Peer Review File

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Reviewer A' s Comments

Major

1. Page 5 line 136- Are platelets in plasma or platelet additive solution? or a mix depending on other factors?

Reply 1: It is a mix and changed over the years, starting with 100% in plasma, transitioning to 100% in platelet additive solution.

Changes in the text (Methods): We added: "Platelets are currently stored in platelet additive solution, but during 2013-2019 platelets were in plasma, platelet additive solution/plasma or platelet additive solution only, changing over the years."

2. Page 5 line 138- what is average or median volume incubated per donation or pool?

Reply 2: 2 x 7.5 ml

Stated in text: "Products are sampled within 2 hours after production and anaerobic and aerobic blood culture bottles are inoculated (7.5 ml each) in a laminar flow cabinet."

3. Page 7 lines 207-212- is it possible to speculate later on what happened in these 7 cases?

Reply 3: It is not clear to us which 7 cases this question refers to. The 16 cases described in line 207-212 are described in Table 1.

4. Page 8 line 224- suggest listing Enterobacterales here as "other Enterobacterales" as Morganella is also within this order.

Reply 4: The text states: "In 5 of these cases, highly virulent pathogens were

cultured from the blood of the patients (Pseudomonas aeruginosa, Morganella spp., S. aureus, Enterobacterales, Candida spp.), which likely were the causative agents." Unfortunately, we don't have information on the exact species in the 'Enterobacterales' case, and thus it could also be Morganella spp.

5. Page 10 line 329- the percentages were hard to estimate from line 329. Suggest adding % behind species name in Figure 2.
Reply 5: We added % in Figure 2, as suggested.

6. Page 11 line 334- why was order level taxonomy used here? was that the ID level or were data lumped?
Reply 6: Data were lumped. We replaced 'Enterobacterales' with the exact bacterial species.
Changes in the text: "We found S. aureus once, Klebsiella oxytoca once, Citrobacter freundii once, all detected before 48 hours, and Campylobacter fetus once, detected just after 48 hours…. "

7. Page 11 lines 363-365- I would suggest citing the actual residual risk per "Number" platelet units or some other descriptor
Reply 7: We added the actual residual risk per distributed platelets.
Changes in the text: "…… thus demonstrate a small residual risk (16 cases per 668,896 distributed platelets ….. "

Minor
1. Page 2 line 52 - suggest "during bacterial screening"
Reply 1: We changed the wording.
Changes in the text: "During 2013-2019, 1,382 out of 432,305 distributed platelets were positive (0.32%) in the bacterial screening, ..."

2. Page 4 line 112- suggest "the TRIP"

Changes in the text: “….. registered by TRIP, the national hemo- and biovigilance office (https://www.tripnet.nl/en/).”

3. Page 4 line 113- suggest citing suggest citing https://www.tripnet.nl/en/

Reply 3: Added.

4. Page 4 line 118- suggest citing TRIP 2018 hemovigalence report https://www.tripnet.nl/wp-content/uploads/2020/08/Trip.HEMO_uitgebreid_ENGdef 2020-4.pdf

Reply 4: Added citation.

5. Page 4 line 125- as above suggest citing TRIP 2018 hemovigilance report https://www.tripnet.nl/wp-content/uploads/2020/08/Trip.HEMO_uitgebreid_ENGdef 2020-4.pdf

Reply 5: Added citation.

6. Page 5 line 145- suggest "released to hospital"

Reply 6: Added.

7. Page 5 line 157- suggest citing as "TrackWise Enterprise Quality Management Software (EQMS)" or more descriptive citation

Reply 7: Changed as suggested.

8. Page 10 line 316- capitalize "Figure"

Reply 8: Changed.

9. Reference 1- suggest adding web link

Reply 9: Added.

10. Reference 8- suggest adding web link

Reply 10: Added.

11. Reference 10- suggest adding web link if possible

Reply 11: Added.

12. Figure 1- suggest italicizing proper genus and species names in legend of Figure 1

Reply 12: Changed as suggested.

13. Figure 2-Suggest italicizing proper genus and species names in Figure 2. It might make sense to add % behind species name for greater clarity

Reply 13: Changed as suggested.

Reviewer B's Comments:

General Comments:

This is an interesting manuscript that reviews the data in the TRIP registry from 2008-2019. It is a very comprehensive review of these data and provides much valuable information. While the manuscript is well-written it is essentially a review of the registry.

Reply: Thank you for your comments. Our paper is not only a review of the TRIP registry, as also late positive cultures already transfused are described in detail, as well as the culture results of our bacterial screening. Thus, data from 2 different organizations are described in our paper: registry data (based on reports from hospitals) are from TRIP and data on bacterial screening are from Sanquin Blood Supply Foundation.

1. The manuscript title could be re-worded to better inform the reader as to the contents.

Reply 1: We think that the (assigned) title covers the content well. Our two approaches (examination of the TRIP registry and examination of cases in which

positively cultured platelets were already transfused), as well as the characterization of the bacterial species found in bacterial screening, together provide information on the residual risk involving contaminated platelets.
However, as discussed below in response to specific comment 2, we will suggest to the Editor to change the title to “Transfusion transmitted bacterial infection (TTBI) involving contaminated platelet concentrates: Residual risk despite intervention strategies.”

2. The text, while focused on bacterial contamination, does not address the use of pathogen reduction in any substantive manner.
Reply 2: We feel that this is logical for an overview of our own data using bacterial screening. Pathogen reduction is not used in The Netherlands and thus not part of our data set. We did briefly discuss pathogen reduction in the Discussion.

3. This is of concern, since while the COVID-19 virus is not blood borne, a future viral pathogen might be and thus bacterial interventions such as those described would be of little value. This concept should be addressed in the text, even if only for a few sentences.
Reply 3: While we are aware of the potential risk of emerging pathogens including viruses, our strategy includes careful and intensive monitoring, and when needed, donor deferral and/or development of novel testing strategies. However, this is outside the scope of this paper on bacterial contamination of platelets.
A few sentences on pathogen reduction are already included in the Discussion.

4. There are no recommendations regarding Delayed Sampling, other than for the authors to say they do not feel the need to delay sampling since the incidence of bacterial contamination they encounter is so low. Do the authors believe there is no possibility of improving on even their impressive statistics? Use of PR technology or delayed sampling might help to close the admittedly narrow gap.
Reply 4: We discussed Delayed Sampling in the Discussion. We do think this may

even further improve the effectiveness of bacterial screening, but it is currently logistically difficult to introduce in our system. Importantly, we do apply large volume sampling in two bottles. We changed the text to better clarify this.

Change in the text: “We have not postponed the time of sampling because this is difficult to introduce due to logistic challenges, and because our current numbers of TTBI are low.”

5. The text is overly long, and it could be cut by 10-20%. With a change in title and some text reorientation, the manuscript would make a useful contribution to the literature by describing their Registry’s results.

Reply 5: We cut some sentences in the Results section (Characterisation of bacterial species found in bacterial screening). We reoriented text in the Results section (Cases with positive bacterial screening after transfusions and a transfusion reaction). We hope that these changes increased the readability. The Author Guidelines did not specify a maximum word count (only for the abstract).

Specific Comments:

1. The title should be changed to better reflect the content- perhaps: Septic Transfusion Reactions Involving Contaminated Platelet Concentrates: A Report of the TRIP Registry 2008-2019. The current title states that there is a residual risk despite intervention, yet on page 14 the authors imply that they do not plan to delay the time of sampling due to their current low numbers. This sends a mixed message to the reader. On the one hand there is a low but still present risk, but on the other hand the risk is so low the authors do not plan to try to lower it even further. Please clarify this issue.

Reply 1: Thank you for your suggestion. The suggested title does not cover the scope of the paper, as discussed in response to an earlier comment above.

A residual risk will always remain due to a limit of detection. After all, we can only sample part of the platelet bag, and not the entire content.

As discussed, we think that large volume delayed sampling is ideal, but delayed

sampling is currently logistically difficult to implement in our system. We hope that the clarification in the text, as described above, takes away the mixed message.

2. The authors do not mention that future threats to the blood supply might be viral. This might be another reason to re-title the manuscript, to have it better reflect that it is primarily, an analysis of the TRIP Registry.
Reply 2: As discussed above, we disagree that our paper is only an analysis of the TRIP Registry. We believed that 'Septic transfusion reaction involving contaminated platelets' is descriptive of sepsis after transfusion due to bacterial contamination of platelets.

However, to address your concern that this wording may suggest coverage of septic transfusion reactions due to viral infection, we could change the title to "Transfusion transmitted bacterial infections (TTBI) involving contaminated platelet concentrates: Residual risk despite intervention strategies." As the original title, for a paper about TTBI and bacterial screening, was assigned by the Editor, we will propose this option to the Editor.

3. The references cited in the manuscript for PR papers are fairly old. Much more current data have been published.
Reply 3: The cited references discuss the mechanisms of pathogen reduction techniques. To address your concern, we added one recent paper on this topic.
"McDonald CP, Bearne J, Aplin K, Sawicka D. Assessing the inactivation capabilities of two commercially available platelet component pathogen inactivation systems: effectiveness at end of shelf life. Vox Sang. 2021;116(4):416-24."

Considering that pathogen reduction is outside the scope of our paper, we believe that the current 6 references on this topic are ample.

Reviewer C's Comments:

General Comments:

This study reports findings from a national hemovigilance database regarding bacterial contamination of platelets (and associated risk), including bacterial culture screening and suspected transfusion reactions.

1. Sampling for bacterial culture is notably performed quite early after collection, which does not allow much time for potentially contaminating bacteria to grow. This may lead to frequent false negative results due to sampling error. A recent study estimated residual risk due to false negative initial culture results using meta-analysis, which showed residual detection rates after primary culture are about 1 in 1,000 to 1 in 11,000 by secondary culture and rapid testing, respectively (Walker et al. Transfusion. 2020.60:2029-2037). It also showed the sensitivity of primary culture is low, at just 31%. The current manuscript details sampling even earlier, which is expected to further reduce sensitivity.

Reply 1: We sample shortly after preparation of the pooled products, but this is 17 to 25 hours after collection of the whole blood, with intermediate storage at room temperature. We added text “(i,e. 17-25 hours after donation)” to clarify.

Changed text: “Buffy coats (BC) are produced from overnight hold whole blood (14-22 hour at room temperature) …” and ”Products are sampled within 2 hours after production (i.e. 17-25 hours after donation) … ...”

2. It seems suspected transfusion reactions in this study were reported passively. It is well known that septic transfusion reactions are underreported and residual risk as defined in this study would therefore appear to be an underestimate of the true clinical risk to patients.

Reply 2: Indeed, we report passively, but the number of reports shows awareness in the clinic for bacterial infection after transfusion. Thus, we expect underestimation to be low.

Regarding the concern about under-reporting it can be stated that participation of well over 95% of the Dutch hospitals is confirmed each year. Although

under-reporting exists in all spontaneous reporting systems, in the TRIP system this is minimized by the collection of non-serious as well as serious reactions, and by registration of reactions and bacteriology findings even in cases where TTBI was not confirmed.

3. Therefore, a bacterial testing strategy with low sensitivity along with likely under recognition or underreporting of suspected septic transfusion reactions, would be expected to result in a falsely low estimate of residual risk to patients. This estimate could lead to a false impression of safety of such an approach.

Reply 3: The meta-analysis by Walker et al. included multiple studies that inoculated only 1 bottle and/or had short incubation time (1 day). Thus, taking into account all parameters of our bacterial screening (a.o. 2 bottles, 7 days culture), we cannot conclude that our bacterial screening has 'low' sensitivity compared to this meta-analysis. Our high reporting rates of (suspected) transfusion reactions in combination with our low numbers of TTBI argue against 'false impression of safety'.

In addition, we also analysed all reports to TRIP where a bacterial species was cultured in the remnant of the transfused platelet concentrate by the hospital during 2008-2019 (Table 4), and identified only 1 additional case of potential septic transfusion reaction due to contaminated platelets, supporting a small residual risk.

Finally, as discussed above, participation by Dutch hospitals in the TRIP reporting system is very high (over 95%).

Additional comments that are either relevant to the above or are relatively minor:

1. Abstract:

-Please add some details about screening methodology and timing of sampling along with expiry.

Reply: We added this to the methods section of the abstract.

Changed text: "Bacterial screening was performed by sampling platelets 17-25 hours

after blood collection, followed by a 7-day incubation of aerobic and anaerobic blood culture bottles in the BacT/ALERT® system."

2. Background:

-Page 3, line 82: Suggest rephrasing to "The FDA guidance for platelet bacterial risk control includes several strategies, some of which use large volume delayed sampling (LVDS)."

Reply: We rephrased the original sentence "The FDA guideline on safety of platelets emphasizes the importance of large volume delayed sampling, as applied in the United Kingdom (8, 9)."

Changed in the text: "Recommended strategies by the FDA guideline on safety of platelets include large volume delayed sampling, as for example applied in the United Kingdom (8, 9)."

-Please specify whether the TRIP database involves passive versus active surveillance.

Reply: added

Changed in the text: "In our country, TRIP (Transfusion and Transplantation Reactions in Patients) registers reports of transfusion reactions of all severity levels (passive surveillance)."

3. Methods

-Does sampling of BC pooled platelets occur at 16-22 hours after collection? This is very early and would be quite prone to false negative results. Similarly, sampling apheresis platelets at 12 hours after collection is very prone to false negative results.

Reply: This is already discussed in the General Commnets (see above). Our approach is very standard; only recently delayed sampling was introduced. The paper is about our screening over the period 2008 to 2019. Based on our hemovigilance results we have very limited false negative results resulting in a clinical TTBI, both for BC pooled platelets and apheresis platelets.

4. Results

-Page 6, line 190: If this TTBI rate is from passive surveillance then it is likely an underestimate of the true rate.

Reply: We report passively, but the total number of reports of post-transfusion bacteremia/sepsis (663 cases during 2008-2019) shows awareness in the clinic for bacterial infection after transfusion. Thus, we expect underestimation to be low, see also reply to General Comments above.

-Page 7, line 212: If this statement is kept, then it should also be stated that a longer delay until sampling for culture could have prevented some cases.

Reply: This is a valid point. We moved the sentence “A longer hold (later release) of platelets would not have prevented these 16 TTBI cases” to the discussion and added the suggested statement in the discussion.

Changes in the text: “However, a longer delay of sampling for culture may have prevented some cases.”

-Page 7, lines 215-223: How were true positive and false positive culture results determined? Please provide clear definitions if this is possible, or if not possible, please state.

Reply: This is a good point. These are ‘confirmed positives’. This is defined in the Methods section: “Confirmed positive results are defined as a positive BacT/ALERT® signal confirmed by a positive culture from the BacT/ALERT® bottle (majority of cases), or a positive Gram staining but a negative culture.”

They are not ‘true positives’, since we don’t resample and reculture the platelet product for confirmation.

We added ‘from the BacT/ALERT® bottle” to clarity and moved this sentence to an earlier paragraph in the Methods section. Also, we specified this in the Results section. Finally, we also added ‘confirmed’ to Table 2.

Change in the text (Methods): “Confirmed positive results are defined as a positive

BacT/ALERT® signal confirmed by a positive culture from the BacT/ALERT® bottle (majority of cases), or a positive Gram staining but a negative culture."

Change in the text (Results): "1,300 pooled BC derived PC (0.32%) and 82 apheresis PC (0.26%) were confirmed positive in the bacterial screening."

-Page 11, line 339: These are probably only detected occasionally because early bacterial screening has poor sensitivity.

Reply: Delayed sampling was only recently introduced; this paper is about our screening over the period 2008 - 2019. The advantage of delayed sampling is discussed in the paper. We added to the discussion "However, a longer delay of sampling for culture may have prevented some cases."

Since many virulent bacteria are relatively fast-growing, there is no reason to expect that delayed sampling would have a much larger impact on detection of virulent bacteria compared to low virulent bacteria. We think that the sentence "Together, these results show that virulent bacteria are detected only occasionally and usually within 48 hours, and thus most before administration." still holds true for our analysed data.

5. Discussion

Page 14, line 462: Would be helpful to include some discussion of clinical efficacy of pathogen reduced platelets (i.e. briefly summarize findings from the key studies and trials).

Reply: Since pathogen reduction is outside the scope of this paper, we discussed this topic only briefly in the discussion. We were asked by a reviewer to shorten the paper, and thus we did not add more discussion on pathogen reduction.

6. Table 3:

"Transfusion reactions" should be "Suspected transfusion reactions" because imputability was often unlikely. Suggest this terminology throughout the manuscript.

Reply: All 20 cases in table 3 were reported transfusion reactions, adverse events

associated with transfusion. However, in only 5/20 cases the transfusion reaction was potentially related to contaminated platelets (imputability in these 5 cases: possible).