Peer Review File

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Reviewer A

Although the usefulness of HLA-compatible platelets has been reported in some studies, few reports have reviewed data from other countries. The authors' data over the past decade clearly show the effectiveness of HLA-matched platelets. In particular, the data comparing the transfusion efficacy of random and HLA-compatible platelets in over 400 cases is valuable and helps to reinforce the importance of supplying HLA-matched platelets. For these reasons, we recommend that this paper be published.
However, some revisions are necessary and are shown below.

Minor
1. Figure 2 should show the CCI cutoff line.
Reply A1: We added the CCI cutoff line as suggested (see Page 15)

2. The method and criteria for HLA antibody testing should be described. (Method section)
Reply A2: The method and criteria for HLA antibody testing varied between centres. We made a summary as advised (See Page 6, Lines 21-25).
Changes in the text: "Investigations for immune-mediated PTR were not mandatory when requesting for HLA-selected platelets. Results of anti-HLA class I antibody and anti-HPA antibody testing were provided by the referring units if available. Platelet serology testing was performed by Luminex assay, enzyme-linked immunosorbent assay, direct and/ or indirect monoclonal antibody immobilization of platelet antigens (MAIPA) assay based methods as decided by the referring centres."

Major
1. Table 1 would be better to show data for women and men. It would also be of value to append the number of HLA antibody tests and positive rate to this table and discuss the differences in antibody positivity rates between men and women.
Reply A3: We added the data in Table 1 as suggested, and the results of men and women were separately presented. The positivity rates of platelet serology testing were described as well (See Page 14).

2. Can you evaluate the relationship between the strength of HLA antibodies and CCI? It has been indicated that weak HLA antibodies do not involve transfusion refractoriness. I believe that this article will be more valuable by discussing it.
Reply A4: We regretted that the results of HLA antibody testing were qualitative only. It was include as a limitation in the discussion section. (See Page 12, Line 24-26)

Changes in the text: “the strength of anti-HLA and anti-HPA antibodies was not tested. It has been reported that weak anti-HLA antibody levels were not associated with PTR(19), that whether HLA-selected platelets benefit these patients remain to be elucidated.”

3. Figure 2 includes some cases that did not increase CCI despite HLA-matched platelets. This paper focuses on HLA-A and -B and ignores the involvement of HLA-C and HPA alloantibodies. These antibodies are also known to cause transfusion refractoriness, therefore it would be better to include discussion.
Reply A5: We included the results of HPA alloantibodies (See Page 9, Line 13-14). HLA-C antigen matching was not considered in the selection of donors. This was included in the discussion as suggested (See Page 11, Line 24-26).
Changes in the text: “One-hour CCI was less than 7,500 $m^2/\mu L$ in 60 HLA-selected platelet transfusions involving 36 patients. Anti-HPA antibody was identified in one patient among 13 who had platelet serology testing performed.” & “It was also recognized that HLA-C antigen alloimmunization is also implicated in immune PTR,(16) but BTS did not routinely provide HLA-C selected platelets to patients.”

Reviewer B

I could not see novelty in the present report, but I believe it can add value to the literature in case the issues of HLA-matched/selected PC in their country are clearly analyzed and the potential solutions are discussed.

For the implementation of an appropriate system, at least the following are necessary:
1) an adequate registry of HLA/HPA- and ABO-typed donors,
2) the testing of patients sera/plasma for the presence of HLA-I and HPA antibodies implemented,
3) education of medical personnel related to use of HLA/HPA-matched/selected PC,
4) a system to confirm the effectiveness of PC transfusion.

The blood donor registry is based on the HKBMDR, with a substantial number of donors who consented to be PC donors, and the number of apheresis donations seems to be increasing.
The major issues are:
1) the need of a more appropriate indication of HLA-matched/selected PC, which depends on the education of physicians, usually requiring cooperation of the national transfusion society to include indication of HLA-matched PC in the guidelines,
2) the need to implement testing to ALL patients with suspect of PTR for the presence of HLA-I and/or HPA antibodies to confirm the immune PTR,

3) the costs associated with the implementation of the above system.
In addition, a discussion on the need (or not) of the cross-match (direct test between donor platelets and patient's serum/plasma or computer (virtual)), and the evaluation of the effectiveness of PC transfusion, which can be achieved only through a good cooperation with hospital physicians, would be necessary. Also, it would be of interest to discuss on the different types of operation that can be implemented in terms of efficiency and effectiveness, such as indicating testing only of patients who did not respond to HLA-matched PC, or selecting antigen-negative, and not "4 out of 4" PC, for patients with confirmed alloantibodies.

Major points:
1. The number of supplied HLA selected PC is very low (only 1,080 units in 10 years?). How many units of PC are supplied yearly in HK?
Reply B1: The information was added (See Page 12, Line 8-11). And we also discussed the alternatives to HLA-selected platelets to support patients with PTR.
Changes in text: "During the study period, BTS issued around 440,000 adult doses of platelets and among them 54,000 units were prepared by apheresis method. HLA-selected platelets, which were the most commonly used products to support patients with PTR locally, only constituted a small proportion of the total platelet supply."

2. Do you perform the cross-match test between donor platelets and patient's plasma/serum? Or is it just a computer cross-match?
Reply B2: Only virtual cross-match was performed (See Page 7, Line 7-8)
Changes in text: Electronic instead of physical platelet crossmatches were performed before issue.

3. Are all HLA selected PC collected by apheresis procedure? What is the rate of apheresis PC units among the total PC supplied in HK? Is it 35%, as described in P6, L11?
Reply B3: All HLA-selected platelets were collected by apheresis method (See Page 6, Line 30). The rate of apheresis platelets constituted 12% of all platelet products supplied in the study period (see Reply B1).

4. What is the rate of antigen-typed and genotyped donors registered at the HKBMDR? How does it affect supply of HLA selected PC?
Reply B4. Donors in HKBMDR were genotyped (See Page 6, Line 33 to Page 7, Line 2).
Changes in text: "Donors recruited before June 2019 had intermediate or high resolution typing of HLA-A and HLA-B antigens whilst those recruited afterwards had high resolution typing performed by next generation sequencing based method"

5. Only 54/147 (36.7%) patients were tested for HLA-I antibodies. Why not all

patients were tested? Does your protocol recommend supply of HLA selected PC when the patient has CCI<7,500 in 2 consecutive transfusions?
Reply B5: Testing of anti-HLA antibody was not mandatory when requesting for HLA-selected platelets (See Page 6, Line 21-22). The low proportion of patients having the test performed is a major problem of the current HLA-selected platelet service, which was discussed in the revised manuscript (See Page 11, Line 31 to Page 12, Line 1).
Changes in text: "Also, the proportion of patients with platelet serology testing was low. This could be partly attributed to the limited access of the tests, which were only restricted to patients who did not respond to HLA-selected platelets in some centres. Another possible reason was a lack of awareness to the investigations for PTR. It is important to differentiate whether PTR is immune related owing to its impact on clinical management."

6. It seems that immune PTR is diagnosed in case CCI-1h < 7,500 is observed after transfusion of random PC. The diagnosis of immune PTR must be done based on the identification of anti-platelet (HLA-I, HPA) antibody.
Reply B6: The causes of immune PTR were described at Page 5, Line 11-14.

7. HLA-I antibodies were not detected in 24% of the patients. Why are HLA selected PC supplied even to patients without HLA-I antibody, and to those not tested for HLA-I antibody? It may be the reason why the authors observed a higher CCI among patients who tested positive for HLA class I antibody. It is well-known that patients with immune PTR respond well to HLA selected PC, but not necessarily those without HLA-I antibody, and those not tested may include non-immune PTR and cases with HPA antibodies, who do not require HLA selected PC. Those with HPA antibodies need transfusion of HPA-matched, but not HLA-matched PC. So, for me it seems to be an obvious observation.
Reply B7: We agree with the comments. And our findings consolidated the importance of immunological workup for PTR. We discussed the need to withdraw the request for HLA-selected platelets if patients did not demonstrate anti-HLA antibodies or did not respond to it (See Page 12, Line 2-6).
Changes in text: Moreover, requests for HLA-selected platelets should be withdrawn by clinicians if anti-HLA antibodies could not be demonstrated. When patients did not respond to HLA- and/ or HPA-selected platelets, they should be supported by random donor platelets. Local guidelines regarding the workup and management of PTR should be developed. The current practice could also be improved by education, audit and a feedback system.

8. In Discussion (P10, L17-18), authors mention that HLA-selected platelets did not bring additional gain to patients who already had 1-hour CCI>7,500 after transfusion of random donor platelets. Are they saying that patients with HLA-I antibodies did respond to transfusion of random donor platelets? Or did those patients have PTR with causes other than immune due to HLA-I antibody?

Reply B8: In some of the requests, patient had suboptimal clinical response to random donor platelet transfusions but not reached the threshold of PTR when requesting for HLA-selected platelets (i.e. 1-hr CCI <7500). Our analysis found that the improvement in 1-hour CCI was significantly less than patients who had PTR (see Page 8, Line 22-27) and thus we concluded that requesting for HLA-selected platelets in patients without PTR should be discouraged (See Page 11, Line 28-30. Refer to reply B9 for the changes in text.

9. What do author mean with "genuine" PTR? The sentences in Abstract (P3, L20-24) and Results (P8, L25-28) are difficult to follow. Please rephrase or explain better.

Reply B9: To avoid confusion we modified our text as suggested. Essentially reply B8 illustrated what we would like to express.

Changes in text:

Abstract – "Improvement in 1-hour CCI (CCI HLA selected platelets – CCI random donor platelets) post-HLA-selected platelet transfusions in patients who had no PTR (1-hour CCIs ≥7,500 $m^2/\mu L$ after random donor platelet transfusions) was 13,316 $m^2/\mu L$ (95% CI: 12,326 – 14,306) less than those with PTR"

Results – "At the time of requesting for HLA-selected platelets, PTR was not substantiated (1-hour CCIs ≥7,500 $m^2/\mu L$ post-random donor platelet transfusions) in 13 patients, whom involved 55 donor-recipient pairs. The mean difference in 1-hour CCIs after HLA-selected and random donor platelet transfusions of these patients was 2,204 ± 5,481 $m^2/\mu L$, which was 11,134 $m^2/\mu L$ (95% CI: 6,122 – 16,135) less than the remaining 364 transfusions given to 129 patients with PTR."

Discussion – First, not all the requests for HLA-selected platelets were justified. As previously discussed, PTR was not established in a significant minority of patients and our data showed that these patients did not benefit from transfusion of HLA-selected platelets.

10. In P9, L19-20 and P9, L23-28, it seems that authors are referring to the same parameter (mean change in 1-hour CCI), so consider using the same description when rephrasing P9, L23-28.

Reply B10: We used mean change in 1-hour CCI when comparing the response to patients with vs. without PTR. We reserved 1-hour CCI after HLA-selected platelet transfusion to assess clinical response in different patient groups, including gender, ABO compatibility and presence of anti-HLA antibodies.

11. In P9, L4-5, it would be interesting to add the "mean change in 1-hour CCI" (as described in P8, L25), for HLA-I antibody-positive and -negative populations.

Reply B11: Mean change in 1-hour CCI in patients who were anti-HLA Ab +ve was 18841±9844 vs 8291±9598. The former was significantly higher 10550 (95% CI 2375 – 14722). We did not report in the manuscript because the results were similar to those of the 1-hour CCI post HLA-selected platelets.

12. In Figure 2, is the presented CCI of random PC the value immediately prior to changing to HLA-selected PC? Please describe.
Reply B12: The CCI after random donor platelet was submitted along with the request for HLA-selected platelets (See Page 6, Line 19-21). HLA-selected platelets would be issued to patients afterwards when available.

13. In Figure 2, it seems only one point CCI is shown for random PC, whereas various points are shown for HLA-selected PC. How does it affect the results? Please show data of patients with CCI-1h>7,500 and those with CCI-1h<7,500 after the random PC transfusion in separate graphs.
Reply B13: Each patient only had one CCI with random donor platelets submitted to BTS but they could receive HLA-selected platelets from more than one donor, so each point on the left bar could join to multiple points on the right bar on the graph. This could induce within-subject correlation when we analysed the CCIs with HLA-selected platelets. We therefore used mixed linear models and allowed a random intercept per patient as adjustment when studying the factors affecting CCIs (See Page 7 Line 27-28).
We separated results of patients with and without PTR with random donor platelets in the updated Figure 1 (Figure 2 of old version) as suggested (See Page 15).

14. In Figure 3B, remove data from those patients with CCI-1h>7,500 with random PC transfusion, or show data in different graphs.
Reply B14: We removed patients who didn't not have PTR when analyzing the factors affecting CCI after HLA-selected platelets as suggested (Page 7 Line 24-26 for method, Page 16 for the graphs).
Change in text: We only selected patients who had PTR to random donor platelets to study the effect of ABO blood group compatibility, patient's sex and the presence of anti-HLA class I antibodies to 1-hour CCI after HLA-selected platelet transfusion.

Minor points
15. In the Introduction, it is necessary to describe that alloantibodies to human platelet antigens (HPA), in addition to HLA, are also responsible for immune PTR.
Reply B15: We modified as recommended (Page 5 Line 12).
Change in text: With repeated transfusions of blood components or multiple pregnancies, patients may be alloimmunized to class I human leukocyte antigens (HLA) or human platelet antigens (HPA) expressed on platelets, resulting in immune-mediated PTR.

16. The description "4 loci" (Abstract, L17 and L29) is not appropriate. They are matched for 2 loci, namely HLA-A and -B. It can be described as "4 out of 4" as in the rest of the text.
Reply 16: We changed to "4 out of 4" as recommended (Abstract, L17 and L28)

17. P6, L15-16: is the CCI formula correct? Is “x1,000” necessary? Please check
Reply 17: We change the unit in the calculation and omit x1000 to avoid confusion (Page 6, Line 14)

18. In Figure 3A, the number of patients is described as “419”, whereas in the text (P8, L30-34), it is counted as “405”. Please check.
Reply 18: We amended the values and now they aligned (Page 9 Line 1-2) and (Page 16 Line 7)

Reviewer C

This article provides data on the effectivity of HLA-matched transfusions. A topic that is written about only very little, so any further understanding would be highly interesting. The article does contain some big limitations in their analyses, which impair interpretation and may even lead to misinterpretation. If these can be addressed properly, the manuscript may be of high interest to the audience of the journal.

Major concerns:
1) The amount of clinical information, including Table 1, of the included population is rather limited. In the first paragraph of the results for example, p8. line 8/9: was this before or during/after enrollment and isn’t this inherent with the study definition? It would be required to describe the included patient (a bit) better to help interpret the data. Figure 3B for example: patients with detectable antibodies do benefit more from HLA-matched transfusion, as expected, as non-immune causes of refractoriness, which are more likely at play in patients without detectable antibodies. More clinical information would be desired to confirm this assumption. The causes (or suspicions) for refractoriness should minimally be included as this study seem to include non-immune refractoriness and immune-refractoriness, which surely will affect study outcome. Figure 2 suggest random donor platelet transfusion data is available (also see remark regarding p8. line 8/9 of the results section), but how much transfusion were random is unknown. If and how much RBC unit were provided and/or potential causes of HLA alloimmunization would also be of relevance when interpreting the results.
Reply C1: We recognized the inherent limitations of the study design. On Page 8 Line 8 we added CCI with random donor platelet transfusion “upon enrolment to the study” to make it clearer. Possible non-immune causes of PTR (i.e. splenomegaly, drugs, infection, etc) were not required when clinicians requested for HLA-selected platelets and therefore such information was not available. The potential HLA sensitizing events (e.g. transfusion/ pregnancy history) were also not available. In the revised manuscript we tried to add more clinical information available. For example, demographics of male and female patients were separately presented in the revised Table 1. The results of anti-HPA antibody testing were

also included (Page 9 Line 12-14).

2) In p9 patients were divided in antibody positive and negative, while actually this discrimination is notoriously difficult and the last decade topic of discussion. Could the authors elaborate what antibody testing they have used? And how the boundary of positivity was defined? Interpretation of some of the figures is hampered as these include both nonimmune- and immune-mediated refractoriness, which will greatly affect interpretation.
Figure 2 for example (informative way of representation) shows that some patients benefit from HLA-matched products while others don't; Could the authors elaborate on this; would these be the patients turning out to be antibody negative? If so, providing that information in figure 2 would improve interpretations.
Furthermore including some more details helps interpretation: when were the random transfusion provided relative to diagnosing the refractoriness and/or HLA-matched transfusion. As the cause for alloimmunization between males and females may be completely different, with the latter frequently having much higher antibody levels, a sensitivity analysis comparing efficacy of HLA-matched platelets between these may be interesting, especially when presented in the context of Fig 3B.
Reply C2: The platelet serology results were submitted by the respective referring unit. We summarized the methods of different centres (Page 6 Line 22-26) and this limitation was highlighted in the discussion (Page 12 Line 22-24).
We illustrated how the predictors affected CCI in Figure 2 of the revised manuscript (Figure 1 in the original version). For example, in Figure 3C patients with anti-HLA antibody detected had higher CCI post-HLA selected platelet transfusions.
We analysed the CCI after HLA-selected platelet transfusion of male and female patients as suggested (Page 8 Line 32-33 and Page 16 Figure 2B. We found that female patients responded better than male patients to HLA-selected platelets. This is likely due to the higher chance of immune PTR in female patients, and this has been discussed in the revised manuscript (Page 11 Line 2-4).

3) The authors show that HLA-matched platelets perform better as compared to random. In our routine, random platelets are shelfed and provided with a first-in-first-out, while HLA-matched transfusion are on demand, thus not stored, and thereby more fresh. Fresh PLTs perform better as stored; could the authors rule out this effect? Please include this information in the manuscript.
Reply C3: This is included as a limitation in the discussion section because it could bias the comparison as mentioned by the reviewer (Page 12 Line 19-21).

Other concerns/remarks:
a) The authors describe 4/4 matching was successful in a remarkable high number of patients does the level of matching affect CCI? Including this analysis would be highly informative for centers that are not able to match HLA 4/4.

Reply C4: As 4/4 were provided exclusively in the study cohort and made us not able to study the relationship between matching and CCI.

b) The discussion is rather shallow and will benefit greatly from more citations and in-depth discussion (e.g. remark 3 should've been discussed).
Figure 3A: the authors show no effect of ABO-matching, while there are numerous reports showing it does. Two of which are cited, but can the authors also discuss and/or explain what causes this discrepancy? Is there a cause for antibody-negative that may be more likely to receive ABO-compatible (as they may not require HLA-matched platelets as often)?
Reply C5: When platelet products were HLA-selected, we could not reproduce the results of higher CCI with ABO compatible platelets. This is also observed in other studies (See Page 11, Line 13-16).
The discussion in the revised manuscript was enriched by including the other possible immune related PTR resulting in suboptimal response to HLA-selected platelets (Page 11 Line 18-26), the current issue of the HLA selected platelet service (Page 11 Line 28 – Page 12 Line 6) as well as the alternatives to HLA selected platelets to support patients with PTR (Page 12 Line 8-17).
Change in text: "It has been reported previously that ABO compatibility might not affect platelet increment if the unit is crossmatch compatible(10). The impact of ABO compatibility in the setting of HLA-selected platelet transfusion warrants further study."

c) The period in which this study is performed is rather long. The authors state "Over the years, increasing number of apheresis platelets were collected and accounted for 35% of adult dose equivalent in year 2020." Are there more things changed over time, such as the isolation of PLTs from the buffy coat, storage media or conditions, HLA-matching etc ? It would be of relevance if the authors elaborate more.
Reply C6: There were no change in the collection/ storage method that would affect the quality and quantity of the platelet products (See Page 6 Line 9-11).
Change in text: There was no change in the method of collection, processing and storage of platelet units that would affect the quantity of platelets per unit and the quality of the product during the study period.

d) The authors describe their HLA matching strategy, but were obtained platelets additionally tested using cross-matching? And were HLA alleles matched merely on sequence level or were Duquesnay' HLA eplets been taking into account?
Reply C7: Only HLA allele matching was considered. The alternative matching method to improve the product availability was discussed (See Page 12 Line 11-16).
Change in text: To increase the availability of products to support alloimmunized patients, crossmatch compatible platelets or antigen negative units could be considered in these patients. Recently, platelet products selected by eplet-based

approach were shown to be non-inferior to standard antigen selected method. This approach might further benefit highly sensitized patients by identifying more units suitable for transfusions.

e) Figure 1: Could the authors include a similar graph with e.g. random transfusion in random patients? Otherwise partially HLA-matched transfusions, or HLA-matched in refractory vs non-refractory patients or something to help create a reference for the provided figure?

Reply C8: The original waterfall plot in Figure 1 was deleted and the information is now presented in Figure 1 of the revised manuscript. We compared the changes in CCI with random and HLA-selected platelets in patients who were refractory and not refractory to random donor platelet transfusion (Page 15 Figure 1).

f) The period in which this study is performed is rather long. The authors state "Over the years, increasing number of apheresis platelets were collected and accounted for 35% of adult dose equivalent in year 2020." Are there more things changed over time, such as the isolation of PLTs from the buffy coat, storage media or conditions, HLA-matching etc ? It would be of relevance if the authors elaborate more.

Same as reply C6