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Craven, B., Lester, W.A., Boyce, S. et al. (13 more authors) (2022) Natural history of PF4 antibodies in vaccine induced immune thrombocytopenia and thrombosis. Blood, 139 (16). pp. 2553-2560. ISSN 0006-4971

https://doi.org/10.1182/blood.2021014684

Natural history of PF4 antibodies in vaccine induced immune thrombocytopenia and thrombosis

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Running title: PF4 antibodies in VITT

Article type: Regular article

Word count: 3701 words (manuscript) 233 words (abstract)

Figures: 7

Tables: 1

References: 25

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Key points

- **72% of patients remain positive for PF4 antibodies at 100 days but differences exist regarding antibody persistence dependent on assay used**
- **Relapse rate is 12.6% , predominantly taking the form of recurrent thrombocytopenia, and all occurred within 90 days of presentation**

Abstract

The COVID-19 pandemic has resulted in the rapid development of a range of vaccines against SARS-CoV-2. Vaccine induced immune thrombocytopenia and thrombosis (VITT) is a rare but life-threatening complication of primarily adenoviral based vaccines, associated with the presence of antibodies to a PF4/polyanion neoepitope, measured by ELISA assays. Presented are serial anti-PF4/polyanion antibodies, platelet and D-dimer measurements in a large cohort of patients and their relation to relapse. 51% of patients using the Stago assay had a persistently positive anti-PF4/polyanion levels 100 days post diagnosis whilst 94% of patients monitored using the Immucor assay remain positive. The median duration of positivity of the PF4 assay is 87 days with 72% of patients remaining positive after a median duration of follow up of 105 days. The use of plasma exchange appeared to reduce anti-PF4/polyanion levels and increase platelet counts in the acute setting more rapidly than other therapies. The rate of relapse in this study was 12.6% with all relapsed cases showing persistently positive PF4 antibodies and falling platelet counts. Only one case had extension of their thrombosis. Overall, despite the persistence of PF4 antibodies in 72% of patients, the rate of relapse is low and does not appear to result in recrudescence of the aggressive clinical picture seen at index presentation. Monitoring of these patients in the UK cohort is ongoing and will aid definition of the natural history of this novel condition.

Introduction

The COVID-19 pandemic began in Wuhan province in China in December 2019 and has led to 285,102,577 infections and 5,441,692 deaths worldwide as of 30th of December 2021 [1]. The rapid spread of this novel infection across the globe led to widespread institution of sweeping non-pharmacological interventions such as mask mandates, travel restrictions and closures of schools and businesses as well as the development of several novel vaccinations against COVID-19 which were developed with unprecedented speed [2–4]. The UK was the first country to roll out the adenoviral vector based ChAdOx1 nCov-19 developed by AstraZeneca in January 2021. As of the 15th of December 2021, 24.9 million people and 24.1 million people have received their first and second doses respectively of the AZ vaccine in the UK [5].

In March 2021, reports emerged simultaneously from Norway, Germany and the UK regarding thrombosis at unusual sites combined with thrombocytopenia in patients who had received the ChAdOx1 vaccine. Subsequently, a novel condition known as vaccine induced immune thrombocytopenia and thrombosis (VITT) was described [6–8]. VITT has also been described following administration of the adenoviral based vaccine Ad26.COV2.S developed by Johnson and Johnson/Janssen [9]. This led to several countries introducing restrictions upon who could receive adenoviral based vaccines, many of them based on age, as the risk has been shown to be higher in people in younger age groups. In the UK, for example, a decision was made to offer ChAdOx1 to only those over 30 on the 7th of April 2021 and subsequently this was increased to only those over 40 on the 7th of May 2021 [10].

A number of large cohort studies have been published describing the initial presentations of both patients with confirmed VITT as well as those with the broader condition of cerebral sinus venous thrombosis post COVID-19 vaccine [11,12], however, to date, only limited descriptions of the natural history of the PF4/polyanion antibodies in VITT exist [13].

Methods

Data was collected on cases that occurred in the UK between January and August 2021. Data was collected from a combination of sources. These sources included daily meetings of the UK Expert Haematology Panel (EHP) which were established as soon as this condition became apparent and helped define and direct the national response; via anonymised electronic forms coordinated by Public Health England; and direct correspondence with local haematologists and laboratories reporting the anti-PF4/polyanion antibody results. All cases were reported to the Medicines and Healthcare products Regulatory Agency (MHRA).

Our focus for this paper was how the PF4 antibody levels changed over time in the VITT patients and whether this had any effect on their rate of relapse. Given their importance, as per the case definition, we have also compared D-dimer and platelet levels over time with that of the PF4 antibody OD values and the impact of therapy on these antibody levels. In an effort to provide as representative a sample of the UK cohort as possible, permissive inclusion criteria were utilised. Cases were included in the longitudinal analysis if they had three or more PF4 ELISAs readings during the course of the study.

Case definition

This dataset is an extension of that previously reported in the UK cohort of patients, where the case definition is previously described [11]. Relapses were defined as recurrent thrombocytopenia following normalisation of platelets which required re-treatment, or extension of existing thrombus, or thrombosis in a new site.

Laboratory tests

The PF4 ELISA assays were performed in specialist haemostasis laboratories in the UK during the period of this study. The centralisation of processing and reporting of PF4 ELISA results was due to many labs in the country having converted their HIT assay to chemiluminescent immunoassays in recent years which are not sensitive for VITT cases. The two assays utilised by specialist laboratories were the Lifecodes PF4 Immucor assay and the Stago Asserachrom HPIA assay. The cut-off for positivity for the Immucor assay was an optical density (OD) reading of 0.4 whilst for the Stago assay this was 0.238. Standard blood counts and clotting assays including Clauss fibrinogen and D-dimer were carried out in the hospital where the patient was being managed. The units for D-dimer were mcg/L or ng/mL fibrinogen equivalent units (FEU) with a cut-off for positivity of 500 FEU. Due to the retrospective nature of the data and the new presentation of this syndrome, the interval between testing, including PF4 ELISA assays, was at the discretion of the treating physician.

Statistical tests

Graphpad Prism (GraphPad Software, San Diego, California USA) was used to illustrate the graphs in this paper. Linear regression modelling was used to compare the change in laboratory values over time and Wilcoxon matched-pairs signed rank tests were used to compare mean PF4/polyanion values at different time points with a p-value of <0.05 taken as the criterion of statistical significance (Graphpad version 9 for Windows). Variables have been described as absolute values, percentages, medians, interquartile ranges and ranges where specified and as appropriate.

Results

Baseline characteristics and treatment received

A total of 148 patients were included in this analysis. 73 (49%) were female. The median age was 49 years of age (range of 20-77). The median platelet count at presentation was 49 x10^9/L (range 9-311) and median D-dimer level was 19,795 mcg/L (FEU) (range 700-119,000). In patients whose platelet levels were normal at admission, all became thrombocytopenic during their admission. Some laboratories' D-dimer assays only detected levels up to a maximum value of 5000 or 20000 mcg/L (FEU), if this was the case those values were taken to be the value quoted although it was understood that this may lead to an underestimate in a minority of cases. 143 (96.6%) patients had thrombosis which was confirmed on imaging. Similar to the larger UK cohort from which this data arises, the mortality rate in this group of patients was 17.5%, in which the therapeutic pathway has been described (figure S1) [11]. Further information on the location of thromboses and the treatments received by patients in this analysis are included in table 1. The median presenting anti-PF4/polyanion OD readings for patients presenting with cerebral venous sinus thrombosis (CVST) or splanchnic vein thromboses were higher than for those patients with DVT or PE alone; 1.29 versus 1.09 and 2.7 versus 2.35 for the Stago and Immucor assays respectively.

Due to the rapid evolution of knowledge around VITT and its management after its discovery, the treatment algorithm for the patients in this study changed throughout the period of observation. Treatment was guided by a daily national multidisciplinary team meeting chaired by the EHP who also produced interim consensus guidelines for the management of VITT which were distributed via the British Society of Haematology website. Plasma exchange (PEX) was reserved for patients who were deemed the most severe by clinical parameters such as widespread thrombosis and adverse laboratory markers such as severe thrombocytopenia[11]. PEX was continued until platelet count was normal. A suggested follow up protocol for patients upon discharge is included in figure S2.

Serial anti-PF4/polyanion readings

The serial anti-PF4/polyanion readings for samples processed via both the Stago and Immucor assays are shown separately in figures 1(a) and 1(b) due to the different cut-off values for positivity with each assay. The X axis in both figures show time from presentation with day 1 being considered the day that the diagnosis of VITT was confirmed via the PF4 ELISA. Due to differences in monitoring between centres as well as early mortality in some patients, serial anti-PF4/polyanion levels (multiple readings beyond 30 days) were available for 70 patients. For this subgroup of patients, the median duration of positive anti-PF4/polyanion readings was 87 days, and 72% of these remained positive after a median duration of follow up of 105 days. As demonstrated below, however, differences exist based on the assay used. This discrepancy is expressed more clearly in figure 2.

61 patients were originally confirmed as having VITT via the Stago assay. Serial data from the Stago assay was available for 33 patients. Of these, 17 (52%) have normalised their PF4/polyanion antibody levels by day 100 with almost all patients showing a clear downward trend as per figure 1(a). The median duration of persistently positive anti-PF4/polyanion OD values in those patients who remained positive was 139 days (range 34-157 days).

87 patients were confirmed as having VITT using the Immucor assay. Serial data was available for 37 of these patients, however, as can be seen in figure 1(b), only two of these patients have normalised their anti-PF4/polyanion antibody values within 100 days and indeed 86% (32/37) of this group remain strongly positive with OD values in excess of 1 at 100 days. The median duration of persistently positive anti-PF4/polyanion OD values in those patients who remained positive was 89 days (range 34-165 days).

Functional testing in the form of heparin induced platelet activation assays were carried out on 67 of the patient samples. 30 (45%) of these were considered positive with platelet activation present at low concentrations of heparin which was abrogated by the addition of heparin in excess. This testing was carried out prior to the description of novel PF4-induced platelet activation (PIPA) and PF4-induced flow cytometry–based platelet activation (PIFPA) assays [14].

Serial anti-PF4/polyanion antibody values related to platelets and D-dimers

There is a clear increase in platelet counts over the first three weeks following presentation (figures 3(a) and 3(b)) with many patients increasing their levels to above the upper limit of normal by the second and third weeks. The increase in platelets occurred at the same time as a decrease in anti-PF4/polyanion OD values from presentation. There was a decrease in D-dimer values which mirror the decrease seen in the anti-PF4/polyanion values over time (figure 4). The D-dimer values in this subgroup went from a median value of 16,480 mcg/L (range 4,160-80,000 mcg/L) at presentation to all values being less than 2000 (the cut-off for probable VITT) by week 4 post presentation.

Serial anti-PF4/polyanion values and platelets following PEX

In the group of nine patients who received PEX and who were monitored using the Stago assay, patients received a median of four PEX treatments (single volume exchanges, range 2-11 treatments). Unfortunately this data was not available for all patients monitored by Immucor who received PEX. Figure 5(a) shows the early reduction in PF4/polyanion antibodies as measured by the Stago assay brought about by PEX compared to age and sex matched control patients who did not receive PEX in figure 5(b). This rapid reduction in PF4/polyanion antibody levels were not seen in those patients who were monitored by the Immucor assay although clinical improvements and laboratory improvements were seen in almost all patients who received PEX. Similar to in figure 1, the low PF4/polyanion antibody levels seen in patients monitored via the Stago assay persisted over time whereas once again the antibodies detected by the Immucor assay appear more persistent.

Similarly, in figure 7, there was a statistically significant difference in platelet counts between those who received PEX and those who did not in the first 14 days post presentation ($p=0.0002$). One patient whose thrombocytopenia was extremely resistant to PEX was considered an outlier and not included in this analysis however this did not change the statistical significance of the finding.

As noted earlier, PEX was introduced as a treatment modality during the UK wave of VITT cases for the most severe cases. Requirement for PEX, therefore, along with mortality and need for neurosurgical or IR intervention can be seen as surrogate markers for disease severity. Taking these three outcomes as a composite, the relative risk (RR) of this outcome for those patients who had a presenting PF4/polyanion OD value of >2 on the Stago assay was 2.09 (95% CI 1.13-3.85, $p=0.01$). The RR for the same outcome using a PF4/polyanion OD value of >2 on the Immucor assay was 2.47 (95%

CI 0.83-7.37, p=0.1). This is suggestive, at least, that a higher presenting PF4/polyanion OD value is associated with a worse outcome.

Relapse

Longer term clinical outcome data on 79 patients, of whom 16 had died, was gathered. Of the remaining 63 patients, there were ten episodes of relapse although three of these were in the same patient yielding a rate of relapse of 12.6%. All but one of these relapses presented as a fall in platelet count rather than extension of thrombosis or recurrence of symptoms. The one exception was a patient who re-presented with recurrence of their thrombocytopenia and extension of their PE one week after being discharged home with normal platelets. One patient also had persistent thrombocytopenia and raised D-dimer with breakthrough thrombosis requiring change of anticoagulation. This latter case was, however, all part of the patient's initial admission and so was not classed as a relapse. Indeed, this was very early post discovery of VITT when treatment pathways were still being developed.

All patients who relapsed had persistently elevated anti-PF4/polyanion antibody levels when it occurred. Representative graphs of trends in anti-PF/polyanion antibody levels, platelet counts and D-dimer levels are provided for one patient who was monitored via Stago and one patient monitored via the Immucor assay in the supplementary material (figure S3 and S4). The median anti-PF4/polyanion OD value at the time of relapse was 1.9 (range 0.69-2.79, positivity cut-off 0.4) for those patients who were monitored using the Immucor assay and 0.77 (range 0.56-3.14, positivity cut-off 0.238) on the Stago assay. The median time from initial presentation to relapse for these cases was 28 days (range 15-90 days). All relapses received further IVIg. In the case of the multiply relapsing patient, the patient also received rituximab and mycophenolate and the patient with recurrent thrombosis did not respond to an initial trial of rituximab and so proceeded to PEX.

Discussion

We sought to describe the ongoing changes in key laboratory parameters, in particular, the PF4/polyanion antibody levels, in VITT patients following ChAdOx1 COVID-19 vaccination as well as how these laboratory parameters may relate to relapse. Furthermore, the aim was to determine the length of time PF4/polyanion antibodies could be detected and if therapy for VITT had any impact on this.

The serial anti-PF4/polyanion readings on the Stago appeared to normalise more quickly than those analysed on Immucor with 86% of patients in the Immucor cohort remaining strongly positive at the time of this analysis with an OD value greater than 1. This may potentially be explained by differences in patient cohorts, for example, more patients receiving PEX in those patients who were monitored using the Stago assay compared to the Immucor assay. Recent descriptions of longitudinal assays from the German cohort would indicate a divergence between their novel PIPA assays and anti-PF4 ELISAs over time [15]. The Stago assay used in the reference laboratory for this study is a polyspecific assay compared to the IgG specific Immucor utilised by the other reference laboratory and differences between these assays, which were developed for HIT, in detecting VITT cases has been well described [16]. The IgG specific assay may be more specific for pathogenesis in HIT but it is possible that the antibodies detected by the Stago assay in VITT are more closely aligned with the functional platelet activation assay described by the German group [17]. It is entirely possible that the patients who tested

negative on the HIPA assay may in fact be positive when a more appropriate VITT specific assay is utilised. It would be instructive to run the PIPA assays on future cases as functional testing with the traditional HIPA assays was less informative.

In the largest cohort of HIT patients followed longitudinally, PF4 antibodies tended to persist for a median time of between 50 and 85 days [18] and so it would appear that the PF4 antibodies found in VITT persist for longer given that the median in this cohort is currently 87 days and follow up is ongoing. This persistence of antibodies is more akin to the natural history of spontaneous HIT-like syndromes [19,20]. The mechanism by which they cause thrombocytopenia and thrombosis seems to ameliorate over time, a feature of spontaneous HIT-like syndromes also [21]. It should be noted also that similar to traditional HIT, the presence of PF4/polyanion antibodies both post COVID-19 vaccination and in the wider population have recently been described and not all of these patients go on to develop overt VITT [22,23]. Despite the persistent positivity on the anti-PF4 ELISA for the VITT patients, there was early and sustained normalisation of platelets and D-dimers as noted below except in patients who relapsed.

The dichotomy in testing methods described above means it is reasonable to consider the advantages and disadvantages of different anti-PF4 ELISAs when interpreting results in the context of VITT. It should be noted, from the offset, that obviously neither assay was developed for the diagnosis of VITT however the authors feel from their experience that either the Immucor or Stago assays may be used to diagnose VITT in the acute setting. It would also be reasonable to consider Immucor testing over Stago in the context of a suspected missed diagnosis as it is possible the result would still be positive months after the acute episode however a negative result in this context would be less informative. Given the closer correlation with novel functional assays and clinical outcomes to date, the Stago assay appears to be preferable for monitoring given the lack of widespread availability of the PIPA assay.

There was a clear rise in platelet count with therapy, corresponding to a reduction in PF4 antibody levels whether measured by the Stago or Immucor assays. In particular, 92.5% of patients had normalised their platelet levels by the third week post presentation. In addition, another key marker of disease activity, D-dimer, which was extremely elevated in all patients on presentation, decreased in conjunction with the anti-PF4/polyanion antibody levels over the first three weeks following presentation and treatment and was <2000 mcg/L (the diagnostic cut-off for probable VITT) in all patients by week four. This is encouraging data that, despite the overwhelming prothrombotic response that we have seen in patients with this condition, it can be reversed with appropriate therapy such as non-heparin anticoagulation, IVIg, steroids and in more severe cases PEX.

PEX was considered as a treatment option for the most severely affected patients including in the index presentation in this cohort. Following identification of the role of PF4/polyanion antibodies in disease pathogenesis along with the observation that patients with extremely low platelet counts ($<30x10^9$/L) and CVST had such a high mortality (>70%)[11] it was increasingly recognised that these patients needed to be treated in an aggressive fashion. This lent weight to the use of PEX in severely affected cases given its ability to rapidly reduce the noxious antibodies in a wide range of other autoimmune conditions.

PEX appeared to promptly reduce the level of PF4/polyanion antibodies when measured by the Stago assay (figure 5) although as noted in the broader cohort the antibodies measured by the Immucor assay appear more persistent (figure 6). This was in spite of a clinical improvement in most patients

who received PEX and improved clinical outcomes as noted previously. Similarly, platelet levels appeared to increase more rapidly than in those who did not receive PEX (figure 7), at least in the important initial two weeks post presentation. However, inferences about therapeutic efficacy were not subject to a clinical trial and potential adverse effects of PEX [24] require monitoring in a centre which has experience managing these patients.

The rate of relapse seen in this study was fortunately low and many of the episodes classified as a relapse re-presented, in fact, with a fall in platelets which were subsequently re-treated due to a lack of knowledge about the natural history of the condition with regards to further thrombotic episodes. As noted in the results, there was extension of thrombus in one case. There does not appear to be a clear cut relationship between change in antibody levels and reduction in platelets. There is a slight uptrend in antibody levels, seen in figure S2(a) in the supplementary material, prior to the drop in platelets however, in figure S2(b) in the supplementary material, there is quite a variation in platelet counts despite a fairly static level of anti-PF4/polyanion antibodies as was characteristic of patients monitored via the Immucor assay. It should be noted, however, that the absolute number of relapses remain small and therefore it is difficult to draw firm conclusions. Although not described during the duration of this study, late relapses could still conceivably occur in the manner of HIT upon re-exposure to an appropriate provoking antigen and so ongoing surveillance is necessary.

Of the many important questions which remain in VITT, two are immediately relevant to the patients' clinical outcomes, that of further COVID-19 vaccinations, given the ongoing pandemic, and duration of anticoagulation. Patients in both our cohort [25] and the German cohort[15,17] have received a second dose of a COVID-19 vaccine (non-adenoviral based vaccines) to date. It will be instructive to follow the response to these patients over time with regards to platelets, anti-PF4/polyanion levels and any further episodes of thrombosis however the evidence to date would suggest a low rate of recurrence. All the patients who received further vaccines in the UK cohort, so far, did so whilst still receiving anticoagulation and have not been associated with recurrence of thrombocytopenia or thrombosis.

Of the 63 patients in this study who are still alive and we have up to date clinical outcomes on, seven have stopped anticoagulation as they had a lower level of thrombus burden or more conventional thrombosis and had normalised their PF4/polyanion antibodies. Many of the patients, however, have multiple sites of thrombosis, thrombosis at unusual sites or persistently positive PF4 antibodies and the current consensus plan would be to continue anticoagulation for a minimum of one year in these patients. This study raises that possibility that a different approach to stopping anticoagulation may be necessary for those patients being monitored with the Immucor assay. Given their persistence, rather than just waiting for their PF4/polyanion antibodies to evanesce, these patients may require a more proactive approach. The low rate of relapse despite antibody persistence suggests a lower level of pathogenicity over time and bodes well for anticoagulation cessation in this cohort.

Acknowledgements: We would like to thank all the haematologists and allied healthcare professionals who contributed to the data collection from sources nationwide to help us attain a greater understanding of this novel and challenging condition.

Authorship contributions: BC and MS originally conceived the study. BC, WL, SB, WT, AK, CD, JH, MM, EBS, SA, PC contributed clinical and follow up data of patients at their respective sites. DS and AC provided laboratory data from the two major reference labs used to process anti-PF4 ELISA assays., SP, WL, MM, BJH and MS were part of the UK EHP which directed the national response to VITT and

assisted with oversight of the data collection. BC wrote the original manuscript and MS provided initial feedback. All authors contributed to and viewed the manuscript before final submission.

Disclosure of conflicts of interest: WL declares speaker honoraria from Boehringer Ingelheim, Bayer, Bristol Myers Squibb, LEO Pharma, Pfizer and has been on an advisory board for Boehringer Ingelheim, Bayer, Bristol Myers Squibb, Daiichi Sankyo, Pfizer. WT declares that he has been on advisory boards for Daiichi Sankyo, Sanofi, Ablynx, speakers fees from Takeda, Bayer, Alexion, Pfizer, Bayer, Portola, Sobi, Novo Nordisk and support to attend educational events from Novo Nordisk and Sobi. SP declares that she has received honoraria for educational lectures, chairing and participation in advisory boards or sponsorship for running courses, from SOBI, Amgen, Alexion, Sanofi, CSL Behring, Novartis, Pharmacosmos and Vifor Pharma, outside the submitted work. All other authors have declared no relevant conflicts of interest.

References

1. COVID Live - Coronavirus Statistics - Worldometer.
2. Baden LR, El Sahly HM, Essink B, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N. Engl. J. Med.* 2021;384(5):403–416.
3. Polack FP, Thomas SJ, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N. Engl. J. Med.* 2020;383(27):2603–2615.
4. Voysey M, Clemens SAC, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *The Lancet*. 2021;397(10269):99–111.
5. Coronavirus vaccine - weekly summary of Yellow Card reporting. *GOV.UK*. .
6. 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):2202–2211.
7. Schultz NH, Sørvoll IH, Michelsen AE, et al. Thrombosis and Thrombocytopenia after ChAdOx1 nCoV-19 Vaccination. *N. Engl. J. Med.* 2021;384(22):2124–2130.
8. Greinacher A, Thiele T, Warkentin TE, et al. Thrombotic Thrombocytopenia after ChAdOx1 nCov-19 Vaccination. *N. Engl. J. Med.* 2021;384(22):2092–2101.
9. Commissioner O of the. Joint CDC and FDA Statement on Johnson & Johnson COVID-19 Vaccine. *FDA*. 2021;
10. MHRA response to JCVI advice on COVID-19 Vaccine AstraZeneca for people aged under 40. *GOV.UK*. .
11. Pavord S, Scully M, Hunt BJ, et al. Clinical Features of Vaccine-Induced Immune Thrombocytopenia and Thrombosis. *N. Engl. J. Med.* 2021;
12. Perry RJ, Tamborska A, Singh B, et al. Cerebral venous thrombosis after vaccination against COVID-19 in the UK: a multicentre cohort study. *Lancet Lond. Engl.* 2021;S0140-6736(21)01608–1.
13. Thaler J, Jilma P, Samadi N, et al. Long-term follow-up after successful treatment of vaccine-induced prothrombotic immune thrombocytopenia. *Thromb. Res.* 2021;207:126–130.
14. Handtke S, Wolff M, Zaninetti C, et al. A flow cytometric assay to detect platelet-activating antibodies in VITT after ChAdOx1 nCov-19 vaccination. *Blood*. 2021;137(26):3656–3659.
15. Schönborn L, Thiele T, Kaderali L, Greinacher A. Decline in Pathogenic Antibodies over Time in VITT. *N. Engl. J. Med.* 2021;0(0):null.
16. 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. JTH*. 2021;19(8):2007–2013.
17. Schönborn L, Thiele T, Kaderali L, et al. Most Anti-PF4 Antibodies in Vaccine-induced Immune Thrombotic Thrombocytopenia are transient. *Blood*. 2022;blood.2021014214.

18. Warkentin TE, Kelton JG. Temporal Aspects of Heparin-Induced Thrombocytopenia. *N. Engl. J. Med.* 2001;344(17):1286–1292.
19. Warkentin TE, Greinacher A. Spontaneous HIT syndrome: Knee replacement, infection, and parallels with vaccine-induced immune thrombotic thrombocytopenia. *Thromb. Res.* 2021;204:40–51.
20. Roberge G, Scarvelis D. Long-term anti-PF4 persistence in autoimmune heparin-induced thrombocytopenia: A glimpse into the natural history of vaccine-induced immune thrombotic thrombocytopenia. *Thromb. Update*. 2021;5:100067.
21. Poudel DR, Ghimire S, Dhital R, Forman DA, Warkentin TE. Spontaneous HIT syndrome post-knee replacement surgery with delayed recovery of thrombocytopenia: a case report and literature review. *Platelets*. 2017;28(6):614–620.
22. Thiele T, Ulm L, Holtfreter S, et al. Frequency of positive anti-PF4/polyanion antibody tests after COVID-19 vaccination with ChAdOx1 nCoV-19 and BNT162b2. *Blood*. 2021;138(4):299–303.
23. Uaprasert N, Watanaboonyongcharoen P, Vichitratchaneekorn R, et al. Prevalence of thrombocytopenia, anti-platelet factor 4 antibodies and D-dimer elevation in Thai people After ChAdOx1 nCoV-19 vaccination. *Res. Pract. Thromb. Haemost.* 2021;5(6):e12580.
24. Shemin D, Briggs D, Greenan M. Complications of therapeutic plasma exchange: a prospective study of 1,727 procedures. *J. Clin. Apheresis*. 2007;22(5):270–276.
25. Lacy J, Pavord S, Brown KE. VITT and Second Doses of Covid-19 Vaccine. *N. Engl. J. Med.* 2021;NEJMc2118507.

Table legends

Table 1. Table demonstrating the sites of thrombosis and major haemorrhage and treatment received by patients. Note patients may have had thrombus in multiple sites as well as receiving multiple treatment modalities. IR=interventional radiology, PEX=plasma exchange.

Figure legends

Figure 1(a) and 1(b). Scatter plots of PF4/polyanion OD values of all patients with confirmed or probable VITT over time. Fig 1(a) includes all patients monitored by the Stago Asserachrom HPIA assay and 1(b) includes all patients monitored by the Immucor Lifecodes PF4 assay. (n=61 and 87 patients respectively for each graph)

Figure 2. Kaplan Meier curve displaying the percentage of patients testing positive on different PF4 assays over time. The positivity cut-off for the Stago and Immucor assays were ODs of 0.238 and 0.4 respectively and an event was recorded as soon as the patient had a negative ELISA. (n=33 and 34 patients for Stago and Immucor respectively)

Figures 3(a) and 3(b) Box and whisker plots of PF4/polyanion OD values as measured by Stago and Immucor assays and platelet counts. The positivity cut-off for the Stago and Immucor assays were ODs of 0.238 and 0.4 respectively with a normal platelet count 150-400x10^9/L. Whiskers extend between maximum and minimum values and the middle line represents the median value with all datapoints displayed as dots. This graph includes only patients for which serial PF4/polyanion and platelet counts were available with some patients having multiple measurements in a given week (n=18 and 22 patients for each graph respectively).

Figure 4 Box and whisker plot of PF4/polyanion OD values as measured by Stago assay and D-dimer. The positivity cut-off for the Stago assay was an OD of 0.238 and the D-dimer normal values were 0-500 mcg/L (FEU). D-dimer values were plotted on a logarithmic scale. Whiskers extend between maximum and minimum values with all datapoints displayed as dots and the middle line representing the median value. This graph includes only patients for which serial PF4/polyanion and D-dimers measurements were available with some patients having multiple measurements in a given week (n=10 patients)

Figure 5(a) and (b) Bar charts of patient's PF4/polyanion OD values over time as measured by the Stago assay based on whether or not they have received PEX. Figure 5(a) shows the median PF4/polyanion OD values for patients who received PEX over time (n=9) compared with age and sex matched control patients who did not receive PEX (n=10). The tails represent interquartile ranges. The positivity cut-off for the Stago assay was an OD of 0.238. Wilcoxon matched-pairs signed rank tests were used to determine statistically significant differences between mean PF4/polyanion OD values at different time points with $p<0.05$ considered to be significant.

Figure 6(a) and 6(b) Bar charts of patient's PF4/polyanion OD values over time as measured by the Immucor assay based on whether or not they have received PEX. Figure 5(a) shows the median PF4/polyanion OD values for patients who received PEX over time (n=9) compared with age and sex matched control patients who did not receive PEX (n=11). The tails represent interquartile ranges. The positivity cut-off for the Immcuor assay was an OD of 0.4. Wilcoxon matched-pairs signed rank tests were used to determine statistically significant differences between mean PF4/polyanion OD values at different time points with $p<0.05$ considered to be significant.

Figure 7 Scatter plot of patient's platelet counts based on whether they received PEX. Normal platelet values 150-400x10^9/L. Linear regression modelling used to plot lines of best fit and compare rates of change with $p<0.05$ considered significant.

Figure 1(a)

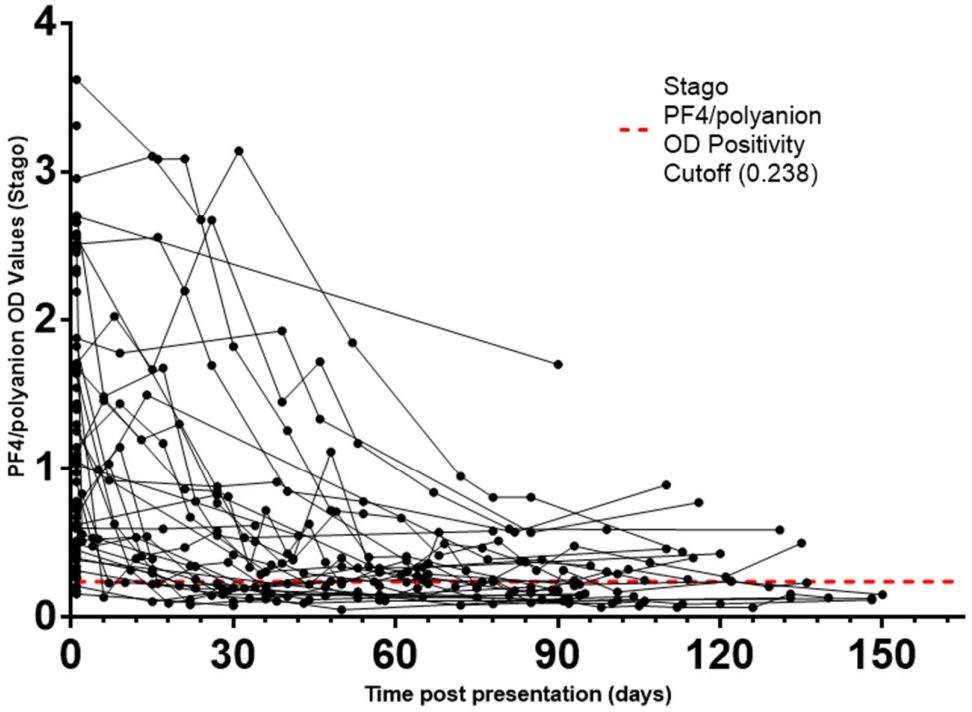

Figure 1(b)

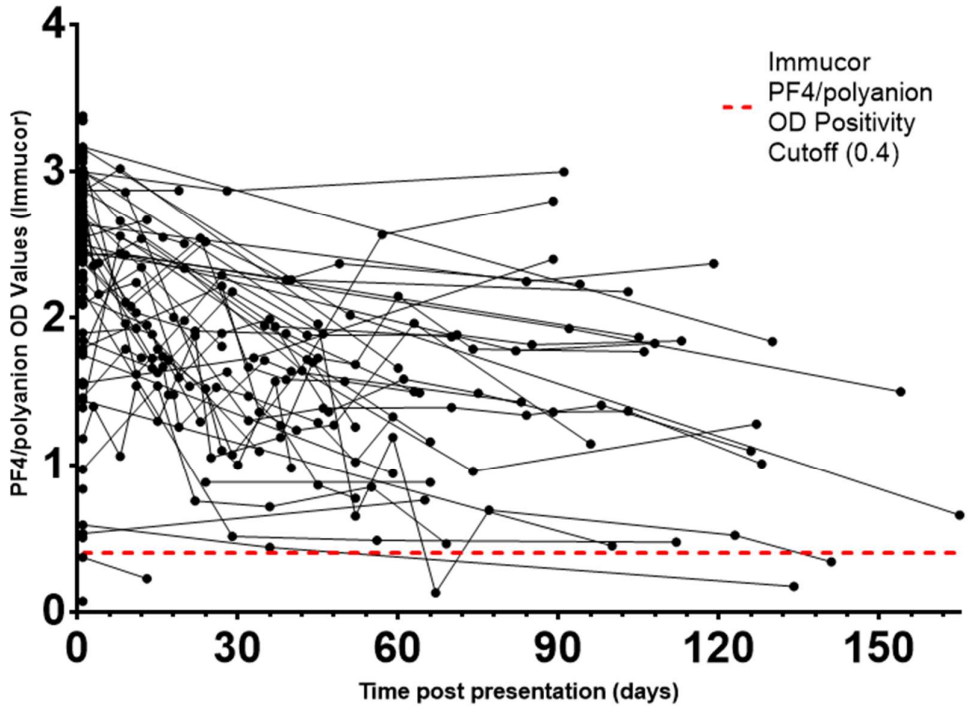

Figure 2

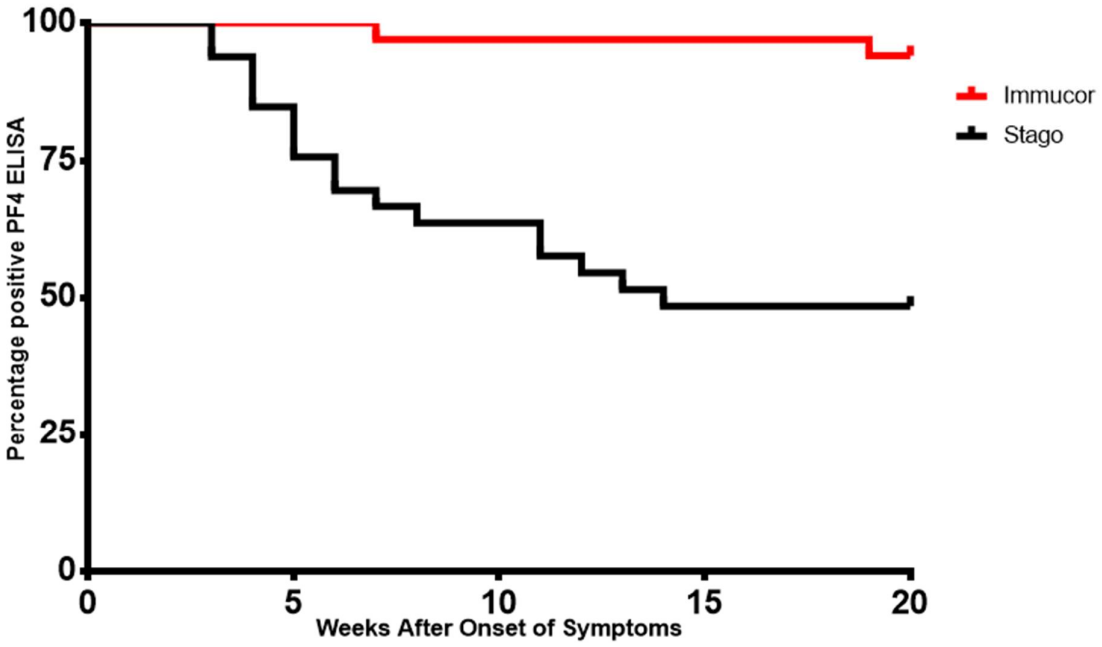
100
75
50
25
0
Percentage positive PF4 ELISA
0
5
10
15
20
Weeks After Onset of Symptoms
Immucor
Stago

Figure 3(a)

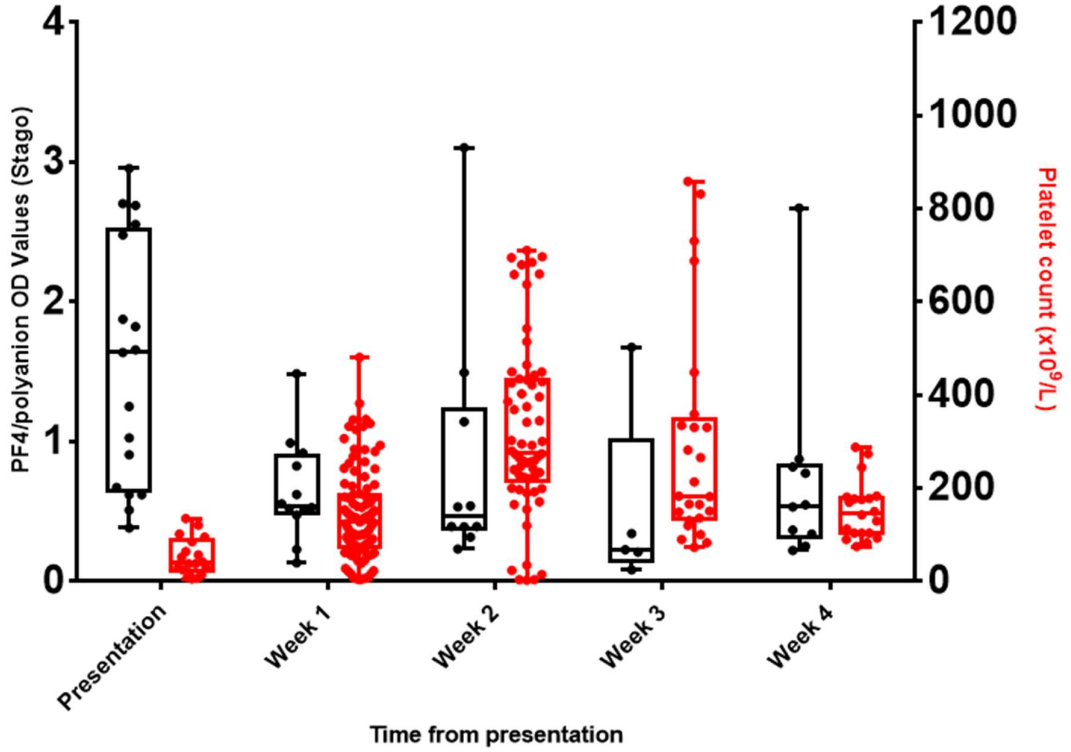

Figure 3(b)

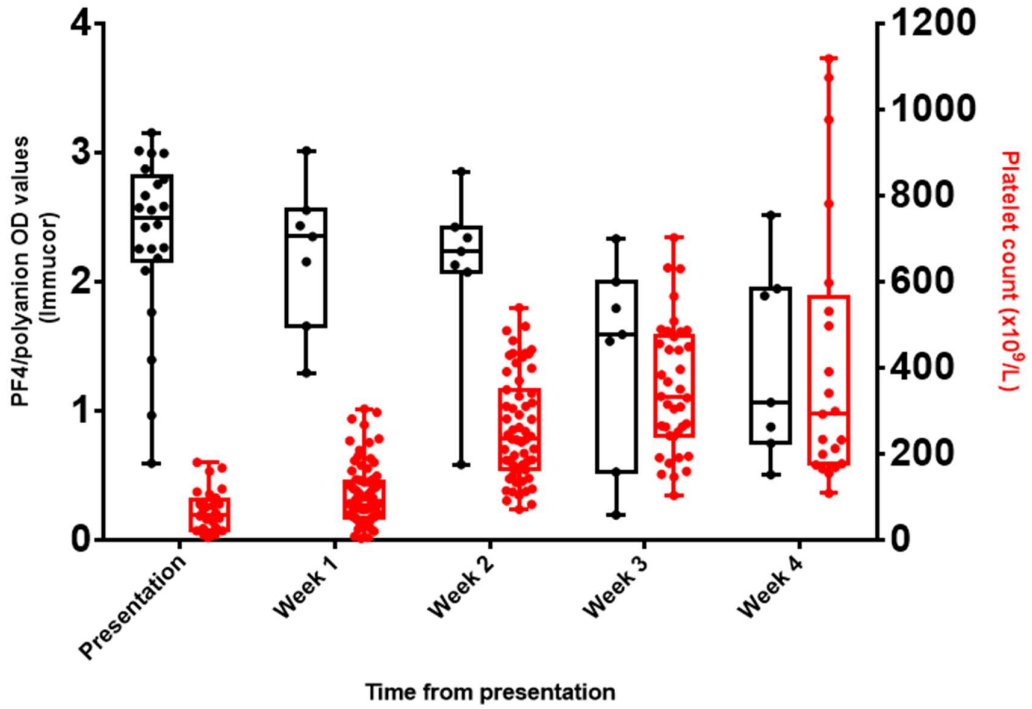

Figure 4

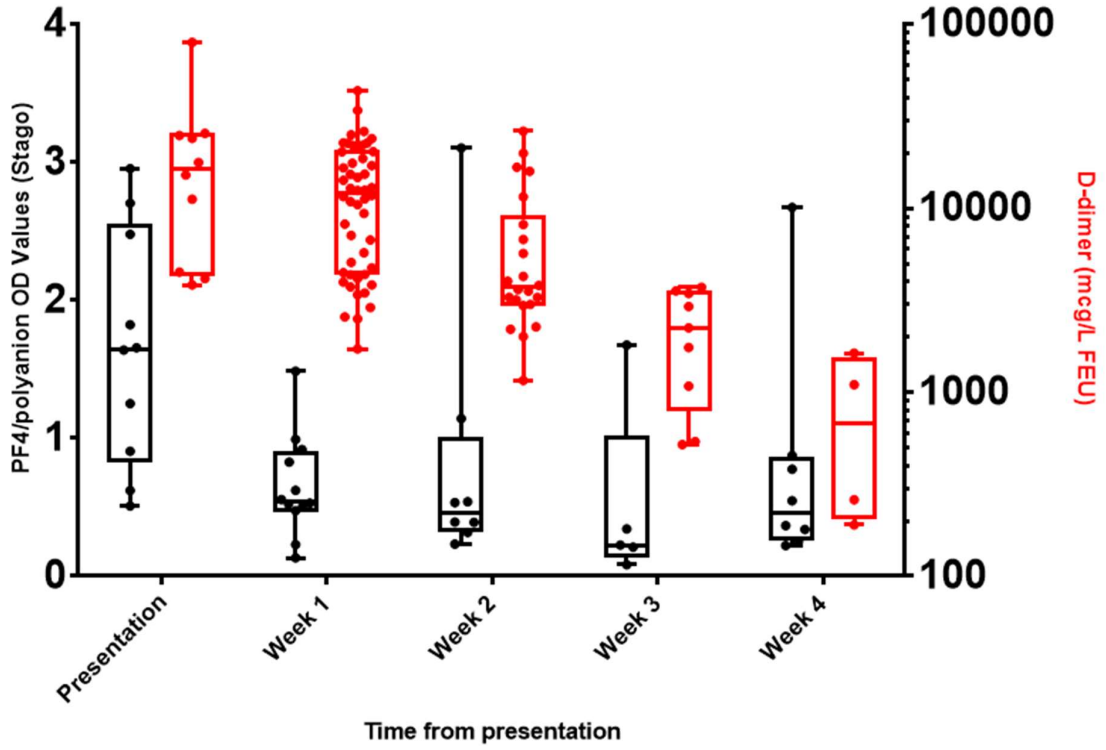
PF4/polyanion OD Values (Stago)
D-dimer (mcg/L FEU)
4
3
2
1
0
100000
10000
1000
100
Presentation
Week 1
Week 2
Week 3
Week 4
Time from presentation

Figure 5(a)

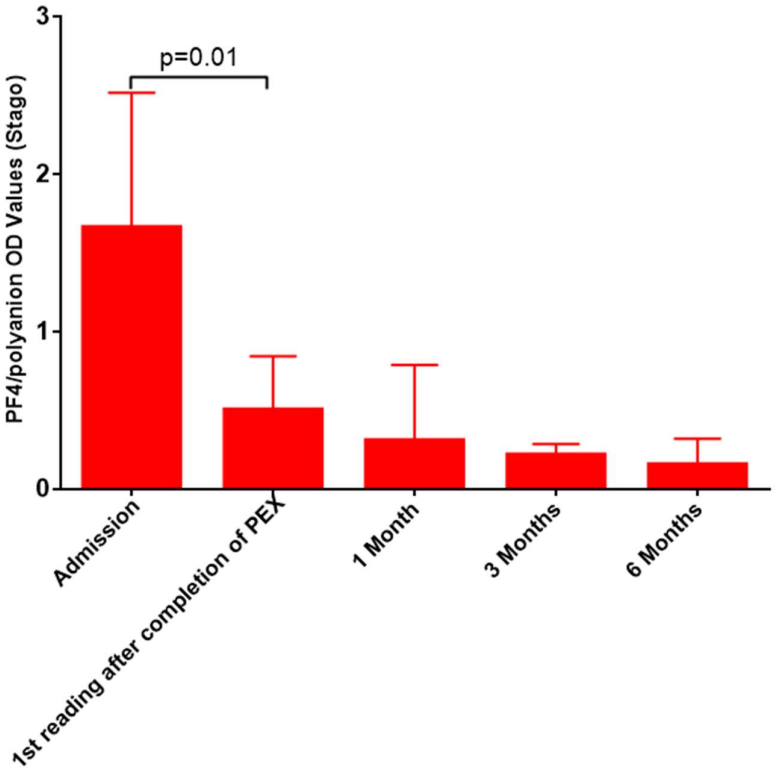

Figure 5(b)

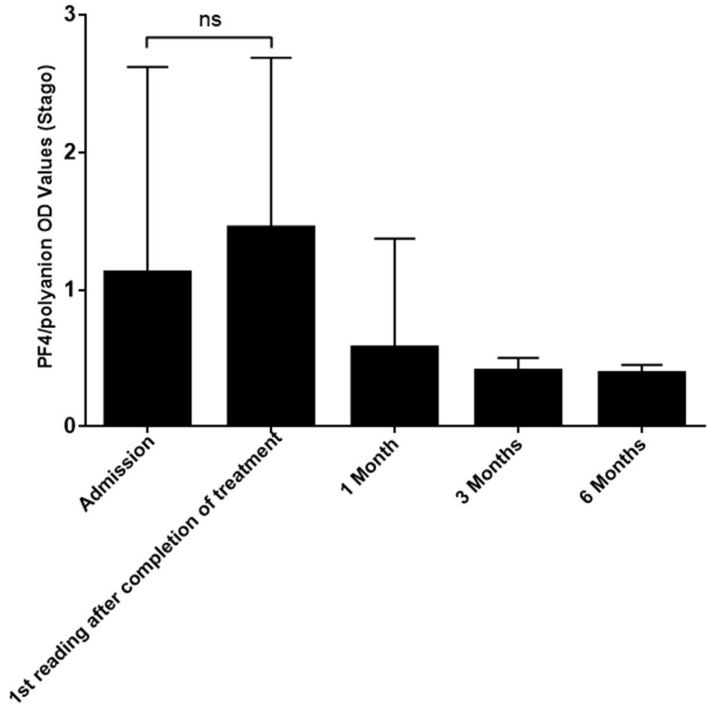

Figure 6(a)

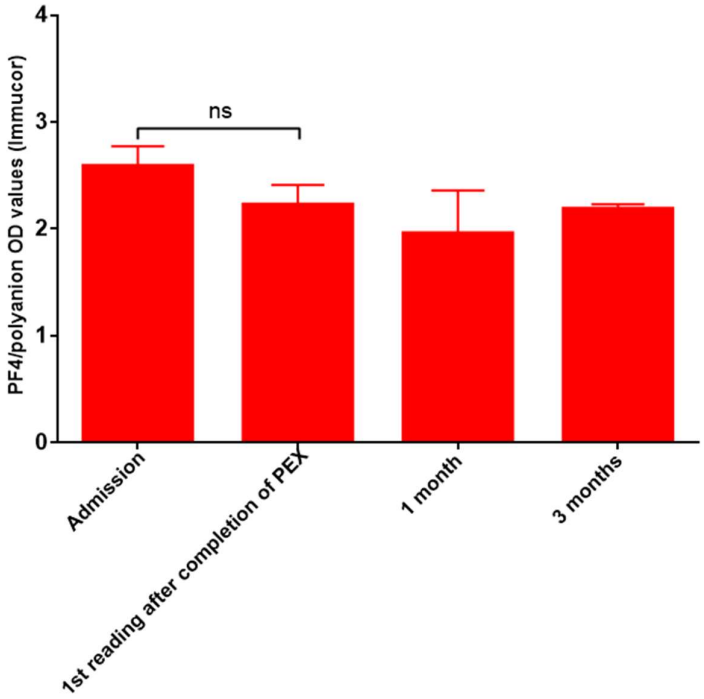

Figure 6(b)

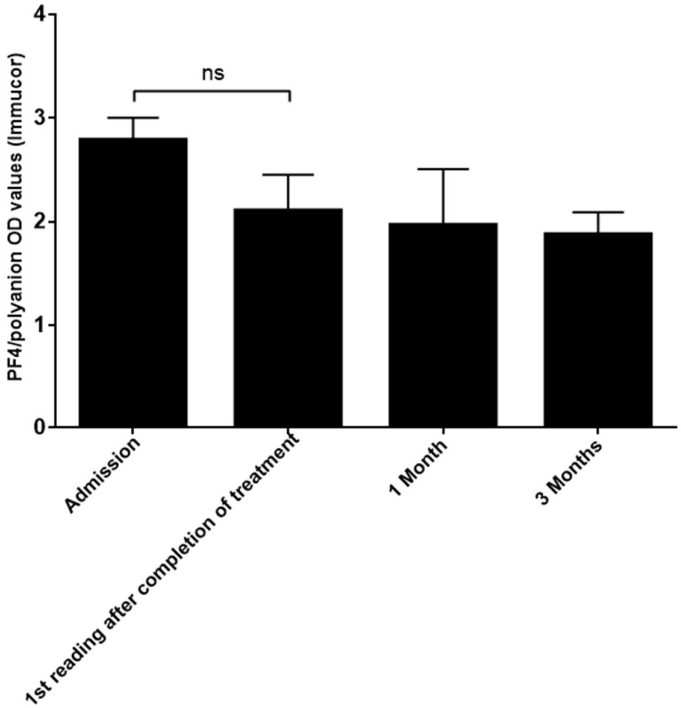

Figure 7

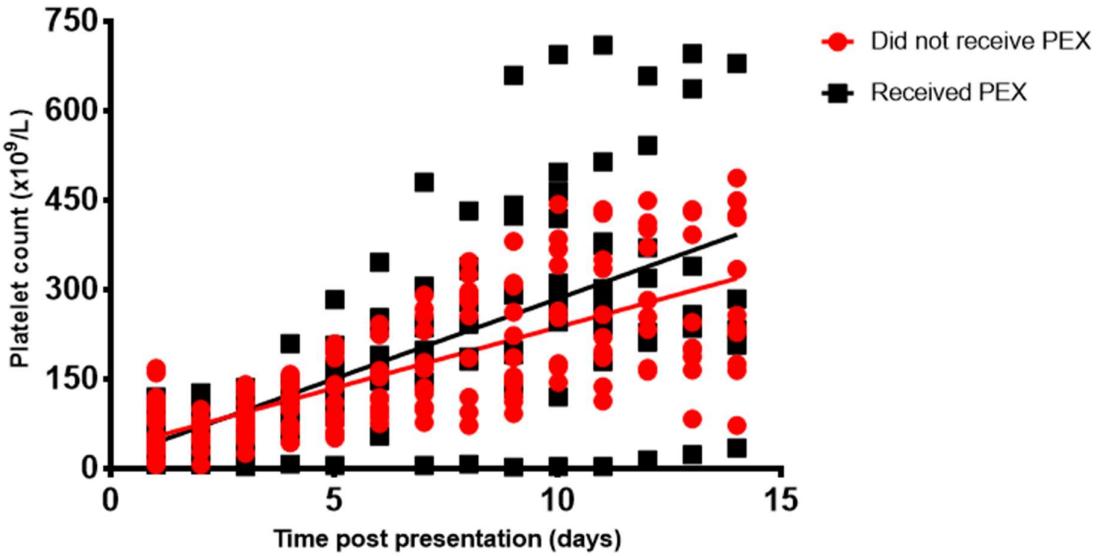
750
600
450
300
150
0
Platelet count (x10^9/L)
0
5
10
15
Time post presentation (days)
Did not receive PEX
Received PEX

Table 1

Table 1	n (%)
Sites of thrombosis	
Cerebral venous sinus thrombosis	67 (45%)
Intracerebral/subarachnoid haemorrhage	35 (23%)
Arterial (limb ischaemia, stroke etc.)	32 (21%)
Lower extremity deep venous thrombosis	17 (11%)
Pulmonary embolus	40 (27%)
Splanchnic vein thrombosis (mesenteric, portal, hepatic etc.)	23 (16%)
Treatment received	
Intravenous immunoglobulin	108 (73%)
Systemic corticosteroids (oral prednisolone/dexamethasone or intravenous methylprednisolone)	85 (57%)
Non-heparin anticoagulation (fondaparinux, argatroban, direct acting oral anticoagulants)	106 (72%)
Low molecular weight heparin	24 (16%)
Unfractionated heparin	8 (5%)
Surgical/IR intervention	19 (13%)
Platelet transfusion	16 (11%)
PEX	18 (12%)