Peer Review File

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Reviewer A

Comment 1: This article aimed to characterize the patients who refuse blood transfusion for religious reasons, which is correctly termed as a "natural experiment". Since at least two decades ago, these anemic patients are being reported to experience higher mortality and morbidity than those who accept blood transfusion. Thus, the main purpose of this manuscript, as from lines 54-57, is an already well-acquired and established matter. It is unclear why the Authors wanted to re-consider it and what is new concerning what is already known.

Reply 1: This paper is submitted as a review and as such reports only on what is already known. It is not a meta-analysis, as specifically stated in the methods and statistical inferences were as reported in original articles. There was no pre-formed hypothesis as expected in a meta-analysis. Conclusions based on the literature review are largely thematic, as stated in the methods and are based on low levels of evidence. Combining such data is problematic particularly with imbalance between larger numbers of case reports of survivors of severe anaemia compared with deaths indicating large reporting bias, as noted in the conclusions.

The reason for the paper is summarized in the introduction: "*This review aims to describe the contribution of religious objection to blood transfusion by Jehovah's Witnesses advancing our understanding of the impact of anaemia, the role of transfusion and the development of patient blood management and alternatives to transfusion.*" Only a minority of these deal with mortality. Indeed, a large number of reports deal with successful management of severe anaemia and suggest it is feasible, whereas a thorough review indicates clear harms. The value in this work is to summarise a very large literature, which has clearly been of interest over decades into an interpretable form.

Comments 2: The Authors have made a tremendous effort in analysing >700 items published until July 2020 and selecting 419 papers among these. Although one does not expect things to have changed much in the last four years, this review might appear slightly outdated.

Reply 2: Repeating the search reveals a further 125 articles to date, highlighting the continued interest in the field. These have included a number of retrospective cohort studies, particularly in cardiac surgery, and two metanalyses in the same fields, each showing the safety and potentially benefits of withholding blood. There remain a large number of case reports. On brief review, these do not meaningfully change the outcomes presented, however have not been included since a similar in-depth approach has not been taken.

Comment 3: In addition, the Authors failed to make any metanalysis nor statistical inference on their big work aimed at confirming their hypothesis that refusing blood transfusion poses higher health risks. They honestly admit that it was not their intention to go beyond a narrative review, but unavoidably the lack of an

attempt to summarize the outcomes of the 419 analysed papers leads this manuscript to become a mere collection of single cases, mostly one independent from the others, that does not provide the reader a clearly cut opinion on whether refusing blood transfusion increases risks. In other words, a narrative review without data.

Reply 3: As noted above, the methodology of this work was thematic analysis, not meta-analysis. This was chosen because the data are too diverse and included studies of mostly low quality. There was no a priori hypothesis. The question merely being what have we learned from the reports to date? The question of whether transfusion refusal increases risks as posed by the reviewer is not clear cut, but is addressed in the very last sentence of the paper – to the extent that this refusal pushes the profession to improved patient blood management it may be beneficial, however refusing life-saving transfusions is clearly harmful.

Comment 4: To let this manuscript acquire scientific consistency for publication, the Authors should provide more data on the 419 articles they have collected. More specifically, they must distinguish the primary items (e.g., the experimental articles) from the secondary items (e.g., reviews, reports, metanalyses, etc), clarify the endpoints (mortality rate, complications?), verify their hypotheses statistically, and validate their conjectures following established international guidelines. Only after doing this, the valuable narrative considerations that presently form the bulk of the manuscript will acquire scientific consistency and readability.

Reply 4: While this methodology is rigorous, the data do not allow such rigorous conclusion to be drawn. Thematic analysis does not pre-suppose the endpoints. The article types have been added in broad terms to guide the reader the quality of the available data.

Minor remarks

Comment 5: I suggest removing terms that might appear unrespectful of the religious beliefs, such as “steadfast” in line 49.

Reply 5: Word has been removed.

Comment 6: In lines 80-81, the sentence appears truncated.

Reply 6: Corrected with the change to:

Most articles were case reports (n=331) or cohort studies (n=61), with case-control studies (n=15), trials (n=1) and reviews (n=11) making up the remainder of the reviewed papers.

Comment 7: The sentence in lines 88-89 is unclear: “calculated dissolved haemoglobin with 100% inspired oxygen accounted for approximately one third of the blood oxygen available for delivery”. Please reformulate the sentence.

Reply 7: Error corrected and sentence clarified: With a haemoglobin of 18g/L, Dicpinigaitis (6) observed that the calculated dissolved plasma oxygen at 100% inspired oxygen accounted for approximately one third of the blood oxygen delivered to the tissues.

Comment 8: Also, the sentence in line 96 is to be reformulated: "Based on the severity of their injuries, a 99% survival was expected, but this assumes availability of blood for transfusion".

Reply8: Revised to:

Based on the severity of their injuries, a 99% survival was expected. Calculation of survival probabilities are based on patients receiving standard of care, including blood transfusion.

English must be revised.

Reviewer B

The article is well structured and I enjoyed the approach done to review the literature used on the methodology.

However, I found necessary to include the most vision of WHO Policy Brief on Patient Blood Management, who guides global perspective on this subject. Some suggestions are included on the article review.

Comment 1: Suggestion

The authors could focus on a more positive conclusion showing the transfusion refusing cohorts have promoted the consideration of different medical approaches when blood transfusion is not an option, expanding the management of anemic patients at large, like the patient blood management new standard of care. It will be in sync with WHO message on its current Policy Brief for PBM.

Reply 1: Re-phrased: Transfusion refusing cohorts have contributed to our understanding of anaemia and management options when transfusion is not an option, while actively promoting patient blood management. However, cohorts of patients have demonstrated increasing mortality when haemoglobin levels are maintained below 70g/L when compared to patients accepting of transfusion.

Comment 2: Suggestion

Many articles reviewing Jehovah's Witnesses position on blood transfusion indicates that their objection is for whole blood and the main 4 blood components (red blood cells, plasma, white cells and platelets). But there is great acceptance of blood fractions (albumin, growth factors, coagulations factors, to list a few).

It could be informative to explain it to differentiate what the patient rejects and what can be acceptable, considering the PBM approach.

Ref: official JW. ORg website - https://www.jw.org/en/medical-library/medical-information/religious-and-ethical-position-medical-therapy/

Reply 2: This was considered during writing. One of the difficulties is that while red cell refusal is generally accepted, some published articles use components as strategies for haemostasis.

The following has been added:

Officially, Jehovah's Witnesses do not accept whole blood (even autologous), cellular or plasma components, but allow fractionated products, such as albumin or immunoglobulin and even marrow transplantation as a matter of choice. However, the literature reports varying practices and individual patients must be involved in their decision making. In the literature this does result in greater heterogeneity when looking at components other than whole blood and red cells.

Comment 3: WHO PBM Policy Brief

It is important to include the WHO Policy Brief that urges PBM Implementation. This creates a new level of recommendation for PBM as standard of care.

Ref: https://www.who.int/publications/i/item/9789240035744

Reply 3: Reference added at line 236.

Comment 4: Suggestion to change phrase

The phrase as it is inclining the reader to consider blood transfusion as better treatment that patient blood management. But is the opposite view of WHO Policy Brief and the many articles recently published showing risks of blood transfusion.

A suggestion to the authors is to rephrase the sentence, like: "By refusing only common blood transfusion, religious objectors can therefore provide an insight into the value of patient blood management approach that do not consider blood transfusions and the only option in the modern advanced health care context."

Reply 4: This suggestion assumes that blood transfusion and PBM are opposite rather than complementary approaches to patient care. Specifically, this review has examined transfusion refusal. While many articles reviewed did include PBM, the variable in common to all was transfusion refusal, not PBM. However, the reviewer's point that many cases have had PBM is true and the phrase has been modified to include that.

By refusing only transfusion, religious objectors can therefore provide an insight into the potential benefit, or lack of benefit of blood transfusion in the modern advanced health care context where the principles of patient blood management are applied.

Comment 5: Reconsider this section conclusions. A literature review of case reports was published to assess survival in patients with extreme anemia, defined by researchers as anemia where hemoglobin levels were less than 2 g/dL. (ESTEVES et al., 2021) Such cases are often dramatic and the risk of death may be considered imminent by many physicians.

The authors conducted a literature search and found 23 published case reports with anemia consistent with extreme anemia. In these 23 reports, there was one death in a patient who received a blood transfusion and four patients had complications after transfusion (posterior reversible encephalopathy syndrome). A total of four patients were not transfused because they refused to receive blood components and obtained other types of treatment, such as erythropoietin, iron, vitamin supplements, among others. All four patients survived.

Despite the limitations of a review of case reports, it can be concluded from the data collected by this review that, in extreme anemias (Hb < 2 g/dL), where the risk of death is high and may be considered imminent by many physicians: transfusion does not guarantee the preservation of life and not transfusing does not lead to certainty of death.

More recently, a systematic review of the literature with meta-analysis of observational studies was published comparing mortality and morbidity in transfusable and non-transfusable patients. (SEEBER et al., 2022)

In this review, non-transfusable patients were defined as those who refused transfusions (regardless of the reason); patients treated in conditions where transfusions were not available (pandemics, disasters, combat environments, countries with limited resources); patients with rare blood types or complex antibody patterns for which transfusions are not available. Transfusable patients were defined as those capable of receiving transfusions regardless of whether or not they had received a transfusion.

A total of 41 studies were included in this systematic review. There was no difference between the groups regarding short-term mortality (within the first 30 days). There was a lower mortality rate in the non-transfusable group regarding 1-year mortality.

Therefore, even though there is no scientifically recognized consensus on what constitutes an imminent risk of death, and there may be subjective and objective criteria that can be discussed, it is clear that there is no definitive proof that transfusion leads to an improvement in mortality and that not transfusing the patient leads to certain death in cases that could potentially be characterized as imminent risk of death.

Ref: Esteves J, Fernandes J, Oliveira-Monteiro P, Almeida M, Nogueira-Silva L, Almeida J. Surviving Extreme Anaemia. Eur J Case Rep Intern Med [Internet]. SMC Media; 2021 Mar 5 [cited 2022 Jun 21];8(LATEST ONLINE). Available from: https://www.ejcrim.com/index.php/EJCRIM/article/view/2357/2586

Seeber P, Döbel KU, Isbister JP, Murray K, Shander A, Trentino KM, et al. Mortality and morbidity in non-transfusable and transfusable patients: A systematic review and meta-analysis. Transfusion (Paris) [Internet]. Transfusion; 2022 Mar 1 [cited 2022 Jun 21];62(3):685–97. Available from: https://pubmed.ncbi.nlm.nih.gov/34967018/

Reply 5: The very thoughtful feedback is appreciated.

The report by Esteves et al also summarised other reported cases of severe anaemia. By nature, case reports are selected by the authors for showing novel, instructive of extraordinary findings. This likely explains why the mortality rate was low and the rate of posterior reversable encephalopathy syndrome (PRES), a rare complication, was high. Indeed, some of these cases were reported because of PRES, an emerging potential complication of transfusion. The cause of PRES generally is unclear, and with endothelial dysfunction being a possible contributor, and potential rapid changes in microvascular flow, there is biological plausibility for transfusion as a cause. However, anaemia induced leukoencephalopathy in anaemia prior to transfusion has also been reported (Kaur eta al. Neurol. India, 2021) and anaemia itself may be a contributing cause. It is true that severe anaemia does not indicate certainty of death without transfusion, and that transfusion guarantees survival, but the data from case reports are inadequate to infer a benefit or lack of it. Case control studies are better for this.

As discussed in this paper, case control studies do suggest increasing mortality associated with non-transfusion, particularly in at risk patient groups. While the meta-analysis from Seeber et al suggests overall safety for a non-transfusion approach using PBM principles, it did not separately analyse patients with very

low haemoglobin levels. A subanalysis of severely anaemic or shocked patients included only two studies, in one of which the comparator arm was selected for having only one unit (in cardiac surgery) and in the other there was a trend to decreased harm with transfusion, despite most people in the study having higher nadir haemoglobins. Therefore, extrapolation to very low haemoglobin levels is not warranted.

The section on anaemia tolerance in light of this additional information (line 99-102):

Despite the very low haemoglobin levels reported in survivors without transfusion, the lowest reported haemoglobins to the best of the author's knowledge were significantly lower. A level of 4g/L in a child with sickle cell disease and acute autoimmune haemolytic anaemia due to ceftriaxone resulted in recovery with severe neurological impairment (11).

The conclusions have also been modified to include the useful review by Seeber et al (Line 292-297):

By corollary, haemoglobin levels above 70g/L are likely to be safe in most circumstances, a finding supported by clinical trials in various settings (1, 2) and meta-analysis of case control studies comparing non-transfusable patients with those able to be transfused (87). Ongoing research into patients refusing transfusion and comparator studies will help further our understanding of the impact of anaemia and coagulopathy, particularly in more severe cases where randomised studies are considered unethical based on current knowledge.

Comment 6: Reconsider the conclusion findings

Reply 6: The final sentence has been amended (line 305-308):

While it encouraged best practice to the point that transfusion is genuinely needed, there is likely a point beyond that where the objection to transfusion causes serious and quantifiable harm and further studies in this cohort will be helpful in defining appropriate limits in the setting of best-practice patient blood management.