Review 1: "Anti-PF4 levels of patients with VITT do not reduce 4 months following AZD1222 vaccination"

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RR:C19 Evidence Scale rating by reviewer:

- **Reliable.** The main study claims are generally justified by its methods and data. The results and conclusions are likely to be similar to the hypothetical ideal study. There are some minor caveats or limitations, but they would/do not change the major claims of the study. The study provides sufficient strength of evidence on its own that its main claims should be considered actionable, with some room for future revision.

Review:**Summary**

This preprint manuscript described a clinical consequence of VITT and a persistent high titer of anti-PF4 following vaccine-induced thrombocytopenia. This is an important topic. The findings contribute to a broader research understanding. The authors found that despite the persistent high titer of anti-PF4, platelet activation was much weaker with patients' serum at the diagnostic time than at the follow-up time. This finding could indicate other potential mechanisms of VITT other than the presence of anti-PF4 antibodies.

Main comments

The manuscripts described a case series of clinical sequelae of 7 patients following the diagnosis of vaccine-induced thrombotic thrombocytopenia (VITT) after AZD1222. In this case series, the researchers observed recurrent thrombocytopenia and persistent symptoms requiring either rituximab, IVIG, or corticosteroid. The authors also performed anti-PF4 antibody and platelet aggregation using the patients' serums and platelets at the time of follow-up (>80 days) since the date of vaccination. A persistently high titer of the anti-PF4 antibody was observed in all patients, except for one who received retreatment with rituximab. Though persistently high titer anti-PF4 antibody was demonstrated, platelet activation with the patient's serum was much weaker with the follow-up serum than with the serum at the time of diagnosis. Since data on clinical sequelae of VITT is lacking, this manuscript describes important clinical data and is of interest in the field. The methods are sound, and the laboratory was performed on standard procedure. The demonstration of persistently high anti-PF4 titer but negative on functional assay at the follow-up time is a major finding

which warrants further research in this area. The manuscript could be published with minor revisions. Few comments are as below.

1. The limitation of a robust conclusion of the findings is the small number of samples used to perform platelet activation. Only 3 samples were available. Whether these samples can represent most VITT cases needs to be elucidated.
2. Anti-PF4 antibody in autoimmune HIT can persist for several months while functional assay has become negative. Therefore, the findings could represent natural history, like that of autoimmune HIT. The author discussed other manners besides a high PF4- antibody titer alone at the time of diagnosis that could cause VITT.
3. The author demonstrated that the levels of FcγRIIa on patients' platelets at the follow-up time were comparable to platelets in healthy control. Therefore, the data of FcγRIIa of patients' platelet at the diagnostic time is potentially informative about whether or not the platelets contributed to the occurrence of VITT.
4. From supplementary figure 1, diagnostic serum of patient 4 caused robust aggregation on its platelets. However, there was no robust aggregation when PF4 was added. In addition, while inpatients 6 and 7 demonstrated no spontaneous platelet activation, robust aggregation occurred when PF4 was added. Are there possible explanations for these findings?
5. The results and the figures should be matched together. The referral of figure 2 and the mention of patient 2 in the result section should be clarified.
6. Similar findings on the persistence of PF4 were published in NEJM (DOI: 10.1056/NEJMc2112760). The reference should be cited.