Peer Review File

Article information: https://dx.doi.org/10.21037/aob-23-26

Reviewer Comments

Reviewer A

Comment 1/ Nomenclature:

- italicize gene and allele names throughout

Reply 1: Reviewing the original manuscript I noticed that all genes and alleles were written in italics but when the manuscript was uploaded on the website the italics were removed. I kept the ISBT nomenclature with italized genes and alleles in this new version of the manuscript.

Comment 2- hrB and hrS are mentioned a few times, HrS once (a typo, probably?): consider including ISBT nomenclature for these antigens at first mention.

Reply 2: Thanks for that. You are correct. HrS was a typo and it is now corrected. ISBT nomenclature for these antigens was included (pages 3, 5 and 6)

Comment 3- RhD-negative and RhD-positive should be avoided and replaced with "D-negative" and "D-positive" (RhD is the protein, while D is the antigen)

Reply 3: Thanks! RhD-negative and RhD-positive were replaced by D-negative and D-positive throughout the article.

Comment 4/ Some paragraphs are a little harder to follow because the sentences are very long. Suggest cutting the long sentences discussing multiple concepts into shorter ones to facilitate reading. Eg. p.3 l.60-65.
Reply 4: I tried to shorten the sentences, especially this one in the beginning of page 3.

Comment 5/ Some word choices or spelling:
- p.2 line 54. Perhaps the antigens are not "defined" as much as "detected" by commercial reagents?
Reply 5. Defined was now replaced by detected

Comment 6- p.3 l.57: the RhesusBase should be spelled as the authors chose to call it "The Human RhesusBase"
Answer: The human Rhesus base was replaced by RhesusBase as recommended.
- p.3 l.67: The antigens do not "miss" but "lack" epitopes.
Reply 6. Thanks for that. Miss was now replaced by lack

Comment 7/ p.3 the concept of "weak" and "partial" antigen are defined

here as opposing concepts. However, in 2007, Daniels G, et al. (Transfus Med. 2007 Apr;17(2):145-6.) eloquently demonstrated that the limit between these two concepts is not simple. In this review, the same nuance should be made clearer. One way to more accurately reflect current understanding could be to underline that these are historical concepts and the overlap of the two could be discussed.

Reply 7: Thanks for this point. Correction was made and the overlap of weak and partial antigens discussed according to Daniels et al. This reference was also included.

Comment 8/ The use of bioinformatics to predict phenotypes is nearly always discussed with the appropriate level of caution. Some studies have shown interesting results, correlating some findings with phenotype. However these approaches still need to demonstrate their capacity to predict (quite a strong term) phenotypes. Bioinformatics may be helpful in assessing new variants, but preferably combined with conventional methods (such as epitope mapping).

- p.4 l 118: "can be used to predict" overestimates what has been reported

Reply 8: The term predict was replaced. Now the sentence reads: “the bioinformatics can be helpful in assessing new variants”.

Comment 9 p.5. l. 150: why mention RhD intraprotein interactions in this sentence but not the more conventional methods (epitope mapping, antigen density)? Recommend removing "RhD intraprotein interactions" here.

Reply 9: Suggestion accepted and RhD intraprotein was removed.

Comment 10- p.8 l.230 "determining the risk" overestimates what has been reported

Reply 10: the sentence was changed and now it reads: “ 3D intraprotein interactions combined with epitope mapping and antigen density have been used in assessing the risk of alloimmunization in carriers of RH variants”

Comment 11- p.8 l.234: overestimates what has been reported. Floch et al showed a correlation between the 3D analysis, including but not limited to protein interactions, correlated with risk of anti-D formation. However, many variants were partial because of extracellular changes, not altered intraprotein interactions.

Reply 11: The sentence was modified to “Floch et al showed that extracellular changes and the alteration of intraprotein interactions were correlated with the risk of anti-D formation”

Comment 12/ p6. It should also be mentioned that autoantibodies can cause hemolysis, which is particularly problematic in patients with SCD, and adds a level of complexity to the interpretation of antibodies in transfused patients with variants.

Reply 12. This information was now included

comment 13/ p.7 l.191. It would be interesting to clarify what is meant by "genotyped donors" here and discuss how this differs from providing blood negative for one antigen when a patient has a partial antigen.

Reply 13: Genotyped donors in this statement was clarified. Now it reads: It is important to emphasize that the selection of compatible blood for a patient with a rare allele also depends on the availability of genotyped donors for RH variant alleles as this request is filled with RH genotype-selected units.

Comment 14/ References. Perhaps add a reference p.7 l.210 (low responders) and p.8 l.218 (donor variants inducing alloimmunization)?

Reply 14: Both references were added. See references 46 and 47

Comment 15/ p.8 l.218 "it is known". Prefer a more moderate wording such as "a study observed that...". While the article the author refers to was a very interesting report, the concept remains to be consolidated by findings from multiple teams before it can be considered a well known "fact".

Reply 15: Thanks for that. The word was replaced as suggested.

Comment 16/ It could be interesting to include a brief discussion of the implication of variants in pregnancy.

Reply 16: The impact of these novel alleles in pregnancy management was included in Conclusion

Reviewer B

Comment 1I suggest to add in the text the correct ISBT nomenclature changing in italics or superscript some blood group antigens, genes and alleles.

Thanks

Reply 1: Thank you! The correct ISBT nomenclature was added in the entire manuscript. Reviewing the original manuscript I noticed that all

genes and alleles were written in italics but when the manuscript was uploaded on the website the italics were removed.

Reviewer C

Comment 1. The manuscript reviews current understanding of the RH system. Enthusiasm is low due to the lack of new information and the extensive reviews that are already published on this topic. No new insights are included here.

Reply 1: An extensive review of the literature was carried out but unfortunately there is little new data on the new RH alleles and their implication in transfusion therapy because the serology is not always detailed in the reports and data from the analysis of the risk of alloimmunization and clinical consequences of alloimmunization have not been reported.

Perhaps this review can encourage investigators to provide more data on the implication in transfusion therapy of these new RH alleles that are being described with the use of new technologies.

Reviewer D

Comment 1. This a good review paper providing analysis and insights to novel RH alleles with limited information in serology and risk of antibody formation.

Reply 1. Thank you! believe this review can stimulate young investigators to demonstrate which new RH alleles are relevant in transfusion therapy.