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Replies to Reviewer A

Comments from Reviewer A

Comment A-1:

The manuscript by Hagino et al gives a nice review of platelet refractoriness with its immune and non-immune causes covered. I think that the title of the manuscript is misleading as it seems that immune and non-immune factors are given equal treatment. Also, incidence is fully covered but management is barely touched upon. For these reasons my first recommendation is to change the title of the manuscript to simply 'Platelet refractoriness: incidence and management. Later I will recommend additional details to strengthen the management section.

Reply A-1: Thank you for the comment. The topic that was initially attributed to us was "non-immune PTR". We referenced articles related to immune PTR in our manuscript because immune and non-immune PTR are overlapping topics. The title was changed to "Incidence and management of non-immune platelet transfusion refractoriness: A narrative review", as recommended by the reviewer. But we could not enter into details of immune PTR, because from our understanding, it will be the topic of another paper in the same issue of the Journal.

Changes in the text:
Page 4 / Lines 52-53
In this article, incidence and management of non-immune PTR are described by referring to existing related literature and our clinical experience.

Page 6 / Lines 87-88
In this article, we describe the practical approaches for the diagnosis and the management of non-immune PTR, by referring to existing related literature and our clinical experience.

Comment A-2:

Abstract:

Comment A-2-1:

1. The authors state that PTR occurs in 30-70% of patients receiving frequent platelet transfusions. Please provide a reference

Reply A-2-1: The references were provided in the introduction section of the original manuscript (Page 5 / Line 72 in the revised manuscript), but not added to the Abstract.

Comment A-2-2:

2. The authors reference anti-platelet antibodies in the abstract and also later in the manuscript. In all cases I think there should be a distinction between anti-platelet antibodies that are specific for platelet antigens and anti-HLA antibodies. I know that HLA is on the surface of platelets, but it is present on nearly every other cell. The convention in naming is to separate platelet antibodies from HLA antibodies.

Reply A-2-2: Corrections were made in the Abstract and the main text, as below.

Changes in the text:
Page 4 / Lines 46-48
When PTR is suspected in patients receiving multiple platelet transfusions, potential causes of non-immune PTR should be promptly investigated prior to assessing alloimmunization profiles including anti-human leukocyte antigen (HLA) antibodies and platelet-specific alloantibodies.

Page 6 / Lines 78-80
Among them, anti-human leukocyte antigen (HLA) antibodies are detected in 80-90% of cases, and antibodies to platelet-specific antigens including human platelet antigens (HPAs) and CD36 isoantigen or isoagglutinin to ABO antigens are the causative in the remaining cases.

Page 20 / Lines 354-355
In case immune PTR is suspected, screening for anti-HLA antibodies is indicated, and if confirmed, transfusion of HLA-matched PC is indicated.

Comment A-2-3:
3. As part of the #2 above, one usually begins screening for anti-HLA antibodies when immune-mediated refractoriness is suspected. As the authors note later, only a small percentage of cases is caused by antibodies specific for platelet antigens.

Reply A-2-3: The information was added to the text, as below.

Changes in the text:
Page 6 / Lines 78-80
Among them, anti-human leukocyte antigen (HLA) antibodies are detected in 80-90% of cases, and antibodies to platelet-specific antigens including human platelet antigens (HPAs) and CD36 isoantigen or isoagglutinin to ABO antigens are the causative in the remaining cases.

Comment A-3:
Introduction:
Comment A-3-1:
1. On the fifth line there is the clause 'Besides them…'. This is not good English usage, particularly in the formal context of a scientific paper.

Reply A-3-1: The sentence was revised as below.

Changes in the text:
Page 5 / Lines 67-68
Moreover, 30 to 40% of cases with platelet transfusions can show inadequate platelet count increment due to various causes (10, 11).

Comment A-3-2:

2. In line seven the author lump hematologic and solid cancers together. I believe most cases of platelet refractoriness is seen with hematologic cancers with solid organ being the exception. If the authors prefer to leave these terms together please add a reference to back up this statement.

Reply A-3-2: The term "solid" was deleted.

Changes in the text:
Page 4 / Lines 39-41
When the post-transfusion platelet count is lower than expected, platelet transfusion refractoriness (PTR) is suspected, which is an important issue especially in patients requiring frequent platelet transfusions such as hematological patients.

Page 5 / Lines 70-71
Typically, PTR can develop in patients with hematological cancers, who usually require repeated platelet transfusions (13-17).

Comment A-3-3:

3. The information about CD36 isoantigen should be referenced separately.

Reply A-3-3: References related to CD36 isoantigen were added to the text, as below.

Changes in the text:
Pages 17-18 / Lines 313-316
Since about 80-90% of immune PTR cases are due to anti-HLA antibodies (11), screening for anti-HLA antibodies is indicated, concomitantly with testing for anti-HPA antibodies. Anti-CD36 isoantibodies, which are found in a higher frequency among Asian and African populations (110), may also cause PTR, so it should be considered when other antibodies are not identified.

Comment A-4:

Diagnosis:

Comment A-4-1:

1. Predicted platelet increase: Please include what the expected increment should be in a patient without platelet refractoriness.

Reply A-4-1: It was added as below.

Changes in the text:
Page 7 / Lines 104-107
Following a platelet transfusion, platelet count can rise with a peak at 10 minutes to one hour and show a gradual decline over 72 hours. Typical dosing of platelet transfusion for an adult is a pool of 6 whole blood derived platelets or one apheresis platelet, by which the platelet count

increment 24-hour post-transfusion in a 70 kg patient is clinically expected to be $30x10^9$/L to $60x10^9$/L.

Comment A-4-2:

2. The authors reference the number of platelets in 1, 5, 10, 15 and 20 units. I think giving an example of just 1 and 5 or 1 and 10 units is sufficient.

Reply A-4-2: It was revised as below.

Changes in the text:

Page 8 / Lines 114-117

In Japan, all PCs transfused are leukoreduced single-donor apheresis-derived, and supplied by the Japanese Red Cross Society (JRCS). In the clinical practice, a 10-unit PC bag (volume of about 200 mL), containing about 2.0×10^{11} platelets, is commonly used, which gives a predicted platelet count increase of about $30x10^9$/L in a patient with a body weight of 70 kg (25, 26).

Comment A-4-3:

3. The authors note that a 1h CCI <7500 indicates an immune based PTR while >7500 indicates non-immune based. I have been convinced that this dogma should be laid to rest based on the following references:

Bishop JF, Matthews JP, Yuen K, McGrath K, Wolf MM, Szer J. The definition of refractoriness to platelet transfusions. Transfus Med 1992;2: 35-41.

Slichter SJ, Davis K, Enright H, Braine H, Gernsheimer T, Kao KJ, Kickler T, Lee E, McFarland J, McCullough J, Rodey G, Schiffer CA, Woodson R. Factors affecting posttransfusion platelet increments, platelet refractoriness, and platelet transfusion intervals in thrombocytopenic patients. Blood 2005;105: 4106-14.

Doughty HA, Murphy MF, Metcalfe P, Rohatiner AZ, Lister TA, Waters AH. Relative importance of immune and non-immune causes of platelet refractoriness. Vox Sang 1994;66: 200-5.

McFarland JG, Anderson AJ, Slichter SJ. Factors influencing the transfusion response to HLA-selected apheresis donor platelets in patients refractory to random platelet concentrates. Br J Haematol 1989;73: 380-6.

Daly PA, Schiffer CA, Aisner J, Wiernik PH. Platelet transfusion therapy. One-hour posttransfusion increments are valuable in predicting the need for HLA-matched preparations. JAMA 1980;243: 435-8.

If a patient is rapidly bleeding or has aggressive DIC then platelets will be consumed rapidly and the CCI at 1h will probably be <7500. Hypersplenism can also cause a significant reduction in the 1h CCI without an immune based reason. The Legler paper referenced uses

a 16-24 h post-platelet count. Please correct this idea in the text and the figure.

Reply A-4-3: The four references were added to the text, as below. And the possibility that PTR cases with rapid bleeding, aggressive DIC or hypersplenism may show low CCI-1h was added to the respective sections, as below.

Changes in the text:
Page 8 / Lines 128-129
CCI-24h higher than 4,500 indicates normal platelet survival *in vivo*, while CCI-1h higher than 7,500 indicates normal platelet recovery *in vivo* (5, 11, 17, 27-29)

Page 11 / Lines 180-181
Of note, aggressive DIC can result in low CCI-1h, resembling immune PTR.

Page 12 / Lines 198-199
It should be noted that hypersplenism can also result in low CCI-1h, resembling immune PTR.

Page 12 / Lines 205-206
Severely active bleeding can also result in low CCI-1h, resembling immune PTR.

Comment A-5:
3-1 Fever
P8 please correct 'causes' to 'cause'
P8 please remove the 'of' from '...most of febrile patients had significantly...'

In general, the authors give many examples of fever and PTR but I would be interested in seeing a comment that distinguishes an association between fever and PTR vs a causal relationship. Does fever somehow cause PTR or do many patients with PTR also have a fever?

Reply A-5: The typo was revised. The sentences related to fever were revised as below.

Changes in the text:
Page 9 / Lines 151-153
Since fever can be secondary to various conditions, such as infection/sepsis, DIC, drug allergy, and HSCT, it is still unclear whether fever is an independent cause of poor response to platelet transfusion (12, 36).

Page 10 / Lines 167-172
In patients with hematological malignancies, the combination of fever, infection and antibiotic therapy has been reported to be the most common cause of PTR (11), but the precise mechanism of this interaction is still unclear (33). In our experience of 224 platelet transfusions in 13 hematological patients with non-immune PTR, the CCI-1h of those who developed post-transfusion fever was comparable to that of patients without fever pre- and post-transfusion, which also suggests uncertain relevance of transfusion-related fever to platelet recovery (31).

Comment A-6:

3-2 DIC

The section on DIC ends by noting that the treatment of the underlying disease is necessary. Since several other diseases are mentioned, please specifically note that resolution of DIC may resolve PTR if that is the only cause for refractoriness.

Reply A-6: According to the comment, some sentences were added in the main text, as below.

Changes in the text:

Page 11 / Lines 176-183

Despite lack of evidence, platelet transfusion is indicated in DIC patients with serious bleeding or those with a platelet count below $50x10^9$/L who need urgent/emergent surgery (51). These patients can be refractory to platelet transfusion mainly due to the increased platelet consumption (11, 29, 33, 52-55). The bleeding tendency is more severe or apparent in patients with leukemia, solid cancer, obstetric disease or severe infection (56). Of note, aggressive DIC can result in low CCI-1h, resembling immune PTR. Since treatment of the underlying cause is the major principle in DIC management, it is anticipated that proper DIC management can alleviate the related platelet consumption and PTR.

Comment A-7:

3-4 Bleeding

As mentioned earlier in this review, a rapidly bleeding patient will consume platelets and should cause a reduced 1h CCI.

The authors reference McFarland et al. (ref #41) in this section to state that bleeding is not an important cause of reduced CCI. However, the McFarland reference found that bleeding did not contribute in a multivariate model that included immune and non-immune causes. When bleeding was considered separately the found:

If transfusion data were analysed according to the presence or absence of certain clinical factors that might adversely affect the transfusion response (infection, fever, bleeding, splenomegaly, or sepsis), there were significantly better responses to HLA well-matched transfusions and improved predictability of the platelet crossmatch tests among patients lacking these factors compared to those with the factors. Not surprisingly. most lymphocytotoxicity-negative patients demonstrated one or more of these clinical factors (P< 0.0001, Fig 4) suggesting that nonimmune mechanisms explained their poor responses to both random-donor as well as the HLA-matched transfusions. Neither bleeding, positive lymphocytotoxic antibody tests against randomdonor lym- phocytes. nor ABO incompatibility between platelet donor and recipient affected the transfusion outcome as evaluated in the multiple regression analysis.

Please make this more nuanced distinction in the manuscript.

Reply A-7: The related sentences were revised as below.

Changes in the text:
Page 12 / Lines 202-207
Clinical bleeding is often listed as a non-immune cause of PTR (62, 63), but it is thought that bleeding itself is a consequence rather than a cause of reduced survival of platelets (22). Actually, in patients who were refractory to pooled random-donor platelet transfusions, fever and splenomegaly, but not bleeding, were found to correlate with the reduced CCI-1h after HLA-matched platelet transfusion (29). Severely active bleeding can also result in low CCI-1h, resembling immune PTR. Therefore, it is important for clinicians to recognize PTR as a sensitive clinical marker for the occurrence of bleeding and impaired patient survival (22).

Comment A-8:
3-5 Drugs
A short list of drugs that are associated with PTR would be a helpful addition. Please see Cohn et al. Platelet transfusion refractoriness: how do I diagnose and manage?

Cohn CS. Hematology Am Soc Hematol Educ Program. 2020 Dec 4;2020(1):527-532. PMID: 33275694

Reply A-8: The list of drugs associated with PTR is already available in Table 1. Please let us know if the Table should be revised. Table legend was revised with the reference included, as below.

Changes in the text:
Page 26 / Lines 613-614
Table legend
Table 1. Causative agents implicated in drug-induced thrombocytopenia (70, 111, 113-116).

Comment A-9:
3-7
The authors state that "…longer storage period of PC 'seems to be' associated with a lower CCI. I think this has been shown often enough that the authors can substitute the stronger phrase:
"…longer storage period of PC is associated with…"

Reply A-9: It was revised as below.

Changes in the text:
Pages 13-14 / Lines 233-234
In hematological patients, longer storage period (up to 5 to 7 days) of PC is associated with a lower CCI compared to shorter storage period (less than 3 days) (82,83).

Comment A-10:

3-8

Under others, please add a sentence or two regarding the data showing that pathogen reduced platelets are associated with increased refractoriness.

Reply A-10: The association between pathogen inactivation and PTR was described in the "Factors related to PC products" section, as below.

Page 13 / Lines 231-232

Recently, pathogen-reduction/inactivation (PI) treatment of PC has also been shown to affect CCI (81).

Changes in the text:

Page 14 / Lines 247-252

PI treatment of donor platelets can negatively affect platelet recovery of recipients. Patients transfused with PI-platelets can have lower CCI-1h and CCI-24h than those with standard platelets, with a relative risk of platelet refractoriness of about 2.7 (91). Therefore, patients receiving PI-platelets require more frequent platelet transfusions than those with standard platelets. Despite the potential benefit of PI treatment in preventing post-transfusion GVHD, it has been recently reported that PI-platelets have no impact on preventing HLA alloimmunization (92).

Comment A-11:

Management

Comment A-11-1:

Please add a reference for TTP and platelet use.

Reply A-11-1: A reference for TTP and platelet use was added and changes were made in the text, as below.

Changes in the text:

Page 17 / Lines 298-301

A large-scale retrospective study by Goel et al. showed that platelet transfusions are associated with higher odds of arterial thrombosis and mortality among patients with TTP and HIT (108). Therefore, clinicians should keep in mind that platelet transfusion has been identified as a potential exacerbator of TTP and HIT.

Comment A-11-2:

3). Select the best treatment:

The authors do not mention the potential difficulty finding an HLA-matched unit and the strategy of using the antibody specificity prediction (ASP) method to select antigen-negative units to avoid the HLA antibodies in the patient. Please add a line or two about the relative advantages of using ASP vs HLA-matched units.

From McFarland et al ref 41 in discussion of results:

If transfusion data were analysed according to the presence or absence of certain clinical factors that might adversely affect the transfusion response (infection, fever, bleeding, splenomegaly, or sepsis), there were significantly better responses to HLA well-matched transfusions and improved predictability of the platelet crossmatch tests among patients lacking these factors compared to those with the factors. Not surprisingly. most lymphocytotoxicity-negative patients demonstrated one or more of these clinical factors (P< 0.0001, Fig 4) suggesting that nonimmune mechanisms explained their poor responses to both random-donor as well as the HLA-matched transfusions. Neither bleeding, positive lymphocytotoxic antibody tests against randomdonor lym- phocytes. nor ABO incompatibility between platelet donor and recipient affected the transfusion outcome as evaluated in the multiple regression analysis.

Reply A-11-2: The descriptions on the difficulty in finding an HLA-matched unit and the relative advantages of using ASP over HLA-matched units were added to the text, as below.

Changes in the text:
Page 18 / Lines 317-321
Since HLA matching of platelets requires recruitment of compatible donors, testing of HLA/HPA antigens/antibodies and crossmatching, HLA-matched platelets are usually expensive and not always available in many countries. Thus, antibody specificity prediction method is applied in some countries as a reasonable way to find “HLA-compatible” platelets. (38, 111) (See the review article on immune PTR in this issue of Annals of Blood.)

Replies to Reviewer B

Comments from Reviewer B

Comment B-1:

1. *The relationship between platelet count and risk of bleeding. This has been analyzed in multiple trials, for example PLADO and TOPPS. Please see reports of recurrent risk analysis.*

Reply B-1: The information regarding the relationship between platelet count and risk of bleeding were added in the introduction section, as below.

Changes in the text:
Page 5 / Lines 55-63
Platelet transfusions are indicated therapeutically for trauma and surgical patients, or prophylactically for patients with thrombocytopenia or platelet dysfunction. Although bleeding is a concern in patients with severe thrombocytopenia, the correlation between platelet count and bleeding risk is unclear. Hence, bleeding risk should be clinically assessed by underlying diseases, prior bleeding episodes, presence of purpura and so on (1). In invasive procedures such as surgery, prophylactic transfusion is used to maintain a platelet count of above $50x10^9$/L until hemostasis is ensured (2, 3). For spinal fluid testing, the British Committee for Standards in Haematology recommends a platelet count of above $50x10^9$/L (4) and the American Society of Clinical Oncology recommends a platelet count of above $20x10^9$/L (5). These thresholds should be used as guides, and it is important to prioritize clinical judgment based on individual patient and disease factors.

Comment B-2:

2. *How important is the platelet count itself, which says nothing about platelet function and the need for platelet transfusion? Do we need to treat all patients with poor increments? Is there a difference between adults and children?*

Reply B-2: If a patient is an adult or a child, we think that bleeding risk should be clinically assessed by underlying diseases, prior bleeding episodes, presence of purpura and so on. As for the change in the text, please see the reply B-1.

Comment B-3:

3. *What exactly is DIC - please expand*

Reply B-3: The description of DIC was added as below.

Changes in the text:
Page 11 / Lines 175-183
DIC is characterized by systemic activation of blood coagulation and increased platelet consumption, which results in life-threatening hemorrhage (50). Despite lack of evidence, platelet transfusion is indicated in DIC patients with serious bleeding or those with a platelet count below $50x10^9$/L who need urgent/emergent surgery (51). These patients can be

refractory to platelet transfusion mainly due to the increased platelet consumption (11, 29, 33, 52-55). The bleeding tendency is more severe or apparent in patients with leukemia, solid cancer, obstetric disease or severe infection (56). Of note, aggressive DIC can result in low CCI-1h, resembling immune PTR. Since treatment of the underlying cause is the major principle in DIC management, it is anticipated that proper DIC management can alleviate the related platelet consumption and PTR.

Comment B-4:

4. *Is there a role for tranexamic acid in patients with non-immune platelet refractoriness?*

Reply B-4: The information regarding tranexamic acid in non-immune PTR was added in the Management section, as below.

Changes in the text:
Page 18 / Lines 322-326
Tranexamic acid, a fibrinolytic inhibitor, has been shown to prevent bleeding and reduce the need of blood transfusion, including PC, in surgical patients, but its role as an alternative to platelet transfusion in PTR cases remains to be investigated (112). The TREATT trial is underway to assess the safety and efficacy of using prophylactic tranexamic acid during a period of intensive chemotherapy and associated thrombocytopenia in hematological patients (113).

Comment B-5:

5. *Is there more variability between platelet donations - for example platelet responsiveness - that is relevant*

Reply B-5: The related information was added in the “Others” section, as below.

Changes in the text:
Page 15 / Lines 260-262
Studies have shown that pooled whole blood-derived PC (WB-PC) and single-donor apheresis PC have similar rates of alloimmunization (20), but that single-donor apheresis PC shows significantly higher 1h-CCI and 18 to 24-h CCI compared to WB-PC (94).

Comment B-6:

6. *What about drip feeds of small volume platelet transfusions, that have sometimes been advocated?*

Reply B-6: The related information was added in the “Others” section, as below.

Changes in the text:
Page 15 / Lines 263-266
Volume-reduced washed platelets may be beneficial for PTR cases because equal volumes can increase platelet count more efficiently than standard platelets (95). Despite the other

advantages of shortened transfusion times and reduced volume loads (96), volume reduction can lead to spontaneous activation and aggregability of platelets (95, 97).

Replies to Reviewer C

Comments from Reviewer C

This article reviews the main causes of non-immune platelet transfusion refractoriness. It is a very interesting issue since little can be done in this situation. However, the article is too superficial, and does not provide data of interest. Authors explain the review is based on their experience but they do not provide data. The approaches for overcoming the non-immune causes of platelet refractoriness are impossible to carry out in many cases.

Reply to Reviewer C: Thank you for the comment. As the reviewer stated, the non-immune PTR is a multifactorial condition where there is little to be done, and no clear algorithm for the management exist, such as for immune PTR. For this reason, we mainly searched the literature and added the few data available in our institution, such as the evaluation of the association between fever and PTR (TAS, in Press), and described the strategy for the appropriate diagnosis and management of the condition. Although the information is not consistent enough, dependent on the lack of data, we hope this can help physicians understand on the difference between immune and non-immune PTR, and on the need to discard non-immune etiologies prior to considering investigation of immune causes.