HEALTH SCIENCES
WITH
PLYMOUTH UNIVERSITY

19th March 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7635-edited[1].doc).

Title: Transfusion related morbidity in premature babies: possible mechanisms and implications for practice.

Author: Keith Collard

Name of Journal: *World Journal of Clinical Pediatrics*

ESPS Manuscript NO: 7635

I have included an abstract of appropriate length and a 100 word Core Tip summary as requested.

I note that reviewers 1 & 2 were satisfied with the original manuscript and recommended acceptance. In contrast reviewer 3 required a number of modifications.

Preamble – The purpose of this review was to examine known potentially adverse changes which occur in paediatric packed red blood cell units during preparation and storage, and to relate these to the specific physiology of the premature newborn. An attempt was then made to relate these to the major complications of prematurity to provide some ideas of potential mechanisms responsible for the link between the receipt of blood transfusions and the development of the complications. There are many facets of the 'storage lesion' which have been well reviewed in the past. The purpose of this review was not to provide a comprehensive review of the storage lesion (which referee 3 appeared to require), but to look at those aspects of the storage lesion which are likely to compromise the premature baby on the basis of the physiology peculiar to the premature baby (as indicated above). Those factors which best fit this scenario are those discussed in detail in this review. Other factors that might contribute are mentioned for the sake of completeness, but not necessarily elaborated upon as there is **no** obvious link to the **specific** physiology of the premature baby. This message was clearly understood by reviewers 1 & 2 but not reviewer 3. The revisions to the manuscript have hopefully spelt this out more clearly.
I also note that Reviewer 3 made **no positive comments** about the work. This is unusual. I also note that many of the comments of referee 3 refer to material which was already present in the original manuscript, but not noticed by the referee. Where this has occurred I have commented on this in my reply.

The manuscript has been modified according to the suggestions of reviewers No 2 and 3. All changes to the original manuscript are highlighted in red:

Revewer No 2 asked only that the reference to Sloan be inserted correctly in the text. This has now been done [Ref 59 p 9].

Comments of Referee 3 and authors replies:

1. Referees comment (1)

An introduction is necessary; something to announce that this is a review with an emphasis on common illnesses of neonatal patients and the potential for relationship with transfusion-related iron overload and oxidative stress. There are many other theories currently being investigated (eg nitric oxide scavenging by free Hb or microparticles (Donadee et al)) , which are insufficiently reviewed in this Manuscript. Your review of NO is rather SNO focused, and the general focus of the field has passed to NO scavenging by free Hb and microparticles. Another important area is the loss of DPG from stored cells and this is not mentioned by the author.

Response.

An introduction has been included in the revised manuscript as requested (pages 5-6). This clarifies the purpose of the review better. The abstract has also been modified to clarify the purpose of the review. The comment regarding Hb scavenging of NO was included in the original manuscript and remains in the revised document with the addition of a reference to the work of Minneci (ref 47) as requested (see page 8). The potential involvement of NO appears at other points in the original manuscript and remains in the revised manuscript (e.g. page 5, page 6). While 2,3 –DPG depletion might interfere with the oxygen carrying ability of transfused RBCs, there is no clinical evidence of adverse effects of 2-3-DPG depletion (Hess *Vox Sang* e-pub Jan 2014)

2. Referees comment (2)

Need to highlight the number of transfusions provided to neonates. Or at least provide a reference for pg1/paragraph1/line1.

Response.

References to publications which refer to numbers of transfusions received by premature babies are included in the introduction (page 5).

3. Referees comment (3)

Last full sentence on page 1 is awkward and cannot be understood by this reader (the clauses appear conflicting): “It also appears to be related to particular changes occurring at 31-32 weeks postconceptional age, and independent of postconceptional age at birth”

Response

This sentence has been modified to make it clearer (page 6) [although it was perfectly clear in the original manuscript].

4. Referees comment (4)

The section regarding BPD and blood transfusions appears to make multiple leaps—connecting BPD and endotracheal infection and a predisposition to infection in patients who have received blood older than 14 days. Is it possible to outline the causal relationship that the authors believe may link these two associated events?

Response.

Some data has been included to strengthen this concept (page 8). This is also alluded to in Section 4. It is well known that iron availability is essential for bacterial growth and factors such as macrophages which limit bacterial growth do so by limiting iron availability.

5. Referees comment (5)

This review appears to highlight the potential that one possible cause, transfusion-related iron overload, is the major contributing factor to neonatal pathology. I believe this should be made clearer in the introduction, and/or even the title. The theme of the review is not made until page 5:

> "This review will investigate the possible link between transfusion-mediated iron overload and oxidative stress and the ability of the premature baby to deal with such a situation, and the implications with regard to the development of the complications of prematurity."

Response.

This has hopefully been rectified in the new introduction and in the revised abstract.

6. Referees comment (6)

If iron is to remain the emphasis, it will be important to highlight studies that demonstrate iron overload occurs in these patients, and discussing a possible pathogenic relationship beyond oxidative stress. Do we know if neonates manifest a change of circulating cytokine levels with transfusion? Do their circulating hepcidin levels change? I recognize that the author presents a thorough discussion of some of the putative mechanisms. Because iron overload and oxidative stress appears to be the major thrust of this paper, I suggest making this clearer in an introduction, and discussing it earlier in the paper, or make reference to section 3 when outlining the potential causal relationships earlier in the paper. Section 5, needs to be moved up front, as well.

Response.

The level of post-transfusional iron overload was discussed in the original manuscript and remains in the modified manuscript (see page 16). This includes all current data on this aspect of blood transfusions in premature babies. It was also discussed in Section 3 of the original manuscript. Now section 4 of the revised manuscript. (see page 11). These remain in the modified manuscript. The importance of iron is also alluded to in the introduction as suggested.

Changes in circulating cytokines was discussed in Section 3 of the original manuscript and remain in the modified manuscript (now Section 4 page 13).

The possible involvement of hepcidin was discussed in the original manuscript in Section 4 (section 5 of this revision see pages 14-15). Further data has been included in the modified manuscript. (see page 15). This adds to what was originally discussed in the original manuscript.
I do not understand the meaning of the term 'moving up front'.

7. Referees comment (7)

Is the cutoff date for the age of blood to be used in transfusions universal? Do you refer only to the UK? You state, "In addition, mannitol and adenine act as preservatives to allow the storage of RBCs up to 35 days for paediatric use and 42 days for adult use [58]." Do all hospitals follow this rule, does the FDA mandate it? Or do most hospitals allow transfusion of 42-day old blood in pediatric patients?

Response.

I have made it clear that these cut-off points refer to the UK only. I am not qualified to discuss what happens elsewhere, and have no knowledge of FDA rules since they do not apply to the UK. (see section 3 page 9).

8. Referees comment (8)

The ARIPI study referenced (71) did NOT transfuse well-defined and far removed blood age groups. They provided blood < 7 days versus standard, which turned out to be 14.6 days.

Response.

The reference to the ARPI study has been modified to reflect this point. (section 4 page 11).

9. Referees comment (9)

There is no mention of the 2008 Koch et al study in NEJM, which triggered many of the recent investigations into the "storage lesion".

Response.

The omission of this major study in the original manuscript was a major oversight by myself. This has been rectified and the importance of this study included in the modified manuscript (section 7 page 19).

10. Referees comment (10)

When discussing the 2008 Koch study, it would also be helpful to reference the review by Dzik, W., Fresh blood for everyone? Balancing availability and quality of stored RBCs. *Transfusion Medicine*, 2008, 18, 260–265.

Response.

I did not refer to this particular paper by Dzik, but to a more recent publication by the same group (Dzik et al Ref 127 in the original manuscript, Ref 132 in the modified manuscript. (section 7 page 19-20).

11. Referees comment (11)

There is no mention of lamb studies examining cell free hemoglobin and nitric oxide scavenging with stored RBC transfusion. See for example 1. Minneci *JCI* 2005; and 2. Baron D., et al., *Anesthesiology* 2012 and Critical Care Medicine 2013. This review is hyperfocused on iron overload only to its detriment.

Response.

The scavenging of NO by free haemoglobin was mentioned in the original manuscript. It remains in the modified manuscript. (It has already been discussed in the response to comment No. 1)

12. Referees comment (12)

Two other important areas need review—the haptoglobin levels of neonates and children (needed for free Hb clearance) and the hemopexin levels (needed for clearance of free heme)-—Reference—Boretti FS et al *JCI* 2009

Response.

These points were covered in Section 4 of the original manuscript, and remain in the modified version (now Section 5).

The additional references required with these modifications are highlighted in red script.

I thank the referees for their comments. I have made an attempt to deal with them in the revised manuscript. I hope I have responded to these comments to your satisfaction.

I look forward to your reply.

Yours sincerely,

Keith Collard