

Old blood, new blood or better stored blood?

Giancarlo Maria Liumbruno¹, James P AuBuchon²

¹*UOC di Immunoematologia e Medicina Trasfusionale, Ospedale "San Giovanni Calibita" Fatebenefratelli, Roma, Italy*

²*Puget Sound Blood Center, Seattle, Washington, USA.*

Blood transfusion has controversial origins shrouded in legend. In ancient times blood was considered "vital lymph" - "*sanguis vita vitae*" - and was probably used as a potion or a tonic, and already the possibility of transferring it from one body to another for therapeutic or rejuvenating purposes had been taken into consideration¹. However, it was only in 1628, the year in which the circulation of blood was scientifically first demonstrated, that transfusing blood became a concrete possibility. The subsequent "experimental" period, a mixture of successes and failures, extended from 1628 to the end of the XVII century and was followed by the so-called "therapeutic" period from the mid 1800s to the present¹. After the anatomic demonstration of blood circulation, nearly 300 years passed before Karl Landsteiner discovered blood groups, the pharmacologist Luigi Sabbatani discovered the anticoagulant properties of sodium citrate¹, and, in 1915, the first red blood cell (RBC) storage solution (a mixture of citrate and glucose) was developed for storing rabbit RBC for use in a heterophile agglutination test for syphilis².

Another revolution in transfusion therapy made it possible to use containers for blood storage^{1,3,4}, although even as far back as the XVII century blood had been stored after adding rectified spirit of wine to it and sealing its container hermetically⁵.

The subsequent history of RBC storage solution development has been one of slow and gradual progress⁶. RBC are still the most widely transfused blood component throughout the world and their story is closely linked with the history of transfusion medicine and the changes in the collection and storage of blood^{6,7}. At present, the most widely used protocol for the storage of RBC (for up to 42 days) is the collection of blood into anticoagulant solutions

(typically citrate-dextrose-phosphate). The red cell concentrates are stored at $4 \pm 2^\circ$ C in a slightly hypertonic additive solution, generally SAGM (sodium, adenine, glucose, mannitol, 376 Osm/L) or a similar solution⁶. Despite this, a definitive protocol that reconciles long-term storage on the one hand and safety and efficacy of the transfusion therapy on the other is still the subject of intense debate and discussion⁸.

Throughout the history of medicine, the beneficial outcomes of allogeneic blood transfusion have been coupled with a wide range of adverse reactions^{9,10}. A recent study in animal models seemed to show that the transfusion of fresh blood is less harmful than the transfusion of stored blood in the context of progressing malignancies¹¹ and a murine RBC storage and transfusion model suggested that the transfusion of older stored RBC produces harmful effects mediated by the pro-inflammatory response associated with increased levels of tissue and circulating iron and with its pro-oxidant effects¹².

Several retrospective studies have reported an increased rate of morbidity and mortality associated with the transfusion of "older" RBC. This increase has not been definitively proven by either recent in-depth analyses of the data from these studies^{7,13} or by randomized prospective studies. The last added study to the sheer volume of publications, a cohort study on 404,959 transfusion episodes conducted using the Scandinavian Donations and Transfusions database, showed a slight excess of mortality in recipients of the oldest RBC, but the risk pattern was more consistent with weak confounding than with an effect of the momentary exposure to stored RBC¹⁴. Although it remains to be determined whether increased storage time *causes* a poorer outcome in the recipient, the storage lesions accumulating over 42 days are unlikely

to be beneficial to recipients. Notwithstanding this, four large randomised clinical trials are now planned or under way in three different populations of patients. The Age of Blood Evaluation (ABLE) study, supported by the Canadian Institutes of Health Research (CIHR), is randomising about 2500 intensive care unit patients to receive, if transfused, either less than 8-day old RBC or standard-issue RBC (2-42 days)¹⁵. In an analogous study, the Age of Red Blood Cells in Premature Infants (ARIPI), also funded by the CIHR, 450 premature infants (=1250 g) will be randomised to receive aliquots of either less than 8-day old RBC or standard-issue RBC (2-42 days)¹⁶. The Red Cell Storage Duration and Outcomes in Cardiac Surgery is randomising 2800 cardiac surgery patients who are 18 years or older to receive, if transfused, RBC that are either less than 14 or more than 20 days old¹⁷. Finally, the National Heart, Lung and Blood Institute Transfusion Medicine and Haemostasis Network Red Cell Storage Duration Study (RECESS) plans to randomise approximately 1800 cardiac surgery patients to receive, if transfused, RBC units that have been stored for 10 days or less or units that have been stored for 21 days or more¹⁸. In addition, several other research teams will use animal models and physiological studies to learn more about storage lesion and what changes RBC undergo during storage, as well as whether these changes affect the blood vessels and tissues once the RBC have been transfused¹⁹.

A brief list of the elements of the so-called "red blood cell storage lesions" includes⁸: morphological changes, slowed metabolism with a decrease in the concentration of adenosine triphosphate (ATP), acidosis with a decrease in the concentration of 2,3-diphosphoglycerate (2,3-DPG), a decrease in nitric oxide binding with haemoglobin, loss of function (usually transient) of cation pumps and consequent loss of intracellular potassium and accumulation of sodium within the cytoplasm, oxidative damage with changes to the structure of band 3 and lipid peroxidation, apoptotic changes with racemisation of membrane phospholipids and loss of parts of the membrane through vesiculation. Some of these changes occur within the first few hours of storage, for example, the decrease in pH or the increases in potassium and lactate; others, however, take days or weeks. Proteomic data of stored RBC have revealed

that storage of these cells is associated with a rearrangement of RBC membranes and an exchange of biologically active proteins between RBC and the storage media and that different storage conditions, such as leucodepletion or an anaerobic environment, have an impact on RBC biology. However, these experiments are only the starting point for more extensive proteomic research in this field, which will undoubtedly help in defining the optimal storage conditions for RBC and, it is to be hoped, add important information to the current controversial discussion on the impact of storage age of RBC on adverse outcomes²⁰.

Although randomised controlled trials will provide the strongest clinical evidence of whether RBC storage duration does or does not affect morbidity and mortality, they are logistically difficult and expensive to conduct, and their results will not be known for several years. In this scenario of suggestive evidence of various adverse consequences in which many actors compete for the role of protagonist in the storage lesion arena and inconclusiveness seems to prevail, Yoshida's group suggested an anaerobic storage protocol that tackles the problem at its source²¹. They proposed storing blood directly in an atmosphere of inert gas at a $pO_2 < 4\%$, using a method that they patented, and suggested that such storage may decrease the RBC storage lesion by protecting against oxidative damage to lipids and proteins. The biological impact of this protocol has been tested with respect to the classical standards (haemolysis and RBC survival at 24 hours post-transfusion) with positive results; slowing in the decrease of 2,3-DPG and ATP was also observed. After the *in vivo* 24-hour recovery phase I trial, the next steps include a larger phase II clinical study that will examine the correlations between the extent and nature of oxidative damage and the 24-hour *in vivo* recovery of stored RBC²¹. In addition to validating the improved RBC quality parameters shown in the phase I trial, Yoshida's group plans to use a prototype anaerobic disposable system with very little or no impact on existing blood banking operations and minimal additional cost to production [Yoshida T, personal communication, XXXIX National Congress of the Italian Society of Transfusion Medicine and Immunohaematology (SIMTI), 10 June 2010, Milan, Italy].

The need for a longer storage period for RBC

which meets standard acceptability criteria is debatable given that only a small proportion of collected units become outdated in most blood collection systems. Furthermore, the majority of these are group AB units and would probably become outdated even with a doubling of storage time given that they can only be transfused to AB recipients. On the other hand, the anaerobic storage approach might reduce the impact of storage on red cells and perhaps prevent a clinically important storage lesion. Furthermore, if the allowable maximum storage time were to be increased by this method, but the approved limit (from regulatory bodies) remained the same, red cell units would be transfused at a relatively earlier time of their "biological storage clock" and perhaps transfer less of the storage lesion's impact to the recipient.

In conclusion, there is no consensus in the literature on possible adverse effects of "older" blood. Most retrospective studies have one or more flaws in their design and/or analyses introducing bias that results in an overestimation of the association between untoward effects and the age of the blood²². A systematic and rational approach, also including proteomic studies, will undoubtedly lead to a better understanding of RBC storage lesions and their clinical effects. While we are waiting for the results of the randomised clinical trials on the clinical effects of aged blood, which may provide convincing evidence and consequently influence policy decisions, the anaerobic storage approach has the potential to enhance the quality of RBC storage and transfusion therapy and will help us to improve and optimise the care of patients in the near future.

References

- 1) Frati P, Montanari Vergallo G, Di Luca NM. Blood transfusion: history, ethic and law. *Med Secoli* 2005; **17**: 769-802.
- 2) Rous P, Turner JW. The preservation of living red blood cells in vitro. *J Exp Med* 1916; **23**: 219-37.
- 3) Robertson OH. Transfusion with preserved red blood cells. *Br Med J* 1918; **1**: 691-5.
- 4) Mollison PL. The introduction of citrate as an anticoagulant and of glucose as a red cell preservative. *Br J Haematol* 2000; **108**: 13-8.
- 5) Marinozzi S, Conforti M. Blood as therapy, therapy through the blood. *Med Secoli* 2005; **17**: 695-720.
- 6) Hess JR. An update on solutions for red cell storage. *Vox Sang* 2006; **91**: 13-9.
- 7) Zimrin AB, Hess JR. Current issues relating to the transfusion of stored red blood cells. *Vox Sang* 2009; **96**: 93-103.
- 8) D'Alessandro A, Liunbruno G, Grazzini G, Lolla Z. Red blood cell storage: the story so far. *Blood Transfus* 2010; **8**: 82-8.
- 9) Klein HG. Immunomodulatory aspects of transfusion: a once and future risk? *Anesthesiology* 1999; **91**: 861-5.
- 10) Vamvakas EC. Why have meta-analyses of randomized controlled trials of the association between non-white-blood-cell reduced allogeneic blood transfusion and postoperative infection produced discordant results? *Vox Sang* 2007; **93**:196-207.
- 11) Atzil S, Arad M, Glasner A, et al. Blood transfusion promotes cancer progression: a critical role for aged erythrocytes. *Anesthesiology* 2008; **109**: 989-97.
- 12) Hod EA, Zhang N, Sokol SA. Transfusion of red blood cells after prolonged storage produces harmful effects that are mediated by iron and inflammation. *Blood* 2010; **115**: 4284-92.
- 13) Lelubre C, Piagnarelli M, Vincent JL. Association between duration of storage of transfused red blood cells and morbidity and mortality in adult patients: myth or reality? *Transfusion* 2009; **49**: 1384-94.
- 14) Edgren G, Kamper-Jørgensen M, Eloranta S, et al. Duration of red blood cell storage and survival of transfused patients. *Transfusion* 2010; **50**: 1183-93.
- 15) Lacroix J. The Age of Blood Evaluation (ABLE) Study—International Standard Randomized Controlled Trial Number Register (ISRCTN). 2008. Available from: <http://www.controlled-trials.com/isrctn/pf/44878718>.
- 16) Fergusson DA. The Age of Red Blood Cells in Premature Infants (ARIP)—International Standard Randomized Controlled Trial Number Register (ISRCTN). 2010. Available from: <http://www.controlledtrials.com/isrctn/pf/65939658>.
- 17) Koch C. The Red Cell Storage Duration and Outcomes in Cardiac Surgery study. 2009. Available from: <http://clinicaltrials.gov/>.
- 18) Assmann SF. The Red Cell Storage Duration Study (RECESS). 2010. Available from: <http://clinicaltrials.gov/>.
- 19) Glynn SA. The red blood cell storage lesion: a method to the madness [editorial]. *Transfusion* 2010; **50**: 1164-9.
- 20) Thiele T, Steil L, Völker U, Greinacher A. Transfusion medicine and proteomics. Alliance or coexistence? *Blood Transfus* 2010; **8** (Suppl 3): s16-25.
- 21) Yoshida T, Shevkopyas SS. Anaerobic storage of red blood cells. *Blood Transfus* 2010; **a cura Redazione**
- 22) Van de Watering LMG. Aging of red cells: risk or benefit? *Vox Sang* 2010; **99** (Suppl 1): 67.

Correspondence: Dr. Giancarlo Maria Liunbruno,
Viale Italia, 19
57126 Livorno, Italy.
e-mail: giancarlo@liunbruno.it
