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This paper reviews the three pathogen inactivation methods for platelet concentrates. There have been other reviews and meta analyses before, but the author has experience with the methods, and the clinical aspect gives it a fresh insight in what it means to actually use pathogen inactivated platelet concentrates. Overall, the paper is well written, is fairly complete (but see my specific comments), and is easy to read.

First of all, I would like to thank the reviewer for taking the time to revise the manuscript and giving me the opportunity to include the necessary modifications to improve the text. All modifications are written in blue.

Specific comments

Comment 1: Page 5, from 5 to 7 days: this is also achievable with other ways of containing bacterial growth, such as BactAlert screening, or post storage dipstick methods (Verax). The statement as it now is, is not untrue, but there are other methods to extend the storage time, and save on outdating

Reply 1:

Certainly, there are other methods to increase the storage time of platelets (PLTs) from 5 to 7 days, such as the culture-based screening tests corresponding to the Pall eBDS and the bioMérieux BacT/ALERT tests and the rapid-detection methods using point-of-issue tests (Verax Pan Genera Detection). However, as I indicate in the introduction (page 5):

"Despite increasingly strict donor selection criteria, advances in laboratory testing and procedures for preventing bacterial contamination, such as donor skin disinfection, diversion of the first milliliters of collected blood and bacterial culture, a small risk of bacterial (1,2), viral (3,4) and parasite (5-9) contamination of platelet (PLT) concentrates still remains", these methods are associated with a small, but real residual risk of bacterial contamination, as referenced in the cited bibliography

(references 1 and 2).

Indeed, the main benefit of PRT is not the extension of PLT storage time, but rather the improvement of PLT safety. These technologies are superior to procedures for detecting bacterial contamination since they can prevent the replication of viruses and parasites as well as bacteria.

Additionally, our research group has previously compared bacterial contamination methods with PRT in a paper published in Transfusion 2014; 54: 158-68 (Girona-Llobera E, Jimenez-Marco T, Galmes-Trueba A, et al. Reducing the financial impact of pathogen inactivation technology for platelet components: our experience.). This manuscript explains in detail the advantages and disadvantages of both methods and their implications on PLT outdating management. This paper is included in the references of the current manuscript in the part describing *reduction in PLT outdating* (reference 20, page 5) for readers to consult if they wish.

Changes in the text:

In order to make this point clearer, the text of the introduction has been slightly modified (page 5): "*Pathogen reduction technology (PRT) for PLTs has the potential to prevent pathogen transmission from donor to patient during PLT transfusion (14). In addition to improving blood safety by preventing the replication of bacteria, viruses and parasites, PRT has other benefits: 1) elimination of the risk of transfusion-transmitted graft-versus-host disease by substituting gamma irradiation with white blood cell inactivation (15); 2) the potential reduction of alloimmunization, which has been described in animal models (16) but still needs to be confirmed by evidence from clinical practice (17); 3) fewer PLT transfusion reactions (18,19); and 4) the improvement of PLT supply by extending the storage time from 5 to 7 days, which results in a substantial reduction in PLT outdating (20, 21)*".

Comment 2: Page 8, possibly weakened response of treated platelets: if the storage time is 7 days, only a small fraction will actually be 7 days old when transfused, so I am not so sure you should focus on that small cohort of platelets. Also, Lozano [Br J Haematol 2011] showed that the PRT platelets had a lower CCI, but this was within their non-inferiority margin, with few to no other untoward effects (bleeding, red cell usage, time to next transfusion). The statement that clinical trials are needed is maybe of scientific interest, but not of practical interest. Ideally, all platelets are transfused

fresh, but that is at a high cost with still a fairly large risk of having no units available, and so compromises need to be made, even if that means that some units at the end of storage have a less than optimal quality. Nonetheless, even these platelets have some function, and that should be considered in the larger picture of providing supportive care.

Reply 2:

Regarding the conclusion of our study (page 8) on the metabolic activity and hemostatic function of Mirasol-PRT-PLTs in which we suggest that further investigations are needed to ascertain if the *in vitro* results of PRT-treated PLTs at the end of the storage time (day 7) correlate with *in vivo* PRT-treated PLT performance, we encourage the readers interested in this study to consult an article in Blood Transfus. 2020;18:280-289 (reference 37). It should also be noted that our *in vitro* study was based on Mirasol PRT-treated PLTs, and Lozano *et al* [Br J Haematol 2011, reference 56 in the manuscript] were investigating the efficacy and safety of Intercept PRT-treated PLTs. As described in the article, different PRT systems have different impacts on PLT functionality, so the results of a study on one PRT method may not necessarily be extrapolatable to another PRT method.

In addition to that, as the reviewer has kindly suggested, although Lozano, *et al* showed that the 1hr CCI was lower for PRT-treated PLTs, albeit not significantly, as it was within the 30% non-inferiority margin, these authors also described that 24-hr CI (P =0.01) and 24-hr CCI (P =0.003) were significant lower for PRT-treated PLTs than the respective values for the control group. Therefore, it is expected that the clinicians, in the routine clinical practice, having not obtained the desired PLT increase after 24 hr post-transfusion, will increase the PLT transfusion frequency in patients with thrombocytopenia who received PLT prophylactically in order to prevent bleeding. This has been shown by other authors describing lower post-transfusion PLT increments after transfusing PRT-treated PLTs (51, 52, 55, 56- 60) and an increase of PLT support or transfusion frequency (52,58,60), and even the occurrence of PLT refractoriness (52, 57). In this regard, please see Table I based on the references from 51-60.

Changes in Table 1:

In order to clarify this point and answer other questions about clinical studies investigating the efficacy and safety of PRT-treated PLTs, I have included more information in Table I (page 14-20). All the additional information is based on a detailed review of the literature (references 51 to 60).

Comment 3: Page 9, implied a higher transfusion frequency: I wonder if that statement is true. As summarized by Rebulla [Transfusion 2020], there is some increase in the transfusion interval (by ~0.5 day), but overall the difference is rather small, at least in my opinion.

Reply 3:

The sentence (page 9), "Consequently, to achieve the necessary PLT increment, the use of PRT-treated PLTs implied a higher transfusion frequency", is based on the studies cited in the references (52,58,60) and the conclusion of the meta-analysis reported by Estcourt *et al.* (reference 47) in which the authors describe that they "found high-quality evidence that pathogen-reduced platelet transfusions increase the risk of platelet refractoriness and the platelet transfusion requirement."

This meta-analysis is mentioned in the following paragraph, summarizing the authors' conclusions in the Cochrane Library: "A meta-analysis of RCTs that evaluated hematology and oncology patients found moderate-quality evidence that PRT-treated PLT transfusion was not associated with statistically significant increments of clinically relevant bleeding and high-quality evidence that it increases PLT requirements (47)."

Changes in the text:

I have also added the references (52,58,60) at the end of the following sentence: "*Consequently, to achieve the necessary PLT increment, the use of PRT-treated PLTs implied a higher transfusion frequency (52,58,60)*".

I have also added the reference 47 to this paragraph to clarify that the statement that the use of PRT-treated PLTs implies a higher transfusion frequency is not based on my opinion but on the meta-analysis carried out by the Cochrane Library authors and the studies already cited: references 52,58,60.

"*A meta-analysis of RCTs that evaluated hematology and oncology patients found*

moderate-quality evidence that PRT-treated PLT transfusion was not associated with statistically significant increments of clinically relevant bleeding and high-quality evidence that it increases PLT requirements (47)"

Besides, as I have already explained in the answer to comment 2, some authors have described lower post-transfusion PLT increments after transfusing PRT-treated PLTs (51, 52, 55, 56- 60) and an increase of PLT support or transfusion frequency (52,58,60) and even the occurrence of PLT refractoriness (52, 57). Please see Table 1 based on the references from 51-60.

Comment 4: Page 9, non-inferior: in fact, many more studies used a non-inferiority design, see Rebulla

Reply 4:

As the reviewer kindly suggests, many other studies have used a non-inferiority design. This has been described in Table 1 in the study design section (please see Table I based on the references from 51-60).

Additionally, I have to apologize for the misunderstanding, as I did not mean that there are only 3 RCTs (IPTAS, EFFIPAP and PREPAReS studies) that used a non-inferiority design, as MIRACLE, HOVON and TESSI studies also used it. What I meant was that three RCTs have investigated whether PLTs treated with PRT are non-inferior to conventional PLTs regarding the prevention of WHO grade 2 or higher bleeding in thrombocytopenic patients with hematological disorders, i.e. three RCTs using a non-inferiority design, whose primary outcome is based on bleeding, described as either "The proportion of patients with WHO grade 2 or higher bleeding" or "The proportion of transfusion-treatment periods in which the patient had grade 2 or higher bleeding". MIRACLE, HOVON and TESSI used 1hr CCI as the primary outcome and not bleeding events. Please see Table 1.

Changes in the text:

In order to clarify this point, the following sentence has been modified (page 9):

"Three RCTs have assessed whether PRT-treated PLTs are non-inferior to conventional PLTs using as a primary outcome the prevention of WHO grade 2 or higher bleeding in thrombocytopenic patients with hematological disorders.**"**

Comment 5: Page 10, the paragraph beginning with Moreover, very few reports: when you talk about safety, you talk about adverse and serious adverse events. This paragraph mostly discusses transfusion frequency. This should be changed. There should be a paragraph on transfusion safety, but it should discuss AEs and SAEs.

Then, you show your own results, with almost double the amount of transfusions in the group of children that receive pathogen inactivated platelets. Very little information is provided on the data that is collected, and the authors must indicate that there is a risk for bias. Were sicker children treated with pathogen inactivated platelets? Is that why they require more platelets? Was the treatment period longer, so that they received more platelets? These factors must be considered before implying that pathogen inactivated platelets increase platelet usage, and therefore some limitations of the data must be provided.

Reply 5:

The paragraph beginning with "Moreover, very few reports" is describing studies on the safety and use of transfused PRT-treated PLTs in a pediatric populations. In fact, this paragraph does describe safety besides PLT uses, as can be seen in the sentences written **in bold**: "Moreover, very few reports have been published about the safety and use of PRT-treated PLTs for transfusion in pediatric patients. A retrospective study in which 240 children received 1,072 Intercept-treated PLTs and 860 conventional PLTs over 21 months **found no safety issues** and red blood cell utilization patterns were similar. Only febrile nonhemolytic and allergic transfusion reactions were reported over the study period, with the number and type of transfusion reactions similar in both groups. However, there was an increased utilization of PLTs in pediatric recipients aged 1–18 years after the transfusion of Intercept–treated PLTs (61). In another retrospective study, 51 children with a mean age of 11 years were transfused with 141 Mirasol–treated PLTs and showed lower post-transfusion PLT counts compared with 86 children receiving 291 standard PLTs. **However, the incidence of bleeding episodes and transfusion-related adverse events was similar in both groups (62)**. Our research group evaluated PLT use in

379 children up to the age of 15 years, who received 4,236 PLTs treated with Mirasol between 2013 and 2017. **While all adverse events in children were mild**, we found a significant increase in PLT transfusions in 132 neonates receiving 458 Mirasol-treated PLTs compared with 99 neonates transfused with 176 standard PLTs. We concluded that additional studies are required to assess the efficacy and safety of PRP-treated PLTs in pediatric patients, especially those requiring chronic transfusion therapy (23)".

For further information about our study of safety and use of transfusion of PRT-treated PLTs in children, I refer the reader to the original manuscript published last year in *Transfusion* whose reference number appears at the end of the paragraph (23 Jimenez-Marco T, Garcia-Recio M, Girona-Llobera E. Use and safety of riboflavin and UV light-treated platelet transfusions in children over a five-year period: focusing on neonates. Transfusion. 2019;59:3580-3588.).

Changes in the text:

However, considering the reviewer´s useful suggestion, I have added a sentence in blue describing in greater detail the words "***found no safety issues"*** of the first study (page 10): *Only febrile nonhemolytic and allergic transfusion reactions were reported over the study period, with the number and type of transfusion reactions similar in both groups.*

Comment 6: Page 10: phototherapy: an n of 11 is really too small. Also, the information was obtained by chart review, so that rashes may have been missed, plus, no chart review was done in the control group. It can still not be excluded that a photoreaction takes place, though the amotosalen is mostly removed once the PRT process is completed. Please provide this context.

Reply 6:

Regarding phototherapy and Intercept pathogen-reduced PLTs in children, considering the reviewer´s suggestion, I have added some additional information.

Changes in the text:

The following phrase has been modified (page 10 and 11):

"*A retrospective study on transfusion reactions in children failed to find any new*

rashes after a manual chart review of 11 newborns undergoing phototherapy and receiving Intercept-treated PLTs, although no chart review was done in the control group (61)."

Comment 7: Page 11: what does "HV1" mean?

Reply 7: I apologize for the error, HV1 corresponds to reference number 63.

Changes in the text:

The text has been corrected (page 11): "*Mirasol, which obtained EC approval in 2007, is currently in use in some European countries (63), as well as in Russia and the Middle East*".

Comment 8: Page 11, limitations: the amotosalen method requires removal of the compound, adding 6 to 16 hours to the production time that should be mentioned. Also, the UV-C method does not cover HIV, which should be corrected in the text. It should be reminded that we probably won't drop any of the NAT testing currently done, so there probably is no real added risk.

Reply 8:

Regarding the comment that the amotosalen method requires removal of the compound, this aspect of Intercept technology is covered in the Pathogen reduction technologies section (page 6): "*After PLT treatment, amotosalen and its photoproducts need to be removed by an in-line compound adsorption device for 6-24 h (25,26).*"

Following the reviewer's suggestion, I have added a comment that the UV-C method does not cover HIV, and included 3 more references (76,77,78) in the section about Limitations of pathogen reduction technologies.

Changes in the text:

The following paragraph has been added in the Limitations of pathogen reduction technologies section (page 12)

"*However, the resistance of HIV to the Theraflex-UV system (76) could be an important limitation. The risk of transfusion-transmitted HIV has decreased*

substantially in most countries, but it can still occur despite negative individual-nucleic acid testing, due to the window period (77), the suppression of viral load and delayed seroconversion after the administration of 'on-demand' pre-exposure prophylaxis (PrEP) or when there is poor adherence to PrEP (78)".