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RBC storage lesion-induced adverse effects: More smoke; is there fire?

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"Red cell transfusion-associated hemolysis in cardiac surgery: An observational cohort study," by Karkouti et al.[1] in this issue is a timely contribution to a growing body of literature on an important, but controversial, topic. That is, do red blood cell (RBC) transfusions *per se*, and transfusions of longer-stored RBC units, in particular, lead to adverse outcomes? The adverse outcomes under consideration are not the usual, better-understood ones, such as those due to transfusion-transmitted infectious diseases (e.g., hepatitis C, human immunodeficiency virus), hemolytic or non-hemolytic transfusion reactions, transfusion-associated acute lung injury, or transfusion-associated circulatory overload. Rather this literature addresses whether there are additional, novel, adverse effects resulting simply from transfusions of refrigerator-stored RBCs themselves. Indeed, one could argue that the origin of all of these types of studies began in 1999 with the surprising results in the TRICC trial,[2] which showed that critically-ill patients had better outcomes, including less mortality, when placed on a restrictive transfusion regimen, rather than a liberal one (i.e., were transfused at a lower hemoglobin threshold, rather than a higher one). These results suggested that there was something "bad" about transfusing "too many" stored RBCs.

Although the underlying mechanisms responsible for these poorer outcomes were not understood, there was at least some suggestion in the older studies that the contaminating white blood cells in the RBC products might play a role. Nonetheless, multiple more recent studies, using leukoreduced RBC products in various patient populations, yielded similar results; that is, a restrictive regimen was equal to, or better than, a liberal one.[3] Taken together, these results suggest that, if there is a problem, it is due to the transfused RBCs themselves. In addition, many retrospective observational studies found a correlation between an increased incidence of adverse effects (e.g., increases in infectious and thrombotic complications, length of stay, and mortality) following transfusion of longer-stored RBC units, as compared to "fresher" ones.[4]

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These findings created great consternation in the Transfusion Medicine community, leading to a spate of randomized, prospective trials comparing transfusions of “fresher” and “older” RBC units, although the latter were actually “standard of care” in most studies.[4] Although, these studies did not identify a benefit from transfusing fresher RBC units, they all suffered from a lack of a mechanistic pathophysiological hypothesis that would inform study design and improve signal-to-noise ratios (if there is a signal!).[4] In addition, the study patients typically received relatively small doses of transfused RBCs,[4,5] although this was not true in all such studies.[6] Interestingly, a recent deeper analysis of the results from the ABLE trial may suggest that older RBC transfusions do, indeed, lead to adverse effects in patients receiving larger doses of these RBCs.[7]

On the other hand, the suggestion that older RBCs were “bad” inspired multiple groups to search for underlying mechanisms that might cause these putative effects. These studies typically focused on consequences of the “RBC storage lesion,” by which refrigerated storage, even in the presence of the most modern preparation methods, anticoagulants, and storage solutions, leads to RBC damage. Multiple aspects of the RBC storage lesion have been described;[8] for regulatory purposes, the most objective consequences of this phenomenon are *ex vivo* hemolysis “in the bag” during refrigerated storage, leading to the infusion of RBC contents and microparticles in the acidic, citrated supernatant, and decreased 24-hour post-transfusion recovery *in vivo* of the transfused RBCs.[9] As such, multiple groups of investigators are studying the potential role(s) of many possible culprits, including free hemoglobin, nitric oxide, microparticles, intravascular hemolysis, extravascular hemolysis, and changes in RBC characteristics that affect hemorheology (e.g., decreased deformability) and oxygen delivery.

Our own group postulated the “iron hypothesis,” which could account for at least some of the putative adverse effects following transfusions of older, stored RBCs.[9–11] In this case, the acute clearance of storage-damaged RBCs by the mononuclear phagocyte system induces catabolism of a bolus of RBCs, releasing abundant amounts of iron that overwhelm the carrying capacity of transferrin, leading to circulating non-transferrin-bound iron (NTBI). NTBI, which is undetectable in normal individuals, induces oxidative stress, adversely affects endothelial cell function, and enhances the virulence of certain pathogens.[9–12] As such, NTBI has the potential to induce thrombotic and infectious sequelae resulting from older, stored RBC transfusions. Indeed, in healthy volunteers, autologous transfusions of 6-week old RBC units, in contrast to fresher units, induces dramatic increases in circulating NTBI.[11,13] This was predominantly due to extravascular hemolysis, as no evidence of intravascular hemolysis was observed.[11,13] The potential danger of transfusing RBCs stored for 5–6 weeks has not been adequately studied in the published randomized prospective trials, because of design considerations, the paucity of observable events, and/or regulatory constraints.[4–6]

The paper by Karkouti et al.[1] adds to this discussion by evaluating measures of intravascular hemolysis (e.g., plasma free hemoglobin) and extravascular hemolysis (e.g., markedly elevated transferrin saturation as an accepted, appropriate surrogate for NTBI)[13] in patients undergoing cardio-pulmonary bypass (CPB). In their retrospective observational study, they enrolled 560 consecutive patients, and data were available and evaluable on 543; of these, 82

patients were transfused with at least one unit of RBCs during CPB.[1] In this cohort of 82 transfused patients, they found elevated plasma free hemoglobin levels as a marker of *ex vivo* and/or *in vivo* hemolysis; however, this finding was not associated with transfusion. Rather, the circulating free hemoglobin detected was presumably due to damage to endogenous and/or transfused RBCs by the CPB procedure; for example, there was a correlation between the length of CPB and the free hemoglobin level. Although others have observed increased plasma free hemoglobin post-transfusion, this presumably results from infusion of free hemoglobin and RBC-derived microparticles in the supernatant of the transfusate that are produced *ex vivo* during storage and/or from intravascular hemolysis of the transfused RBCs *in vivo*.[14,15] For example, transient changes in endothelial function and increases in blood pressure, presumably due to nitric oxide scavenging, have been seen post-transfusion, but these changes are typically acute and self-limited.[14,15] If due to infusion of free hemoglobin, one would expect to see a dose-response, but the patients in the current study only received a small transfusion load (i.e., a median of one RBC unit) in the setting of ongoing, CPB-induced hemolysis.[1] In addition, shed blood in the operative field was re-infused; free hemoglobin and additional damage to these (endogenous) shed RBCs caused by the collection and washing procedures may also affect their results.[16] Thus, it is possible that this ongoing, procedure-related hemolysis swamped out any observable effects on cell-free hemoglobin levels caused by *ex vivo* and/or intravascular hemolysis of the relatively small number of transfused RBCs. Nonetheless, these results are relevant to other published clinical trials in the setting of ongoing hemolysis,[5,6] suggesting caution in generalizing the results to other patient populations without underlying hemolysis.

In contrast, Karkouti et al. did find evidence of extravascular hemolysis in their patients who specifically received RBC transfusions, as particularly exemplified by markedly increased levels of transferrin saturation, as a surrogate for NTBI.[1] Because this was observed more frequently in their transfused patients, it presumably results from the effects of the RBC storage lesion. These data are in accord with our “iron hypothesis” and agree with results seen in healthy human volunteers.[11,13] In addition, in the RECESS trial, which also predominantly studied CPB patients, serum bilirubin levels were significantly higher in the group receiving “older” RBC transfusions, suggesting increased extravascular hemolysis in this group; unfortunately, NTBI levels were not measured in this study.[5]

Although the current paper was a single site, retrospective, observational study, it was carefully performed and the authors’ conclusions were modest and appropriate. As just one example of their attention to detail, in an effort to minimize any artifactual “intravascular” hemolysis that would confound their results, their patient blood samples were taken from indwelling arterial catheters.

Nonetheless, there are limitations of the current study, most of which were well recognized by the authors. For example, this study was not designed to detect adverse outcomes of RBC transfusions; rather, its “sample size was one of convenience” as part of an ongoing quality assurance study. Because it was not planned in advance, there were no *a priori* calculated power analyses or sample size determinations for specific outcomes. In addition, the cohort of evaluable transfused patients was small, with only 82 of 543 evaluable patients receiving even one RBC transfusion. In addition, based on animal and other human studies, if RBC

transfusions do induce adverse effects, it is reasonable to expect a dose-response relationship;[10] nonetheless, their patients received few transfusions during CPB (i.e., a median of one unit). Because of the small study size, they could also not investigate the role of RBC storage age, or other aspects of RBC storage quality. Furthermore, the study was neither large enough, nor was designed, to detect any adverse consequences of RBC transfusion. Thus, although they did identify elevated transferrin saturation levels, which could predispose patients to infectious complications, these levels, by themselves, are not an adverse effect.

Finally, samples for hemolysis testing were only obtained 15–30 minutes after completion of CPB and no serial samples were obtained. In addition, no information was provided concerning the interval between completion of the transfusion(s) and sample collection, although CPB duration was 104 +/− 42 minutes (i.e., ~1–2.5 hours). Studies in healthy volunteers suggest that increases in transferrin saturation (and NTBI) typically become apparent 1–2 hours post-transfusion, peak at 8–10 hours post-transfusion, and return toward baseline by 24 hours post-transfusion.[13] Because of the lack of serial sampling, the incidence of elevated transferrin saturation levels in the transfused patients studied by Karkouti et al. could even be higher.

In summary, the current paper suggests that RBC transfusions produce evidence of extravascular hemolysis, particularly with respect to measures of iron status. Although they did not evaluate storage age, it is reasonable to expect, based on other studies, that these biomarkers would increase in proportion to increasing RBC storage interval. Their finding of markers of intravascular hemolysis in CPB patients, whether or not they received transfusions, suggest that care needs to be taken when interpreting the results in studies of patient populations with ongoing intravascular hemolysis at baseline (e.g., CPB in the RECESS trial;[5] sickle cell anemia and/or active malaria in the TOTAL trial).[6]

Recent publications suggest that transfusions of RBCs, which are stored near the 42-day outdate mandated by the United States Food & Drug Administration, produce significantly elevated levels of NTBI in healthy volunteers.[11,13] These types of transfusions may also produce adverse effects in patients.[17] When these observations are combined with the suggestion that larger doses of longer-stored RBCs may be required to produce adverse effects,[7] this indicates that a fair amount of “smoke” still surrounds this controversial issue. Therefore, the question remains whether a “fire” may also be present. We believe that carefully-designed, mechanistically-inspired clinical trials in appropriate patient populations, which evaluate transfusions of significant doses of RBCs at the extremes of the full storage interval, are required to answer this question.

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