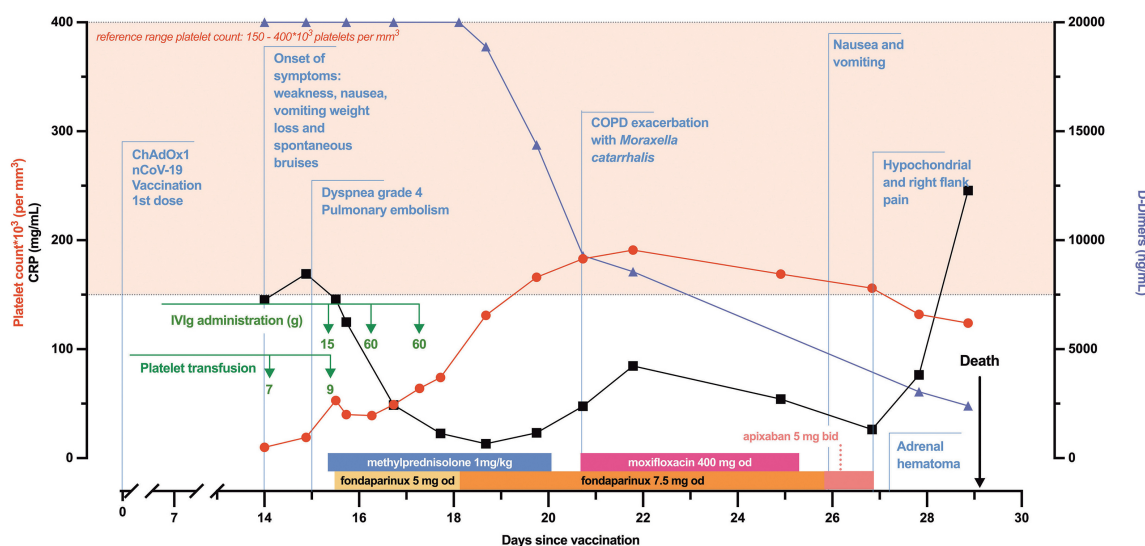


## Fatal exacerbation of ChadOx1-nCoV-19-induced thrombotic thrombocytopenia syndrome after initial successful therapy with intravenous immunoglobulins - a rationale for monitoring immunoglobulin G levels

The present report describes a vaccine-induced thrombotic thrombocytopenia (VITT) case with fatal exacerbation after initial improvement following initial intravenous immunoglobulin (IVIg) administration and anticoagulation. An 83-year-old woman presented at the emergency room with an alteration of her general condition. She presented with symptoms of weakness, nausea, vomiting, weight loss and spontaneous bruises without any obvious reason, 14 days after having received her first dose of ChadOx1 nCov-19. According to our medical records, she did not receive heparin or derivative during the previous 4 months. Clinical examination unraveled bruising on the upper limbs. Computer tomography (CT) of thorax and abdomen was normal. Oxygen saturation was 98% at admission and the patient was tested negative for SARS-CoV-2 infection as assessed by reverse-transcriptase polymerase chain reaction (RT-PCR). The initial laboratory investigations on the day of admission revealed that the patient was suffering from marked thrombocytopenia (i.e., platelet count of 10,000 per mm<sup>3</sup>), dramatically increased D-dimers plasma levels (i.e., > 20,000 ng/mL) and slightly low plasma fibrinogen (i.e., 179 mg/dL) (Figure 1). She was transfused with a platelet concentrate (roughly 3.5x10<sup>11</sup> platelets) on the day of admission.

During the night, she suffered from dyspnea grade NYHA 4. Pulmonary ventilation and perfusion (V/Q) scan was performed and disclosed bilateral pulmonary

embolism. Anti-PF4 immunoglobulin G (IgG) antibodies (i.e. 1.80 AU/mL, Figure 1) were detected on day 1 post-admission using a PF4/polyvinylsulfonate rapid assay (HemosIL® AcuStar HIT IgG assay, Instrumentation Laboratory Belgium NV, Zaventem, Belgium). The diagnosis of VITT was confirmed using a heparin-induced multi-electrode aggregometry method.<sup>1</sup> In face of the clinical picture, i.e., thrombocytopenia and thrombosis, with the presence of anti-PF4 antibodies and positive platelet activation tests within 30 days after vaccination with ChadOx1 nCov-19, VITT was diagnosed.<sup>2</sup> The patient therefore promptly received 15 grams of IVIg (Privigen®, CSL Behring GmbH, Marburg, Germany) and methylprednisolone 1 mg/kg. A second platelet concentrate (roughly 4.5x10<sup>11</sup> platelets) was administered to allow initiation of anticoagulation as the platelet count was still below 30,000 per mm<sup>3</sup>. The platelet count rapidly improved, i.e., 53,000 per mm<sup>3</sup>, and anticoagulation was started with fondaparinux 5 mg once a day (od) subcutaneously from day 1 to day 3 (taking into account renal failure, i.e., Cockcroft-Gault creatinine clearance <50 mL/min). She received additional IVIg on day 2 and 3, at the dose of 60 grams per day for a total IVIg dose of 135 grams corresponding to 1.7 grams of IVIg per kg administered over a period of 48 hours. Fondaparinux dose was increased to 7.5 mg od from day 4 to day 11 since renal function improved. On day 6, the patient was stabilized, and her global health status was improved as witnessed by normalized platelet count, decrease in D-dimers (i.e., from > 20,000 ng/mL at admission to 14,380 ng/mL) and C-reactive protein (CRP) (from 145 mg/dL at admission to 23 mg/dL). Later that day, however, oxygen saturation dropped below 80%. Cough with sputum production was noted and exacerbation of COPD with *Moraxella catarrhalis* infection was diagnosed. Oxygen



Fibrinogen (mg/dL)	179	154	65	172	269	433	619	601	Ref. range: 180 - 400
Anti-PF4 - LIFECODES PF4 IgG (OD)	3.34		3.33		3.20		3.52		Pos. if > 0.40
Anti-PF4 - HemosIL® AcuStar HIT IgG (AU/mL)	1.80		1.34				0.76	1.25	Pos. if > 1.00
PF4-SRA (% platelet activation w/o heparin)	90		35		36		79		Pos. if > 20
Immunoglobulin G (g/L)	6.8		37.1		23.8		11.3		Ref. range: 7.0 - 16.0

Figure 1. Clinical and laboratory data of the case. CRP: C-reactive protein; COPD: chronic obstructive pulmonary disease; HIT: heparin-induced thrombocytopenia; od: once daily.

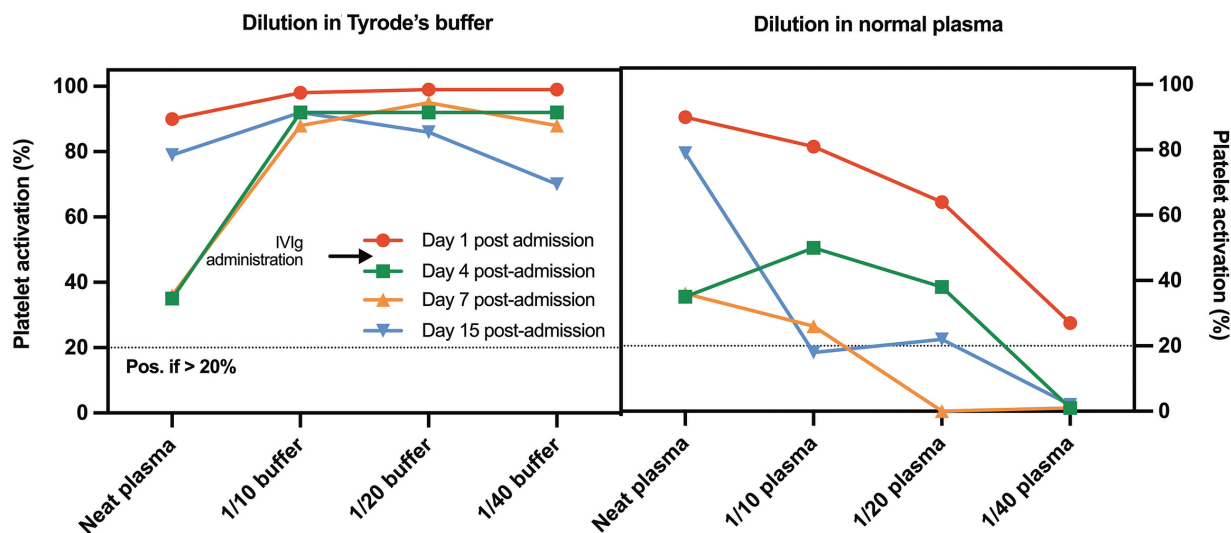


Figure 2. Dilution experiments on samples collected at day 1 post-admission (diagnosis), days 4 post-admission (after IVIg administration), day 7 post-admission (marked clinical improvement with platelet count normalization) and day 15 post-admission (deterioration of patient's status and death). Dilutions were made in normal heated plasma (containing normal immunoglobulin G [IgG] level) or in modified Tyrode's buffer at 1/10, 1/20, 1/40 dilution ratios. Platelet activation was assessed without heparin in platelet-activating anti-platelet factor 4-serotonin-release assay (PF4-SRA) and results are expressed in percentage of serotonin release. IVIG: intravenous immunoglobulin.

supplementation was then started (2 liters per minute) combined with oral moxifloxacin 400 mg od for 5 days. On day 12 post-admission, anticoagulation was switched from fondaparinux to apixaban 5 mg twice a day (bid).

Unfortunately, the clinical status worsened on day 12 post-admission with a de novo reduction of platelet count. Abdominal CT scan showed right adrenal hematoma with left adrenal infiltrate, which is a usual presentation of adrenal infarction, as described in autoimmune heparin-induced thrombocytopenia and also recently in VITT.<sup>4,5</sup> Four units of 500 IU/mL of prothrombin complex concentrate were administered on day 14 as an attempt to control the adrenal hematoma, but she died later that day from hypovolemic shock probably secondary to adrenal hemorrhage. A causal adrenal infarction may have existed but could not be confirmed as neither an injected CT scan nor an autopsy was performed. Nevertheless, adrenal insufficiency was not documented and cortisol levels on day 14 was still in the upper range (i.e., 21 µg/dL, normal range: 6.2-18 µg/dL). Moreover, it must be noted that although apixaban was last administered on day 13 in the morning and was never reintroduced, its plasma level on day 14 was 353 ng/mL (usual Ctrough range: 22-177 ng/mL) and was still 132 ng/mL on day 15. The accumulation of apixaban may thus have contributed to, or even triggered, this bleeding event. The initial infusion of platelet concentrates may also have contributed to disease progression, as well as to the pulmonary embolism observed at diagnosis. An immediate treatment with IVIg, as now recommended by the American Society of Hematology,<sup>6</sup> could have been beneficial but the presence of active bruising, the absence of documented thrombosis and the marked thrombocytopenia guided our therapeutic choice at that time. This case further highlights how VITT is a dynamical condition, which should not be discounted in a recently vaccinated patient with only thrombocytopenia and increased D-dimer levels, even in the absence of documented thrombosis, and that IVIg should be considered promptly, along with anti-PF4 testing and thrombosis screening.

The rapid fatal outcome, occurring 2 weeks after VITT diagnosis while an improvement was noticed, raised the question of an early relapse or an exacerbation of the initial event. In an attempt to understand the possible cause(s), additional laboratory investigations were performed, as reported in Figure 1. Blood samples collected post-admission on day 1 (diagnosis), day 4 (after IVIg administration), day 7 (marked clinical improvement with platelet count normalization) and day 15 (deterioration of patient's status, leading to death) were selected to assess time-related changes of anti-PF4 antibodies, platelet activation with PF4-SRA<sup>7</sup> and total IgG levels.

Result of the enzyme-linked immunosorbent assay (ELISA) with immobilized PF4/PVS complexes (LIFE-CODES PF4 IgG, Immucor Lifecodes, Jette, Belgium) remained strongly positive during the whole hospital stay with OD >3.00 measured with all samples collected from day 1 to day 15. Positive results were also obtained using a modified in-house ELISA in which the wells are only coated by PF4,<sup>8</sup> demonstrating the presence of IgG antibodies that bound PF4 alone (data not shown). Such characteristics of VITT antibodies, shared with those of highly pathogenic auto-immune heparin-induced thrombocytopenia (HIT) antibodies,<sup>9</sup> indicate a different specificity and affinity towards PF4 compared to classical HIT antibodies, and explain why their detection by HIT-dedicated immunoassays may be inadequate.<sup>7</sup> The fact that anti-PF4 IgG antibodies were detected using a PF4/polyvinylsulfonate rapid assay (HemosIL<sup>®</sup> AcuStar HIT IgG assay) is particular since this assay failed to detect anti-PF4 antibodies in most of the reported VITT cases. Nevertheless, the HemosIL<sup>®</sup> AcuStar HIT IgG assay rarely gives weak positive results in patients with likely VITT diagnosis, as stated by Platon et al., who reported two positive results out of 31 patients, all of whom had anti-PF4 IgG detected by ELISA.<sup>10</sup>

PF4 serotonin-release assay (PF4-SRA) was performed with the same samples, as previously described.<sup>7</sup> Platelet activation, measured through maximal serotonin release, was 90% in the absence of heparin on day 1, decreased by more than 50% after IVIg administration (day 4,

35%) and remained low when the patient was getting better (day 7, 36%). In contrast, platelet activation returned to a high level at the time of clinical deterioration, day 15 (79%). Time related changes of total IgG plasma levels mirrored those of PF4-SRA (Figure 1). While the total IgG level was rather low at diagnosis, it rose above 30 g/L after IVIg administration and then rapidly decreased, as observed on day 15, with a return to normal values for a healthy adult. These data support the hypothesis that the rebound of platelet activation observed in PF4-SRA at the time of clinical deterioration could be due to a rapid elimination of the infused immunoglobulins and the loss of their competing effect with platelet activating anti-PF4 antibodies on platelet FcγRIIa.

Additional experiments were performed with PF4-SRA to consolidate our hypothesis that the levels of IgG can explain the exacerbation. The same samples were diluted either in normal plasma (containing normal IgG level) or in modified Tyrode's buffer at 1/10, 1/20, 1/40 dilution ratios. When samples were diluted in normal plasma, platelet activation decreased proportionally with the dilution, and was completely abolished at 1/40, except for the day 1 sample, which still slightly activate platelets under these conditions (Figure 2). In contrast, when the samples were diluted in Tyrode's buffer, platelet activation remained high (near 100%) for the samples collected on day 1 and day 15 but increased for those obtained at days 5 and 7, approaching 100% (Figure 2). These results strongly support that platelet activation by VITT antibodies was inhibited by normal IgG, and that lowering the concentrations of normal IgG led to the reappearance of platelet activation by loss of competition between the IVIg and anti-PF4 IgG.

This case supports the concept that proper monitoring using an appropriate functional assay could help in the clinical decision making since PF4-SRA mirrored with the clinical evolution of the patient. Such an observation has also been made in a recent study, which demonstrated that platelet activation by VITT antibodies was inhibited in patients treated with IVIg.<sup>11</sup> Nevertheless, this needs to be confirmed. Interestingly, the inhibitory effect of normal polyclonal IgG on the platelet activation induced by PF4-specific antibodies could also vary from one patient to another, as previously demonstrated in HIT patients.<sup>12</sup> It is also important to note that administration of IVIg reduces the activation of platelets as assessed by the PF4-SRA. This has major consequences when collecting samples for confirmation of VITT diagnosis and dilution in appropriate buffer, as we did in our experiments, could be recommended to assess the competitive interaction between anti-PF4 IgG and IVIg. However, this test lacks worldwide availability, and cannot easily be used for emergency patient monitoring. Therefore, as total IgG concentration measured in the patient inversely correlated with platelet activation in PF4-SRA, quantitatively assaying anti-PF4 IgG antibodies levels and total IgG concentration in the patient's plasma could help to identify situations where the competition between normal polyclonal IgG and anti-PF4 IgG on FcγRIIa may switch in favor of the platelet activating antibodies.<sup>15</sup>

Even though data on IVIg clearance parameters and target concentrations are lacking for such a very peculiar condition, a rapid decrease in total IgG concentrations within the normal range (i.e., 7-16 g/L)<sup>14</sup> could alert to possible therapeutic escape, and the need for re-administration of IVIg, especially in a situation where anti-PF4 IgG remains high. In the patient, total IgG concentration

was reduced by half within 8 days, which is substantially faster than the median half-life of 30 days generally reported in the literature for IVIg.<sup>15</sup> Although further studies are needed to understand the accelerated clearance and to assess the clinical relevance of total IgG measurement to monitor the efficacy of IVIg, it appears a very affordable tool in medical practice in combination with anti-PF4 IgG antibodies testing.

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Contributions: JD and CV analyzed the results, wrote the first draft of the manuscript and designed the figures; JR, YG, CP, CV provided and analyzed the results and revised the manuscript; TL analyzed the results and thoroughly revised the manuscript; JF provided and analyzed the results and revised the manuscript; FP, EG and VM managed the patient and thoroughly revised the manuscript; J-MD analyzed the results; FM designed and supervised the experiments, provided and analyzed the data, and interpreted the results.

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CHU UCL Namur (OM070) was consulted to decide if reporting this case is ethically acceptable. This Ethical Committee (decision number: 27-21) was in favor of the publication of this case and did not see any ethical issue in publishing this case report. All data has been anonymized.

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