Platelet Factor 4

Subjects: Hematology

Contributor: Emmanuel J Favaloro

Antibodies against platelet factor 4 (PF4), a protein released from alpha-granules of activated platelets, may cause a number of pathophysiological conditions. The most commonly known is heparin-induced thrombocytopenia (HIT), which develops in a small proportion of people treated with the anticoagulant drug heparin.

Antibodies COVID-19 Platelet factor 4

1. Introduction

Platelet factor 4 (PF4) is a 70-amino acid protein that is stored in alpha granules of platelets and is released on platelet activation [1]. PF4 is cationic, or positively charged, and forms tetramers at physiological pH and ionic strength. Upon normal physiological platelet activation, PF4 is released as a complex with a chondroitin sulfate proteoglycan carrier and disappears rapidly from the plasma as it translocates to higher affinity heparan sulfate on endothelial cells, inhibiting local antithrombin activity and thus promoting coagulation [1]. In addition to its role in hemostasis, PF4, also known as chemokine CXCL4 (chemokine [C–X–C motif] ligand 4), has many other biological effects, which may also depend on its association with extracellular glycosaminoglycans (GAGs).

Under some conditions, pathological platelet activation can occur subsequent to the development of autoantibodies against PF4. Typically, for a particular pathophysiology, these anti-PF4 antibodies arise against PF4 in complex with negatively charged ‘species’ (i.e., molecules and polyanions), of which several candidate species exist [1]. For example, in the condition known as heparin-induced thrombocytopenia (HIT), anti-PF4 antibodies arise against PF4 in complex with heparin (i.e., anti-PF4/H antibodies are formed). These anti-PF4/H antibodies can pathologically activate platelets in a proportion of susceptible patients, leading to platelet aggregation, associated thrombocytopenia, and thrombosis at sites of vessel occlusion. In other anti-PF4 antibody pathophysiologies, the ‘associated PF4 cofactor’ may or may not be heparin and may or may not be known

2. Anti-PF4 Antibodies in COVID-19 Patients

COVID-19 (Coronavirus Disease 2019) is a now well-recognized pandemic caused by infection with the virus SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2). COVID-19 is a clearly prothrombotic disorder that involves multiple hemostasis pathways of interest, including platelet activation [2]. However, thrombosis in COVID-19 patients is multi-factorial, and platelets only play a part in a larger coagulopathic process. Unlike in HIT patients, platelet counts in COVID-19 patients are not usually very low, and so these patients are considered only mildly thrombocytopenic. In COVID-19, multiple hemostatic pathways can be affected, including primary

hemostasis (platelets and von Willebrand factor [VWF]), secondary hemostasis ('coagulation'), and fibrinolysis. Moreover, anti-PF4 antibodies do not arise in the majority of COVID-19 patients [3] and so cannot be considered a major driver of COVID-19-associated coagulopathy.

Nevertheless, of relevance to the current review, beside direct platelet activation resulting from direct interaction of SARS-CoV-2 spike protein with platelet receptors [4], 'HIT-like' events may occur in a small proportion of patients with COVID-19, and there have been several reports of anti-PF4 antibodies in COVID-19 patients, as recently reviewed by some of us [3]. In some cases, these were identified as involving heparin (i.e., anti-PF4/H antibodies were identified); however, in other cases, they did not involve prior heparin exposure (i.e., they were not anti-PF4/H antibodies, and so can be considered anti-PF4/X antibodies). Indeed, in some reports, the addition of therapeutic heparin levels in an assay can be shown to decrease antibody detection by immunological assays or inhibit platelet activation in functional assays, further confirming that these are not anti-PF4/H antibodies. Nevertheless, these events cannot really be considered as a form of 'spontaneous HIT-like syndrome', since SARS-CoV-2 is a likely trigger in at least some of these patients. Nevertheless, they do likely represent an analogous entity to the HIT-like syndromes mentioned in the preceding section, albeit associated with a particular viral infection, being SARS-CoV-2 [5]. Whether the anionic species associated with PF4 (i.e., the 'X' in PF4/X) is part of the virus, part of an associated co-bacterial infection, or simply arises due to platelet activation and consequent complex formation is still unknown.

In summary, a small proportion of COVID-19 patients may have anti-PF4/X antibodies, only a fraction of which can be identified as anti-PF4/H antibodies, with the remainder representing antibodies against PF4, potentially in complex with an as yet unknown 'anionic species' ('X') [3][6][7]. From the laboratory testing perspective, these will be anti-PF4 ELISA antibody-positive by immunological assessment, and SRA testing may show platelet activation in either presence or absence of added therapeutic heparin, depending on whether antibody development was due to heparin exposure (PF4/H complexes) or not (PF4/X complexes). In either case, a high dose (supra-therapeutic) of heparin should show inhibition of platelet activation/aggregation.

3. Anti-PF4 Antibodies in VITT/TTS

The COVID-19 pandemic has led to the rapid production and deployment of a large number of COVID-19 vaccines [8][9]. Notably, some minor adverse reactions may be anticipated for any vaccination program. Unfortunately, occasional rare and potentially fatal adverse events may also arise. One such event appears to arise in a small proportion of individuals vaccinated with COVID-19 adenovirus-based vaccines. Termed VITT (for vaccine-induced [immune] thrombotic thrombocytopenia) by the workers who first reported on the associated pathophysiological events [10][11][12], the term TTS (for thrombotic thrombocytopenia syndrome) may preferentially be used by government reporting agencies (for example, the FDA [Food and Drug Administration] in the USA, the EMA [European Medicines Agency] in Europe, and the TGA [Therapeutic Good Administration] in Australia) [8]. VITT and TTS after COVID-19 vaccine use essentially represent the 'same' condition, albeit that the specific case definition used to define VITT or TTS may result in the recognition of different patient cohorts. Even within the entity described as VITT, different patient cohorts may be identified, according to the diagnostic pathway used [13]. In a

recent review, some half-dozen diagnostic pathways were identified as being recommended by various expert groups [13]. Whilst the pathways in general aimed to accurately identify VITT patients, differences in the approach could lead to inclusion or exclusion of some cases relative to a different diagnostic approach. For example, some diagnostic pathways restricted the inclusion of cases up to 28 days post-vaccine exposure, whereas others captured cases up to 42 days post-exposure [13]. Some diagnostic pathways placed stronger emphasis on D-dimer measurements than others, and some diagnostic pathways restricted case capture only to patients with thrombocytopenia, whereas some pathways included case capture for patients without thrombocytopenia but with a substantive fall in platelet counts, akin to 4Ts in HIT assessment.

VITT/TTS also has a recognized prevalence, albeit potentially different according to the vaccine with which it occurs. For example, for the AstraZeneca vaccine (also known as ChAdOx1 nCoV-19, AZD1222, Vaxzevria), the prevalence is around 1 in 80,000 doses or between 10 and 15 cases per million doses (**Figure 1**). For the Janssen (Johnson & Johnson) vaccine (alternatively known as Ad26.COV2.S or JNJ-78436735), the prevalence seems to be lower, perhaps 1 in 500,000 doses, or ~2 cases/million doses [8]. The prevalence of VITT/TTS will also differ according to the case definition and the diagnostic pathway chosen, as further outlined above, of which there are many [13]. For example, a total of only 58 cases of TTS worldwide were identified by one recent systematic review as of 23 August 2021, as based on WHO criteria for TTS identification [14]. In contrast, a separate review performed by one of us [15] identified at least 83 cases of VITT worldwide as of a much earlier date, i.e., 27 May 2021, and based on clinical presentations and results of laboratory tests. Thus, the systematic review would significantly underestimate the number of TTS cases worldwide, which currently stand at >150 for Australia alone, according to a recent TGA report (**Figure 1**).

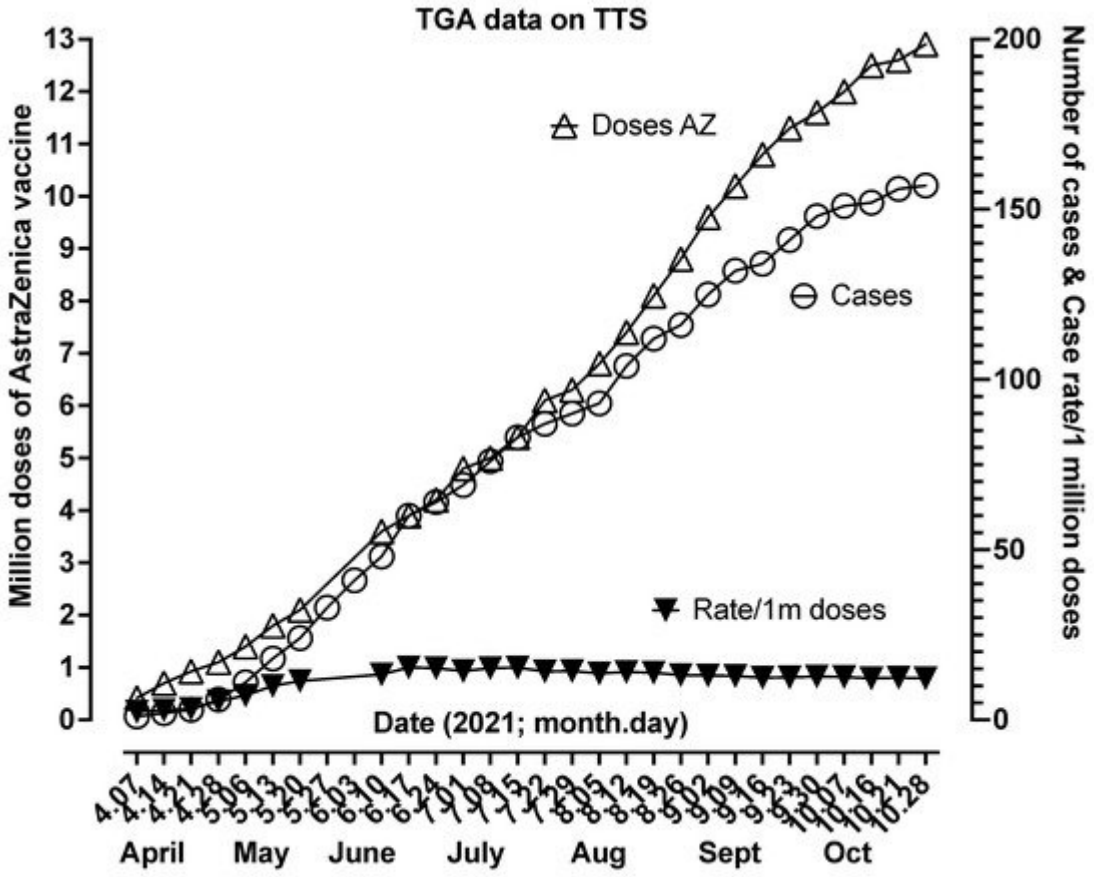

Figure 1. Cases of TTS as reported weekly by the Australian TGA from the first case reported at the beginning of April 2021 until the end of October 2021. The figure shows the cumulative number of AstraZeneca (AZ) doses administered, the cumulative number of TTS cases, and the TTS rate per million AZ doses. From an initial slow case attainment, the rate seemed to stabilize from June to October at around 13–15/million doses.

Of major relevance to the current review, VITT/TTS is also characterized by the presence of anti-PF4 antibodies, or more likely anti-PF4/X antibodies, with X being an unknown anionic 'species' at present (perhaps, heparan sulfate proteoglycans or vaccine components such as adenovirus-derived hexon) [16][17]. Again, these anti-PF4 antibodies can be detected by immunological assays, but unlike in HIT, not all such assays can identify these antibodies with sufficient diagnostic sensitivity. Indeed, only the ELISA-based techniques can consistently identify anti-PF4 (or anti-PF4/X) antibodies in VITT/TTS, with all other immunological methods, including rapid assays, either not detecting the antibodies or only detecting these in a minor proportion of patients (i.e., typically below 30%) [6]. This tends to support the concept that the 'X' in anti-PF4/X for VITT/TTS is not heparin. Perhaps also interesting here is that the in vitro use of heparin in laboratory testing tends to reduce the level of detected antibodies in immunological assays for the majority of VITT patients and tends to inhibit platelet activation in functional assays, essentially proving that the species in complex with PF4 (at least, in the majority of patients) is not heparin. An example of this for two patients from the Westmead Hospital laboratory is provided in **Figure 2**. However, therapeutic heparin does not inhibit platelet activation in all VITT patients [18][19], and so this does not provide an infallible distinction from HITT.

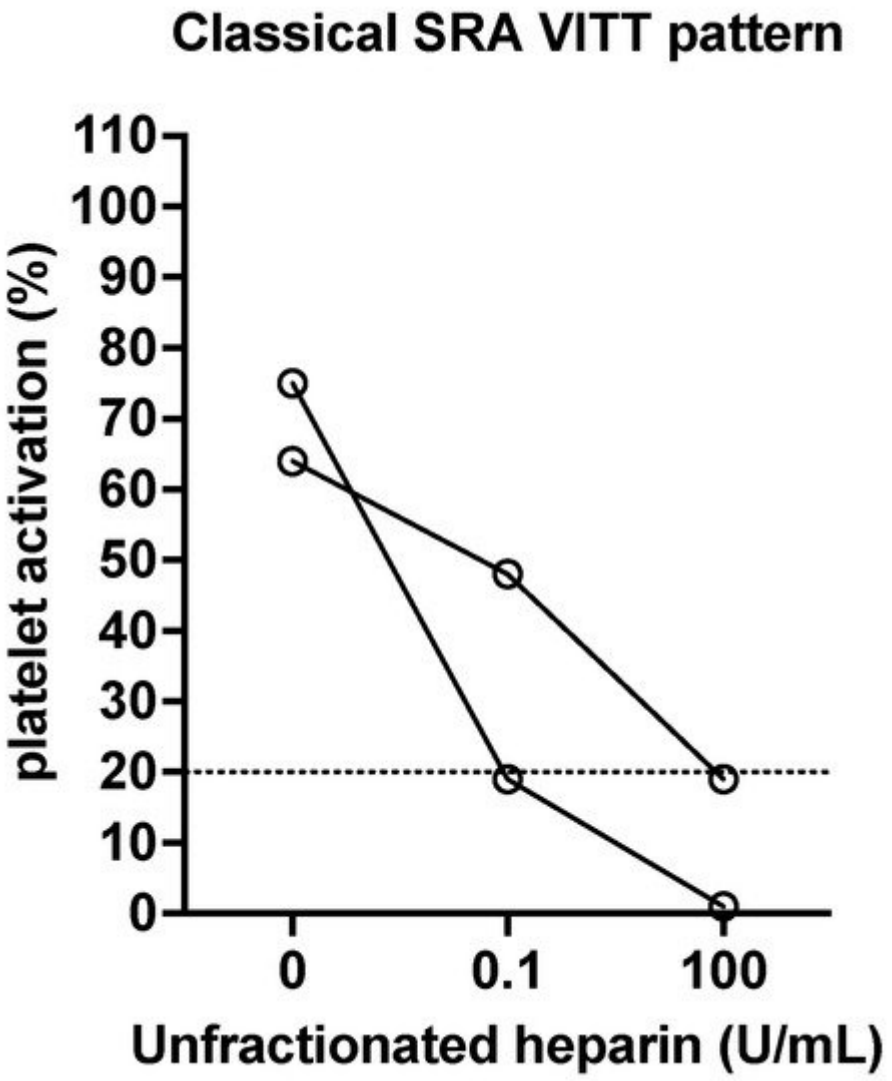

Figure 2. Two examples of a classical pattern expected for VITT-positive (Westmead Hospital) cases assessed by SRA. Platelet activation should occur in the absence of added heparin. In the presence of added heparin at a therapeutic level (0.1 U/mL final concentration in these examples; 0.5 U/mL may lead to even greater inhibition), platelet activation, measured as serotonin release, may be inhibited. In the presence of added heparin at a supra-therapeutic level (100 U/mL final concentration in these examples), platelet activation, measured as serotonin release, is further inhibited (i.e., very little or no release occurs). Occasionally, however, platelet activation may also occur in the presence of added therapeutic heparin, thereby challenging the discrimination between VITT and HITT.

References

1. Prechel, M.M.; Walenga, J.M. Emphasis on the Role of PF4 in the Incidence, Pathophysiology and Treatment of Heparin Induced Thrombocytopenia. Thromb. J. 2013, 11, 7.

2. Lippi, G.; Sanchis-Gomar, F.; Favaloro, E.J.; Lavie, C.J.; Henry, B.M. Coronavirus Disease 2019-Associated Coagulopathy. Mayo Clin. Proc. 2021, 96, 203–217.
3. Favaloro, E.J.; Henry, B.M.; Lippi, G. The complicated relationships of heparin-induced thrombocytopenia and platelet factor 4 antibodies with COVID-19. Int. J. Lab. Hematol. 2021, 43, 547–558.
4. Cipok, M.; Tomer, A.; Elalamy, I.; Kirgner, I.; Dror, N.; Kay, S.; Deutsch, V.R. Pathogenic heparin-induced thrombocytopenia and thrombosis (HIT) antibodies determined by rapid functional flow cytometry. Eur. J. Haematol. 2019, 103, 225–233.
5. Warkentin, T.E.; Greinacher, A. Spontaneous HIT syndrome: Knee replacement, infection, and parallels with vaccine-induced immune thrombotic thrombocytopenia. Thromb. Res. 2021, 204, 40–51.
6. Appelbaum, J.; Arnold, D.M.; Kelton, J.G.; Gernsheimer, T.B.; Jevtic, S.D.; Ivetic, N.; Smith, J.W.; Nazy, I. SARS-CoV-2 spike-dependent platelet activation in COVID-19 vaccine-induced thrombocytopenia. Blood Adv. 2021.
7. Pascreau, T.; Ballester, M.C.; Van Dreden, P.; Zia-Chahabi, S.; Zuber, B.; Choucair, J.; Bironien, R.; Farfour, E.; Vasse, M. The high frequency of anti-PF4/heparin antibodies in patients with COVID-19 is neither related to heparin treatment or to an increased incidence of thrombosis. Clin. Chem. Lab. Med. 2021, 59, e405–e408.
8. Favaloro, E.J.; Pasalic, L. COVID-19 vaccine induced (immune) thrombotic thrombocytopenia (VITT)/thrombosis with thrombocytopenia syndrome (TTS): An update. Aust. J. Med. Sci. 2021, 42, 86–93.
9. World Health Organisation. COVID-19 Vaccines. Available online: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/covid-19-vaccines (accessed on 23 December 2021).
10. Greinacher, A.; Thiele, T.; Warkentin, T.E.; Weisser, K.; Kyrle, P.A.; Eichinger, S. Thrombotic Thrombocytopenia after ChAdOx1 nCov-19 Vaccination. N. Engl. J. Med. 2021, 384, 2092–2101.
11. Schultz, N.H.; Sørvoll, I.H.; Michelsen, A.E.; Munthe, L.A.; Lund-Johansen, F.; Ahlen, M.T.; Wiedmann, M.; Aamodt, A.H.; Skattør, T.H.; Tjønnfjord, G.E.; et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. N. Engl. J. Med. 2021, 384, 2124–2130.
12. Scully, M.; Singh, D.; Lown, R.; Poles, A.; Solomon, T.; Levi, M.; Goldblatt, D.; Kotoucek, P.; Thomas, W.; Lester, W. Pathologic Antibodies to Platelet Factor 4 after ChAdOx1 nCoV-19 Vaccination. N. Engl. J. Med. 2021, 384, 2202–2211.
13. Favaloro, E.J.; Pasalic, L.; Lippi, G. Review and evolution of guidelines for diagnosis of COVID-19 vaccine induced thrombotic thrombocytopenia (VITT). Clin. Chem. Lab. Med. 2021, 60, 7–17.

14. Hafeez, M.U.; Ikram, M.; Shafiq, Z.; Sarfraz, A.; Sarfraz, Z.; Jaiswal, V.; Sarfraz, M.; Chérrez-Ojeda, I. COVID-19 Vaccine-Associated Thrombosis with Thrombocytopenia Syndrome (TTS): A Systematic Review and Post Hoc Analysis. Clin. Appl. Thromb. Hemost. 2021, 27.
15. Favaloro, E.J. Laboratory testing for suspected COVID-19 vaccine-induced (immune) thrombotic thrombocytopenia. Int. J. Lab. Hematol. 2021, 43, 559–570.
16. Goldman, M.; Hermans, C. Thrombotic thrombocytopenia associated with COVID-19 infection or vaccination: Possible paths to platelet factor 4 autoimmunity. PLoS Med. 2021, 18, e1003648.
17. Greinacher, A.; Selleng, K.; Palankar, R.; Wesche, J.; Handtke, S.; Wolff, M.; Aurich, K.; Lalk, M.; Methling, K.; Volker, U.; et al. Insights in ChAdOx1 nCov-19 Vaccine-induced Immune Thrombotic Thrombocytopenia (VITT). Blood 2021, 138, 2256–2268.
18. Vayne, C.; Rollin, J.; Gruel, Y.; Pouplard, C.; Galinat, H.; Huet, O.; Mémier, V.; Geeraerts, T.; Marlu, R.; Pernod, G.; et al. PF4 Immunoassays in Vaccine-Induced Thrombotic Thrombocytopenia. N. Engl. J. Med. 2021, 385, 376–378.
19. Singh, B.; Kanack, A.; Bayas, A.; George, G.; Abou-Ismail, M.Y.; Kohlhagen, M.; Christ, M.; Naumann, M.; Moser, K.; Smock, K.; et al. Anti-PF4 VITT antibodies are oligoclonal and variably inhibited by heparin. medRxiv 2021.