The 3-phase evolution of stored red blood cells and the clinical trials: an obvious relationship

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Dear Sir,

Recent clinical trials testing the effect of erythrocyte concentrates (ECs) stored for various periods of time on transfused patients arrived at the conclusion that the transfusion of ECs fresher than standard of care is not beneficial for patients[1,2]. However, they do not provide data on long-term stored ECs; nor were they powered to collect such data[2,3]. Whereas the shelf life of ECs has been fixed by gold standards (haemolysis at the end of storage and red blood cell [RBCs] survival rates in the recipient's circulation), a detailed analysis of *ex vivo* RBC ageing has brought new insights beyond a recurrent 14-day limit. First of all, let's focus on the biology and biochemistry of stored RBCs.

During storage in different known additive solutions, RBCs accumulate storage lesions[2-4]. These lesions impact various aspects of the cell ranging from energy metabolite depletion to cell morphology in a cascade of events. They can be classified as either reversible or irreversible, the former term referring to lesions that are reversed either *in vivo* once transfused or *in vitro* after adapted treatments[3]. Reversible lesions include defects in metabolism and protein activity, whereas irreversible lesions include accumulation of metabolites, protein oxidation, shape change, microvesiculation and haemolysis. A detailed review of all these lesions show that they are time dependent and linked together. Reversible lesions (mainly related to metabolism) mostly happen in the first two weeks of storage, and those irreversible lesions only after the fourth week of storage. The 28-35-day stage is the most critical limit in EC storage since irreversible lesions, which are *per se* permanent, start accumulating. Last but not least, two confounding factors should also be taken into account: firstly, ECs contain the whole spectrum of the donor's RBCs, from just-matured cells to close-to-senescence RBCs, and secondly, there are more and more data showing the importance of donor's characteristics on the quality of stored RBCs.

In published clinical studies, the mean age for long-term storage is 26±8 days (including data from Heddle *et al.*[1])[3]. Because of the design of these trials, ECs that contain irreversible lesions are not really taken into account[2,3]. Even though most routine transfusions involve ECs around 17±8 days (in our institution), 35 day-old ECs are still transfused. In critically ill patients, these products stored for more than 35 days were associated with increased morbidity and mortality compared to ECs transfused before 21 days of storage, and the length of stay was increased in both ECs stored for more than 28 and 35 days[5]. These observations are consistent with biochemical data and the accumulation of irreversible lesions.

In summary, three phases are observed during storage. The first transition involving reversible lesions occurs around the second week and the second transition involving irreversible lesions around 4-5 weeks of storage. The clinical trials have focused on the first stage and offer reassurance on RBC transfusion. However, the existence of irreversible lesions should be considered because they might be harmful in some circumstances (in an intensive care unit setting, older patients, etc.). Two issues will have to be considered. First, improve the RBC quality markers (in relation to transfusion efficiency) so as to be able to capture the exact level of RBC storage lesions. Second, find the strategy to postpone or reduce irreversible lesions that might be the reason of adverse clinical outcomes. Biological and biochemical data analyses (including omics and system biology) will contribute to better characterise ECs, to improve storage, and to select the right product for the right patient. Because for our patients, we can still do better.

The Authors declare no conflicts of interest.

References

1) Heddle NM, Cook RJ, Arnold DM, et al. Effect of short-term vs. long-term blood storage on mortality after transfusion. New Engl J Med 2016; **375**: 1937-45.
2) Glynn SA, Klein HG, Ness PM. The red blood cell storage lesion: the end of the beginning. Transfusion 2016; **56**: 1462-8.
3) Prudent M, Tissot JD, Lion N. In vitro assays and clinical trials in red blood cell aging: lost in translation. Transfus Apher Sci 2015; **52**: 270-6.
4) Paglia G, D'Alessandro A, Rolfsson O, et al. Biomarkers defining the metabolic age of red blood cells during cold storage. Blood 2016; **128**: E43-50.
5) Goel R, Johnson DJ, Scott AV, et al. Red blood cells stored 35 days or more are associated with adverse outcomes in high-risk patients. Transfusion 2016; **56**: 1690-8.

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