Red blood cell components: Meeting the quantitative and qualitative transfusion needs

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Summary

Red blood cell (RBC) transfusion is a very common therapeutic intervention. However, because of multiple recent studies improving our understanding of appropriate transfusion scenarios, the total number of RBC units transfused per year is actually decreasing in the developed world and there are no longer major shortages of RBC products for general use. Nonetheless, there are an increasing number of "special" uses, which can put strains on the blood supply for particular types of products; these may produce shortages of specific types of RBCs or require collections targeting certain types of donors. This review will focus on several broad topics, including providing some examples of "special" settings that require, or could require, special types of RBC products.

Introduction

Red blood cell (RBC) transfusion is one of the most common therapeutic interventions in hospitalized patients; in particular, in the United States, approximately 15 million units are transfused annually into approximately 5 million recipients [1]. Nonetheless, due to multiple clinical trials comparing clinical efficacy outcomes of liberal versus restrictive transfusion protocols [e.g., the Transfusion Requirements in Critical Care (TRICC) trial [2]], the total number of RBC units transfused per year is actually decreasing in the United States [3]. Thus, there are no longer major shortages of RBC products for general use. In addition, multiple other studies have been published, or are underway, identifying the best "transfusion trigger" and the best indications for RBC transfusions (e.g., ref. [4]); the conclusions of some of these studies are sure to lead to decreases in RBC utilization in...
certain specific settings. However, other such studies may actually increase the utilization of RBC products [5]. Some of the latter studies may also identify "special" uses, which can put strains on the blood supply for particular types of products; as a result, these may produce shortages of specific types of RBCs or require targeted collections. One example of this issue encompasses using group O, Rh-negative RBCs for uncrossmatched emergency transfusions [6]. Another example involves patients with sickle cell disease, in which Rh-genotypically matched RBCs can be provided to patients with existing alloantibodies recognizing unusual Rh antigens; similarly these types of RBC units can be provided to other such patients to prevent potential alloimmunization [7,8].

In addition, there is a broad consensus that having better RBC products and better indications for RBC transfusions would decrease the number of transfusions required, in general, and in the chronic transfusion setting (e.g., for patients with sickle cell disease or beta-thalassemia), in particular [9]. Decreased numbers of RBC transfusions would also limit a recipient's exposure to various acute and chronic adverse effects [e.g., transfusion-associated acute lung injury (TRALI), alloimmunization, hemolytic transfusion reactions, iron overload, transfusion-transmitted infections]. Therefore, although this review will focus on several broad topics, which are outlined below, there are complex interrelationships between these somewhat artificially separated topics. In addition, this review will identify some examples of "special" settings that require, or could require, special types of RBC products.

Why do we transfuse RBCs?

To provide the appropriate context, it is important to understand the indications for RBC transfusions. The most common, generally accepted, rationale is to improve oxygen delivery and, concomitantly, improve carbon dioxide removal. In addition, RBC transfusions, particularly exchange transfusions, are used to replace or dilute "bad" circulating RBCs, such as in the setting of acute chest syndrome in patients with sickle cell disease. A similar rationale is used in patients with malaria or babesiosis, who, if they present with high levels of parasitemia, are at-risk for significant morbidity and mortality. A final example in this category includes neonates with clinically severe hemolytic disease of the fetus and newborn.

An additional, less appreciated indication for RBC transfusions is to promote hemostasis. Thus, RBC transfusions can improve hemostasis in anemic, thrombocytopenic patients, even in the absence of platelet transfusions, presumably by improving laminar flow and decreasing the width of the "cell-free zone," thereby increasing the probability that the circulating platelets will interact with the vessel wall [10–12]. It is also possible that, following prolonged RBC storage, phosphatidylserine-expressing RBCs and RBC-derived microparticles, may enhance hemostasis (see below). In addition, recent studies highlighted the beneficial role that RBCs play in clot architecture [13,14]. Nonetheless, as will be seen below, this is a two-edged sword, and transfusions of refrigerator storage-damaged RBCs may actually enhance pathological thrombosis.

Finally, chronic RBC transfusions can repress endogenous erythropoiesis, either in settings of ineffective erythropoiesis (e.g., in beta-thalassemia and myelodysplastic syndrome) or those involving production of abnormal RBCs (e.g., in sickle cell disease). Again, this approach can have negative consequences, particularly if the transfused RBCs have a suboptimal lifespan; the major adverse outcome in this regard is chronic iron overload, potentially producing significant organ dysfunction.

What are the consequences of transfusing RBCs?

Alloimmunization

Although RBC transfusions are therapeutically beneficial, they are not without risk. For example, because it is not yet possible to transfuse patients with genotypically completely identical RBCs (other than in the setting of autologous transfusion or in the very rare case of the donor being an identical twin), a major potential consequence of RBC transfusion is alloimmunization to blood group antigens. Alloimmunization can result in acute or delayed hemolytic transfusion reactions [15], potentially producing significant morbidity and mortality. In addition, it is more difficult, time consuming, and costly to identify compatible RBC units for alloimmunized patients. Other than phenotype/genotype matching, it is not at all clear how to prevent alloimmunization, and the overall phenomenon remains poorly understood; nonetheless, there is general agreement that the probability of alloimmunization increases somewhat in proportion to the number of units a patient receives over their lifetime [16,17]. In addition, abundant evidence in animal models demonstrates that certain types of inflammation in the recipient enhance alloimmunization following RBC transfusion [18]. However, it remains controversial whether this is relevant in humans [16,19–21]. Furthermore, in accord with our understanding of basic immunology, blood group alloantigens need to be presented by appropriate human leukocyte antigens (HLA) of the major histocompatibility

Glossary

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<th>Term</th>
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<td>RBC</td>
<td>red blood cell</td>
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<td>TRICC</td>
<td>transfusion requirements in critical care</td>
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<td>TRALI</td>
<td>transfusion-related graft-versus-host disease</td>
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<td>HLA</td>
<td>human leukocyte antigen(s)</td>
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<td>glucose-6-phosphate dehydrogenase</td>
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complex to enable the relevant immune response [22]. Finally, much anecdotal evidence supports the concept that some patients are “responders,” whereas others can be classified as “non-responders;” nonetheless, the biological basis for this phenomenon is not understood, including whether or not genetic and/or environmental factors are relevant [17,21]. Given the general truism (with some caveats, such as HLA type) that a recipient will not produce an alloimmune response to an antigen they have not been exposed to, the most effective way to prevent blood group alloimmunization is to minimize or prevent exposure to foreign blood group alloantigens [23]. To this end, many groups advocate providing antigen-matched RBC transfusions, not only for ABO and Rh(D), but also for various “minor” blood group antigens, particularly in the chronic transfusion setting [7,8]. Nonetheless, there is not yet general agreement regarding the number of antigens to match for, and whether RBC phenotyping is sufficient or genotyping is required. However, in certain patient populations (e.g., sickle cell disease patients with “unusual” Rh genotypes), genotyping donors and recipients may be the only way to prevent alloimmunization to clinically-significant blood group antigens and/or to identify compatible donors for previously alloimmunized patients. Various approaches have been used or proposed to increase the probability of identifying rare donors for these situations, including increasing donor recruitment in demographically-relevant populations, routine screening for rare antigen types, routine genotyping of repeat donors, and using “buddy programs” to pair appropriate dedicated donors with identified recipients. In addition, in the future, if “blood pharmping” becomes safe and cost-effective, it may even be possible to produce commercially-relevant amounts of RBCs from induced pluripotent stem cells using, for example, donors of important, but rare, genotypes. However, it is important to remember that there may be genetic linkages between the types of blood group antigens present on the RBC surface and polymorphisms in cytosolic proteins inside the same RBC. As one example of this scenario, donors of African descent, whose RBCs may be particularly useful for transfusion because of matching for “rare” Rh genotypes and other relevant blood group antigens, may also be glucose-6-phosphate dehydrogenase (G6PD)-deficient [24], presumably due to evolutionary-assembly selection in malaria-endemic regions. Although it is not yet completely clear whether G6PD-deficient RBCs store poorly, this may become a contraindication to their future use in transfusion therapy [25]. Similarly, these donors are more likely to carry “sickle trait,” which is a contraindication for transfusion into recipients with sickle cell disease and may also affect RBC storage quality [26]. Although data are not yet available, it is also possible that selecting donors based on blood group antigen status may select for donor populations that are enriched for other RBC polymorphisms (e.g. alpha-thalassemia trait, beta-thalassemia trait, hemoglobin C, etc.); it is not yet clear whether these polymorphisms affect RBC storage quality and/or post-transfusion recovery and lifespan.

Finally, some evidence suggests that alloimmunization to RBC blood group antigens, even following transfusion of leukoreduced RBC products, can negatively affect the success of hematopoietic stem cell transplantation [27]. These results suggest that antigen cross-presentation may be a real, but unappreciated, mechanism producing this effect.

**Non-hemolytic transfusion reactions**

Other presumably immunologically-mediated adverse consequences of RBC transfusion include allergic, anaphylactic, and febrile transfusion reactions, and TRALI. Based on the understanding that, at least part of, the mechanism underlying febrile transfusion reactions relates to cytokines and other molecules produced by, or released from, contaminating white blood cells in the stored RBC units, pre-storage leukoreduction was introduced. Although there are also other benefits of leukoreduction, it did produce a significant decrease in the incidence of febrile transfusion reactions [28]. As a result, leukoreduction is universally applied in Europe and Canada, and is virtually universal in the United States. Another potential benefit of leukoreduction is that, by removing lymphocytes, it decreases the risk of transfusion-associated graft-versus-host disease; nonetheless, in at-risk recipients (e.g., with severe immunosuppression or immunodeficiency), irradiation of RBC products is required to eliminate this risk completely [29]. Although a complete understanding of the donor and recipient factors responsible for allergic transfusion reactions remains elusive, there is general agreement that the inciting agents are in the plasma remaining in packed RBC units [30,31]. Thus, in patients with severe, intractable allergic reactions, providing washed RBCs typically provides relief [31]. There is a more detailed understanding of the mechanisms underlying anaphylactic transfusion reactions, which typically occur in individuals who are genetically deficient in important plasma proteins (e.g., IgA or haptoglobin) and develop IgE antibodies with these specificities [30,32]. Preventing these potentially catastrophic events typically involves obtaining blood products from similar types of donors (e.g., with IgA deficiency or anhaptoglobineaemia), although washed RBCs from random donors may suffice. Over the last several years, a great deal of experimental evidence, in humans and animal models, has elucidated much about the recipient and donor factors important in TRALI pathogenesis [33,34]. In particular, anti-HLA antibodies in donor plasma are important in causing many cases of TRALI. Because women often develop anti-HLA antibodies during pregnancy, some have advocated excluding female plasma products (e.g., fresh frozen plasma) from the blood supply, particularly because most TRALI cases were seen following plasma transfusions. Indeed, after implementing this approach, there was a dramatic decrease in TRALI incidence [34]. Because relatively little plasma...
remains in packed RBC products, particularly when using modern storage solution methods, and because TRALI is uncommon following RBC transfusion (although now more common than following plasma infusion), there has not been any enthusiasm for restricting the RBC blood supply to male donors. Nonetheless, there has been discussion about screening all donors for the presence of anti-HLA antibodies. Similarly, one could argue that washing RBC products would eliminate the risk of TRALI (albeit, by introducing other logistical issues).

**Transfusion-transmitted infections**

Over the last 30 years there has been tremendous progress in enhancing the safety of the blood supply by virtually eliminating the risks, at least in the developed world, of many transfusion-transmitted infections, such as those caused by human immunodeficiency virus and hepatitis C. In addition, (near) universal leukoreduction is relevant to transfusion-transmitted infection with cytomegalovirus (CMV) and has made RBC transfusions “CMV-safe,” if they are not yet considered to be “CMV-negative.” Nonetheless, there remains a residual risk of transfusion-transmitted infections when using donor populations from endemic areas, particularly for viruses, such as West Nile Virus and those causing dengue fever, and for parasites such as malaria and Babesia, which are infections of RBCs, and Chagas disease. It is, at least theoretically, possible to screen the blood supply for these agents, and there is a significant amount of ongoing research in this area. However, only the donor screening history is currently used in practice, particularly for malaria, as it is not yet felt to be cost-effective to introduce laboratory tests for universal screening.

**Is “old blood” bad? Is “fresh blood” better?**

Because refrigerated storage produces the “RBC storage lesion,” there has been much discussion regarding the consequences, if any, of transfusing RBCs after longer storage durations (i.e., “old” RBCs). As a result, over the last several years, there has been a significant amount of interest, and a significant amount of controversy, regarding issues related to the “age of blood.” Despite the controversy, there is general agreement that potassium and lactate levels increase, and that pH decreases, in RBC units as a function of storage time. Because neonates are believed to be incapable of effectively handling acute, large potassium loads, transfusions in these patients, particularly in the surgical setting, often involve using “fresh” blood, typically with less than 7 days of storage. Nonetheless, this approach produces an interesting conundrum, because neonatologists typically request “pediatric sized” transfusions from a single, repetitively-accessed RBC unit, using sterile technique; this approach does limit donor exposure, which is a holdover from the time when transfusion-transmitted infections were of great concern. However, although the first such transfusion is certainly “fresh,” the final such transfusion in a multiply-transfused patient would more than likely be “old” [35].

In addition, although there is general agreement that the RBC storage lesion is a real entity, and that the 24-hour post-transfusion recovery with old blood is less than that with fresh blood [36], there is no agreement regarding whether transfusions of old blood produce any acute or chronic adverse consequences. This issue has been addressed in many retrospective observational studies [37], multiple bench research studies [38,39], and several prospective, randomized clinical trials. Nonetheless, the largest and most carefully designed of the latter [40–42] addressed the question of whether fresh blood was better than standard issue, or whether “old blood was bad” [43,44]. Recent reviews and meta-analyses conclude that this still remains an open question [43,45]. Nonetheless, based on the claims (albeit controversial) that old blood may be bad, some have invoked the “precautionary principle” to suggest decreasing the storage outdate from 42 days to 35; additional evidence in vitro suggests that maximal storage duration should be decreased even further [46]. Nonetheless, significantly changing the regulatory outdate would produce significant logistical issues, which may compromise the integrity of the blood supply [47]. One concern regarding the use of “old” RBCs for transfusion relates to acute and/or chronic iron toxicity/overload, for which some pre-clinical and clinical evidence exists [38,48]. Thus, transfusions of 40–42 day old RBCs lead to rapid clearance of a substantial portion of refrigerator storage-damaged RBCs, thereby producing acute increases in circulating non-transferrin bound iron, both in healthy volunteers and certain patient populations [49,50]. This is a concern because high levels of circulating non-transferrin bound iron can produce oxidative damage of multiple cell types, and can enhance infection by ferrophilic pathogens [38,48].

In addition to concerns about acute iron toxicity, chronic RBC transfusions induce chronic iron overload (i.e., “transfusion-induced hemosiderosis”), which can produce significant organ dysfunction, particularly in transfused patients with sickle cell disease or beta-thalassemia. Due to this concern, these patients typically receive iron chelation therapy [51]. Although chronic RBC transfusions per se induce chronic iron overload, it seems reasonable to suggest that only using RBC units that produce excellent 24-hour post-transfusion recovery and RBC lifespan would lead to fewer transfusions overall and produce less chronic (and acute) iron overload. As an analogy, in the past, there was interest in collecting and transfusing “neocytes;” that is, RBCs of a younger age in vivo [52,53], particularly for application to the chronic transfusion setting (e.g., sickle cell disease and beta-thalassemia). Indeed, even after 42 days of refrigerated storage, neocytes exhibited surprisingly good 24-hour post-transfusion recovery and impressive RBC lifespan [52]. Another application of this approach could be in patients with the myelodysplastic syndrome, who are at-risk for iron overload, from both ineffective erythropoiesis and transfusion therapy, and who currently benefit from iron chelation therapy. Indeed,
RBC transfusion-induced exacerbation of iron overload may contribute to leukemic transformation in these patients; thus, they may benefit from transfusions of “high quality” RBCs with longer post-transfusion circulatory lifespan [54]. Nonetheless, despite promising results, neocyte transfusions never became a clinical standard of practice. In addition, it remains an open question whether transfusing “fresh” RBC units (i.e., with shorter storage age), by providing better 24-hour transfusion recovery, thereby mimicking at least some aspects of neocytes, would decrease both transfusion requirements and the extent of chronic iron overload.

In animal models, transfusions of “old” RBCs clearly induced acute pro-inflammatory cytokine responses [38]. Whether this occurs in human transfusion recipients is more controversial; inflammatory responses were seen in some, but not all, studies [48-50,55,56]. However, if a post-transfusion inflammatory response is induced in humans, then this could, for example, enhance alloimmunization risk (see above) or contribute to the cytokine storm seen in sepsis [38]. Nonetheless, conclusions regarding this possible adverse effect will need to await future studies.

Finally, the benefits in promoting therapeutic hemostasis were described above as a positive effect of RBC transfusions. However, observational clinical studies suggest that transfusions of old RBCs may enhance the risk of pathological thromboembolism [57]. Indeed, as RBCs “age” during refrigerated storage, they expose more surface phosphatidylserine ex vivo and produce more phosphatidylserine-expressing microparticles [58]. Because cell surface phosphatidylserine enhances coagulation, this putative “hypercoagulability” may actually be a good thing in trauma patients who are bleeding, but may produce adverse outcomes in patients with thrombotic diatheses.

How can we optimize the collected RBCs?

A major research focus in transfusion medicine aims to “make better products” [9]. As a first step, one can imagine incrementally improving the quality of RBCs that are currently collected. For example, with current storage methods, we know that some hemolysis occurs ex vivo leading to the infusion of free hemoglobin, which itself can produce adverse effects, for example, by scavenging nitric oxide, thereby resulting in transient vasoconstriction [59]. Similarly, storage-induced decreased or absent 2,3-diphosphoglycerate affects oxygen delivery by the transfused RBCs [60]. Finally, the rapid clearance of a population of storage-damaged “effete” RBCs, if nothing else, diminishes the dose of RBCs delivered by transfusion. As such, a minimal goal is to prepare and process donor RBCs for transfusion that behave as much as possible as very fresh RBCs (e.g., as if they were transfused “arm to arm”). Continuing development of improved RBC storage solutions [61,62] and rejuvenation methods [63] are bringing us closer to this goal. In addition, some groups advocate more universal washing of stored RBC products [55] because of its ability to remove free hemoglobin, microparticles, and, perhaps, some of the effete RBCs; however, there is at least some concern that washing can itself damage stored RBCs, thereby diminishing post-transfusion recovery [64]. As an alternative approach, relatively few studies have addressed the “quality” or suitability of the RBC donors themselves, even though we have known for approximately 50 years that RBCs from some human donors store poorly, whereas those of others store surprisingly well [36,65]. In addition, these characteristics appear to be stable in individual donors, suggesting that they may be due to genetic, dietary, and/or environmental factors. In particular, data from murine models demonstrate that genetic factors can affect RBC storage quality [15].

To this end, abundant evidence suggests that refrigerated storage induces an oxidative stress and that this accounts, at least in part, for the mechanism(s) underlying the RBC storage lesion [66,67]. As such, one might expect RBCs from individuals who have damaging polymorphisms in RBC enzymes and proteins important in resisting oxidative stress would store poorly, and that these individuals would be “poor storers.” As an example, RBCs from individuals with G6PD deficiency are highly sensitive to oxidative stress; however, it remains controversial whether their refrigerator-stored RBCs are of lower quality, with suboptimal 24-hour post-transfusion recovery [25,68]. If they were, one might recommend that these individuals be excluded from the donor pool, or, at least, be identified in the donor pool [69]. As a more extreme suggestion, one can speculate whether antioxidant treatment of the donor (or of the collected unit; e.g., with Vitamin C and E), whether or not they are G6PD-deficient, would yield RBCs that were better able to withstand refrigerated storage [70-72]. Similarly, preliminary pre-clinical studies suggest that other genetic (e.g., sickle cell trait) and environmental (e.g., iron deficiency) variables may affect RBC storage quality [26,73]. Thus, a potentially fruitful avenue of future research could address the question of whether identifying (or modifying) various donor characteristics can yield better RBC products, even using current storage methods. Some of these interventions could also improve donor health (i.e., “protect our donors”) by, for example, identifying individuals with G6PD deficiency and educating them to the potential risks they face, or by preventing and treating iron deficiency in otherwise healthy volunteer donors.

Can we build even better RBCs? That is, how to “make [even] better products.”

Although a bit speculative, it is not unreasonable to try to “make [even] better products” by approaching the pharmacological ideal of developing homogeneous, highly reproducible, and virtually identical RBC units, as if they were Pharmaceuticals. Indeed, RBCs for transfusion have been proposed to be included on the WHO Model List of Essential Medicines [74]. One could
even imagine, given the current emphasis on personalized medicine, that a quiver of different RBC products could be developed for different indications. However, from the discussion above, it is clear that there is a significant degree of variability based on donor variation in their genetics, environment, diet, etc. and on variations in RBC processing methods [75]. Nonetheless, some significant advances have been made in this regard, as described above, including, for example, (near)-universal leukoreduction. To this end, the remainder of this section will deal with some more speculative ideas on this topic.

As one example, focusing on current technology, one might desire the "ideal" RBC product to be fresh (to yield maximal post-transfusion recovery), leukoreduced (to prevent febrile reactions, HLA alloimmunization, etc.), irradiated (to prevent transfusion-associated graft-versus-host disease), washed (to prevent plasma-dependent allergic reactions and TRALI), and lacking in any genetic predispositions that could cause post-transfusion complications [e.g., genetically identical for all relevant blood group antigens (to prevent alloimmunization and hemolytic transfusion reactions) and lacking Hemoglobin S (relevant for exchange transfusions in patients with sickle cell disease)]. Interestingly, in very select instances, and where clinically indicated, this ideal can be achieved currently.

An alternative approach (which would use the existing donor population, but a different collection method) could conceivably produce neocytes (see above), which store better at refrigerated temperatures [52], perhaps because they better handle storage-induced stress ex vivo. They also exhibit better post-transfusion recovery and RBC lifespan in vivo [52]. In addition, significant advances have been made in producing mature, enucleated RBCs in vitro using tissue culture methods (i.e., "blood pharming") [76,77]. If some additional technical hurdles can be overcome (e.g., "pharmed" RBCs currently produce hemoglobin F, not hemoglobin A), and scaled-up production can be made cost-effective, then perhaps even better RBC products can be made available. For example, even if the mechanism(s) underlying the RBC storage lesion is not yet understood, perhaps induced pluripotent stem cells could be prepared from "super storers," to enable banking of RBCs that better resist refrigerator-induced damage. As another example, perhaps induced pluripotent stem cells could be engineered to exhibit high levels of endogenous antioxidants. As a final example, perhaps transfusing RBCs containing hemoglobin variants with altered oxygen binding properties would actually improve oxygen delivery in specific circumstances.

Finally, in addition to considering RBCs as pharmaceuticals, several novel approaches have suggested using RBCs as carriers that can target the delivery of other pharmaceuticals in unique and specific ways [78]. Combining this concept with "blood pharming" would allow for the opportunity to modify induced pluripotent stem cells using recombinant methods to produce truly unique and tailored RBC products.

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CURRENT QUESTIONS IN TRANSFUSION MEDICINE

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