10.1182/blood.2021011958 [published Online First: 2021/04/29]

Table 1 – Baseline characteristics and laboratory markers of 4 patients presenting with cerebral venous sinus thrombosis following AstraZeneca/Oxford University ChAdOx1 nCoV-19 vaccine

	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**
Sex	Female	Female	Female	Female
Age	46	41	43	46
Past Medical History	Migraine	Migraine	Hypertension DVT	Migraine
Clinical presentation	headache, collapse, hemiparesis	Headaches, Vomiting, abdominal pain	shortness of breath, collapse headache, paraesthesia	headache
No. of days post vaccine	14	9	28	9
Thrombosis	CVST	CVST Branch portal vein thrombus PE	CVST Pulmonary saddle embolus	CVST Hepatic vein thrombus Portal vein thrombus
Bleeding	Subarachnoid haemorrhage	None	Subarachnoid haemorrhage	None
Admission Bloods				
HemosIL AcuStar HIT-IgG* (<1u/ml)	0.05 (neg)	0.03 (neg)	0.04 (neg)	0.02 (neg)
Immucor ELISA PF4 HIT-IgG (<0.4OD)	**2.48**	**2.19**	**2.35**	**2.18**
HYPHEN BioMed ELISA PF4 HIT-IgG (OD) (<0.4OD)	**1.67**	**1.89**	**1.92**	**1.78**
Platelets (150-400 $x10^9$)	**39**	**125**	**161**	**57**
D Dimer (<500 ng/ml) FEU	**>20,000**	**>20,000**	**>20,000**	**>20,000**

Fibrinogen (1.9-4.3g/L)	**0.7**	4.26	2.75	**1.4**
Troponin (ng/L) (<19.8)	4.3	**54.8**	4.6	1.6
PT (12.8-17.4 secs)	14.7	13.5	14.8	12.0
APTT (25.0-35.0secs)	**43.2**	28.5	26.2	39.0
DRVVT ratio	Negative	Negative	Negative	Negative
Antithrombin (70-130 IU/dL)	107	110	123	114
Protein C activity (70-130IU/dL)	72	**155**	65	**144**
Free Protein S antigen (70-130IU/dL)	68.8	119.0	102.8	92.7
Factor VIII (50-150 IU/dL)	64.3	**172.7**	59.2	**193**
VWF:Ag (50-150 IU/dL)	**220**	**202.8**	**226.3**	**220.1**
VWF:Rco (50-150 IU/dL)	**235**	**192.2**	**236.1**	**178.1**
Plasminogen activity (70-130 IU/dL)	72	101	84	94
PAI-1 (ng/mL) *§	**10.1**	**12.9**	**38.3**	**25.8**
E-selectin*§	6.9	**14.0**	9.9	7.2
ICAM-1*§	78.8	138.6	129.7	130.1
VCAM-1*§	**1192.8**	760.3	**1605.8**	**1276.5**
Thrombomodulin*§	4.9	3.9	**6.0**	**5.9**
P-selectin*§	33.1	**37.3**	**82.4**	**59.7**
C3 (0.79–1.52mg/dL)	1.25	**0.23**	0.79	1.06
C4 (0.16-0.38 mg/dL)	**0.10**	0.35	**0.12**	**0.25**
C3a (ng/mL) #§	**289.4**	151.8	**570.2**	80.4
C5b-9 (ng/mL)#§	184.8	194.9	**468.9**	**254.2**

*Mean (range) levels in 8 control plasma samples tested in parallel: PAI-1: 4.1 (2.9-6.7); E-selectin: 7.5 (2.8-13.5); ICAM-1: 98.8 (77.0-148.0); VCAM-1: 671.9 (190.7-1076);

Thrombomodulin: 5.1 (4.8-5.8); P-selectin: 26.5 (0-34.7) ng/mL.

[#]Mean (range) in control plasma as defined by manufacturer: C3a, 129.6 (33.8-268.1) and C5b-9, 147 (75-219) ng/mL. Values in bold are abnormal.

[§]Performed in post-admission bloods.

PT= Prothrombin time; APTT: activated partial thromboplastin time; DRVVT= Dilute Russell's viper venom time; ELISA: Enzyme-linked immunosorbent assay; ICAM-1: intercellular adhesion molecule-1; PAI-1: plasminogen activator inhibitor-1; PE: Pulmonary Embolus; PT: prothrombin time; VCAM-1: vascular cell adhesion molecule-1; VWF:Ag : Von Willebrand Antigen; VWF:Rco : Von Willebrand factor ristocetin factor

Figure 1 Legend

Figure 1: Cranial images from four patients with cerebral venous sinus thrombosis-vaccine-induced immune thrombotic thrombocytopenia

(a) Initial non-contrast CT 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 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) and (d): MR susceptibility-weighted imaging (SWI) sequence performed two 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.

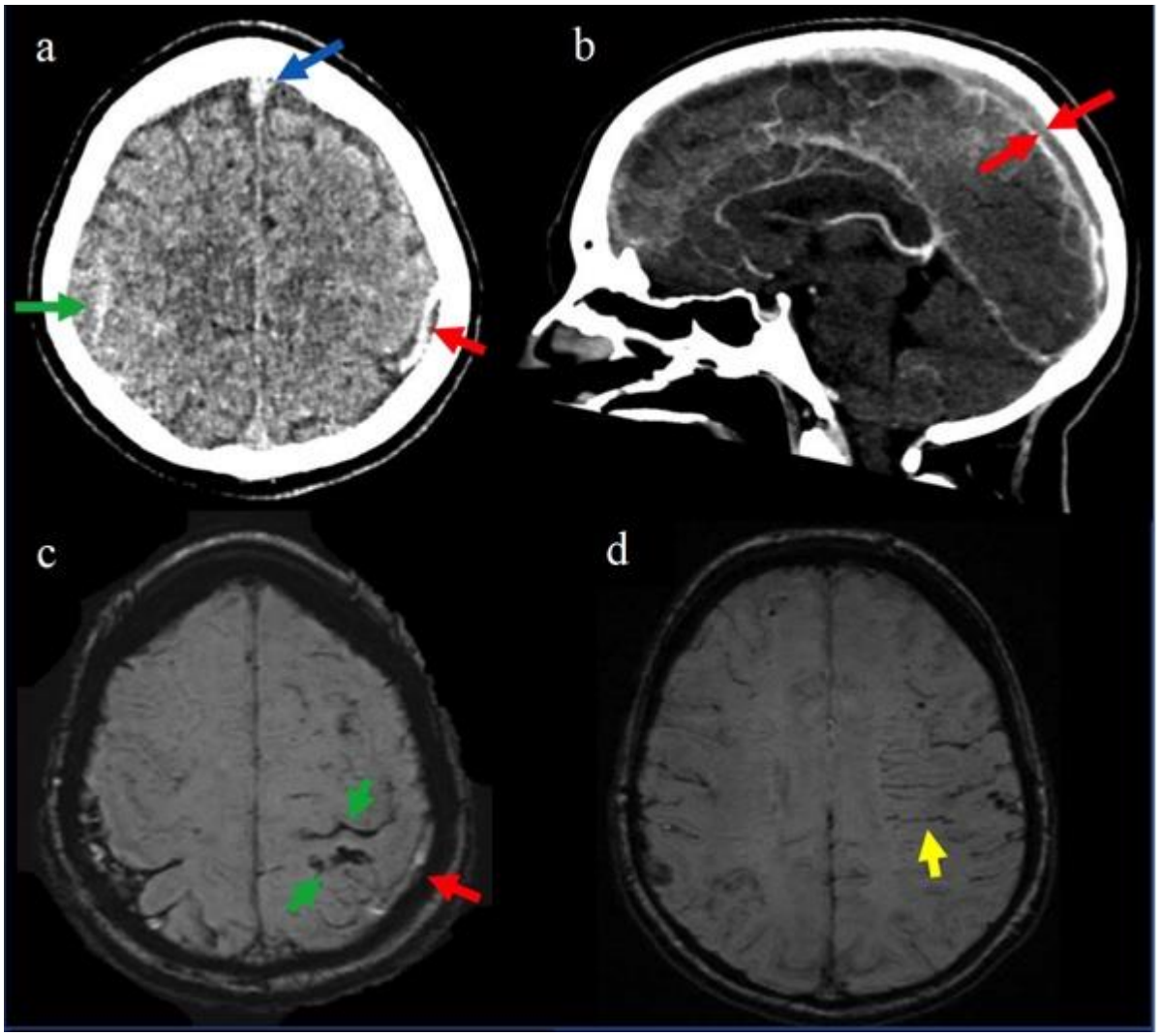
(e) Initial CT venogram head performed on admission for Patient 2. A large thrombus is seen within the mid-superior sagittal sinus where it is expanded (red arrow). It extends anteriorly with no contrast opacification anteriorly.

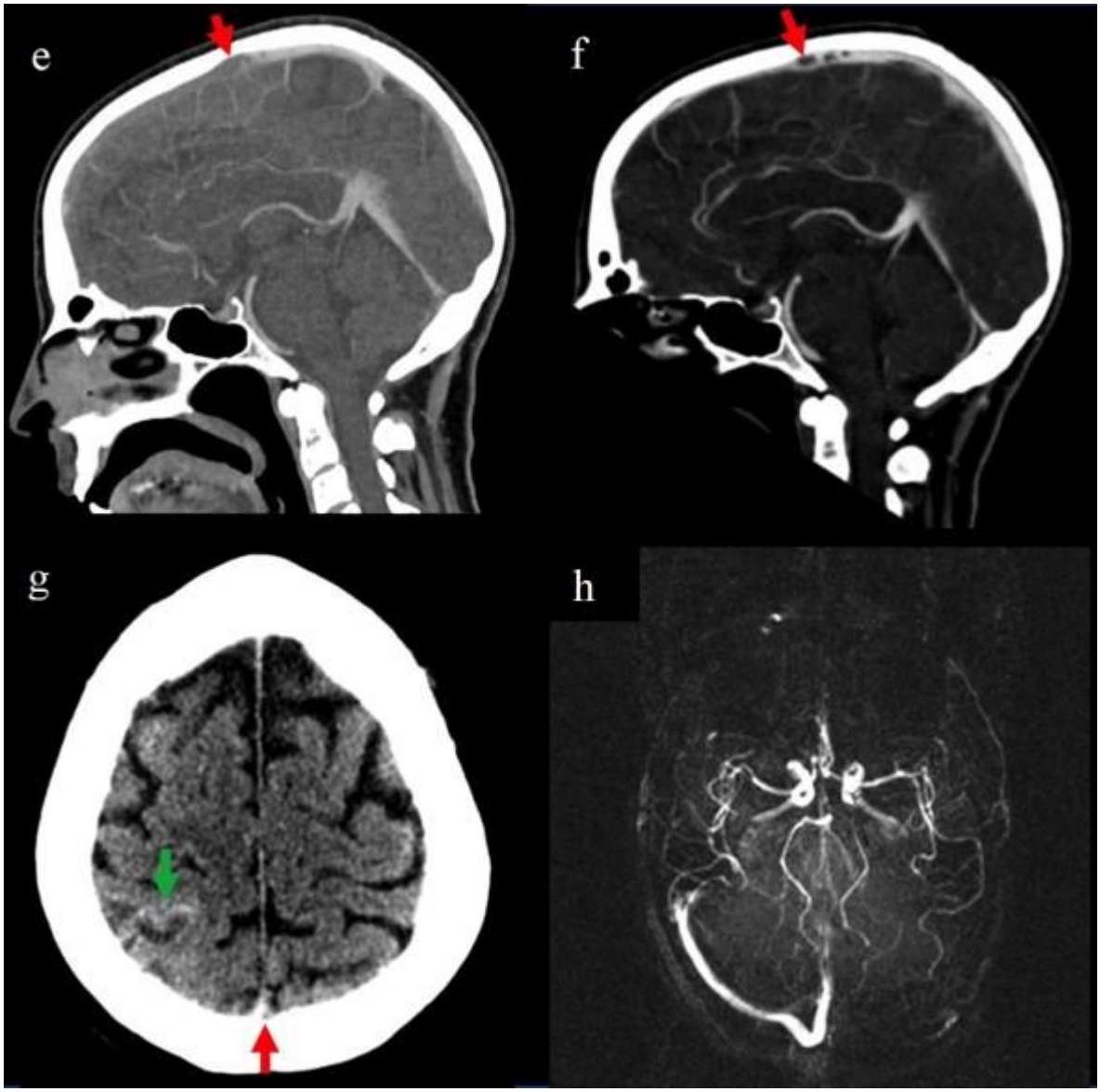
(f) CT venogram performed at 2 weeks for Patient 2 shows interval improvement with a reduction in size of the thrombus (red arrow) at the mid-superior sagittal sinus and contrast visible anteriorly.

(g) Patient 3 initial unenhanced CT performed at 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 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.

Figure 1

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