Transfusion of rare cryopreserved red blood cell units stored at −80°C: the French experience

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The technology allowing freezing of RBC units has been available for many decades. The high-glycerol method for RBC storage at −80°C is predominantly used. Several studies have shown satisfactory results regarding the in vitro viability and function of cryopreserved RBCs. RBC freezing is nowadays mostly encountered in rare blood programs and military deployments. Preservation time of frozen RBCs appears to be virtually indefinite, but most countries apply a 10-year outdate. There is no mandatory time restriction in France. The National Rare Blood Bank currently includes 962 (17.5%) RBC units aged 10 years or more and 153 (2.8%) aged 20 years or more. Since 1994, 1957 RBC units have been thawed and transfused, among which 118 were aged 10 years or more and 8 were aged 20 years or more. Discarding RBC units older than 10 years may be highly sensitive for very rare blood groups, e.g., U−, of which approximately 30 percent of the cryopreserved units are aged 10 years or more. However, the lack of nucleic acid testing for HIV and HCV may be problematic for old RBC units drawn from donors who were not subsequently tested for these markers, which is now mandatory in most countries. Regarding the 118 transfused RBC units older than 10 years, no evidence of hemolysis of thawed RBCs and no transfusion reaction, clinical or biologic hemolysis, or transfusion ineffectiveness was reported, either by any of the parties involved in the transfusion supply of rare RBC units or through the French hemovigilance program, which requires a mandatory report of any transfusion reaction. It has recently been suggested to extend the 10-year restriction in some countries. Considering our experience and observational data, we may consider it safe and efficient to transfuse rare frozen RBC units older than 10 years. An international consensus for RBC cryopreservation time should ideally be established. *Immunohematology* 2009;25:13–17.

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Humans have experimented with blood transfusions for more than 300 years and attempted to preserve human blood since the early 1900s. The first modern approach to blood storage, with a citrate-glucose solution, was stimulated by World War I. Long-term storage of RBCs using glycerol as a cryopreservative was initially implemented in the early 1950s. Two methods have been described: the low-glycerol method, for freezing units in liquid nitrogen at −196°C, and the high-glycerol method for storage of RBCs at −80°C. The technology that is currently predominantly used is the high-glycerol technique, which was initially developed in the 1960s and early 1970s by the American Red Cross Research Laboratory (Washington, DC) and the U.S. Naval Blood Research Laboratory (Boston, MA).

The technology allowing freezing of RBC units has been available for more than four decades. In addition to long-term storage, RBC cryopreservation has other advantages, which are mainly related to the necessary washing procedure for glycerol removal once units are thawed. Indeed, washing of thawed RBCs eliminates most cell debris, WBCs, platelets, cytokines, residual plasma, and free hemoglobin. However, RBC cryopreservation has never reached the expected popularity; it is still infrequently used, especially because of the labor-intensiveness and expense of the procedure, as well as the RBC loss through the process and limited shelf life of thawed RBC units. As a result, stockpiling of frozen inventory RBCs is today mostly encountered in rare blood program management and military deployments.

**Organization of the Rare Blood Program in France**

French regulations stipulate that a blood group is regarded as rare if its prevalence is 4 in 1000 or less (i.e., ≤1 in 250) in the general population. A national rare blood donor database was implemented in our country in the late 1960s to ensure the transfusion and obstetric safety of patients with rare blood phenotypes. A national rare blood bank was set up in the early 1980s. Potential rare blood donors from the national database are highly encouraged to donate blood on a routine basis for the National Rare Blood Bank. This facility is located in Créteil (a few kilometers from Paris) and is comanaged by the National Institute of Blood Transfusion (INTS, Paris) and the French Blood Establishment (EFS Ile de France, Paris). The confirmation of the rare blood type, registration of new people with a rare blood group in the national database, and selection and delivery agreement of rare blood units are exclusively carried out by the National Reference Laboratory for Blood Groups (CNRGS, Paris), a department of the INTS, Paris. To date, 9508 individuals (patients and donors) are enlisted in the national registry of people with rare blood phenotypes or genotypes.

Rare blood units from the National Rare Blood Bank are frozen according to the Cohn method, using glycerol in an ionic medium to achieve a final glycerol concentration of 40 percent (wt/vol), the so-called high-glycerol method. RBCs are subsequently stored at a mean temperature of −80°C.
(range –65° to –90°C). Since late 2005, the Haemonetics Automated Cell Processor (ACP) 215 closed-circuit instrument (Haemonetics Corp., Braintree, MA) has been systematically used to glycerolize and deglycerolize human RBCs, allowing for a 7-day shelf life for thawed units when stored at 2° to 6°C in a saline-adenine-glucose-mannitol (SAGM) additive solution. Older RBC units are handled with the Cobe 2991 Cell Processor (COBE BCT, Lakewood, CO), with a 24-hour shelf life for thawed units resuspended in SAGM. According to the French current rules, the quality control of the thawed RBC units consists in the hemoglobin measurement (≥235 g/unit), hematocrit determination (50% to 80% for RBC units handled in an open circuit, 40% to 70% for those handled in a closed circuit), extracellular hemoglobin measurement (≤1.2% of total hemoglobin), and extracellular glycerol measurement (≤1 g/unit). Both open-circuit and closed-circuit technologies have been validated. It was shown for the open-circuit and closed-circuit procedures that the postthawing mean extracellular hemoglobin was 0.21 percent (0.06% to 0.57%) and 0.16 percent (0.11% to 0.25%), and the extracellular glycerol was 0.14 g (0.02 to 0.73 g) and 0.15 g (0.11 to 0.19 g), respectively. Random sampling is used for the routine quality control of the thawed units according to the NF-ISO-2889 standard.

The National Rare Blood Bank currently includes 5503 cryopreserved blood units, from 1630 blood donors. From 2001 to 2005, an annual average of 161 rare blood units were transfused (i.e., 2.53 per 10^6 inhabitants per year) for 2001 to 2005, an annual average of 161 rare blood units were transfused (i.e., 2.53 per 10^6 inhabitants per year) for an average annual average of 28 patients and 70 transfusion episodes; 149 rare blood units were transfused in 2006 and 236 in 2007.

The Preservation Time of Rare Blood Units

Rare blood, by its very nature, is considered a rare resource, and its long-term storage is necessary. The preservation time of frozen RBC units appears to be virtually indefinite. However, the question of a maximum period of validity has often been raised. In September 1987, the Food and Drug Administration in the United States approved the change from a 3- to a 10-year outdated period for glycerol-frozen RBCs stored at –80°C. Most other countries, e.g., China and South Africa, currently apply this 10-year outdated. The European guidelines from the Guide to the Preparation, Use and Quality Assurance of Blood Components state that storage of frozen RBC units is possible for at least 10 years, provided that an adequate temperature can be guaranteed (–60° to –80°C for the high-glycerol cryopreservation procedure). The Technical Manual of the AABB, 16th edition (2008), states that “frozen RBCs must be stored at temperatures colder than –65°C and will expire after 10 years. Rare frozen units may be used beyond the expiration date, but only after medical review and approval that are based on the patient’s needs and the availability of other rare compatible units.”

Several studies have been performed to investigate the in vitro recovery and quality of deglycerolized RBCs. Valeri et al. reported that RBCs frozen with 40 percent to 45 percent (wt/vol) glycerol could be stored at –80°C for up to 37 years, with mean in vitro freeze-thaw-wash recovery values of 75 percent, without affecting the RBC 2,3 DPG level and with hemolysis after 24 hours of postwash storage at 4°C usually less than 1 percent. All units tested were sterile. Moreover, frozen RBC units that were stored for as long as 21 years and subsequently thawed, washed, and stored at 4°C for 24 hours have been shown to demonstrate an in vivo 24-hour posttransfusion survival value greater than 75 percent. In 2004, a similar study was performed to investigate the in vitro quality of RBCs frozen with 40 percent (wt/vol) glycerol at –80°C for 14 years, deglycerolized with the Haemonetics ACP 215 instrument, and stored at 4°C in AS-1 or AS-3 for up to 3 weeks. Acceptable results were achieved (mean in vitro recovery value of 80 ± 7%), similar to those previously described, although deglycerolized RBCs in AS-1 exhibited significantly higher hemolysis than those in AS-3 after storage at 4°C for 7 to 21 days. Another study in 2004 examined RBC units that had been cryopreserved in 40 percent (wt/vol) glycerol and stored at –80°C for up to 22 years. Postthawed RBCs had acceptable mean freeze-thaw-wash recovery and normal oxygen transport function, RBC morphology, RBC indices, methemoglobin, and osmotic fragility. Interestingly, it was also shown that the in vitro viability and function of cryopreserved RBCs was not dependent on the length of frozen storage or post-thaw storage at 4°C, but was influenced by the length of storage at 4°C before cryopreservation. The more quickly the RBC units are frozen, the better are the in vitro viability and function of the thawed RBCs.

Despite the conclusions from these different studies advocating the possibility of storing RBCs well over 10 years, the 10-year outdated has remained since 1978 in the United States and is still largely applied in rare blood banks throughout the world.

Storage and Transfusion of Frozen RBCs Beyond 10 Years: The French Experience

Age of frozen RBC units and thawed transfused RBC units: statistical data

Between 1994 and 2007, a mean of 628 rare blood units per year were frozen in the National Rare Blood Bank. After blood donation, RBC units are frozen as rapidly as possible, ideally within 7 days. However, freezing blood up to the 42-day maximum period of validity is still possible for exceedingly rare types. Since 2004, 13.2 percent of RBC units have been frozen within 7 days of blood donation, 88.2 percent within 14 days, and 97.0 percent within 21 days.

Despite European recommendations, no official mandatory time limit regarding the validity period of rare frozen RBC units exists in France. The National Rare Blood Bank...
currently includes 962 (17.5%) RBC units aged 10 years or more and 153 (2.8%) aged 20 years or more (Fig. 1). The average age of rare frozen RBC units is 6.1 years (maximum 33.5 years). Since 1994, 1957 rare RBC units have been thawed and transfused, among which 118 (6.0%) were aged 10 years or more and 8 (0.4%) aged 20 years or more (Fig. 1). The average age of rare transfused RBC units is 3.6 years (maximum 23.8 years).

Fig. 1. Age of frozen RBC units in the National Rare Blood Bank and age of transfused rare RBC units.

Rare RBC units older than 10 years

Discarding rare blood units older than 10 years may be, ethically speaking, highly sensitive, as there are usually no alternative resources for the specified patients. If we decided to discard all rare blood units aged 10 years or more, this would represent the destruction of 17.5 percent of the current French rare frozen RBC stock, which could be very problematic for some rare blood phenotypes. For example, 240 Vel– (VEL:-1) rare RBC units are now cryopreserved, among which 64 (26.7%) are aged 10 years or more. For the Kp(b–) rare specificity (KEL:-4), 75 (32.8%) are aged 10 years or more. For the U– rare specificity (MNS:-3,-4,-5), which is quite frequently requested in France for patients of Afro-Caribbean ancestry suffering from sickle cell disease, 3,7 113 (29.7%) are aged 10 years or more.

Long-term preservation of rare RBC units and nucleic acid testing in blood donors

A specific problem for long-term preservation of rare RBC units concerns testing for mandatory markers for transfusion-transmitted diseases according to the current national regulations. In France, the current mandatory and systematic serologic markers are anti-HIV-1/anti-HIV-2, anti-HCV, AgHBs and anti-HBc for HBV, and anti-HTLV-I/anti-HTLV-II. In addition, nucleic acid testing (NAT) for HIV and HCV has been required since July 2001. For either serologic markers or NAT, RBC units drawn before their respective official implementation date are considered valid, provided that a blood donation or blood sample has been subsequently tested, according to a so-called retroactive process. In our country, however, no testing is performed for that purpose on fresh samples from a biologic resource center. Presently, 4562 (82.9%) of the RBC units stored within the National Rare Blood Bank have been tested for all legal mandatory markers, including NAT, either in real terms or retroactively. RBC units confirmed by a retroactive NAT testing are considered fully valid for transfusion according to the French regulations. Another issue is the 941 (17.1%) rare RBC units for which the corresponding donors have never been subsequently tested for NAT. RBC units aged 10 years or more represent 54.9 percent of these 941 blood donations. These RBC units may also be used for transfusion, provided there is no alternative solution. Our current policy, however, is to progressively discard such units that were drawn from donors who were never subsequently tested for HIV NAT and HCV NAT. Nevertheless, this attitude requires a very special care for RBC units with an exceedingly rare phenotype, e.g., Jk(a–b–) (JK:-1,-2), Hr– (RH:-18), Hr– (RH:-34), Hy– (DO:-4), or Jr(a–). There are no specific rules in France about an optimal inventory for a given rare blood type. However, the following criteria are usually taken into account when a decision has to be made about the destruction of rare RBC units from a donor who was never tested for NAT: alive or deceased blood donor; prevalence of the rare blood phenotype within the general population; and number of available frozen RBC units from other donors with the same rare blood group, including D, C, E, c, k, Fy/a/Fy/b, Jk/s/Jk/b, S/s antigen match. If we consider the example of the Jr(a–) rare blood group, nine Jr(a–) blood donors are known in France, and the current stock of Jr(a–) rare blood units is 15, among which 6 units (40%) are aged 10 years or more. HIV and HCV NAT have never been performed in five of these nine Jr(a–) donors, which affects 9 (60%) of the 15 Jr(a–) blood units. As a result, it does not seem reasonable to discard 60 percent of the Jr(a–) RBC units from the national stock, considering the highly sensitive ethical aspect of such a decision.

Rare RBC transfused units older than 10 years and the French hemovigilance data

Between 1994 and 2007, 118 RBC units older than 10 years were transfused. For every transfusion episode that involved an RBC unit aged 10 years or more, the CNRGS medical staff questioned the National Rare Blood Bank to learn whether a macroscopic hemolysis of the RBC supernatant was visible after the thawing-washing procedure. In addition, no extracellular hemoglobin measurement greater than 1.2 percent of the total hemoglobin (threshold corresponding to the current French guidelines) has been found to date for thawed RBC units, according to the quality control scheme of the National Rare Blood Bank. The CNRGS staff has also asked whether the posttransfusion
hemoglobin rise in the recipient was compatible with the one expected and whether there was any reported clinical or biologic transfusion reaction. Regarding the transfusion of the 118 units that were older than 10 years, no evidence for hemolysis of thawed RBCs and no transfusion reaction, clinical or biologic hemolysis, or transfusion ineffectiveness was reported that could be related to long-term storage beyond 10 years.

Transfusion of RBCs older than 10 years has always been legal in France. As a consequence, it did not appear necessary to perform a full prospective or retrospective study justifying the possible use of such RBCs. However, we may consider our observational data about the transfusion safety and efficiency of RBC units older than 10 years reliable for two main reasons.

The first reason lies in the close communication among all parties involved in the transfusion supply of rare RBC units, probably much stronger and closer than that of a standard blood transfusion. Indeed, every single rare RBC unit, delivery of which is exclusively authorized by the CNRGS, is strictly time-followed. The patient is carefully and closely monitored, and the transfusion outcome is subsequently discussed between the medical staff in charge of the patient and the CNRGS staff.

The second reason is the presence of an active hemovigilance program in France since 1994, the rules of which are clearly defined according to the French regulations. The report of every adverse transfusion reaction by anyone within the transfusion chain is strictly mandatory, whatever the intensity, seriousness, and imputability of the reaction. A transfusion reaction is defined in France as an “unexpected or undesired event, due or susceptible to be due to the transfusion of a blood product.” The precise list of the clinical or biologic manifestations that may occur during or after a blood transfusion and that must lead to a hemovigilance report is legally stipulated. Any unexpected or undesired clinical manifestation has to be reported. These include hemoglobinuria; positive direct antiglobulin test; hemolytic anemia; and failure of expected hemoglobin increment, estimated through the hemoglobin measurement performed within 24 hours after transfusion.

Between 1994 and 2007, no transfusion reaction was reported for any of the 118 rare RBC units older than 10 years that were transfused. As a result, our observational data may provide new arguments for the safe and efficient use of very long-term cryopreserved RBC units.

**Conclusions**

Between 1994 and 2007, 118 rare blood units aged 10 years or more (6.0%) and 8 (0.4%) aged 20 years or more were transfused in France. The maximum age of a rare RBC transfused unit was approximately 24 years. No transfusion reactions potentially linked to the long-term preservation of blood units older than 10 years were reported, either by the medical staff involved in the careful monitoring of the patients or through the French hemovigilance program, which requires a strict mandatory report of every transfusion adverse reaction, whatever its intensity or imputability. It has been quite recently suggested to extend the 10-year restriction for frozen RBC units in the United States. Based on our experience and observational data, we may consider it to be safe and efficient to transfuse rare frozen RBC units older than 10 years. Such a policy allows keeping a larger stock of frozen RBC units for the rarest phenotypes, to urgently meet the national or even international requests for rare blood. However, the question of a maximum time limit for rare frozen blood storage is still raised. Indefinite storage, theoretically possible, may be difficult because of ever-growing quality management requirements and constraints. The period of validity of rare frozen RBC units should ideally be the same for all rare blood banks throughout the world. This point deserves consideration by the ISBT Working Party on Rare Donors to establish an international consensus.

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