



## Scientific Comment

# Reticulocytes and the storage lesion<sup>☆</sup>



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Much is still being elucidated about the impact of red blood cell (RBC) storage time on disturbances in erythrocyte physiology, denominated 'storage lesion'. Longer storage times have proved to lead to dysregulation of several metabolic pathways, including changes in pH, decreases in adenosine triphosphate and 2,3-diphosphoglycerate (2,3-DPG), and altered RBC deformability.<sup>1</sup> However, the actual repercussions on the recipients of these 'older' units and possible deleterious consequences of the storage lesion have not yet been adequately proved.

Possibly, younger RBCs lead to better transfusion yields, increasing the interval between transfusions, which would be a real advantage for patients in regular chronic transfusion schemes as in the case of patients with hemoglobinopathies; however, this is also a very contradictory point. Longer storage times could possibly lead to augmented inflammation caused by hemolysis and higher concentrations of extracellular vesicles, with consequent free-iron and heme availability, higher generation of reactive oxygen species (ROS) and depletion of available nitric oxide.<sup>2</sup> Observational studies have demonstrated possible associations between longer storage time and greater predisposition to infections, organ failure and even death.<sup>3</sup> Despite these observations, recent studies have failed to demonstrate enhanced clearance of these RBCs or higher rates of transfusion or disease-related adverse events<sup>4</sup>; policies regarding this aspect still vary among institutions.<sup>5</sup> There may be lower yields with the use of 'older' RBCs however, these would not necessarily be malefic or lead to clinical complications. The studies themselves are difficult to assess as several

other factors may affect this judgment and compromise the quality of the data, such as adequate definitions of 'old' and 'young' RBCs, the fractionation methods employed, donor status and clinical conditions, and the severity of the recipient's disease.<sup>6</sup>

In reality, storage time is not thought to represent the most important or even the single parameter of this effect and today much consideration is placed regarding the intrinsic characteristics of the donor, such as, for example, his basal inflammatory state or individual genetic characteristics. In fact, recent studies have diverged as to the idea that donor factors, such as genetic background and social habits such as smoking or exercise, may also play an important role in the occurrence of possible effects of the storage lesion.<sup>7–9</sup>

The study of Urbina and Palomino published in this issue of Hematology, Transfusion and Cell Therapy<sup>10</sup> shows that a fraction of the reticulocytes contained in donor blood remain quiescent in RBC concentrates and end their maturation when cultured *in vitro*. This effect, despite decreasing progressively, is observed even after long periods of storage and leads to the hypothesis that this maturation may occur *in vivo* after transfusion, in the circulation of the recipient.

The hypothesis that the concentration and maturation profiles of the reticulocytes present in the RBC concentrates may further vary according to donor characteristics is plausible. There may even be genetic patterns that favor this situation of quiescence and subsequent reticulocyte maturation, also contributing in some way to improved transfusion yield and time to re-transfusion. However, even if this were proved, the

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<sup>☆</sup> See paper by Urbina and Palomino on pages [143–150].

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evaluation of how much the recipient's microenvironment could influence this maturation and the clearance of these cells – an important factor to be considered in the case of chronic inflammation of patients with sickle cell anemia, for example – would remain necessary.

Another point of interest arising from the findings of this study is the importance of reticulocytes in the production of RBCs *in vitro* for transfusion purposes. Considering the good viability of reticulocytes observed during storage, stem cell cultures could perhaps be discontinued at this stage, leading to a shorter culture time, a lower chance of bacterial contamination and facilitated logistics for large-scale production. Another advantage would be the lower expression of antigens in younger erythrocytes, a positive factor for the use of reticulocytes in patients previously alloimmunized or at risk of developing RBC alloantibodies.

Therefore, the observation of the reticulocyte quiescence pattern in RBC concentrates and the subsequent maturation of reticulocytes opens new areas to be explored in future studies. Not only does the need to define the exact biochemical mechanisms involved in this process remain, but how reticulocytes guarantee this quiescence and in a certain way may be 'protected' from the storage lesion must also be established. Consequently, could the discovery of these possible molecular pathways of 'rejuvenation' be manipulated and edited in mature erythrocytes? And to what extent could we concentrate these reticulocytes in the future, improving transfusion yield in the case of patients theoretically more vulnerable to storage lesions? The incorporation of metabolomics and proteomics techniques in the field of transfusion medicine will certainly help in this sense, but it also indicates that we are still many leagues (and studies) away from these definitive answers.

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### Conflicts of interest

The author declares no conflicts of interest.

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