The controversy over the age of blood: what do the clinical trials really teach us?

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Abstract

Red blood cell transfusions have been used in clinical practice for decades and represent the most common therapeutic procedure performed in hospitalised patients. Depending on the storage solution and national regulatory requirements, red blood cells can be stored in the refrigerator up to 42 days before transfusion. We reviewed five of the most recent randomised clinical trials that examined clinical outcomes in specific patient populations. Although these studies provide some comfort regarding our current standard of care, they do not address whether the oldest blood is associated with harm in certain patient populations.

Keywords: blood transfusion, storage lesion, red blood cell, haemolysis, non-transferrin-bound iron.

Introduction

All medications have side effects. Although red blood cell (RBC) transfusions have been used in clinical practice for decades and represent the most common therapeutic procedure performed on hospitalised patients[1], one might expect them to have unintended side effects as well. We are more familiar with the risks posed from infectious disease transmission (e.g., HIV, hepatitis) and transfusion reactions (e.g., haemolytic allergic, febrile, acute lung injury, circulatory overload); however, those resulting from the "RBC storage lesion" are less well characterised. Depending on the storage solution and national regulatory requirements, RBCs can be refrigerator stored for up to 42 days before transfusion[2]. During refrigeration, RBCs undergo multiple metabolic and structural changes, collectively termed the "RBC storage lesion"[3]. Although the clinical outcomes resulting from the RBC storage lesion are debatable, everyone agrees that refrigerator damage *in vitro* does induce haemolysis *in vivo* of some of the storage-damaged RBCs[4]. In various animal models (e.g., mice, guinea pigs, dogs, sheep), under certain clinical circumstances, the haemolysis of transfused, storage-damaged RBCs produces adverse effects[5-7]. In addition, in healthy human volunteers, there is increasing haemolysis *in vivo* of transfused RBCs in proportion to increasing storage time before transfusion[4]; nonetheless, there was no definitive evidence of clinical harm in these research subjects.

A provocative observational study[8] concluded that transfusions of RBCs stored for more than two weeks were associated with significantly increased risks of post-operative complications after cardiac surgery, accompanied by increased short-term and long-term mortality. Although not the first such observational study examining the effect of prolonged RBC storage on clinical outcomes, this one initiated a flurry of well-designed clinical trials to examine this question more closely. Based on the conclusions from the five major clinical trials published since then, which primarily addressed the potential benefit of "fresher" RBCs, some have argued that "additional trials do not appear warranted at this time"[9], because no benefit of a fresher blood policy was demonstrated. However, we believe that one must examine these clinical trials more closely before concluding that the current standards do not have unintended consequences in certain clinical settings. To this end, we herein review the five major clinical trials published to date and discuss the strengths and limitations of each.

The Age of Red Blood Cells in Premature Infants (ARIPI) randomised trial

The ARIPI trial of fresh red blood cell transfusions in premature infants[10] was a double-blind randomised controlled trial in 377 premature infants with birth weights less than 1,250 grams admitted to six Canadian tertiary-care neonatal intensive care units (ICUs) between May 2006 and June 2011. The objective was to determine if RBCs stored for seven days or less, as compared with the standard of care, decreased rates of major nosocomial infection and organ dysfunction in neonatal ICU patients requiring at least one RBC transfusion. The overall conclusion was that a fresher RBC transfusion policy did not improve outcomes in premature, very low-birth-weight infants. However, we caution against concluding that the use of older RBCs is safe in this population, because this was not tested in the ARIPI trial; this is of special interest given the typical practice of reserving one unit for transfusion to the same neonate over the course of their hospitalisation, providing older and older aliquots as time goes on. In particular, the median age of "standard of care" RBCs in the Canadian sites participating in the ARIPI trial

was 14.6 days, whereas other studies in neonates[11,12] suggest that there is very little evidence of haemolysis from transfusing RBCs stored for less than two weeks. Because the standard of care arm in the ARIPI trial represented reasonably fresh RBCs, it is perhaps more justifiable, based on this study, to conclude that a "really fresh" RBC policy is not superior to a "fresh" RBC policy in neonates.

The Age of Blood Evaluation (ABLE) trial

The ABLE trial on the age of transfused blood in critically ill adults[13] was a multicentre randomised controlled trial comparing RBCs stored for less than eight days with standard-issue RBCs. Critically ill adults, 18 years of age or older, from tertiary-care ICUs at 64 centres were enrolled. Patients were eligible if a first RBC transfusion was prescribed within seven days after admission to the ICU and if they were expected to require invasive or non-invasive mechanical ventilation for at least 48 hours. The study interventions aimed to compare RBCs stored for less than eight days (mean 6.1±4.9 days) to standard-issue RBCs (mean 22.0±8.4 days). This study did not find any benefit attributable to the transfusion of fresh RBCs in critically ill patients. Although the primary outcome was clinically relevant and important for both patients and clinical decision makers, the study was not designed to answer the question of "Is old blood bad?" but, rather, "Is fresh blood better than the standard of care?"[13]. We recently demonstrated that circulating non-transferrin-bound iron (i.e., circulating iron not bound to the physiological iron transport protein, transferrin), a marker of excessive acute haemolysis, is not produced in transfused healthy volunteers until after 35 days of RBC storage[14,15]. Furthermore, evidence suggests that non-transferrin-bound iron can potentially mediate at least some of the adverse effects of transfusion[5-7,16,17]. Taken together, if non-transferrin-bound iron is a mediator of adverse effects resulting from the RBC storage lesion, it would be difficult to observe a clinical difference between fresh and standard-issue RBCs, unless the standard-issue RBCs were particularly old (e.g., 35-42 days). Thus, although this study provides some comfort regarding the current standard of care, it does not address the question as to whether RBCs stored up until the last week allowable result in harm in this vulnerable population.

The Informing Fresh *vs* Old Red Cell Management (INFORM) trial

The INFORM trial on the effect of short-term *vs* long-term blood storage on mortality after transfusion[18], conducted from April 2012 to October 2015, was a large, multicentre, pragmatic, randomised trial involving a general population of hospitalised patients aged 18 years and older who required a red cell transfusion. The 31,497 patients were randomised in a 1:2 ratio to receive the freshest or the oldest RBC units available. The median storage duration in the "old" transfusion storage group was 23.6 days. There was no difference observed in the primary outcome, comparing the rate of death between the patients transfused with the freshest or oldest available RBCs. Although this was the largest study performed to date, like the ABLE study[13], it was not designed to answer the question of whether the oldest RBCs (e.g., 35-42 days) are harmful in specific vulnerable patient populations. To confound this study further, by including multiple clinical conditions, an adverse effect in one sub-population may mask a beneficial effect of older RBCs in another. For example, if older RBCs do, in fact, function in a pro-coagulant fashion, this aspect may benefit a bleeding patient, but harm a patient with a thrombotic diathesis[3]. Based on this study, on average, fresher blood was not superior to older blood (although the median age of the older blood was approximately 24 days); however, in a given patient, it does not address whether a 42-day old unit will result in harm if transfused. In contrast, multiple animal studies suggest longer-stored RBCs have the potential to be harmful in certain circumstances[5-7,17,19]. Furthermore, a recent, albeit observational, study suggests that RBCs stored for 35-42 days are, indeed, associated with increased morbidity and mortality[20]. Although the INFORM trial is a large study, one must be careful in concluding that "old blood is safe" given these limitations.

The Red Cell Storage Duration Study (RECESS) trial

The RECESS trial on the effects of red-cell storage duration on patients undergoing cardiac surgery[21] was a multicentre, prospective, randomised clinical trial that compared clinical outcomes after cardiac surgery in patients who received transfused RBCs stored for ten days or less ("fresh" blood) or for 21 days or more ("old" blood). Running from 2010 to 2014, participants were 12 years of age or older undergoing complex cardiac surgery and likely to receive transfused blood. The primary outcome was the change in Multiple Organ Dysfunction Score (MODS; range 0-24, with higher scores indicating more severe organ dysfunction) from the pre-operative score to the highest composite score through Day 7 or the time of death or discharge. There was no difference in MODS observed between transfusions of fresh or old RBCs. It is very important to note that the cardiac surgery patients in this study were on bypass for a median time of 140 minutes. Cardiac bypass is associated with a significant amount of haemolysis from RBC damage caused by the pump[22,23].

The incremental haemolysis from the transfusion of one or two units of stored RBCs is expected to be small compared to the effects of the underlying haemolysis due to the bypass pump. Thus, to the extent that haemolysis of transfused storage-damaged RBCs is responsible for the adverse effects of transfusion, the results of this study are not generalisable to other patient populations without baseline haemolysis from bypass. Although this study may not be generalisable to other patient populations, it does answer an important clinical question raised by Koch *et al.*[8]. Based on that study[8], cardiac surgeons had an argument for demanding fresher blood for their cardiac surgery patients. The RECESS trial suggests that in patients on bypass, with significant pump-induced haemolysis, the additional effect of haemolysis from transfusion of older RBCs does not pose a significant clinical risk and the current standard of care is acceptable.

The Tissue Oxygenation by Transfusion in severe anaemia with Lactic Acidosis (TOTAL) trial

The TOTAL trial on the effect of transfusion of red blood cells with longer *vs* shorter storage duration on elevated blood lactate levels in children with severe anaemia[24] was a randomised non-inferiority trial of 290 children (aged 6-60 months) with severe anaemia, most with malaria or sickle cell disease, presenting between February 2013 through May 2015 in Uganda. The objective was to determine if older RBC units (25-35 days) were non-inferior to fresher RBC units (1-10 days) with regards to tissue oxygenation, as measured by reduction in blood lactate levels and improvement in cerebral tissue oxygen saturation. The primary outcome was the proportion of patients with a lactate level of 3 mmol/L or lower by eight hours after transfusion using a margin of non-inferiority equal to an absolute difference of 25%. Among children with lactic acidosis due to severe anaemia, transfusion of older RBCs did not result in inferior reduction of elevated blood lactate levels. Again, it must be noted that, analogous to the RECESS trial, virtually all the patients in the TOTAL trial had significant ongoing baseline haemolysis from their underlying illness (almost all had malaria and/or sickle cell disease, with a haemoglobin level on hospital admission of 5 g/dL or lower). Thus, to the extent that haemolysis is responsible for the adverse effects of transfusing older RBCs, this baseline haemolysis would make it very difficult to see a clinical difference in transfusion efficacy between fresh and older blood. Furthermore, this study could not test the hypothesis that the oldest blood, that is blood stored for more than 35 days, is responsible for adverse effects, because the standard of care in Uganda limits the outdate to 35 days. This policy is similar to other countries and institutions, which have reduced the maximum outdate due to the precautionary principle, such as the United Kingdom, the Republic of Ireland, the Netherlands, and the Blood Bank of the National Institutes of Health[25,26]. Given the difficulty of performing randomised clinical trials to test RBCs stored exclusively for 35-42 days, and the successful experience of some countries in limiting the allowable outdate, some would invoke the precautionary principle to argue that the maximum outdate should simply be changed to 35 days universally[15].

Conclusions

Although the clinical trials completed to date provide answers to clinically-relevant questions, they have yet to answer the question of whether transfusing RBCs after 35-42 days of storage is safe. One must be careful to generalise from patient populations with baseline hemolysis from an underlying disease to other patient populations. This raises the issue that, in some patients, older RBCs are not expected to be harmful; furthermore, one can even imagine patient populations in which older RBCs would be beneficial. Thus, a more nuanced approach is necessary before concluding that no further clinical studies are necessary[9]. In addition, none of the completed trials were designed on the basis of a mechanistic understanding of why transfusions of older RBCs might produce adverse outcomes (e.g., production of non-transferrin-bound iron can enhance biofilm formation of "ferrophilic" pathogens)[27]. Without a mechanistic basis, there remains the concern that the clinical trial design might include too much "noise" to reveal the "signal" of concern (e.g., a primary outcome of all infections may result in a different conclusion than using a primary outcome of biofilm-related infections from "ferrophilic" pathogens). Finally, although the clinical studies published to date provide some comfort regarding our current standard of care, the specific patient populations that would benefit from a fresher RBC strategy, or from avoidance of the oldest RBCs, remain to be fully explored.

The Authors declare no conflicts of interest.

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