Supplementary material

Prothrombotic immune thrombocytopenia after COVID-19 vaccine

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Case studies

Patient 1

The 63-year-old woman was admitted with severe headache, somnolence, dysphasia, right-sided hemiparesis and arterial hypertension 11 days after vaccination. She was previously healthy without known concomitant disease or medication. She had never been exposed to heparin. Since the day after vaccination, she had felt increasingly uncomfortable with flu-like symptoms and headache. Computed tomography showed intracerebral bleeding with ventricular compression and midline shift (Figure S1). Platelet transfusion was given before craniotomy and evacuation of the hematoma because of severe thrombocytopenia. CT angiography confirmed sigmoid and transverse sinus thrombosis.

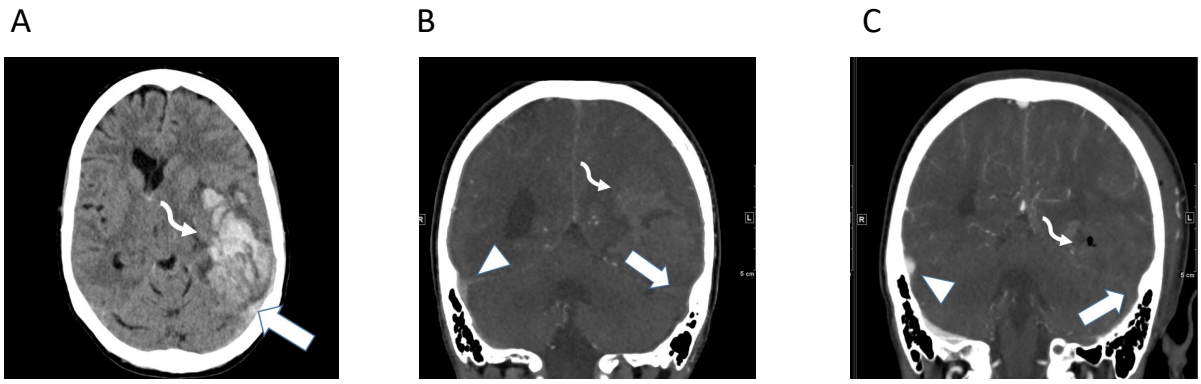

Figure S1 CVST in patient 1. (A) Computed tomography (CT) upon admission showed intraparenchymal hemorrhage (curved arrow) with suspected thrombosis of left transversal sinus (white arrow). (B) Early phase CT angiography before surgery (day 1). Faint filling of right transverse sinus (white arrowhead) without filling of left transverse sinus (white arrow). Extensive hemorrhage of left temporal lobe (curved arrow). (C) Late phase of CT-angiography after trephination. Normal filling of right transverse sinus (white arrowhead) without filling of left transverse sinus (white arrow). Air after evacuation of hematoma (curved arrow).

Thrombocytopenia persisted and the patient developed acute renal failure (Table S1). Signs of thrombotic microangiopathy (TMA) were noted including schistocytes, elevated LDH and absent haptoglobin. ADAMTS-13 activity was normal (92%) ruling out classical thrombotic thrombocytopenia purpura (TTP). Lupus anticoagulant and deficiency in antithrombin, protein C or S were also excluded. Heparin-induced thrombocytopenia (HIT) screening with the chemiluminescence immunoassay (CLIA) was negative upon admission but positive later on with enzyme-linked immunosorbent assays (ELISA). The patient received full-dose heparin (starting day 1) and plasma exchange (days 2 and 3) because of TMA but did not improve. Therapy was then switched to eculizumab 900 mg weekly (day 4) with heparin being continued, resulting in continuous improvement of TMA

and thrombocytopenia (see main article). The patient had no further thromboembolic event. At the time of this report (day 42), she is breathing spontaneously while, still being comatose after cessation of sedation. She has been off dialysis for 1 week and the kidney function is recovering.

Patient 2

This 67-year-old woman presented with severe headache and thrombocytopenia of 40 /nl to a remote hospital 8 days after vaccination. The neurological status was normal, as was a cranial CT scan. A CT angiography, however, revealed thrombi in the aortic arch, left common carotid artery and in the descendent aorta, and the patient was referred to our institution. Cranial MRI confirmed small cortical embolic infarctions in the left posterior circulation and in the right parietal cortex (Figure S2). She had mild hypercholesterinemia. D-Dimer was markedly elevated and anti-PF4 antibodies positive. She received anticoagulation with argatroban (starting dose 1 µg/kg per min) tailored to plasma levels of 0.5-1.0 µg/ml, and IVIG (1 g/kg on days 1 and 2). D-Dimer promptly decreased, and platelet count recovered by day 7. She had no further clinical events, was switched to oral anticoagulation with apixaban and discharged on day 15 without any clinical symptoms.

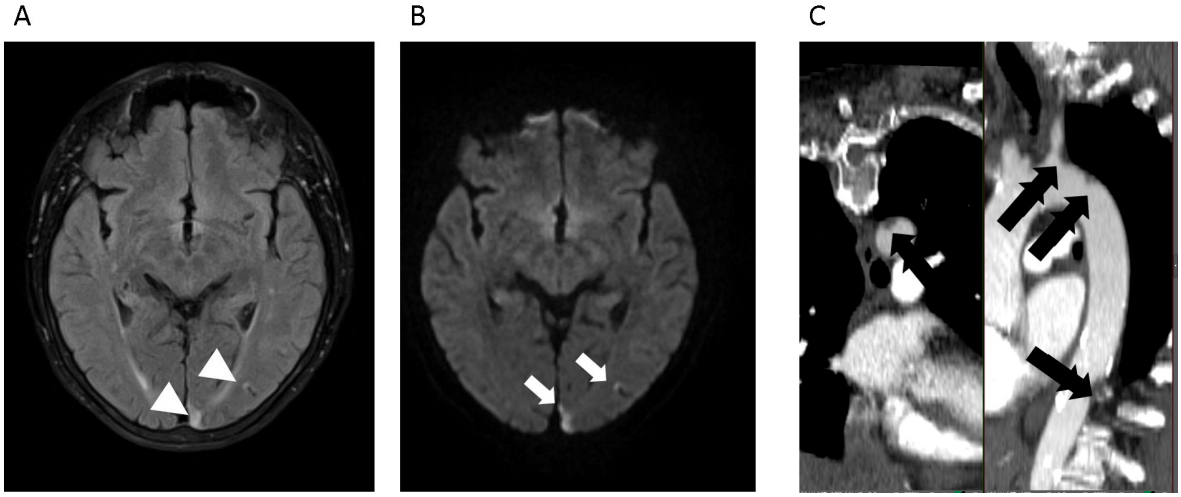

Figure S2. Thromboembolic manifestations in patient 2. (A) MRI showed cortical lesions in FLAIR (white arrowheads). (B) Diffusion weighted imaging confirmed cortical ischemia of embolic origin (white arrows). (C) Arterial phase of lung CT depicted multiple thrombi in the aorta and at the origin of the left common carotid artery (black double arrows).

Patient 3

The 41-year-old woman presented to the emergency room because of headache and visual disturbance 5 days after vaccination. The neurological status was normal. Imaging excluded ischemic brain lesions. Contrast enhanced MR angiography with high time resolution showed normal cerebral vasculature without any filling defect especially in the cranial veins (data not shown). She had mild thrombocytopenia, markedly elevated D-

Dimer and positive anti-PF4 antibodies. She received anticoagulation with argatroban. Headache and visual disturbance improved within 24 h. D-Dimer decreased, and platelet count recovered by day 8. She had no further clinical events, was switched to oral anticoagulation with apixaban and discharged on day 15 free of symptoms.

Patient 4

The 61-year-old woman presented to a remote hospital because of severe fatigue 9 days after vaccination. Petechial bleeding was noted. Platelets were decreased to 12 /nl, and D-Dimer was markedly elevated. CVST was ruled out by cranial MRI. Argatroban, IVIG and tranexamic acid (because of petechial bleeding) were given. When the platelet count had risen to 113 /nl on day 6, she suddenly reported abdominal pain. Abdominal ultrasound and CT scan revealed extended splanchnic vein thrombosis with complete obstruction of portal, splenic, and mesenteric veins (Figure S3). She received alteplase (50 mg bolus, 50 mg infusion over 24 h) and was referred to our institution. She still had signs of complement consumption (CH_{50} <10%, ref. range 32-58%) as well as moderate signs of hemolysis, and therefore received eculizumab (900 mg i.v. weekly, starting day 8). Platelet count improved and D-Dimer dropped over the next days (see main article). Follow up contrast enhanced ultrasound assessments revealed a continuous improvement with almost complete recanalization of the portal and splenic veins as well as partial recanalization of the mesenteric veins. She had no further thromboembolic event and is still recovering at the time of this report.

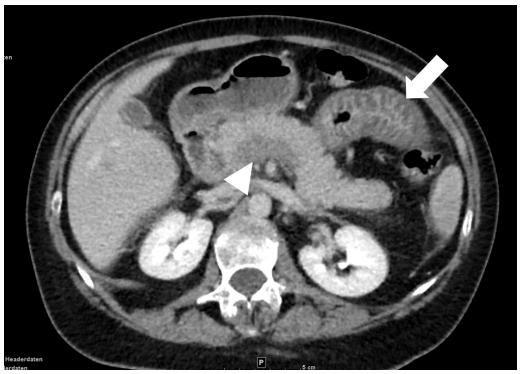

Figure S3. Splanchnic vein thrombosis in patient 4. Venous phase CT confirmed thrombosis of the dilated portal vein (white arrowhead) with poorly enhanced bowel wall as sign of initial ischemia (white arrow).

Patient 5

The 61-year-old woman developed nocturnal headache 8 days after vaccination. Upon awakening in the next morning, she presented dysarthria, left-sided hemiplegia and conjugated gaze palsy to the left (NIH stroke scale 17). A cranial CT demonstrated infarction of the right middle cerebral artery (MCA) territory (Alberta stroke program early CT score 6, Figure S4). CT angiography disclosed a longitudinal right-sided internal carotid artery

(ICA) and MCA M1 occlusion, which was removed by mechanical thrombectomy and extracranial ICA stenting with residual peripheral M2 occlusion (Thrombolysis in cerebral infarction scale 2B). Follow-up CT imaging demonstrated hemorrhagic transformation and partial anterior cerebral artery (ACA) infarction. Due to a 7 mm midline shift and corresponding cranial pressure signs, urgent decompression hemicraniectomy was performed. Additionally, there was a long venous thrombosis on the left arm from the elbow to the lower third of the proximal humerus, as well as a partial thrombosis of the left-sided internal jugular vein. Due to thrombocytopenia, the patient received two units of platelet concentrate. When anti-PF4 antibodies became available, she received IVIG (1 g/kg on two consecutive days), dexamethasone and argatroban (target level 0.4-0.8 µg/ml). While the platelet count was rising, she developed acute right-sided popliteal artery occlusion on day 8, confirmed by duplex sonography and CT angiography. Thrombectomy was performed and argatroban target concentration increased to 0.5-1.0 µg/ml. At the time of this report (day 15), she was breathing spontaneously, still being comatose despite termination of sedation for 2 days.

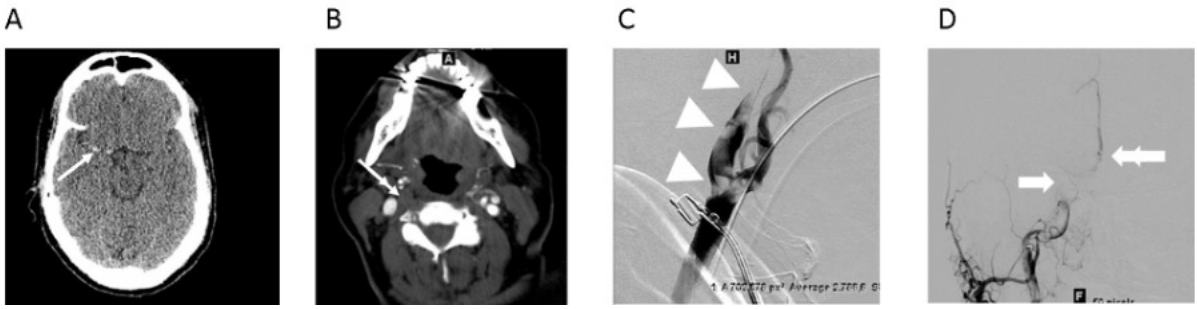

Figure S4 (A) Cranial CT showed dense middle cerebral artery consistent with thrombus (white arrow). (B) CT angiography disclosed occluded right internal carotid artery (double arrow). (C) Digital subtraction angiography confirmed multiple thrombi in the right cervical part of internal carotid artery (arrowheads). (D) Occlusion of the distal internal carotid artery and the middle cerebral artery (large white arrow) with thrombotic material in the anterior cerebral artery (big white double arrow) was seen intracranially.

Table S1: Selected laboratory data

Patient 1	Reference interval	Day 1	Day 3	Day 8	Day 15
Leukocyte count (/nl)	3.9-10.2	5.9	21.2	20.9	20.7
Hemoglobin (g/dl)	12.0-15.6	13.7	7.9	9.2	9.2
Platelet count (/nl)	150-370	27	62	106	191
Schistocytes (%)	<1	1.4	3.5	1.9	0.8
Fibrinogen (g/l)	1.8-3.5	1.7	4.0	6.3	5.9
D-Dimer (mg/l)	<0.5	>35.2	10.6	8.0	5.2
Creatinine (µmol/l)	45-84	42	149	300	189
CRP (mg/l)	<5	11.9	79.5	56.7	14.8
LDH (U/l)	<247	1083	1112	455	336
Haptoglobin (g/l)	0.3-2.0	<0.1	<0.1	1.86	1.2

Patient 2	Reference interval	Day 1	Day 4	Day 8	Day 15
Leukocyte count (/nl)	3.9-10.2	7.2	7.7	7.2	6.0
Hemoglobin (g/dl)	12.0-15.6	11.9	11.8	11.9	11.0
Platelet count (/nl)	150-370	40	111	207	227
Schistocytes (%)	<1	0.7			
Fibrinogen (g/l)	1.8-3.5	2.7			
D-Dimer (mg/l)	<0.5	>35.2	16.7	5.6	2.6
Creatinine (µmol/l)	45-84	69	66	74	71
CRP (mg/l)	<5	189	18	11	25
LDH (U/l)	<247	247	178		

Patient 3	Reference interval	Day 1	Day 3	Day 8	Day 15
Leukocyte count (/nl)	3.9-10.2	6.8	5.1	6.3	7.8
Hemoglobin (g/dl)	12.0-15.6	12.9	13.1	13.7	13.2
Platelet count (/nl)	150-370	105	131	210	204
Schistocytes (%)	<1	0.0			
Fibrinogen (g/l)	1.8-3.5	3.6	3.6	3.0	3.1
D-Dimer (mg/l)	<0.5	22.4	0.9	0.5	<0.2
Creatinine (µmol/l)	45-84	69	63	71	
CRP (mg/l)	<5	109	64	1.9	
LDH (U/l)	<247	198			
CH_{50} (%)	32-58	<10			

Patient 4		Day 1	Day 6	Day 7	Day 10
Leukocyte count (/nl)	3.9-10.2	5.3	8.4	7.5	5.8
Hemoglobin (g/dl)	12.0-15.6	13.9	11.0	10.0	9.3
Platelet count (/nl)	150-370	12	113	115	199
Schistocytes (%)		"very few"			
Fibrinogen (g/l)	1.8-3.5	1.1	2.1	2.1	3.7
D-Dimer (mg/l)	<0.5	>35.2	15.9	28.5	5.1
CRP (mg/l)	<5	28	35		
LDH (U/l)	<247	353	174		155
CH_{50} (%)	32-58			<10	

Patient 5		Day 1	Day 3	Day 8	Day 15
Leukocyte count (/nl)	3.9-10.2	10.8	10.6	14.5	10.1
Hemoglobin (g/dl)	12.0-15.6	12.6	8.4	8.7	9.3
Platelet count (/nl)	150-370	62	25	172	313
Schistocytes (%)			0.8		
Fibrinogen (g/l)	1.8-3.5		0.9	4.9	6.8
D-Dimer (mg/l)	<0.5		>35.2	9.0	16.8
CRP (mg/l)	<5	71	115	184	73
LDH (U/l)	<247		360		
CH_{50} (%)	32-58		>60		

Anti-PF4 antibodies

Emerging experience exchanged in a German, Austrian and Swiss Thrombosis and Hemostasis Society (GTH) expert panel suggested discrepant results between different anti-PF4 immunoassays.

The **CLIA** (ACL AcuStar HIT IgG, Werfen, Munich, Germany) was negative in all tested samples. In this assay, anti-PF4 antibodies are captured by PF4 bound to polyvinyl sulfonate (PVS) adsorbed to magnetic beads. After separation of the microparticles, isoluminol-labeled anti-human IgG is added and detected.[1] The assay was performed according to the manufacturer's instructions with test results of 1.0 U/mL or greater considered positive.

ELISA from two different manufacturers tested positive in all samples with very similar results: Zymutest HIT IgG (Hyphen BioMed, CoaChrom, Maria Enzersdorf, Austria) and Lifecodes PF4 IgG (Immucor GTI Diagnostics, Rodermark, Germany). In the Zymutest assay, unfractionated heparin is immobilized on microwells, and PF4 is provided by the patient plasma and by a platelet lysate within the reaction mix during the assay procedure.[2] In the Lifecodes assay, PF4/PVS is immobilized on microwells.[2] Both assays were performed according to the manufacturer's instructions, including negative and positive controls and confirmatory inhibition of the reaction in the presence of high concentrations of heparin. Results of the Zymutest IgG assay are reported in OD throughout the main paper.

Flow cytometry

Flow cytometry was performed as described with minor modifications.[3] Donor platelets from type 0 donors were freshly isolated and pre-incubated with AZD1222 or saline in the absence or presence of heparin (100 IU/ml) for 30 min. Serum samples were subsequently added. After 30 min, fluorescein isothiocyanate-labelled anti-human immunoglobulin G was added to detect antibody binding. The drug-to-saline mean fluorescence intensity (MFI) ratio was calculated. Ratios above 2.0 were considered positive. Healthy donor serum was used a negative control and known drug-dependent antibodies as positive controls.

Modified heparin-induced platelet aggregation (HIPA)

HIPA tests were performed as described with minor modifications.[4] Each sample was tested with washed platelets from four different donors in the absence (buffer alone) or presence of heparin (0.2 or 100 IU/ml). For the modified test, AZD1222 was added. Reactions were placed in microtiter wells containing spherical stir bars and stirred at approximately 500 rpm. Wells were visually examined in 5-minute intervals for loss of turbidity. Sera were interpreted as reactive (positive) if a shift from turbidity to transparency occurred within 40 min in at least two suspensions. Each test included positive and negative control sera. Results are depicted in Fig. S5.

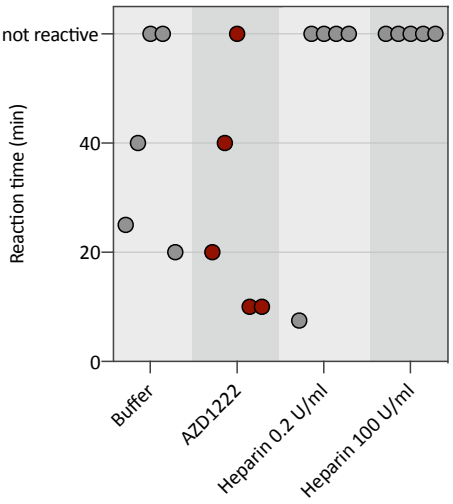

Figure S5 Modified HIPA test. Dots represent mean time to aggregation within patients (n=5).

Supplement references

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3. Chen S, Cooper N, Muller M, Bein G, Sachs UJ. Piperacillin-dependent anti-platelet antibodies are a relevant, easy to confirm differential diagnosis in patients with rapid-onset thrombocytopenia. *Br J Haematol*. 2020;190(5):e320-e321. 10.1111/bjh.16899

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