Red blood cell storage and clinical outcomes: new insights

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The Good

Transfusion of packed red blood cells (RBCs) is a life-saving intervention for millions of chronically or massively transfused recipients worldwide every year. After over a century of improvements, ten years ago a highly-debated retrospective clinical paper[1] suggested the potential negative association between storage "age of blood" and transfusion outcomes. This controversial observation fuelled the debate about the potential clinical relevance of the so-called storage lesion(s), a wide series of biochemical and morphological alterations RBCs undergo during storage in the blood bank (as extensively reviewed[2-5]). Ten years later, a series of comprehensive randomised clinical trials (RCTs) have come to an end, providing reassuring evidence about the lack of a detectable difference between fresh blood and standard of care at the limits of the statistical power of these studies[6-10]. This translates into the appreciation of the fact that the general standard of care will not be improved by preferentially issuing fresh blood[11], at least to the specific categories of recipients enrolled in those RCTs. Many have noted the limitations of the RCTs (including several contributors to this thematic issue of Blood Transfusion). Limitations relate, for example, to the lack of comparison of fresh blood products *vs* products close to the end of their shelf-life (35 days or older) owing to ethical concerns hampering the design and feasibility of such studies. It may be provocatively argued that "if we do not deem ethical to design a study where half of the recipients will only receive >35 day old blood, then we should not transfuse the oldest blood to actual patients as well", as recent studies seem to suggest[12]. Still, it is undeniable that RCTs reassured the field to such an extent that it became reasonable to conclude that, quoting American Association of Blood Banks (AABB) guidelines, "a restrictive transfusion threshold is safe in most clinical settings and the current blood banking practices of using standard-issue blood should be continued"[11]. In other terms, current practices are for the most part as safe and effective as they have ever been in the history of Transfusion Medicine. Nevertheless, it ought to be noted that, as Zimring and Spitalnik suggest in this issue[13], "when approximately 80 million RBC units are transfused annually worldwide, even vanishingly small (transfusion-associated negative) events, if they are real, can affect actual human lives; it then becomes a question of ethics and economics whether it is *worthwhile* to study and attempt to prevent them".

It is a matter of pride for all the members of the international Transfusion Medicine community to note that, despite the reassuring evidence coming from RCTs, the field still fancies the opportunity to define an international agenda to pursue the amelioration of blood storage strategies. An example of this critical commitment by decision makers in the United States and Italy is represented by the recent 2016 meetings at the National Heart, Lung, Blood Institutes, Food and Drug Administration and Italian National Blood Center, where some of the leading experts in the field gathered to identify current issues associated with blood storage, and shared strategies to address such issues.

The Bad

While clinical trials have informed us about the substantial safety of current transfusion practices at large, laboratory sciences, especially omics technologies, have contributed insights into the reason why the transfusion therapy may mediate, in a minimum but not negligible number of cases, untoward transfusion-related events (e.g. transfusion-related acute lung injury [TRALI], transfusion-related immunomodulation [TRIM]) or aggravate underlying conditions (e.g. sepsis[14]). Improving our understanding of the storage lesion at a molecular level is a critical step towards the introduction of improved blood processing and storage guidelines. Many groups have contributed to document the energy and oxidative lesions targeting stored RBCs (as extensively reviewed by several authors in this issue). RBC energy and redox metabolic reprogramming during storage in the blood bank has been associated with the processes of vesiculation, impaired morphology and functionality (e.g. gas transport and off-loading), as well as *in vivo* survival in animal models and humans (as extensively reviewed in this issue and elsewhere)[2-5]. Protein[15] and metabolic markers[16] of the RBC storage lesion have been proposed by us and others. The metabolic phenotype of stored RBCs follows a specific 3-stage sequence, as gleaned through multivariate analysis of metabolomics data

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from different storage additives (as detailed by Prudent and Colleagues, Bordar, and us in this issue). We now understand that RBC metabolic reprogramming during storage in the blood bank is a biochemical necessity driven by refrigeration and excess oxidative stress, hence the necessity to restore reducing equivalents in order to counteract oxidative stress to functional proteins, such as haemoglobins and anti-oxidant enzymes (e.g. peroxiredoxin 2). Energy and redox homeostasis in stored RBCs are intertwined to such an extent that storage additives may be designed to boost either or both metabolic necessities, such as in the case of alkaline additives or hypoxic storage of erythrocyte concentrates (as discussed in this issue). For the interested reader, this thematic issue offers the opportunity to get a glimpse of the recent advancements in this field, as well as to get a general overview of the main technologies that contributed to our making the most significant strides forward in this research endeavour (i.e. omics technologies*)*.

The Ugly

The apparent disconnection between laboratory science and clinical trials has been increasingly explained in the past 12 months by the small scale of laboratory omics studies performed to date and by the necessity to investigate the biology of the donor and the recipient along with the evolution of the storage lesion *per se*; a "Copernican revolution" we had anticipated in 2009[17-19]. As pointed out by some of the contributors to this issue, until recently donor and recipient biology had often been overlooked in laboratory and clinical studies of the RBC storage lesion. While the clinical relevance of the storage lesion(s) remains a matter of debate, large-scale studies such as the REDS III Omics initiative will tackle this relevant issue in the coming years.

To further support the statements above, it is worthwhile recalling the 2008 study by Dumont and Aubuchon in which the results were published from a large retrospective study of radiolabelled RBC recoveries in autologous healthy volunteers (n=641)[20]. Results indicated that end of storage RBCs had recoveries averaging around 82.4±6.7%, with some donors showing 24-h *in vivo* survival as low as 35-40%[20]. These numbers are also suggestive that, on average, approximately 17% of the RBCs in a transfused unit are lost during storage and transfusion to healthy volunteers[20] (as pointed out by Mays and Hess in this issue). These numbers would theoretically be even worse if the biology of actual recipients were taken into account, in that heterologous chronically or massively transfused recipients would respond differently to blood transfusion than autologous healthy volunteer recipients owing to their repeated exposure to allogeneic cells or the underlying pro-inflammatory/metabolically-deranged physiology, respectively (as pointed out in this issue by several groups).

The Bad, the Good and the Ugly: old blood, new blood or better stored blood all over again

You may have noticed that paragraph subtitles in this editorial are a tribute to Sergio Leone's masterpiece "The Good, the Bad and the Ugly" which celebrates its 50th anniversary. The title of the film has entered the English language as an idiomatic expression, one that is typically used to describe something by referring to its upsides (the Good), downsides (the Bad), and the parts that could, or should have been done better, but were not (the Ugly). Besides the poetic license of the comparison to Sergio Leone's title, the whole field seems to have lost interest in the "new blood - old blood" diatribe, and rather agrees on the necessity to welcome the opportunity omics/laboratory studies have provided us with to further improve storage quality[21]. For the foreseeable future, small molecule/protein pre-storage markers of the lesion may inform us about the possibility of designing specific storage strategies for a given blood product for which the biology of the donor is already known, before matching it to the biology and specific clinical indications for the recipient. Alternatively, as suggested by Yoshida and Colleagues[22] in this issue, strategies such as hypoxic storage may exploit biochemical constraints to normalise inter-donor variability and provide more homogeneous blood products to the community.

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