With or without you: a tale about oxygen removal from stored, packed erythrocytes

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In the present issue of Blood Transfusion, Longo and colleagues from Zolla's group[1] in Italy describe the outcome of a pilot proteomics study, whereby the authors confirmed that prolonged anaerobic storage of leucoreduced packed red blood cells, for the whole storage period under standard storage conditions at 4 °C, was characterized by less haemolysis during storage, improved erythrocyte membrane proteome homeostasis by decreasing the likelihood of protein fragmentation and aggregation, as well as red blood cell microvesiculation in comparison with the controls blood units which had been stored under standard aerobic storage conditions. They also showed that only during the last days of storage did deoxygenated packed erythrocytes had better resistance to osmotic stress than had control packed erythrocytes. Two-dimensional gel electrophoretic approaches confirmed that deoxygenation of packed erythrocytes prevented membrane accumulation of peroxiredoxin-2, a key anti-oxidant protein and a supposed biomarker of red blood cell aging *in vitro.* Proteomics, an important research tool in transfusion blood products, can provide information on the production and storage processes of packed red blood cell products and finally aid quality assurance in transfusion medicine[2]. Zolla's team, which is well established in the field of proteomics and especially in transfusion medicine, for the first time provides a proteomics analysis of deoxygenated packed red cells.

Packed red blood cells are the most widely transfused blood products throughout the world. However, despite a century of continuous improvements in the field of transfusion medicine, there are still concerns about the safety and effectiveness of packed red blood cells stored for more than 2 weeks (older *vs* young blood units). While definitive clinical evidence is still lacking and randomised clinical trials are either underway or inconclusive, hints derived from retrospective evaluation of the literature have been largely questioned[3].

In parallel, a significant body of laboratory evidence has increasingly shown a wide series of biochemical and morphological alterations to packed red blood cells in the blood bank, a phenomenon generally referred to as "storage lesions". The most well characterized erythrocyte storage lesions are the loss of viability, potassium leakage to the supernatant, the decrease of adenosine triphosphate, S-nitroso haemoglobin and 2,3-diphosphoglycerate concentrations, the shape transformation from biconcave disks to echinocytes and finally to irreversible spheroechinocytes, membrane changes due to microvesiculation (as *ex vivo* aging) and oxidative membrane and/or cytoskeleton protein and lipid damage[2,4-11]. These lesions impair red blood cell physiology, morphology and function and, as the laboratory evidence suggests, ultimately might compromise the effectiveness of transfusion therapies based upon the administration of packed erythrocyte units that have been stored for longer times - especially those used after the middle of the storage period. In addition, the viability of packed red blood cells, post transfusion, was reduced after a 24-hour recovery and it is suspected as one of the possible causes of clinically observed complications of erythrocyte transfusions, especially in critically ill or multi-transfused patients. The viability of erythrocytes is typically measured as the fraction of packed red cells that, at the end of the storage period, are able to circulate through the recipient[12].

While some storage lesions are reversible to some extent, such as the progressive depletion of high energy phosphate compounds (namely, adenosine triphospate and 2,3-diphosphoglycerate), others are not, such as those involving reactive oxygen species (ROS), or protein carbonylation, which in turn affect the protein and the lipid fractions, especially in non-leucoreduced units[5].

On this background, alternative storage strategies have been proposed over the years with the aim of preserving packed erythrocytes better and longer and preventing the accumulation of oxidative stress. In 2007 Yoshida *et al.* published data about packed red blood cells maintained without oxygen throughout the whole duration of the storage period in order to deal with oxidative stress triggering phenomena[13]. Within this framework, anaerobic storage of packed red blood cells was demonstrated to be a viable strategy for preserving stored erythrocytes for 6 weeks, being better than standard practice, and might also offer the opportunity to extend the shelf-life of packed red cells contrasting the anaerobic storage that would be coupled to alternative

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additive solutions[14]. The rationale underpinning the theoretical effectiveness of anaerobic storage is based on two main biochemical hypotheses. The first is that oxygen removal directly tackles oxidative stress by eliminating the main fuel for pro-oxidant reactions, thus preventing ROS-generating Fenton and Haber-Weiss reactions. The second concerns the role of deoxygenation in promoting glycolytic enzyme activity, through a delicate oxygen-dependent process that involves competitive binding of deoxyhaemoglobin to the cytosolic domain of band 3, which in turn prompts the release-activation of otherwise bound-inhibited glycolytic enzymes in the very same site. To put it differently, deoxygenation ends up promoting metabolic fluxes through the Embden-Meyerhof pathway, which in turn promotes replenishment of high energy phosphate compound reservoirs and thus prolonged survival of red blood cells in laboratory experiments.

While clinical trials are about to give some further clarification of the efficacy of anaerobically stored erythrocyte concentrates[10], anaerobic storage of packed red blood cells has attracted the attention of researcher's worldwide[15]. However, anaerobically stored packed red blood cells must be considered with caution among blood donors with a high prevalence of sickle cell haemoglobinopathy due to sickling of erythrocytes as a result of the deliberate deoxygenaration[16]. Controversies about this approach are basically related to the purported beneficial effects being associated with alkalosis resulting from carbon dioxide removal during deoxygenation[17], an aspect to be pondered in addition to the potential deleterious effects of deoxygenation on certain key anti-oxidant pathways. Indeed, recent mass spectrometry-based metabolomics approaches have highlighted further, as of yet underestimated, effects of prolonged anaerobic storage of packed red blood cells, the most evident being the impairment of anti-oxidant defences, resulting from oxygen removal promoting metabolic fluxes through the glycolytic pathway at the expense of the anti-oxidant NADPH-generating pentose phosphate pathway[18]. In this view, early results[18] seem to suggest that, while deoxygenation *per se* may not be harmful to erythrocytes, it might worth further assessing the effects of reoxygenation on red blood cells exposed to prolonged anaerobiosis.

However, it is also worth stressing that encouraging laboratory evidence has been provided over the years about the beneficial effects of anaerobic storage in terms of preserving adenosine triphosphate and 2,3-diphosphoglycerate, protecting against haemolysis, promoting *in vivo* recovery at 24 hour after transfusion[10,14-15,18], delaying phosphatidyl serine exposure for about 3 weeks, reducing microvesiculation and improving morphology scores[10,19].

Although further studies are essential and clinical evidence is still lacking, these results are encouraging in that they complement currently available knowledge on anaerobic storage of packed red blood cells and set a proteomics standard (two dimensional gel electrophoretic approaches red blood cell membrane protein spot numbers) to rapidly highlight the effectiveness of emerging alternative storage strategies from a laboratory science perspective.

More studies in all fields of erythrocyte biology under anaerobic and classical aerobic conditions, especially with proteomics techniques, are necessary to understand protein-protein interactions, aggregate ones and signalling or death/clearance components during the storage of packed red blood cells. A low costlautomated anaerobic protocol for blood bank conditions is also needed in addition to cost/benefit or cost/effectiveness studies for blood bankers.

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