REVIEW

Donor-variation effect on red blood cell storage lesion: A close relationship emerges

Vassilis L. Tzounakas[1], Anastasios G. Kriebardis[2], Issidora S. Papassideri[1] and Marianna H. Antonelou[1]

[1] Department of Cell Biology and Biophysics, Faculty of Biology, NKUA, Athens, Greece
[2] Department of Medical Laboratories, Faculty of Health and Caring Professions, Technological and Educational Institute of Athens, Greece

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Although the molecular pathways leading to the progressive deterioration of stored red blood cells (RBC storage lesion) and the clinical relevance of storage-induced changes remain uncertain, substantial donor-specific variability in RBC performance during storage, and posttransfusion has been established ("donor-variation effect"). In-bag hemolysis and numerous properties of the RBC units that may affect transfusion efficacy have proved to be strongly donor-specific. Donor-variation effect may lead to the production of highly unequal blood labile products even when similar storage strategy and duration are applied. Genetic, undiagnosed/subclinical medical conditions and lifestyle factors that affect RBC characteristics at baseline, including RBC lifespan, energy metabolism, and sensitivity to oxidative stress, are all likely to influence the storage capacity of individual donors' cells, although not evident by the donor's health or hematological status at blood donation. Consequently, baseline characteristics of the donors, such as membrane peroxiredoxin-2 and serum uric acid concentration, have been proposed as candidate biomarkers of storage quality. This review article focuses on specific factors that might contribute to the donor-variation effect and emphasizes the emerging need for using omics-based technologies in association with in vitro and in vivo transfusion models and clinical trials to discover biomarkers of storage quality and posttransfusion recovery in donor blood.

Keywords:
Biomarkers / Donor-variation / Omics / Red blood cells / Storage lesion

1 Red blood cells are affected by storage—Are the recipients affected by storage lesion?

Blood products are vital components of health care. Red blood cells (RBCs) transfusion supports fetal medicine and neonatal intensive care, trauma, and high-risk obstetric care, all forms of surgery, cancer, and degenerative conditions and provides comfort when other treatments are no longer appropriate.

RBCs undergo time-dependent deterioration in several aspects of their physiology during storage in standard blood bank conditions, collectively named as "RBC storage lesion." In this context, functionally important disturbances in energy and redox metabolism, rheology, and finally, in RBC aging and removal signaling are apparent after examination of RBC concentrates in vitro by both targeted and integrated, omics-based approaches [1]. Indeed, time-dependent changes in the expression/accumulation and post-translational modifications of hemoglobin (Hb), supernatant and membrane proteins and lipids, including fragmentation, oxidation/carbonylation, oligomerization, destabilization, and phosphorylation have been reported as candidate

Correspondence: Marianna H. Antonelou, Department of Cell Biology and Biophysics, Faculty of Biology, University of Athens (NKUA), Panepistimiopolis, Athens 15784, Greece
E-mail: manton@biol.uoa.gr

Abbreviations: **2,3-DPG**, 2,3-diphosphoglycerate; **ATP**, adenosine triphosphate; **BPGM**, bisphosphoglycerate mutase; **G6PD**, glucose-6-phosphate dehydrogenase; **GR**, glutathione reductase; **GSSG**, oxidized glutathione; **Hb**, hemoglobin; **MCV**, mean cell volume; **MP**, microparticle; **pRBC**, packed RBC; **Prx2**, peroxiredoxin-2; **ps**, phosphatidylserine; **RBC**, red blood cell; **SCT**, sickle cell trait; **Tm**, thalassemia carriers

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biomarkers of storage quality and posttransfusion effects in RBC units [2–6]. Omics studies have expanded knowledge on the metabolic, biomechanical, and oxidative effects of storage on RBCs and their molecular basis as a function of storage duration, strategy, and probable clinical relevance [7–10].

Although the clinical importance of the RBC storage lesion is poorly understood, some of the irreversible changes, like hemolysis, potassium release, and microparticles (MPs) accumulation, are associated with reduced posttransfusion survival/efficacy and increased risk of adverse reactions in the recipients [11–13]. Processing strategies such as leukofiltration, pathogen inactivation, irradiation, additive solutions, and rejuvenation have been applied to minimize the RBC storage lesion and/or its associated deleterious effects in the recipient, including immune responses and the risk of febrile nonhemolytic transfusion reaction or graft versus host disease [14, 15]. Integrated metabolomics and proteomic studies have contributed to better understand the influence of storage conditions and strategies on RBC quality and survival [16]. According to these findings, some interventions improve the biochemical and biomechanical profile of stored RBCs (e.g. additive solutions or leukoreduction) [17, 18], while others seem to negatively affect the ion and energy homeostasis, membrane preservation, and viability (e.g. irradiation) [19].

There is not yet the same degree of consensus among the members of the scientific community as to the impact of transfusing fresh versus old RBCs on patient-important outcomes (morbidity, mortality), since the literature contains clinical studies reporting conflicting results. Animal models of transfusion [20] and several retrospective, observational cohort studies [21, 22] suggested that the duration of storage is an independent risk factor in patient outcome, although in some cases risk pattern seemed consistent with weak confounding rather than exposure to stored RBCs [23, 24]. In the opposite direction, recently conducted prospective, randomized, controlled clinical trials (TOTAL, ARIPI, ABLE etc.) have shown that fresh RBCs do not improve transfusion outcomes compared to standard issue or old (>21 days) RBCs in several groups of recipients [25–28]. A recent metaanalysis using data from 12 different clinical trials provided moderate and low certainty for the impact of transfusing fresh RBCs in mortality and adverse effects, respectively [29]. Debate upon the possible clinical effects of transfusing young or old stored RBCs will probably not end until the performance of clinical trials (i) with strict and narrow RBCs age cut-off falling into line of in vitro aging (in which "old" units are characterized by the prevalence of irreversible, severe damages) [30], (ii) with increased power necessary to disclose potential harmful effects confined to the last days of storage [31], and (iii) of clinical trials that focus on narrow and discrete recipient groups, such as trauma patients [32].

2 Donor-specific characteristics account for the variability observed in RBC storage quality and posttransfusion performance

"Donor-variation effect" refers to substantial donor-to-donor differences in blood storage quality and posttransfusion recovery that have been noticed since 1969 [33, 34]. In fact, the two "gold quality standards," namely in-bag hemolysis rate and 24-h in vivo recovery, that have successfully guided the development of the RBC storage systems for six decades, exhibit large donor-dependent end-of-storage variability. Although preanalytical issues [35] and storage conditions [14, 36] might affect in-bag hemolysis, interdonor variability seems to be the most significant contributing factor even when all other parameters are taken into consideration [37]. The correlation of end-of-storage hemolysis with currently undetermined donor-specific factors refers to the whole range of hemolysis levels and not only to the pathologically high values exhibited by approximately 1% of the donors, as shown in a randomized, paired cross-over study [38]. End-of-storage viability of stored RBCs fluctuates from less than 60 to more than 95% among individual units [39], while RBC recovery, which varies considerably among donors [40], is a constant attribute of individual donors under a variety of storage conditions, as shown by cross-over and repeated donation studies [41].

Apart from in-bag-hemolysis and recovery, several properties of stored RBCs including adenosine triphosphate (ATP) levels [42], oxygen transport efficacy [43], cellular fragility, metabolic rate [44], and accumulation of oxidative stress biomarkers, like peroxiredoxin-2 (Prx2) [36], are donor-related (Table 1). Moreover, the sensitivity of packed RBCs (pRBCs) to oxidative and calcium stressors [45], and the generation of metabolites and RBC-derived MPs [3, 46] are also associated with donor-specific factors exhibiting wide interdonor variation. In the absence of an obvious explanation for the wide interdonor variability observed in the MPs accumulation, intrinsic genetic factors and lifestyle aspects, such as ABO blood group, age, sex, or diet have been suggested as probable contributors [46]. This suggestion is probably true for all of the above-mentioned variables, since baseline donor-to-donor differences in blood homeostasis that concern or influence RBC properties might not be neutral in respect to their effect on RBC storability. To mention one example, recently reported data suggest that variation in the basal levels of RBC calcium channels' abundance and activity among healthy subjects, which is regulated by plasma-borne factors like glutamate and glycine, is associated with intracellular Ca^{2+} accumulation levels and probably with interdonor variation in RBC volume, intracellular pH and Hb-oxygen affinity [47]. Notably, donor variation has not been considered as an aspect of the storage lesion in the clinical trials conducted so far to answer the fresh versus old stored RBCs controversy. These studies have made the assumption that all RBC

Table 1. Overview of studies connecting storage lesion parameters to donor variability

Storage parameter or recovery	Donor-specific characteristic or RBC unit processing-associated parameter	Reference
Hemolysis	Duration of storage	[34]
	Suspending solution	[14]
	Leukoreduction	[36]
	Phosphatidylserine exposure	[153]
	Sickle cell trait (hemolysis during leukofiltration)	[87]
	Sex	[65, 67]
	Age (donor's)	[67]
	Addition of progesterone	[68]
	G6PD (Posttransfusion hemolysis)	[98]
Post-transfusion recovery	Duration of storage	[34]
	Suspending solution	
	Leukoreduction	
	G6PD	[100]
Osmotic fragility	Sickle cell trait	[84]
	HbC trait	
	Addition of progesterone	[68]
	Osmotic fragility	[155]
Mechanical fragility	Sex	[65]
Carbon monoxide	Smoking	[123]
Lipid peroxidation	Prx2	[161]
GSH and GSH/GSSG	GSH	[154]
Intracellular calcium	Plasma uric acid	[57]
Shape transformation	Plasma uric acid	
Band 3 clustering	Plasma uric acid	
Annexin V release	RBC age upon donation	[53]
Glycophorin A	RBC age upon donation	
Cell adhesion molecules	RBC age upon donation	
Total Hb content	Sex	[56]
	Age	
	Body weight	
RBC indexes	RBC indexes	[155]
Supernatant potassium	Familial pseudohyperkalemia	[51]
Supernatant nitrate/nitrite	Plasma nitrate/nitrite	[155]
Supernatant clusterin	Plasma clusterin	
Uric acid-dependent antioxidant capacity of the supernatant	Uric acid-dependent antioxidant capacity of the plasma	
Supernatant total antioxidant capacity	Plasma uric acid	[57]

concentrates produced and stored according to the standard operating procedures and guidelines are identical [48]. However, substantial intraunit differences exist and donor characteristics may account for some aspects of the young versus old results reported.

3 Genetic factors determine subclinical interdonor differences in RBC physiology

3.1 Introduction

Several aspects of RBC physiology that are presumably associated with their storability in blood bank conditions are genetically determined. For example, a study performed on twins has revealed that the GSH content of human RBCs is a heritable trait [49]. Moreover, mutations in RBC membrane proteins as in the case of hereditary spherocytosis might affect the progression of RBC storage lesion in terms of cell fragility [50]. Genetic contexts that define normal-range variation in RBC lifespan, geometry, structural stability, metabolism, or pro/antioxidant factors equilibrium among eligible blood donors might not affect blood homeostasis in vivo but may be of importance inside the stored pRBC unit, where additional stressful stimuli are present. In support of this theory, clinically silent familial pseudohyperkalemia has been reported to increase potassium accumulation in the supernatant of the pRBC units already during the first period of storage [51]. The mean age of RBCs at the time of donation, which varies from 38 to 60 days [52], is linked to the degree of storage lesion [53]. Therefore, it is reasonable to suggest that genetic

variables associated with accelerating RBC senescence or oxidative stresses are natural determinants of storability.

3.2 Is storage lesion affected by sex-associated differences in RBC characteristics?

Several biological sex-dependent differences exist in RBC and soluble blood components. Hematocrit, Hb concentration, RBC count [54], iron homeostasis indexes (folate, ferritin, transferrin) [54], and the major antioxidant factor uric acid [55] are usually lower in females compared to men. Considering that donor-to-donor variation in Hb concentration is preserved during the storage of RBCs [56] and that serum uric acid levels have been associated in part with the quality of storage [57], some baseline differences might also affect, in a sex-dependent manner, the storability of RBCs. Female RBCs are less susceptible to oxidative, osmotic, and mechanical stress stimuli in vitro, exhibiting lower levels of hemolysis [58] and lipid peroxidation [59] in both mice and humans. Female sex hormones, including estradiol, probably contribute to these attributes [60, 61]. Estradiol promotes GSH generation in plasma and RBCs [62], decreases ATP leakage from RBCs and lowers nitric oxide generation from endothelium [63]. Finally, lower levels of RBC membrane vesiculation and a different overall blood cells-borne MP generation profile have been reported in women compared to men [64].

In terms of RBC storage lesion, Raval and colleagues have demonstrated that premenopausal female RBCs exhibit lower in-bag hemolysis and mechanical fragility under various blood bank conditions compared to male RBCs [65], probably because the regular loss of blood through menstruation leads to a younger RBC population in women [66]. By analyzing blood bank quality control data for 20 818 RBC concentrates it was found that units from female donors exhibit lower hemolysis than male donations [67] (Table 1). Moreover, progesterone supplement in pRBC units has been reported to reduce the osmotic fragility and spontaneous lysis of stored RBCs, suggesting a protective effect of progesterone on the membrane [68]. On the contrary, Daly and colleagues did not report any significant differences between male and female stored RBCs in terms of mechanical properties and deformability [69].

3.3 RBC modifications in heterozygous carriers of beta-thalassemia might affect storage lesion development

According to the donor deferral criteria, heterozygous carriers of thalassemia and Hb variants with normal Hb levels (>12.5 g/dL) are not excluded from becoming regular blood donors. RBCs of heterozygous carriers of beta thalassemia mutations (Tm) are quite different compared to the general population, in a manner that might be advantageous or disadvantageous for the RBC storage lesion.

On one hand, lower production of functional Hb and slight excess of soluble alpha-globin chains in Tm-RBCs lead to low Hb concentration, slightly enhanced hemolysis and oxidative stress and a cascade of directly or indirectly associated changes [70] including membrane loss, impaired rheology [71], defective spectrin self-association [72], aberrant binding of Hb to skeletal components, cellular dehydration, membrane rigidity [73], or defected deformability [74], increased band 3 phosphorylation [75] and caspase-3 activation [76], which exposes band 3 to increased risk of proteolysis [77]. Membrane remodeling [70] and aberrant activity of membrane pumps and transporters [78] lead to loss of band 3 metabolic modulation, depletion of energy sources, senescence and signaling of RBC removal. Collectively, these findings suggest that Tm-RBCs have a handicap as donated biological material, in terms of their structural and functional integrity, which might predispose them to increased oxidative lesions and hemolysis during storage.

On the other hand, the Tm-RBCs exhibit lower aggregability [79] compared to the non-Tm-RBCs, while the altered ion homeostasis [78] leads to higher intracellular K^+ but loss of osmotically active intracellular components and cell shrinkage [80]. As a result of high surface-to-volume ratio [81], the Tm-RBCs are resistant to osmotic lysis [80]. Considering the aggravating effect of storage on RBCs aggregability [82] and osmotic fragility [83], these intrinsic characteristics of Tm-RBCs would be a competitive advantage over normal donated RBCs. Moreover, the sustained level of oxidative stress in vivo may trigger the intracellular antioxidant pathways to a better response against the oxidative challenges of storage.

In the same context, low osmotic fragility and higher 2,3-diphosphoglycerate (2,3-DPG) preservation have been reported for stored RBCs donated by sickle cell trait (SCT) and HbC trait donors, without incident of sickling during storage [84]. Conversely, for currently unknown reasons, leukoreduction of SCT-RBC units is less efficient compared to normal units [85, 86] and the SCT-RBCs are susceptible to hemolysis during this procedure [87].

3.4 Glucose-6-phosphate dehydrogenase (G6PD) deficiency undermines redox homeostasis in RBCs

A number of genetic enzymopathies that affect RBC metabolism and redox homeostasis have been described [71]. Metabolic impairments and lack of sufficient energy cause chronic nonspherocytic hemolytic anemia of variable severity with shortened lifespan of the RBCs and increased levels of oxidative stress [88]. In a context of clinically detected anemia, the carriers are excluded from blood donation but nonanemic donors with glutathione reductase (GR) or bisphosphoglycerate mutase (BPGM) deficiencies for example, might be regular blood donors. In that case, GR-deficient RBCs will inevitably exhibit low levels of GSH and consequently decreased baseline levels of antioxidant capacity, and

BPGM-deficient RBCs will exhibit low 2,3-DPG levels but increased affinity of Hb for oxygen and ATP generation [88]. This combination might lead to loss of band 3 physiological modulation during storage but also to better preservation of ATP-dependent processes like ion homeostasis.

G6PD deficiency represents the most common enzyme genetic defect with more than 400 million of people affected worldwide [89]. According to the World Health Organization's guidelines, blood can be accepted from G6PD-deficient individuals without a history of hemolytic crisis, provided that it shall not be transfused in groups of susceptible recipients, such as neonates. The main characteristic of G6PD-deficient RBCs is the low levels of GSH and insufficient anti-oxidant defenses, leading to acute hemolytic anemia following exposure to prooxidant drugs, infections and fava beans [90]. As a result, lipid peroxidation, affected RBC deformability [91], protein carbonylation [92], sensitivity to oxidative damage, and destabilization of the RBC membrane [93] have been reported in G6PD-deficient RBCs in vivo. In addition, G6PD-deficient RBCs are more susceptible to eryptosis after osmotic, oxidative, calcium, or energy depletion stress [94].

Considering that storage per se represents an exogenous oxidative stimulus for the RBCs [95], inherent defects in the antioxidant capacity are expected to affect their storability [96] and probably condition RBCs for enhanced destruction after transfusion [97, 98]. On the contrary, G6PD-deficient RBCs represent a younger erythrocyte population [99] that is probably resistant to the accelerated aging effect of storage. Although lower 24 h posttransfusion recovery of stored G6PD-deficient RBCs was reported about fifty years ago [100], contradictory results on posttransfusion hemolysis have been reported in clinical studies [97, 98]. In general, deleterious effects of G6PD-deficient RBCs have been reported in neonates, hemolytic G6PD patients, multitransfused patients, and recipients on oxidative medication [98, 101] but not in adult recipients [102, 103], highlighting another critical issue of transfusion medicine, namely the recipient-variation. However, it is currently unknown whether the above-mentioned adverse posttransfusion effects are associated with a putative poor storability of the G6PD-deficient RBCs compared to the G6PD-normal RBCs.

4 Nongenetic factors may contribute to the donor-variation effect

4.1 Organismal aging affects RBCs' age and redox status at the time of donation

Aging is an inevitable biological process characterized by a general decline in physiological functions and redox homeostasis, which result from accumulated lesions in various cellular components [104]. In many countries volunteers are eligible for blood donation until the age of 65, however in other countries no upper age limit for blood donation exists, provided that the donors fulfill the established eligibility criteria and have no restrictions or limitations to their activities. Consequently, the degree of age-associated changes in RBC senescence and oxidative stress parameters that have been observed in the past, imply an effect of donor's age on RBC storability. Indeed, the oxidative and metabolic stresses that are generated during storage might disturb or accelerate the physiological senescence of stored RBCs [1].

Under the influence of many systemic factors, erythrocyte aging is accelerated in old individuals [105]. In fact, similar remodeling in RBC membrane has been observed during cellular and organismal aging, including increased binding of autologous IgGs to band 3 [106], calpain translocation to the membrane and band 3 proteolysis [107]. Normal hematocrit is usually associated with increased reticulocyte number in older people [108], implying the existence of a younger RBC population that is, however, susceptible to RBC lysis. Disturbed mechanical properties and deformability [109] or increased susceptibility to oxidative stress [110] might explain these findings. In support, compromised plasma [111] and RBC antioxidant capacity has been reported during human aging, as evidenced by low levels of GSH [110], antioxidant enzymes activity [112], and clusterin [113] in association with pathologically increased levels of oxidative lesions in RBC lipid and protein components [104, 114]. Human aging is negatively correlated with the concentration of Hb in fresh or stored RBCs [56] but positively correlated with the activity of RBC plasma membrane redox system, as a probable protective mechanism for plasma dehydroascorbic acid reduction under increased oxidative stress [111]. There is evidence that RBC vesiculation in fresh blood and in-bag hemolysis increase as a function of donor age [64, 67, 115] probably in association with donor sex [64, 67]. However at clinical level, donor age does not seem to affect mortality of transfused patients as recently shown by a retrospective, observational study using a large cohort (>180 000) of recipients [116].

Age- and sex-associated effects on the average RBC life span may address in part the large heterogeneity observed in the mean age of RBCs among eligible blood donors [52]. Although stored RBCs degrade over time through more than one mechanisms of cellular injury [36], including disturbance in redox and metabolism homeostasis [1], RBCs' age at the time of donation probably affects the quality of stored RBC concentrates [53, 117]. In terms of posttransfusion efficacy, the effectiveness of transfused young RBCs ("neocytes") seems to be less than predicted by early in vitro and in vivo studies [118, 119] and varies among patients [120] probably because neocytes are more sensitive to the presence or absence of survival signals compared to older cells [121].

4.2 Donor-specific lifestyle characteristics define in part the physiological profile of donated RBCs and, probably, their storage capacity

Information on the lifestyle of donors is not regularly recorded by blood donation services. However, some

physiological aspects of blood that directly or indirectly affect RBC storability might be related with smoking, diet, or other lifestyle characteristics of the donor.

Although smoking is not considered an exclusion criterion for blood donation [122], it exposes individuals to excessive amounts of oxidants and toxins that harm RBCs and soluble components of the blood. It has been reported that the main source for the pathologically increased levels of carbon monoxide (CO) found inside the pRBC units is smoking (Table 1) and that the elimination half-life of CO after the last smoked cigarette varies significantly among donors [123]. Smokers' RBCs are characterized by decreased deformability [124], increased osmotic fragility [125], susceptibility to oxidant-induced hemolysis [126], and reduced folate levels [54]. The enzymatic antioxidant activity [127] and the concentration of nonenzymatic antioxidant components like GSH and clusterin in RBCs [113, 127] have been found decreased compared to nonsmoking subjects. The plasma of smokers is further characterized by accumulation of reactive oxygen or nitrogen species and advanced glycation end products [125, 128], lipid peroxidation [127], protein carbonylation [129, 130], and low ascorbate levels [131]. Contradictory results from other studies have shown that the sustained levels of oxidative stress in the blood of smokers trigger the RBC antioxidant systems, thus protecting RBCs form hemolysis [132] and endothelial cells from hydrogen peroxide-induced damages [133]. These effects might be modified by other factors of interdonor variability, including the amount of cigarette consumption and donor age as shown by Hulea et al. [134], highlighting the cross-talk between smoking, organismal aging, and probably other parameters in defining the physiological status of donor RBCs.

Dietary habits, consumption of fatty meals before donation and body weight may also affect blood characteristics. Body weight correlates with total Hb concentration inside the unit of donated pRBCs [56]. Moreover, high fat meals and diet have been reported to affect plasma antioxidants [135], distribution of sex hormones between plasma and blood cells [136] and RBC membrane [137], probably by exposing RBCs to high amount of glucose and prooxidant factors [138]. Indeed RBCs from obese or overweight healthy individuals are characterized by increased ROS generation, susceptibility to peroxidation and radical-induced hemolysis, decreased GSH/GSSG (oxidized glutathione) ratio, decreased deformability [139] and abnormalities in the sodium/potassium pump resulting in disturbed ion homeostasis [140]. Pathological blood rheology, as a probable result of modifications in RBC aggregation and volume [141, 142] has also been reported in obese, apparently healthy donors. Thus, blood donated by obese donors might be disadvantageous regarding storage effect on redox homeostasis or structural integrity of RBCs. Volunteers with a body mass index >35 are not generally considered for blood donation; however, overweight volunteers are eligible blood donors. Obesity is common among donors in countries like USA [143] and obese people are often advised to donate blood in order to gain certain health benefits.

Regarding alcohol consumption, while red wine polyphenols exert an antioxidant effect on RBCs [144], chronic alcohol consumption has been associated with increased mean cell volume (MCV) [145], that continues to rise even upon cessation of drinking [145] as well as with increased glutathione peroxidase activity in the plasma [146]. Finally, regular moderate exercise is considered beneficial for redox homeostasis but acute and strenuous exercise has the opposite effect [147]. Indeed, ROS production and antioxidant capacity are negatively affected after acute exercise [148] but chronic repetition of exercise training may lead to the reduction of exercise-induced oxidative stress [149]. Although the same stimulus is necessary to allow an upregulation in endogenous antioxidant defenses, decreased RBC deformability, aggravation of the plasma antioxidant levels and increased lipoperoxidation have been measured in association with endurance exercise training [150]. Exercise can further affect the average age of the circulating RBCs by causing intravascular hemolysis mainly of senescent cells. The enrichment of peripheral blood in younger RBCs is associated with increased metabolic activity, oxygen release, and cellular deformability in trained athletes [151].

5 Studying the "donor-variation" effect

5.1 Donor and recipient variation effects on transfusion outcomes in the era of personalized medicine

The evolving field of personalized and precision medicine that refers to individualized treatment regimen tailored for the benefit of a single patient, presupposes the assessment of individual variability and the identification of biomarkers that shape individual responses to treatments, by precision approaches including *N-of-1* trials and Omics technologies [152]. In the field of transfusion medicine, it is currently clear that physiological differences between apparently "equal" donors might be associated with the variability observed in the quality of the blood labile product. Indeed, percentage of phosphatidylserine-exposing circulating RBCs has been found correlated to in-bag hemolysis [153], while pRBCs prepared by donors with high serum uric acid levels (natural antioxidant factor) exhibited significantly better levels of spheroechinocytosis and intracellular calcium accumulation as well as of supernatant antioxidant activity compared to donors with low serum uric acid [57]. Apart from these "inter-parameter" correlations between fresh and stored blood, it has been recently revealed that baseline donor-to-donor variation in Hb [56], GSH, and GSH/GSSG ratio levels [154], as well as MCV, RBC osmotic fragility, and plasma nitrate/nitrite and antioxidant capacity [155], is well preserved during storage of donated RBCs, and thus, can be safely predicted (Table 1). Furthermore, interdonor differences might become more evident posttransfusion [156] when cellular and soluble components of high physiological variability cross-talk with

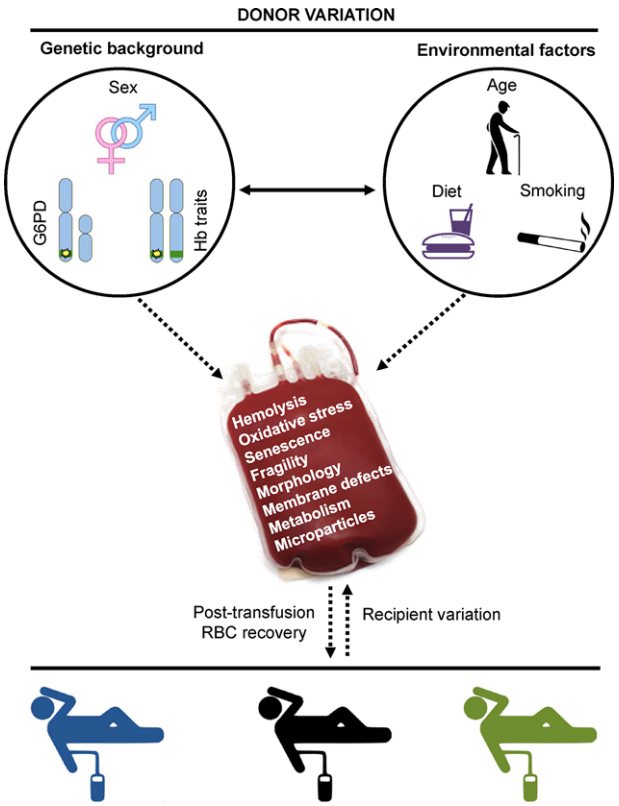

Figure 1. The donor-variation effect. According to the "double hit" hypothesis it is functionally associated with the recipient-variation effect.

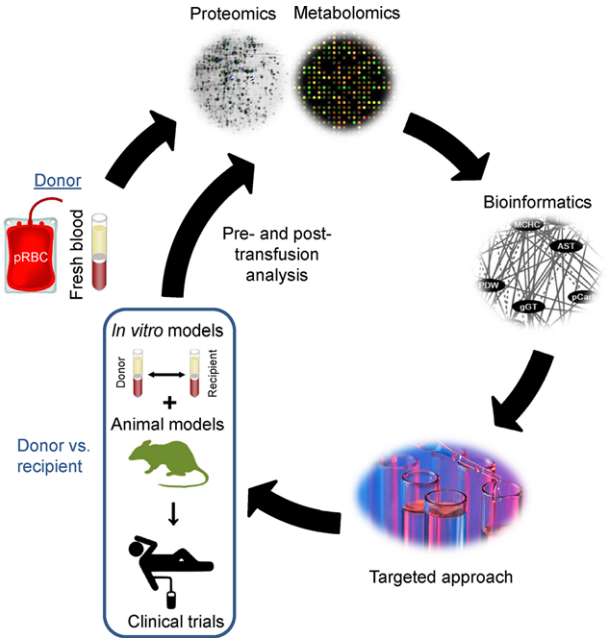

Figure 2. Currently proposed experimental approach for studying the donor-variation effect. This research work plan starts from donor analysis (upper left) and continues clockwise.

pathophysiologic factors exhibiting considerable diversity in the recipient (Fig. 1). This meeting of two extremely variable worlds determines the transfusion efficacy and transfusion-associated morbidity.

5.2 The resolution power of omics technologies might help elucidating donor variation effect on RBC storability and recovery

Omics precision technologies have revealed that the extent of individual donor variability is larger than that considered at first. To mention some examples, a remarkable high variability in the protein content of plasma, plasma MPs and exosomes from different healthy donors was shown that might be relevant for complement and coagulation signal-transduction cascades and acute phase responses after their interaction with cellular systems in the recipient [157]. An equal variability has been reported in several oxidized species, glucose-6-phosphate, dihydroxyacetone phosphate, nicotinamide adenine dinucleotide, and other 132 biochemicals among "equal" RBC concentrates by high resolution metabolomics analysis [158, 159].

Omics approaches have revealed that numerous biological parameters differ among healthy blood donors and change in RBC concentrates over storage. The power of omics in revealing integrated profiles of blood samples might lead to the identification of the biological mechanisms by which donor-specific factors affect the quality of stored RBC components, and consequently, to the identification of reliable biomarkers of RBCs storability and posttransfusion performance for donor screening (Fig. 2). The same tools have proved to be very effective in revealing molecular hematological factors functionally associated with the pathophysiology of various diseases [16, 160]. In this context, it was revealed that donors showing increased membrane binding of the redox marker Prx2 in fresh RBCs exhibit a corresponding increase of membrane lipoperoxidation during storage [161], suggesting that donor-specific redox status or susceptibility to oxidative injuries might influence the rate of storage lesion development (Table 1). On the other side, it should be noted that proteomic techniques are time-consuming and although their accuracy has been improved the last years, interlaboratory reproducibility problems, mainly associated with preanalytical variables, still exist [35].

Maps for distinct group of donors can be generated by processing of omics-derived data through currently available elegant bioinformatics tools, in order to achieve a more systemic approach to RBC storage and transfusion biology in the light of donor-to-donor differences [162]. To this purpose, either strictly defined groups of similar healthy donors (e.g. G6PD-deficient versus G6PD efficient donors) or nonpooled approaches need to be adopted in omics assays. Information derived by integrated proteomics studies has already been used to construct interactomics of human RBCs and

to probe critical protein changes and biomarkers of severity in blood disorders [163]. Similar networks of correlations between donor-specific baseline and storage physiological variables arisen by conventional, small-scale analyses revealed that donor variation is a multivariable phenomenon in transfusion biology that could be, however, sorted out by paired fresh blood versus stored RBC data acquisition [155]. The evaluation of variables emerging as candidate biomarkers of storability by these integrated approaches might be performed by targeted, small-scale biological and hematological assays, like hemolysis estimation or Western blotting, in independent groups of donors.

5.3 In vitro and in vivo models of transfusion and clinical trials meet the need of unraveling the clinical relevance of interdonor variability at baseline and in RBC storage measures

The prominent advantage of obtaining large amounts of in vitro data stresses emphatically the considerable lack of knowledge on their clinical relevance. Consequently, the last step of this circular research plan should address posttransfusion issues, by examining donor-specific factors in vivo and storage quality biomarkers that are functionally associated with the RBC performance and effects, through in vitro (human) and in vivo (animal) models of transfusion. The combined posttransfusion data would eventually fuel clinical trials.

In vitro models that simulate recipient context and transfusion-associated stresses by incubating donor pRBCs units with recipient blood under certain, controlled conditions [164], offer the possibility to study large number of human samples and specific donor-recipient pair settings, after taking into consideration donor/recipient-variation effects. However, they are characterized by lack of the RBCs natural environment and probable in vitro artifacts compared to the in situ state [165]. As a result they can be used as tools to provide supplemental information to data obtained from animal models.

Animal models of RBC storage/transfusion offer the opportunity to study the effects of storage duration, strategy, and pRBC volume on transfusion efficacy and effects, in tightly controlled and specific clinical conditions in vivo but their findings are usually based on the examination of a small number of animals and usually, donor-variation effects are not reported. Interestingly, similar to donor-to-donor variation in storage of human RBCs, genetically distinct strains of mice have a wide range of RBC storage biology regarding both metabolome and poststorage circulation. In a murine model of RBC storage/transfusion that combined high resolution metabolomics and in vivo recoveries, lipid peroxidation, and metabolites that differed in fresh blood were found associated with poor 24-h recovery, allowing screening of donors at time of collection [166]. However, animal models may be less relevant to human transfusion medicine, since animals are not directly equivalent to human patients, clinical settings, and typical clinical practice [167], and consequently they have very limited capacity to be transferred to human settings. Through these methodological strengths and weaknesses, the combination of the data acquired by the posttransfusion examination tools would be very helpful in guiding future clinical trials.

In clinical trials human samples are tested in situ. They would address all the questions set during clinical practice but unfortunately, their design is a really tough task. The age and volume of the transfused blood are hard to keep constant, broad clinical settings are usually used, control-recipients with matching comorbidities and pharmaceutical supplementation are usually missing and extremes of RBC storage duration have not been considered [30]. Future trials should be designed in the light of knowledge that stored RBC concentrates are a nonuniform product, based upon donor-to-donor variation in red blood cell storage biology.

The biological systems are very complex and the clinical outcomes of transfusions are often unpredictable based on theoretical effects. The multireactivity of transfused RBCs with the majority of body tissues lead us back to the need of high throughput approaches to study transfusion outcomes, taking into account the recipient-variation effect. The examination of the "double hit" hypothesis (donated blood—recipient's pathological characteristics) [58] falls within the "precision" transfusion medicine concept (Fig. 1).

6 The ultimate benefit

Intensive focusing on storage time as the sole measure of storage lesion has led to practically ignoring the inherent variability of donated RBC properties. However, during the last decade a turn towards pair studying of donors in vivo and during storage of RBCs has resulted in the identification of factors with the potential for being considered as storage quality biomarkers in large-scale studies (Table 1). The identification of donor-specific storability biomarkers would expedite the development and refinement of the storage systems and allow the effective management of donated blood by blood bank services according to the donor-associated storability characteristics, in order to gain the optimum from each donor in favour of the patients. In the generally accepted context that "no one donor is in excess," labile products of poor storability would proceed earlier to the recipients or be stored under the currently optimum conditions. In contrast, labile products of good storability would be stored for longer or proceed to certain patients groups, that are vulnerable to the unintended consequences of the transfusion therapy, e.g. infants, critically ill patients, or thalassemia patients who can become iron overloaded by repeated transfusions. At present, physicians choose RBCs units of shorter storage time for transfusing, e.g. infants. However, an "old" unit from a good "storer" might be better than a "fresh" one from a donor with poor storability. This individualization would improve care and

markedly reduce health system cost. The currently proposed holistic methodological approach for studying the donor variation effect at three levels, namely at the time of donation, during storage and after transfusion could move transfusion medicine towards a more personalized era when each individual's genetic and physiological signature will be assessed and taken into account. Future in vitro and in vivo studies of transfusion and clinical trials should consider emerging, accumulating data reporting donor-to-donor variation in red blood cell basal and storage biology and assess transfusion outcome as the synergistic result of recipient variation as well.

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