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Review

Insights into red blood cell storage lesion: Toward a new appreciation

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ABSTRACT

Red blood cell storage lesion (RSL) is a multifaceted biological phenomenon. It refers to deterioration in RBC quality that is characterized by lethal and sub-lethal, reversible and irreversible defects. RSL is influenced by prestorage variables and it might be associated with variable clinical outcomes. Optimal biopreservation conditions are expected to offer maximum levels of RBC survival and acceptable functionality and bioreactivity in-bag and *in vivo*; consequently, full appraisal of RSL requires understanding of how RSL changes interact with each other and with the recipient. Recent technological innovation in MS-based omics, imaging, cytometry, small particle and systems biology has offered better understanding of RSL contributing factors and effects. A number of elegant *in vivo* and *in vitro* studies have paved the way for the identification of quality control biomarkers useful to predict RSL profile and posttransfusion performance. Moreover, screening tools for the early detection of good or poor "storers" and donors have been developed. In the light of new perspectives, storage time is not the touchstone to rule on the quality of a packed RBC unit. At least by a biochemical standpoint, the metabolic aging pattern during storage may not correspond to the currently fresh/old distinction of stored RBCs. Finally, although each unit of RBCs is probably unique, a metabolic signature of RSL across storage variables might exist. Moving forward from traditional hematologic measures to integrated information on structure, composition, biochemistry and interactions collected in bag and *in vivo* will allow identification of points for intervention in a transfusion meaningful context.

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1. RBC storage lesion

Storage of blood and blood components permits the separation of blood donation from transfusion in time and space ensuring better control of safety, adequacy and "management" of blood supply. Depending upon the preservative solution used, red blood cells (RBCs) can be stored for up to 42 or 49 days in the US and in Europe, respectively. However, with age in blood bags a clear deterioration is detected in RBC quality and functionality, collectively referred to as "RBC storage lesion" (RSL). RSL is the result of storage under *ex vivo*, artificial conditions of low temperature, immobilization and low presentation of natural cellular and plasma environment. Moreover, in the absence of clearance mechanisms, healthy stored RBCs coexist with senescent or severely damaged cells and cellular debris. RSL concerns all components of the stored biological material, namely, RBCs *per se*, supernatant with residual plasma and residual non-erythroid cells of the donor. Regarding RBCs, storage affects their homeostasis at three intimately connected levels, those of energy metabolism, redox metabolism and cell membrane, resulting in a well defined "phenotype" of morphologic, structural, and functional alterations. This phenotype is characterized by loss of normal shape, decrease in pH, glutathione (GSH), S-NO-Hb, phospholipids and cholesterol, depletion of 2,3-diphosphoglycerate (DPG) and adenosine triphosphate (ATP) but increase in removal signaling, ROS and protein heterogeneity, in addition to accumulation of free Hb, heme, lactate, GSSG, malondialdehyde, potassium, inflammatory lipids and extracellular vesicles (EVs), including microvesicles (MVs), in the supernatant [1,2].

Defected ATP-centered metabolism and oxidative stress are major driving forces in the development of RSL. High-energy phosphate compound metabolites serve as energy tokens to be spent on the preservation of membrane stability and thus, RBC survival [3]. As a result, RBCs lose energy and their valuable deformability, physiological surface area, topography and shape during storage. Some RBCs undergo hemolysis or eryptosis [4]. While the physiologically aged RBCs are mainly engulfed by macrophages in the splenic red pulp, liver or marrow [5,6] there is evidence that a small population of stored RBCs with increased calcium permeability is preferentially captured in a CD47-independent manner by macrophages and dendritic cells in the marginal zone of the spleen, much alike the capture of apoptotic nucleated cells [7]. Stored RBCs become older in a weird way that promotes recognition and premature removal by the surveillance systems of the recipient, and others become less adjustable to the physiological levels of stress in which their circulating counterparts respond efficiently. This last aspect of RSL is hard to be assessed; however, it is clinically relevant to the effectiveness and the adverse effects of transfusion, similarly to the obvious, measurable facets of RSL. Despite being heavily affected by storage, alterations related to metabolism activity, such as DPG, ATP and cation imbalances are rapidly reversible upon transfusion in the bloodstream of the recipients [8]. On the other side, protein and lipid modifications (degradation, oxidation, etc.) and changes related to RBC morphology, such as surface area loss and spheroechinocytosis, are not restored after transfusion of pRBCs to the recipient. Conversely, they may get worse posttransfusion.

2. Potential contribution of the bio-active components of RSL

Each unit of stored RBCs contains a wide range of cellular, sub-cellular and soluble components which might affect the clinical outcomes of the transfusion [9]. Loss of RBC integrity leads to increased extracellular potassium concentration that increases the risk of hyperkalemia-induced arrhythmia in susceptible recipients and free-Hb that is a potent pro-oxidant factor. Free-Hb, Hb-containing EVs and senescent RBCs *per se* are effective scavengers of nitric oxide (NO) and inhibitors of NO generation post transfusion [10,11]. Storage MVs can flow closer to the endothelium than stored RBCs, bringing Hb near the sites of NO synthesis [12]. Reduced NO bioavailability might lead to inhibition of vasodilatory response and blood flow, insufficient tissue oxygenation in the microcirculation and end-organ injury [10,13], especially in recipients with endothelial dysfunction issues [14].

Storage EVs carry antigens and potent signaling molecules (including IgGs, PS, tissue factor, complement components, Hb, heme, cytokines and chemokines) that in transfusion settings might affect coagulation, angiogenesis, inflammation and immune responses through complex interactions with blood cells, endothelium and clearance systems [15–18]. As shown by *in vitro* models of transfusion, EVs derived from long-term stored RBCs may serve as a platform for the coagulation cascade [19], although this effect may occur only in specific recipient context [20]. Notably, there is evidence pointing to the opposite, anticoagulant activity of RBC EVs [21]. In a similar way, EVs and bioactive lipids from RBC units can activate neutrophils, as a piece of the pathogenesis of TRALI [22], and induce production of predominantly proinflammatory cytokines and chemokines in peripheral blood mononuclear cells [23], although anti-inflammatory and immunosuppressive properties have been also reported for them [24]. Other soluble mediators of the supernatant (probably extracellular protein-bound RNA species) might also account for monocyte suppression *in vitro* [25]. EVs can effectively deliver vesicular material to innocent bystander cells. Indeed, transferring of RBC EVs-bound CD59 surface protein to CD59-deficient RBCs has been reported *in vivo* after transfusion in patients with paroxysmal nocturnal hemoglobinuria [26], and transferring of heme from sickle RBC EVs to vascular endothelial cells leading to endothelium activation and injury has been shown in sickle cell disease [27].

Moreover, transfusion of a large population of senescent, eryptotic or severely injured RBCs may overwhelm the mononuclear phagocyte system and the capacities of intracellular and extracellular iron chaperones working for the metabolism of ingested RBCs and iron-rich Hb. According to the "iron hypothesis", the increased intracellular free iron levels enhance the production and secretion of proinflammatory cytokines by phagocytes and thus, the severity of the systemic inflammatory response syndrome. In addition, excess of free iron exported from phagocytes might lead to increased NTBI which can induce oxidative damage