

Peer Review File

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Reviewer A

This manuscript reviews the cases of anti-CD36 antibodies causing platelet refractoriness or FNAIT in the literature and also details the various methods of detecting the anti-CD36 antibodies. Overall, the paper flows well and is interesting. CD36 antibodies are rare; however, they may be overlooked and more effort may be needed to detect them particularly in Asian and African populations. The paper requires a major revision mainly due to the need for multiple figures describing the CD36 detection methods described. The methods described are difficult to conceptualize and would be greatly enhanced by adding a figure describing the mechanism of action for each.

Reply: Thanks for pointing out this issue. Multiple figures for describing the mechanism of every method are added.

Changes in the text: Figures 1-4 are added as advised.

Small points are as follows:

1) Table 1: patient #23, should be FNAIT instead of FANIT

Reply: The reviewer is correct.

Changes in the text: We have changed “FANIT” to “FNAIT” in Table 1 as advised.

2) Page 3 line 75: please replace pathomechanism with pathophysiology

Reply: We agree with the suggestion of the reviewer.

Changes in the text: We have modified our text as advised (see Page 3, line 76).

3) Page 4, line 98: Please replace practicability with practicality

Reply: We agree with the suggestion of the reviewer.

Changes in the text: We have modified our text as advised (see Page 4, line 101).

4) Page 4, line 101: Please add “for” to “However, MPHA was only used to test for anti-CD36 ...”

Reply: The reviewer is correct.

Changes in the text: We have modified our text as advised (see Page 4, line 104).

Reviewer B

This manuscript reviews the development and description of the various methods available for anti-CD36 detection. Anti-CD36, also known as anti-NAK is descriptive in Asian and African populations in association with PTR, FNAIT and TRALI. This manuscript is very good and deserves to be publishing with minor suggestions. I suggest the following modifications:

1) The introduction of this work is clear; however a concept should be introduced about CD36 or the Nak glycoprotein.

Reply: Indeed. The concept about CD36 and Nak(a) antigen is important.

Changes in the text: We have modified our text as advised (see Page 3, lines 68 to70).

2) A table should be included, describing the advantages and disadvantages of the different methods available.

Reply: Agree. A table describing the advantages and disadvantages of different methods available for anti-CD36 detecting is added.

Changes in the text: We have modified our text as advised (see **Table 2**)

Reviewer C

This is an interesting review that provides an overview on the various techniques and their limitations with detecting CD36 antibodies. This review highlights the challenges for accurately detecting these antibodies that cause immune thrombocytopenic disorders in Asian and African populations.

1. Page 3, line 62.

<http://www.ebi.ac.uk/idp/hpa/> is now a retired database. Authors should include the current HPA database:

<https://www.versiti.org/medical-professionals/precision-medicine-expertise/platelet-antigen-database/hpa-gene-database>

Reply: We agree with the suggestion of the reviewer.

Changes in the text: We have included the current HPA database (<https://www.versiti.org/medical-professionals/precision-medicine-expertise/platelet-antigen-database/hpa-gene-database>) as advised (see Page 3, lines 61 to 62).

2. Page 14.

Authors should provide a more detailed explanation or description of Table 1 either in the legend or in the text, that these are a collection of PTR and FNAIT cases whereby anti-CD36 antibodies were implicated and the methods used for antibody detection.

Reply: We agree with the suggestion of the reviewer.

Changes in the text: We added more detailed explanation or description in the legend of **Table 1** and in the text (see Page 3, lines 82 to 83).

3. Page 5, line 122.

Authors state that the method described above is known as MAIPA or MACE but in fact the two assays differ. Both are antigen capture assays, but MACE differs in that the mAbs are immobilized (instead of anti-mouse IgG) and therefore not incubated with the patient serum and whole platelets. Modification of this explanation is required with inclusion of reference: Matsushashi M & Tsuno NH. Laboratory testing for the diagnosis of immune-mediated thrombocytopenia. Annals of Blood 2018 3:41

Reply: Thanks for pointing out this issue.

Changes in the text: We have modified the text according the reference (24) : Matsushashi M & Tsuno NH. Laboratory testing for the diagnosis of immune-mediated thrombocytopenia.

Annals of Blood 2018 3:41(see Page 5, lines 126 to 129)

4. Page 6, line 158.

“HP-CD36 cells were first reacted”. Addition of word “were” is required.

Reply: The reviewer is correct.

Changes in the text: We have modified our text as advised (see Page 6, line 165).

5. Page 7, line 170.

Authors state that positive results was obtained by ACA despite negative results by MAIPA for patients #21, #22 and #23. Authors could perhaps add ACA to Table 1 for these patients to make this clearer.

Reply: The reviewer is correct.

Changes in the text: We have added “ACA” to **Table 1** as advised.

6. Page 7, line 189.

“For example, as shown in Table 1...MAIPA results were negative”. This sentence might be better placed in line 187, before “However, the stability and heterogeneity...taken into consideration.” This would be less confusing for the reader.

Reply: The reviewer is correct.

Changes in the text: We have modified our text as advised (see Pages 7 and 8, lines 194 to 196).

Reviewer D

1. Line 97-98, “stripping of HLA class I antigens from the platelet surface with chloroquine (14)”. as the authors know, chloroquine treatment actually denatures the class I HLA immune-epitopes, but does not actually “strip/remove the HLA from the platelet.

Reply: agree

Changes in the title: The sentence “stripping of HLA class I antigens from the platelet surface with chloroquine (14)” is changed to “Nordhagen et al. denatured HLA antigenic

determinants to produce HLA α 1 platelets by treatment with chloroquine”

2. Line 191-192, “...purified platelet antigens (HPAs)”: I am quite certain that the PAKLx assay uses beads coated with platelet glycoprotein-specific monoclonal antibodies that have captured platelet glycoproteins from platelet lysates with various HPA types. These are not “purified HPAs”. It could be different for CD36. Please check this and correct the manuscript accordingly as required.

Reply: agree

Changes in the title: The sentence “...purified platelet antigens (HPAs)” is changed to “These fluorescence-labelled beads as targets are immobilized with platelet lysate-derived glycoproteins to capture and identify antibodies against HPA, HLA Class I or CD36 ”. (Line 191-193).