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Utilization and quality of cryopreserved red blood cells in transfusion medicine

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Cryopreserved (frozen) red blood cells have been used in transfusion medicine since the Vietnam war. The main method to freeze the red blood cells is by usage of glycerol. Although the usage of cryopreserved red blood cells was promising due to the prolonged storage time and the limited cellular deterioration at subzero temperatures, its usage have been hampered due to the more complex and labour intensive procedure and the limited shelf life of thawed products. Since the FDA approval of a closed (de) glycerolization procedure in 2002, allowing a prolonged postthaw storage of red blood cells up to 21 days at 2–6°C, cryopreserved red blood cells have become a more utilized blood product. Currently, cryopreserved red blood cells are mainly used in military operations and to stock red blood cells with rare phenotypes. Yet, cryopreserved red blood cells could also be useful to replenish temporary blood shortages, to prolong storage time before autologous transfusion and for IgA-deficient patients. This review describes the main methods to cryopreserve red blood cells, explores the quality of this blood product and highlights clinical settings in which cryopreserved red blood cells are or could be utilized.

Key words: cryopreservation, deglycerolization, glycerol, red blood cells, transfusion.

Introduction

The ability to store red blood cells (RBCs) outside the body has been regarded as a life-saving practice for many years. More recently, the usage of refrigerated stored RBCs in transfusion medicine has been under extensive evaluation. During refrigerated storage, RBCs progressively deteriorate [1] and infusion of prolonged stored RBCs has been linked to adverse clinical outcome in terms of postoperative infections, length of hospital stay, multiple organ failure and mortality [2]. Although the majority of these studies are prone to bias due to pitfalls in design and methodology [3], concerns regarding the infusion of prolonged stored RBCs still remains and a restrictive transfusion strategy is currently being favoured [4, 5].

The latter concerns have also revived the interest in RBC cryopreservation. Storage of RBCs at ultra-low temperatures halts the cellular metabolism and subsequently prevents the progressive cellular deterioration that has been linked to adverse clinical outcome. Cryopreservation appeared a promising approach for maintaining RBCs viable for prolonged periods of time. Yet, the clinical applicability of cryopreserved RBCs (commonly known as frozen RBCs) was hampered by the expensive, time-consuming and less efficient nature as well as by the unfamiliarity with the use of cryopreserved RBCs [6, 7]. However, ongoing scientific and technological advancement have made cryopreserved RBCs less expensive, less time-consuming and more utilizable for clinical practice. In this review, the utilization of cryopreserved RBCs in modern transfusion practice will be discussed.

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Cryopreservation methods

Storage of RBCs at ultra-low temperatures ceases the biological activity of RBCs, which enables them to be preserved for prolonged periods of time [8]. In order to minimize freezing damage, cryoprotective additives are pivotal. Along the years, different non-permeating and permeating additives for the cryopreservation of RBCs have been investigated. Non-permeating additives such as hydroxyethyl starch and polyvinylpyrrolidone, as well as a variety of glycols and sugars appeared promising because it was proposed that removal from thawed RBCs prior to transfusion was not required [9]. Yet to date, non-permeating additives have not been licensed or used in clinical practice [6, 7].

The permeating additive glycerol has been used to freeze RBCs since the early fifties. The concentration of glycerol that is necessary to protect the RBCs is dependent on the cooling rate and the storage temperature [10]. At slow cooling rates, ice crystals will form extracellular. As ice forms, the solute content of the unfrozen fraction becomes more concentrated. The resulting osmotic imbalance causes fluid to move out of the RBC and intracellular dehydration, volume reduction and pH changes occurs. At rapid cooling rates however, the RBC cytoplasm becomes super-cooled and intracellular ice formation occurs, which subsequently can lead to mechanical damage (Fig. 1). Glycerol protects the RBCs by limiting ice crystal formation while minimizing solute effects and cellular dehydration during freezing [7].

To date, there are two freezing methods approved for the storage of RBCs [11, 12]. On the one hand, RBCs can be frozen rapidly (i.e. >100°C/min) by the low-glycerol method (LGM). With this method, RBCs are frozen with a final concentration of approximately 20% glycerol (wt/vol) and stored at temperatures below −140°C. On the other hand, RBCs can be frozen slowly (i.e. ~1–3°C/min) by the high-glycerol method (HGM). With this method, RBCs are frozen with a final concentration of approximately 40% glycerol (wt/vol) and stored at temperatures between −60°C and −80°C. Overall, RBC preservation can be extended to at least 10 years if the correct storage temperature is guaranteed.

Frozen RBC units are preferentially thawed in a shaking water bath of about 36 to 42°C. Thawing should be done rapidly in order to prevent ice crystal growth (the so-called recrystallization) upon warming [13]. Once thawed, a deglycerolization washing procedure is performed to reduce the glycerol content in the RBCs prior to infusion. This is necessary, since incomplete deglycerolized RBCs will swell and lyse upon infusion, resulting in high concentrations of free haemoglobin, which may cause renal failure [14, 15]. The deglycerolization washing process also removes detrimental substances such as bioactive lipids, cytokines, potassium and free haemoglobin from the RBC unit, as well as reduces the number of residual leucocytes to 1×10^7 per unit and its immunogenicity [16–19]. Reducing the amount of leucocytes in the RBC unit before freezing is preferable since cytokines and enzymes derived from activated leucocytes can compromise the postthaw quality of RBCs. However, RBC freezing alone will not reduce residual leucocyte counts to the currently recommended EU limit of less than 1×10^6 leucocytes per unit. Buffy-coat depletion before

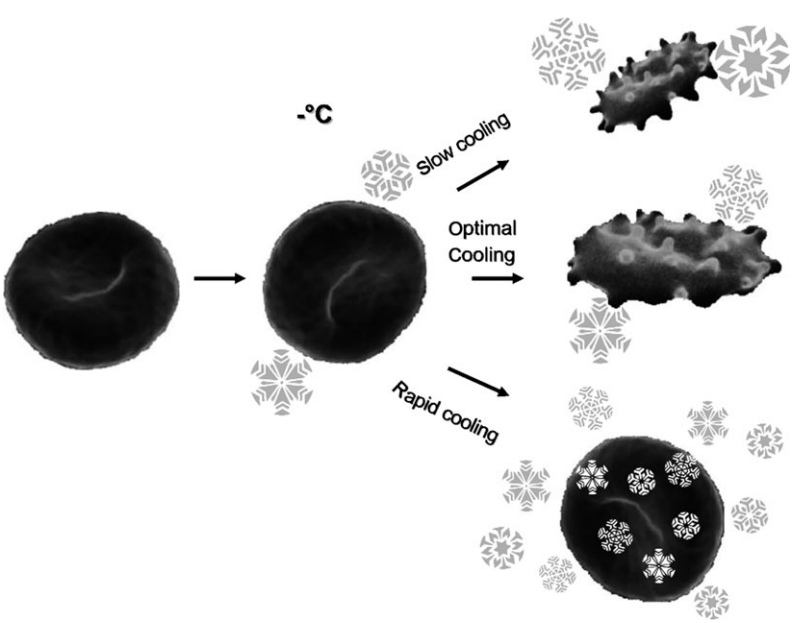

Fig 1 RBC changes in response to cooling rate. The snowflakes indicate the presence of ice crystals in the extra- and/or intracellular environment. Printed with permission of Scott *et al.* [7].

cryopreservation does reduces the leucocyte count to a mean of 1–3 $\times$ 10^6 leucocytes per unit after deglycerolization, but in order to achieve the limits for leucocyte count, leucodepletion by filtration is needed. The deglycerolization washing process does cause some osmotic stress to the RBC which results in cellular losses [20]. This cell loss is more pronounced in the HGM frozen group (~10–20%), because these RBCs require more extensive washing due to the higher glycerol concentration. It has been suggested that this cell loss preferentially involves older RBC populations [21]. Yet, no difference in the density profile (i.e. cell density is associated with cell age) was observed between LGM- and HGM-treated RBCs after deglycerolization or after postthaw storage [20], indicating no selective removal of aged RBCs by the cryopreservation procedure.

The use of cryopreserved RBCs in transfusion medicine was long-time hampered by the limited postthaw storage time. Initially, the open character of the glycerolization and deglycerolization procedure limited the postthaw storage time to 24 h due to the potential risk of bacterial contamination. However, the introduction of an automated closed system to glycerolize and deglycerolize RBCs (Haemonetics ACP-215 device) [22] has extended the postthaw storage time of HGM frozen RBCs and thereby its usability. Nowadays, HGM frozen RBCs can be postthaw stored up to 7 days in saline–adenine–glucose–mannitol (SAGM) solution and up to 14 to 21 days in additive solution-3 (AS-3) [23–26]. Unlike the HGM frozen RBCs, the LGM frozen RBCs cannot be processed in a closed system because the PVC tubing, that is required for sterile connection is not resistant to liquid nitrogen. As a result, LGM frozen RBCs can only be stored up to 24 h postthaw. Furthermore, storage of HGM frozen RBCs at −80°C in mechanical freezers is relatively easy and the temperature can be easily maintained during transportation on dry ice. Although transient warming has raised concerns about the quality of RBC in inventory [27], this issue is negligible for HGM frozen RBCs. HGM frozen cells are not very sensitive to temperature changes and can even be thawed and refrozen with minimal effect on RBC quality [11]. Furthermore, the use of strict protocols and temperature monitoring during both storage and transportation makes the use of HGM frozen RBCs relatively robust. HGM frozen RBCs have also been shown to be more stable than LGM frozen RBCs during postthaw storage [8, 20]. These advantages, together with the possibility to perform the (de) glycerolization process in a closed system, have made the HGM procedure the most applied freezing method for RBC transfusion.

Quality of HGM cryopreserved RBCs

Cryopreservation prolongs the longevity of RBCs. However, once thawed the shelf life of RBCs is limited. The deglycerolized RBCs have to meet certain guidelines, most of which are similar to the guidelines for leucodepleted refrigerated stored RBCs [11, 12]. Yet, these guidelines do not specifically reflect the ability of the RBCs to function after infusion. The duration of frozen storage *per se* minimally attributes to cellular damage [28, 29]. However, refrigerated stored RBCs need to be frozen as soon as possible in order to limit storage-induced lesions. According to the AABB technical manual [11], RBCs collected in citrate–phosphate–dextrose–adenine (CPDA-1) need to be frozen within 6 days, whereas in Europe refrigerated stored RBCs (in SAGM) are preferably frozen within 7 days after collection [12] and fresh leucodepleted whole blood (in CPD) is frozen within 24 h after collection [21, 30]. It is possible to freeze prolonged or outdated refrigerated stored RBCs, provided that the RBCs have been rejuvenated prior to freezing in order to restore the metabolic status (i.e. adenosine triphosphate, 2,3-diphosphoglycerate and haemoglobin p50 values) of the cell [31–33]. After frozen storage and subsequent deglycerolization, outdated rejuvenated RBCs can be refrigerated stored up to 7 days at 2–6°C in AS–3 [22].

During frozen storage, the adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) content is preserved. Yet, the length of prefreezing storage time at 4°C is a predictor of the ATP and 2,3-DPG content after deglycerolization [22, 29]. The RBC ATP content is important for the overall functioning of the cell. Loss of ATP has been associated with rigid cell membranes, echinocyte shape change, exposure of phosphatidylserine on the RBC surface, loss of vasodilatation control and decreased RBC viability [34–38]. Similar to refrigerated stored RBC, the ATP content of cryopreserved RBCs gradually declines during postthaw storage at 4°C.

Binding of 2,3-DPG to haemoglobin induces a conformation state which facilitates the release of oxygen from the haemoglobin *in vivo*. Loss of 2,3-DPG *in vivo* will increase haemoglobin‘s affinity for oxygen, which may hamper the oxygen delivery to the tissues. A considerable loss of 2,3-DPG content is observed already after 1 week of refrigerated RBC storage [1]. By limiting the prefreezing storage time, high 2,3-DPG concentrations can be obtained postthaw [39]. During postthaw storage, the 2,3-DPG concentration declines similar to the 2,3-DPG concentration observed in refrigerated stored RBC [1]. Nevertheless, the RBC 2,3-DPG content is replenished *in vivo* within hours following infusion [40, 41] and transfusing

RBCs with low 2,3-DPG content has not been shown to affect tissue oxygenation [42].

The ability of RBCs to deform, aggregate and adhere to the vascular endothelium (rheologic properties) are important determinants of the oxygenation of the microvascular environment [43]. Alterations in the RBC rheologic properties may hinder or obstruct the blood flow in the microcirculation, leading to impaired tissue perfusion, ischaemia or even infarction [44–46]. In the microcirculation, the ability of RBCs to deform enables these cells to adapt their size to squeeze through narrow capillaries. A high RBC flexibility and a rapid recovery to the normal shape are essential factors for maintaining adequate tissue perfusion and cell survival [47, 48]. The HGM procedure was shown not to be detrimental to the deformability of RBCs [49].

Red blood cells are able to form linear aggregates (Rouleaux) or more complex three-dimensional aggregates in regions with low shear flow or at stasis. Under normal physiological conditions, the rise in blood flow rate is sufficient to disperse these aggregates. However, in certain pathological conditions stronger and or larger aggregates are formed which are more resistant to dispersion by the blood flow [50]. Enhanced aggregation has been observed in a variety of pathological states such as cardiovascular disease, diabetes mellitus, renal failure, sepsis, thalassaemia and sickle cell disease [45, 51, 52]. In contrast, RBCs with low aggregation tendencies could be less beneficial in directing leucocytes and possibly platelets to the damaged vessel wall [53]. The HGM procedure does affect the aggregation profile of RBCs; deglycerolized RBCs have lower aggregation tendencies compared to fresh and refrigerated stored RBCs [49]. Presumably, the increased RBC volume after deglycerolization suppresses cell contact and subsequently aggregation formation [54]. RBC swelling is most likely to be reversed upon transfusion due to the high colloid osmotic pressure of plasma [55].

The adherence of RBCs to vascular endothelium is under physiological conditions negligible. Yet, structural changes in the RBC membrane may promote adherence to endothelial cells (EC) and impair the microcirculatory blood flow [56, 57]. Phosphatidyl serine (PS) expression on the RBC surface mediates adherence of RBCs to ECs and triggers RBC clearance from the circulation by macrophages [58, 59]. In contrast, surface expression of CD-47 prevents normally that RBCs are being engulfed by macrophages [60, 61]. In general, PS exposure and loss of CD-47 expression on the RBC surface as well as membrane microvesiculation are all determining factors for the life span of the RBCs [62, 63]. One day postthaw, the HGM frozen RBCs show no significant changes in PS exposure, CD-47 expression and membrane microvesiculation compared to prefreeze levels when the prefreezing storage time is limited to 2–3 days. However, PS exposure and microvesiculation increases when longer prefreezing storage times (13–14 days) are used [39].

The osmotic fragility of RBCs reflects the membrane's ability to maintain structural integrity. Although the osmotic fragility test is not reliable in predicting the function of RBCs, it is still an effective measure to demonstrate the susceptibility of RBCs to osmotic stress [64]. Cryopreserved RBCs show a higher osmotic fragility compared to fresh and refrigerated stored RBCs [29, 49]. The freeze-thaw process alters the RBC cation homoeostasis, which results in cell swelling and subsequently alterations in cell morphology [21, 25, 65]. Probably, the higher cell volume makes the RBCs more prone to haemolysis upon concomitant swelling when subjected to osmotic stress in the osmotic fragility test [64, 66].

Recently, it was shown that transfusion of cryopreserved RBCs, which were stored for 2 days in SAGM, increases the level of extravascular haemolysis 2 h after transfusion. This was shown by the increased levels of transferrin bound serum ion [67]. Nevertheless, the transferrin saturation level increase was still 8–9 times lower compared to that of volunteers who were transfused with 42-day refrigerated stored RBCs, indicating that upon transfusion cryopreserved RBCs haemolyse less than prolonged refrigerated stored RBCs. In addition, cryopreserved RBCs have a high 24-h *in vivo* survival [23, 24], which indicates that these blood products can be used safely. It was even indicated that cryopreserved RBCs have superior tissue oxygenation and biochemical profile compared to refrigerated stored RBCs [68, 69].

Utilization of cryopreserved RBCs

Cryopreserved RBCs are primarily used in situations where the RBC availability is limited or unpredictable. Yet, cryopreserved RBCs may be useful in a variety of clinical settings.

Storage of rare RBCs

Cryopreservation has been used to store RBCs with rare blood phenotypes. Although the definition of 'rare' varies from country to country, in general, a blood group is regarded as rare if the RBC phenotype has a frequency of approximately 1 in 100–1000 or less in the general population [70]. Refrigerated stored RBCs are normally shipped to the glycerolization–freezing location and frozen as soon as possible, ideally within 6 to 7 days after donation. Cryopreservation of RBCs even beyond the regulated expiration date is still possible for exceedingly rare RBC phenotypes, provided that the RBCs have been rejuvenated prior to freezing [7].

Over the years, numerous countries in Europe, America and Asia have set up frozen rare RBC banks [70–72], usually frozen with the LGM method. In Europe, the blood banks in for instance Amsterdam, Birmingham and Paris house a large collection of cryopreserved rare RBC units. Most cryopreserved rare RBC units are for national use. Nevertheless, when no compatible blood can be found via the national blood banks, it is general practice to appeal to countries abroad and the rare deglycerolized RBC unit is shipped at 4°C to the location of transfusion.

Although usage of cryopreserved rare RBCs has been lifesaving to a variety of patients, its usage is costly and international shipment has been cumbersome due to the limited postthaw storage time of LGM frozen RBCs. Because of the ability to perform the (de) glycerolization procedure in a closed system, thereby prolonging the postthaw outdating period, most blood banks are currently using the HGM method for the cryopreservation of rare RBCs. The longer outdating period makes cryopreserved rare RBCs more utilizable for clinical practice.

Military blood bank

In military combat massive blood loss is a major cause of death [73, 74]. Having RBCs, plasma and platelets available in the military theatre at all times is therefore of vital importance. An inventory of RBCs is difficult to maintain in combat areas due to the unpredictable demand and the limited shelf life of refrigerated stored RBCs. Yet, cryopreserved RBCs are a valuable blood resource due to the prolonged storage times. HGM cryopreserved RBCs have been used in the military theatre since the Vietnam War [75, 76]. Although back then the processing of cryopreserved RBCs was still in its early stages, it was already concluded that usage of cryopreserved RBCs in combat casualty care was technically feasible and clinically acceptable. Ongoing scientific and technological advancement (especially the introduction of the closed system processing in 2002) have made cryopreserved RBCs become a more utilized blood product in modern military operations [8, 30, 77, 78]. The Dutch military has been using HGM cryopreserved RBCs and −80°C frozen plasma and platelets in theatre operations with great success [30, 79]. In the period of 2006–2012, a total of 2175 cryopreserved RBC units have been transfused in Afghanistan without any shortages or transfusion reactions reported [80]. Hence, the Dutch military blood bank uses only type-O leucodepleted RBCs in the theatre, in order to improve the effectiveness and decrease the possibility of ABO mismatches. The absence of transfusion reactions may be related to the fact that whole blood is leucodepleted, and RBCs are frozen within 24 h after donation [30, 78].

Cryopreserved RBC units are transported to combat areas in insulated boxes filled with dry ice. These RBCs are frozen in polyvinyl chloride plastic (PVC) bags, packed in a vacuum-sealed over-wrap bag and stored in rigid cardboard boxes. Using this, the breakage incidence of cryopreserved RBC units subjected to transportation has been reduced to 0·4% [80]. Subsequently, all in theatre storage, thawing and washing procedures are performed in a temperature-controlled environment. Thawed and washed RBCs are ultimately suspended in AS-3 and can be refrigerated stored at 2–6°C for a maximum of 14 days.

Usage of cryopreserved RBCs in combat casualty care could help to improve inventory control. As shown by the Dutch military 41% of the refrigerated stored RBCs and 51% of the cryopreserved RBCs transported to Afghanistan were transfused, which is much more efficient compared to the missions in the Gulf War, Bosnia and Kosovo, where, respectively 0·3, 1·7 and 15% of the refrigerated stored RBCs provided were transfused by the US military [76, 80]. This is because cryopreserved RBC units can be prepared either on demand or in advance, thereby providing a continuous RBC supply independent of logistics. Freshly thawed cryopreserved RBCs may even be advantageous since its availability within 90–120 min can prevent that massively transfused patients are only transfused with old RBC units. Notably, the US military have increased their logistics to the operational medical treatment facilities in 2008 to decrease the average age of refrigerated stored RBC from 32 days to 23 days for the treatment of severely bleeding trauma patients [81].

Blood shortages

Blood shortages due to natural or civil disasters, as well as due to seasonal shortages, can pose a major health challenge. A main strategy of the emergency procedures of blood centres is to mobilize stocks of refrigerated stored RBCs through coordination? with nearby blood centres [82]. Usually, blood centres will have a 2- to 3-day supply of refrigerated stored RBC units on hand. This strategy can compensate for RBC shortages, as long as the local stocks of the nearby blood centres are replenished appropriately. After a disaster, the influx of blood donations is often increased because of the altruistic response of the public. Although this influx of blood donations potentially could be used to replenish local stocks, part of the donated blood is often not used due to a higher frequency of positive screening tests in first-time donors and due to inadequately processing procedures [83, 84]. Therefore, blood centres that do send refrigerated stored RBC units could face the risk of becoming under-supplied themselves.

Managing a frozen RBC reserve can be useful in emergencies scenarios. Cryopreserved RBCs could serve as a bridge-over supply during short-term RBC deficits (due to redirecting of cryopreserved RBCs to emergency hospitals) until support by the blood centres are re-established. Despite the complexity and costs of implementing and maintaining a frozen RBC reserve, it is advocated that the benefits of self-sufficiency outweighs the disadvantages [85].

Autologous transfusion

Cryopreserved RBCs have occasionally been used in preoperative autologous transfusion practices [86]. In general, preoperative autologous RBC transfusion offers advantage above allogeneic RBC transfusions in that it prevents immunosuppression and infectious disease transmission, while it might reduce postoperative infections and subsequently length of hospital stay [87, 88]. During the last couple of years, the use of preoperative autologous RBCs has been questioned and its demand has declined. This is predominantly due to the improved safety of allogeneic RBCs, as well as due to the organizational and logistical constraints, the higher disposal rate and the more costly nature associated with autologous RBCs transfusions [89]. Also, the beneficial effect of preoperative autologous RBC transfusion has been compromised by the short-time interval between the last donation and the planned surgical procedure. As a result, patients often develop anaemia before the surgery and are more likely to receive a RBC transfusion [89, 90]. In order to avoid anaemia, RBCs need to be harvested months in advance of the expected use so that the haemoglobin level of the patient can be restored. Yet, prolongation of the time period between the last donation and the surgical procedure is hampered by the short storage time of refrigerated stored RBCs. In contrast, cryopreservation enables storage of RBCs for years, which allows RBCs to be donated far in advance of the surgical procedure [91] without affecting its quality. Although the benefit of preoperative autologous RBC transfusions is currently questioned, new pathogens keep emerging which could potentially threat the safety of allogenic RBC transfusions [92, 93] and the implementation of cryopreservation could make preoperative autologous RBC transfusions become a viable alternative.

Usage of washed RBCs

Cryopreserved RBCs may also be helpful as part of the routine RBC inventory because of its proposed advantages over prolonged refrigerated stored RBCs. Hence, transfusion of prolonged refrigerated stored RBCs is associated with occurrences of transfusion related acute lung injury (TRALI) and systemic inflammatory response syndrome (SIRS) [6, 94]. Although the aetiology of TRALI and SIRS remains incompletely understood, it is recognized that substances which accumulate in the supernatant of refrigerated stored RBCs are involved in the pathogenesis of these syndromes [95, 96]. Ultimately, these syndromes will lead to an increased hospitalization and subsequently higher burden on the healthcare costs. In contrast, washed (cryopreserved) RBCs contain hardly any plasma and biologically active substances in the supernatant, which make them an ideal blood product to prevent TRALI and SIRS [94].

Washed RBCs are also useful for patients with immunoglobulin A (IgA) deficiency. These patients usually have undetectable IgA and high titre of class-specific anti-IgA. Transfusion of only a small amount of RBCs in these patients can cause severe anaphylactic reactions due to the presence of residual IgA [97]. Since cryopreserved RBCs are extensively washed postthaw, these blood products are ideal for patients with IgA deficiency.

Cryopreserved RBC in standard routine?

One of the challenges with understanding the current utilization of cryopreserved RBCs has been the lack of reported data. Most reports have focused either on the military use of cryopreserved RBCs or on the cryopreservation method, and blood use or clinical data is often not reported. Nevertheless, in situations of disturbed logistics, austere environments or to reduce immunosuppression after infusion, cryopreserved RBC could serve as a viable alternative for refrigerated stored RBC in clinical practice.

Initially, cryopreserved RBCs have been infrequently implemented in transfusion medicine because of the more expensive nature of this preservation method. Although cryopreserved RBCs are more expensive, the cost difference with regard to refrigerated stored RBCs is often overrated. This is because the costs of treating and managing adverse events of refrigerated stored RBCs are not taken into account, indicating that the total cost of a refrigerated stored RBC unit would be higher than currently is represented [98].

During hypothermic storage, red cells progressively age and become less functional. Although RBCs are allowed to be stored for 42 days at 2–6°C, it has been suggested to shorten the shelf life to 14 days [94]. The use of cryopreserved RBCs besides refrigerated stored RBCs, could be very useful in managing the inventory crisis that could occur due to a restriction of the storage age of refrigerated stored RBCs. Also, the use of cryopreserved RBCs may expand as a result of a change in RBC supply and demand, owing to a shift of increasingly older patient

population [99, 100]. Frozen storage would allow RBCs to be stored beyond the expiration date, which will limit the wastage rate and mitigate the effects of sudden and predicted increases in RBC demand.

Conclusion

Cryopreserved RBCs have been used in transfusion practice since the early sixties. Initially, these transfusion products were resource intensive. However, during the years, scientific and technological advancement have made cryopreserved RBCs more utilizable for clinical practice. In particular, the introduction of the sterile (de) glycerolization processing of HGM cryopreserved RBC in 2002 has eased the clinical applicability of cryopreserved RBCs. To date, cryopreserved RBCs are still infrequently implemented in transfusion medicine. Yet, cryopreserved RBCs could be useful in a variety of clinical settings, as have been demonstrated in the military setting for the treatment of trauma patients. Overall, cryopreserved RBCs are safe, in compliance with international regulations and guidelines and can be used effectively, which may now inspire clinical settings to implement cryopreserved RBCs in the support of their routine inventory.

Conflict of interest

The authors declare no conflict of interests.

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