State of art

The storage lesions: From past to future

Les lésions de stockage : entre peurs rétrospectives et perspectives

J.-D. Tissot [a,b,*], M. Bardyn [a], G. Sonego [a], M. Abonnenc [a], M. Prudent [a,b]

[a] Transfusion interrégionale CRS, Laboratoire de Recherche sur les Produits Sanguins, 2, route de la Corniche, CH-1066 Epalinges, Switzerland
[b] Faculté de biologie et de médecine, université de Lausanne, Lausanne, Switzerland

Abstract

Red blood cell (RBC) concentrates are stored in additive solutions at 4 °C for up to 42 days, whereas platelets concentrates (PCs) are stored at 22 °C with continuous agitation for up to 5 to 7 days, according national regulations, and the use or not of pathogen inactivation procedures. Storage induces cellular lesion and alters either RBC or platelet metabolism, and is associated with protein alterations. Some age-related alterations prove reversible, while other changes are irreversible, notably following protein oxidation. It is likely that any irreversible damage affects the blood component quality and thus the transfusion efficiency. Nevertheless, there still exists a debate surrounding the impact of storage lesions, for both RBCs and PCs. Uncertainty is not completely resolved. Several studies show a tendency for poorer outcomes to occur in patients receiving older blood products; however, no clear significant association has yet been demonstrated. The present short review aims to promote a better understanding of the occurrence of storage lesions, with particular emphasis on biochemical modifications opening discussions of the future advancement of blood transfusion processes. The paper is also an advocacy for the implementation of an independent international organization in charge of planning and controlling clinical studies in transfusion medicine, in order to base transfusion medicine practices both on security principles, but also on clinical evidences.
© 2017 Elsevier Masson SAS. All rights reserved.

Keywords: Ageing; Blood cells; Microparticles; Platelets; Oxidation; Proteomics; Red blood cells; Storage; Transfusion

Résumé

Les concentrés de globules rouges sont conservés dans des solutions additives à 4 °C, pour une durée allant généralement jusqu'à 42 jours, alors que les concentrés plaquettaires sont conservés à température ambiante, pour des périodes allant jusqu'à 5 ou 7 jours, selon les législations, et suivant l'usage de procédés de réduction des pathogènes. Le stockage induit obligatoirement des lésions cellulaires touchant leur métabolisme ainsi que des altérations protéiques, notamment au niveau oxydatif. Il est probable que les lésions, lorsqu'elles sont irréversibles affectent tant la qualité des produits sanguins transfusés que leur efficacité. Néanmoins, un doute persiste sur l'importance clinique des lésions de stockages, malgré un certain nombre d'études cliniques qui se veulent rassurantes. Cet article a pour but de faire une brève revue synthétique des lésions de stockage et de les mettre en perspective face aux obligations que les services/établissements de transfusion devront assumer vis-à-vis notamment des autorités sanitaires délivrant l'autorisation de mise sur le marché et dans leur participation aux études cliniques. Il est aussi un plaidoyer pour que la recherche clinique soit coordonnée et organisée afin que les pratiques puissent être basées non seulement sur des arguments sécuritaires, mais surtout sur des évidences cliniques solides.
© 2017 Elsevier Masson SAS. Tous droits réservés.

Mots clés : Ageing ; Blood cells ; Microparticles ; Platelets ; Oxidation ; Proteomics ; Red blood cells ; Storage ; Transfusion

1. Introduction

If blood components (BCs) have to be introduced on the market in 2017 as novel therapeutics, who will accept that red

* Corresponding author. Jean-Daniel Tissot, transfusion interrégionale CRS, route de la Corniche 2, 1066 Epalinges, Switzerland.
    E-mail address: jean-daniel.tissot@itransfusion.ch (J.-D. Tissot).

blood cells (RBCs) can be stored at 4 °C without any proofs of the absence of clinical consequences of conservation of blood outside physiological condition. The same is true when considering platelets, which are stored in really non-physiological conditions. Both RBCs and platelets are generated by nature in order to survive at 37 °C in the presence of plasma and without any anticoagulant. Who can remember, in 2017, that most of the conditions used to prepare and to store blood components were derived from empirical as well as practical approaches established by pioneer's decades before quality controls and validation procedures were put in place and controlled by external organisations such as the ANSM, Swissmedic or the Paul Erlich Institute? Most of our practices in transfusion medicine are based on historical approaches not necessarily scientifically sounded. For example, the reason why platelets cannot be stored at 4 °C without inducing lesions that did not allows re-circulation is quite recent [1–3]. Normal platelets are produced by megakaryoctes in the bone marrow (as well as in the lungs [4,5]): they circulate within blood, at 37 °C, in presence of plasma and many other cells like leukocytes, RBCs and endothelial cells. Therefore, who can admit that they can be stored at room temperatures with constant agitation in the presence of relatively low concentrations of plasma and in the presence of additive solutions? Nevertheless, this practice is considered as normal and is the standard approach [6,7]. If nowadays such an approach would be proposed to the health authorities, it is all but sure that, according to the detected storage lesions that have been identified, authorities will allow placing on the market such BCs. The real world is quite different. The retrospective regard is like a wink on the distance that is clearly apparent between the requirements that are nowadays considered as normal procedures and the historical pragmatism that prevailed in the last century: regardless of the considerations taken into account, BCs are on the market. Nevertheless, both fundamental, translational and clinical studies are needed in the near future to provide better care to our patients and to provide strong evidence to the health authorities that the processes are under control. In this context, this paper puts in perspective the RBC as well as the platelet storage lesions. Many different physiological and biochemical pathways are affected by storage, and stored BC are clearly very different when compared to freshly drown blood. Therefore, most of clinicians are lost in translation because experimental data and clinical data may be interpreted in divergent manners. Finally, the development of "new" products is hampered, due to all validation steps and procedures that are needed even if based on current practices, without consideration of the biology of cells that will be transfused. All these aspects are hot topics in transfusion medicine.

2. The past

The history of storage lesions is parallel to that of transfusion medicine (reviewed in [8]). Early blood transfusion was a surgical act involving the dissection of veins or arteries in both the donor and the recipient. End-to-end anastomosis of blood vessels was commonly performed. Transfusions were thus performed arm-to-arm, meaning that donors had to be present next to the patients. The first direct blood transfusion (arm-to-arm) of World War I (WWI) was performed by a French physician in 1914. Interestingly, the donor and recipient bloods were not cross-matched to ensure their group compatibility. Indeed, the watchword was to accept the risk of blood incompatibility issues rather than let people die from massive haemorrhage. Following the advance of anticoagulation and short-term preservation procedures, blood was bottled and transfused with no necessary contact between the donor and the patient. Blood preservation saw another substantial improvement in 1943: preservative solutions with different sodium citrate/citric acid ratios were tested. Blood storage for four weeks at 3 °C–7 °C and end-storage haemolysis were examined. Several biochemical parameters-such as spontaneous haemolysis, osmotic fragility, pH, glucose, potassium, and formation of methemoglobin were evaluated. These studies lead to the conclusion that acidified citrate-glucose (ACD) preservative solutions were satisfactory for blood storage. ACD blood preservative solutions allowed blood storage for three weeks (based on 24 h post-transfusion survival) and remained in use until the introduction of phosphate, which allowed blood storage for up to four weeks with increased levels of 2,3-DPG in 1957 [9].

In 1952, the utilization of a closed system of plastic bags for blood collection, preservation in ACD, and transfusion was proposed. Compared to glass bottles, the plastic bag system had the obvious advantages of reduced bacterial contamination (no contact with air), lighter weight, shock resistance, and ease of storage in refrigerators. During the war of Korea, the use of plastic bags for blood transfusion was proposed. Blood stored in such bags had lower plasma potassium levels than blood stored in glass bottles, perhaps due to the presence of diethylhexyl-phthalate (DEHP) released from blood bags limiting microvesiculation of RBC membranes [10]. The next improvements of blood storage involved the introduction of adenine as a constituent of preservative solutions. Citrate phosphate dextrose adenine solutions (CPDA-1 and CPDA-2) were licensed for use in the United States during the late 70s/early 80s, although transfusion medicine had already shifted to the use of packed RBC concentrates (RBCCs). The use of whole blood progressively become obsolete. The first additive solution used for storage of packed RBC units was sodium-adenine-glucose (SAG) [11], which was further modified by addition of mannitol (becoming SAGM) to reduce end-storage haemolysis. Other additive solutions were derived from the original SAG and are currently used worldwide, including additive solution (AS)-1, AS-3, and AS-5 in the United States and Canada, and MAP in Japan. The most recent improvement in blood transfusion involves the removal of leukocytes before or during whole blood processing. Leukoreduction is performed by removal of the buffy coat layer after whole blood centrifugation and/or leukofiltration [12].

Platelet concentrates (PCs) are produced either from whole blood or by apheresis (reviewed in [6,7]). However, taken into consideration the various protocols that are used for manufacturing PCs, several hundreds of different types of PCs are one the market, if we combine all possibilities provided by the type of

Table 1
Processing and storage lesions in banked BCs.

	Lesions (including storage and processing such as pathogen inactivation)	Potential markers	
		Quality control	Clinic
RBC concentrates			
Metabolism	Loss of metabolic modulation (2,3-DPG, ATP, urate...)	+	+
	Accumulation of lactate and pH drop	++	++
	Ion leakage (K^+, Fe^{3+})	+	+++
	Decrease of antioxidant defences	+	+
Macromolecules	Loss of ATP-dependent protein function	−	+
	Protein oxidation (sulfenic acid, carbonylation)/degradation	+	+
	Membrane proteins (band3 dimerization, delocalisation such as preoxiredoxin-2)	+	+
	Haemolysis	+++	+++
	Lipid oxidation	−	+
Phenotype	Exposure of senescence markers (phosphatidylserine)	+	+++
	Shape change/spherocytic shift	++	+
	Number of RBCs	+++	+++
Function	Reduced deformability	+++	+++
	Microvesiculation, release of lipids	++	++
	Aggregation properties	++	++
Platelet concentrates			
Metabolism	Metabolic shifts	+	−
	pH drop	++	++
	Accumulation of lactate	+	+
	Antioxydant drop	+++	+
Macromolecules	Protein relocalization	+	−
	Protein oxidation (cysteine oxidation and carbonylation)	+	−
	Protein activity	−	+
	mRNA, miRNA, mtDNA	++	−
Phenotype	Activation markers	+	++
	Exposure of senescence markers (phosphatidylserine)	+	+
	Shape change/size	+	+
	Number of platelets	+++	+++
Function	Deformability (decrease HSR...)	++	+
	Increase adhesion properties	−	++
	Loss of COAT platelets	+	++
	Variations (agonist-dependent) in aggregation properties	−	++
	α-degranulation	+	+

donation, the type of centrifugation (platelet rich plasma, buffy coats), the possibility of pooling, of leukoreduction, the addition of storage solutions and the introduction of pathogen inactivation technologies [7,13–15].

3. The present

Many efforts have contributed to improving blood transfusion processes, including advancements in procedures for collection, storage, and infusion. Nowadays, BCs are obtained either by processing whole blood donations or by collecting individual components via apheresis-driven donation of the required fraction(s). Managing each BC type separately allows optimized storage of each component in accordance with its intrinsic properties. Moreover, the various labile BCs can be transfused independently of each other, as each can be used to treat different pathologies. The dogma that was prevalent over the last decades was to only transfuse patients either with PC, RBCC or fresh frozen plasma (FFP) depending of the clinical situation. However, with the progresses of modern medicine, more and more "resuscitation packs" are used in emergency rooms: they usually encompass four red RBCCs, four FFP units, and 4–6 PC

units. These constituents are combined with the goal of recovering whole blood properties [16]. Definitively, the "reconstituted whole blood" presently used in emergency wards is totally different when compared with non-sorted fresh blood that was transfused arm to arm. Even if modern processes offer considerable improvements in terms of quality and immunohaematology and safety, they also introduces some caveats that can be underestimated or mistakenly ignored, notably those related to storage lesions. These lesions are summarized in Table 1.

3.1. Red blood cells storage lesions

RBCC are stored (42–49 days at 4 °C) in additive solutions, after leukoreduction or not, exhibit various types of lesions [17–22]. They notably compass a shift in metabolism and protein activity, modifications of protein integrity, phenotype and morphological properties of RBC [23–29]. In addition, metabolism dysregulation induces a cascade of events related to protein function impairment, proteasome inhibition, oxidized protein accumulation at the RBC membrane and their elimination through microvesiculation [23,30,31]. These

microvesicles released during storage have been reported to have pro-coagulant properties [32–37].

3.2. Platelets storage lesions

PCs account for near ten percent of all BCs that are transfused. They are accountable for more than twenty five percent of the reported adverse events. Besides issues linked to patients themselves, who may be particularly at risk of side effects depending of their underlying illness, there are many aspects directly related to platelet collection, preparation and storage that predispose to adverse events [7]. Platelets that are stored for a shorter time (5 to 7 days, at 22 °C) also suffer from lesions, related to the processing or the aging [38]. Loss of platelet quality during storage has been extensively characterized over the past decade but novel concepts are still emerging that could explain the functional decline. Manual and automated processes with distinct additive solutions affect whole blood-derived PC differently [39]. Platelet storage lesions include apparition of platelet activation markers, morphological changes, mitochondrial dysfunction, loss of GPIbα and α-granule secretion [13,40,41]. The application of pathogen inactivation techniques on PCs tends to accelerate the apparition and extent of platelet storage lesions [38]. In addition, oxidative damages are observed that can be prevented by urate [42]. However, so far, none of these in vitro parameters has been correlated with in vivo recovery and function.

3.3. Potential implications of storage lesions

There is a huge variety of identified storage lesions, occurring both for RBCCs and PCs. However, most of the non-specialist are lost when interpreting research data, and by confronting them with the clinical reality as well as with clinical data: in summary, we are totally lost in translation [43]. What is scientifically sounded, and what is clinically relevant: what are the parameters to be considered? Thus, amongst others, scavenging of nitric oxide by free haemoglobin or other microparticles might reduce vascular relaxation, extravascular haemolysis following damaged RBC elimination and the risk of bacterial growth or immune response, generation of bioactive lipids involved in TRALI, release of biological response modifiers, preactivation of platelets and in vivo efficacy, and so forth [44]. These effects are linked to in vitro parameters that change during the processing and the storage of BCs. Moreover, they are influenced by the donors' characteristics [45,46]. The lesions take different paths to damage the cells. The storage lesions are usually the consequence a cascade of events that starts with metabolism dysregulation to protein activity dysfunction, and cellular and functional lesions [23,45]. The impact of blood processing is another example where it can directly alter the cells [45], in a storage-independent manner. For instance, the photochemical treatment of PCs induces a drop in antioxidant power [40], the modification of proteins [38] as well as an increase of the storage lesions.

Even if the storage lesion open avenues for basic research, and for the understanding of biological processes, the clinical correlations between storage lesions and clinical outcome do not remain totally clear [47–49]. Several studies have reported the potential toxicity of transfused BCs (beyond immunological complications notably due the mismatch in blood groups or to the presence of various cytokines), the used of "older" blood is probably not "dangerous" [49], but certainly should be used with caution in particular clinical situations that certainly should be identified [23,48]. As proposed by Prudent et al., three phases may be considered when regarding RBC storage lesions [23,43]. The analysis of in vitro data highlights the presence of reversible and irreversible storage lesions and demonstrates that RBCs exhibit two limits during storage: one around 2 weeks and another one around 4 weeks of storage. Of particular importance, the first lesions to appear, i.e. the reversible ones, are per se reversible once transfused, whereas the irreversible lesions are not. In clinical trials, the RBCCs age cut-off for short-term storage is in general fewer than 14 days (11 ± 4 days) and more disperse for long-term-stored RBCCs (17 ± 13 days), regardless the clinical outcomes. Taking together, RBCCs age cut-off in clinical trials does not totally fall into line of in vitro aging data, whereas it is the key criteria in clinical studies. Long-term-stored RBCCs considered in clinical trials are not probably old enough to answer the question: "Does transfusion of long-term-stored RBCCs (older than 4 weeks) result in worse clinical outcomes?" The age of erythrocyte concentrates RBCCs in transfusion medicine and the adverse outcomes when transfusing long-term-stored RBCCs are highly controversial issues. Whereas the definition of a short-term-stored RBCC or a long-term-stored RBCC is unclear in clinical trials, data based on in vitro storage assays can help defining a limit in addition of the expiration date.

4. The future

4.1. An advocacy for the implementation of clinical studies

Transfusion of RBCCs efficiency is quite difficult to evaluate because this therapy was introduced before evidence-based medicine was formally established. Transfusion is almost unanimously considered a lifesaving procedure, with many advertisements promoting blood donation stating that "blood saves lives". However, this cannot be taken at face value. It must also be considered that, in rare cases, transfusion may harm or even kill the recipient [50–53]. Moreover, the increasing availability of alternative treatments mean that transfusion is no longer a simple matter of "life or death", but more often a situation in which transfusion provides a more effective or rapid treatment [54–57]. Specialists in transfusion medicine tend to argue that transfusion is beneficial, while other physicians cast more doubts on the procedure and express concerns regarding on transfusion-linked hazards. It is important to achieve a realistic view of the benefit-hazard ratio of transfusion processes. This is the reason why, in this paper, we take to opportunity to make a "plaidoyer" for the creation of an international organization in charge of planning and controlling clinical studies in transfusion medicine. We urgently ask the community of transfusion medicine to think about the future and to propose solutions allowing to combine efficiency
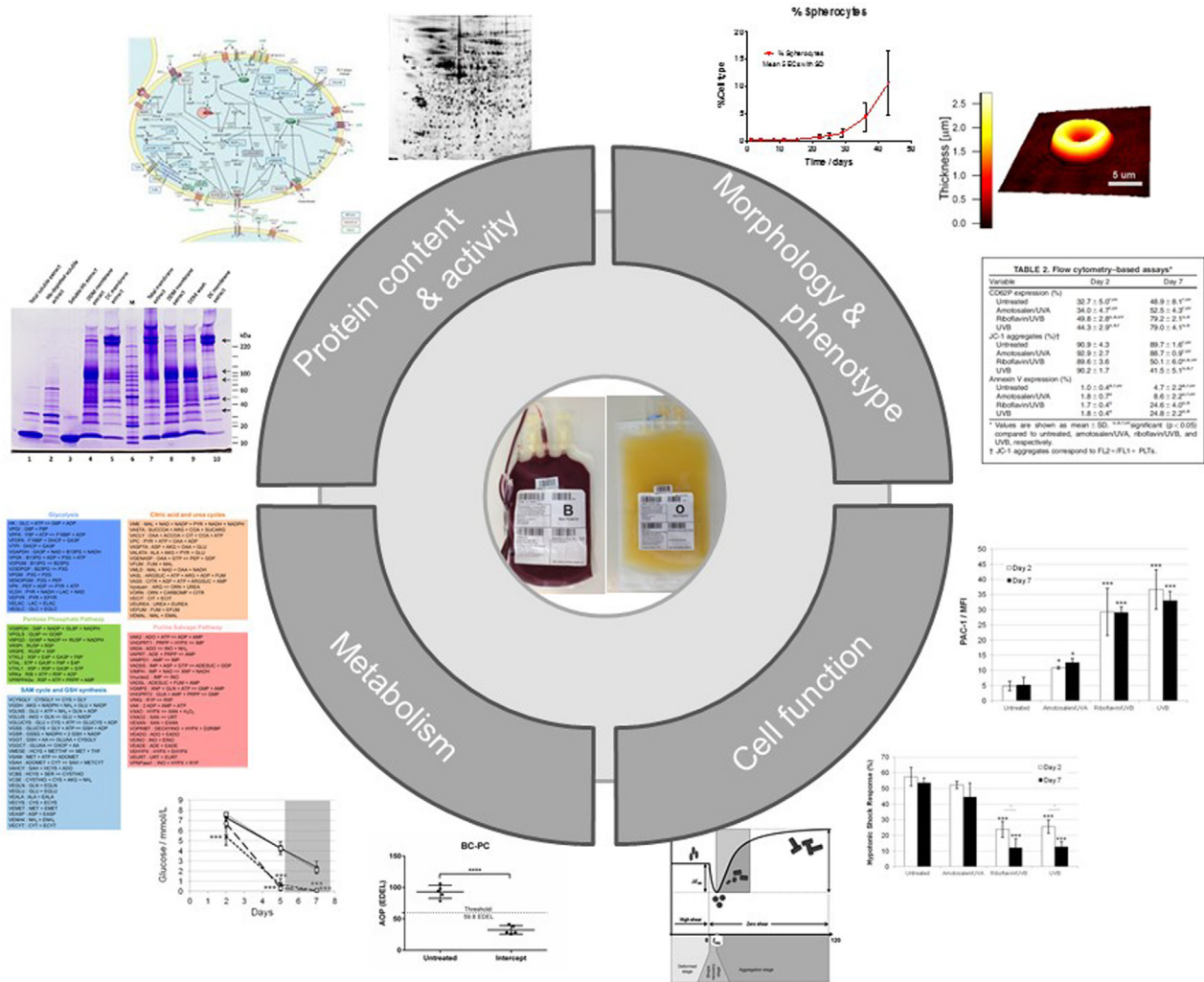
Fig. 1. Illustration of research strategy and targets for the in vitro improvement of the efficiency and safety of BCs. Metabolism: selected pathways of RBC metabolism modelization, glucose consumption and antioxidant level in PCs. Protein content and activity: distribution of RBC and platelet proteins in one dimension gel (SDS-PAGE) and two dimensional gel electrophoresis (2D-PAGE), and impacted proteins in pathogen-inactivated PCs. Morphology and phenotype: proportion of spherocytes in aged RBCCs, discoid shape of a red blood cells, phenotype of stored platelets. Cell function: activation of integrin and hypotonic shock response data in platelets, aggregation of red blood cells [17,30,38,40,41,84,85]. This scheme does not include biological effects on animal models, human cells or in vivo experiments such as survival.

and quality in clinical trials [58–62]. Most often, the number of patients included in published trials is quite (or even too) small. The non-inferiority hypothesis is frequently the only end point that is tested [63]. Finally, the financial support of these studies is commonly that provided by the few industrials still remaining on the market. Taken together, it appears obvious that the organization of methodologically sounded and ethically founded trials is very difficult. The design of clinical studies should be underground the control of international associations, providing coordination, methodological support. Of course, the achievement of such a proposal will largely depend on large and substantial public as well as private funding.

4.2. Parameters to be considered

What would be the parameters to consider in the XXIst century to evaluate the quality of the storage taking into consideration the donor (sex, age, habits, genotype, phenotype,

frequency of donation), the procedures used to prepare BCs? The advancements in the characterization of PCs and RBCCs have been impressive during the last two decades. There is a plethora of parameters (Fig. 1) that can be evaluated. Of note, there is still a lot of markers to be discover as recently evidenced by the potential of redox proteomics for instance [64]: such parameters may prove useful to follow processing BCs or ex vivo aging phenomena. Of course, all of these parameters have to be used in regard of each study design. Indeed, clinical trials cannot record all the parameters that might be followed in research on BCs. Moreover, the parameter has to be quantitative. Therefore, in order to reconsider the processing of blood in the light of the recent developments, in vitro data might be definitively considered and the reduction of storage lesions should be the target beyond the famous haemolysis upper limit or the platelet count, the swirling and pH in PCs. Nevertheless, the usefulness of each parameter considered as "a gold standard parameter" should be critically evaluated and re-evaluated in each study. They should
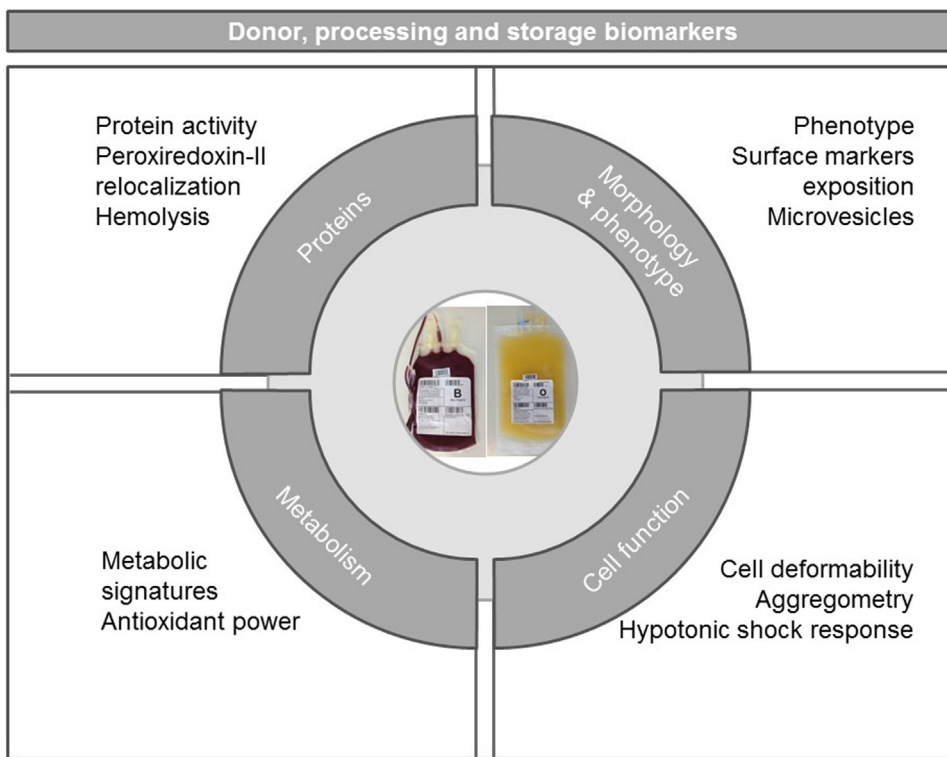
Fig. 2. Key parameters of the storage lesions: a virtuous circle to be considered.

not be used only because they are considered as requirement by health authorities. This is particularly evident when considering the pH value. It is considered as a marker of storage of PCs and is measured in blood banks on a sample of PC at expiry, as part of routine quality control programs. Platelets become irreversibly damaged when the pH falls below 6.4. However, the relevance of measuring the pH value as a quality control has been questioned [65]. In a multicenter study published on behalf of the Biomedical Excellence for Safer Transfusion (BEST), very few pH failures were identified in any of the centres participating in the study confirming that this measurement is a poor indicator of platelet quality [66]. However, a significant variability in the distribution of pH values between centres was observed as a result of differences in processing methods. Interestingly, the donor's characteristics, namely age and gender, influence the pH values distribution suggesting that certain donor's characteristics are associated with poor platelet storage.

In the future, donor's characteristics are key parameters to take into consideration while assessing the quality of BCs. Platelet lesions have been extensively characterized at the metabolite and protein levels [13,38,40,41,67]. More recently, miRNAs — important regulators of cellular functions — have been investigated in platelets subjected or not to pathogen inactivation techniques [68–70]. Alteration of platelet miRNAs has been reported upon pathogen inactivation treatments [68,69]. Dahiya et al. provided the first-time insights into the miRNA-mRNA interactions underlying mitochondrial dysfunction in stored platelets [71]. Further investigations need to be performed to evaluate microRNAs as potential markers of platelet quality.

By consequence, the development and improvement of BC quality and safety should consider markers that reflect the damages at different levels, i.e. chemical, biological and cellular levels (Fig. 2). Indeed, the haemolysis is a clear marker but it does not represent the functionality of the remaining "healthy" RBCs that might be recorded by deformability assays or metabolic signatures. Such approach implies the use of several analytical methods and the expertise in different fields. To cite a few: proteomics [72–76] combined with mass spectrometry-based research and all omic sciences, biochemistry and protein activity, molecular biology (mRNA, mtDNA), flow cytometry and functional assays (deformability, aggregation) and microscopy. This strategy will enable to broadly picture out the lesions and lesion events [77]. Then, the correction of those will be guided and driven by all the recorded data.

5. Perspectives

The key paradox of modern transfusion medicine is related to the fact that donated blood is not directly transfused from donors to patients, but rather undergoes processing for at least 1–2 days and storage for various length of time. BCs are products deriving from blood, but are all but "normal" circulating blood!

Shall we personalize transfusion medicine or shall we do our best to offer BCs without any storage lesions? How should we consider security in transfusion medicine? The answer is not obvious [78]. How should we consider the costs of security? Certainly, transfusion medicine has the world record of the costs of quality-adjusted life year (QALY), particularly when different procedures are added [79–83]. How should we consider transfusion medicine in the field of personalized medicine and in the context of blood management? In reality, transfusion medicine is already a form of personalized medicine: the donor is selected

according to its ABO and Rh (RH1) blood groups. Additional personalized items are frequently taken into consideration such as other blood groups notably in the RH, KEL, DUFY, KID or MNS, the absence of biomarkers for CMV, HAV, PARVO B19, HEV or selection according to their HLA serotype or genotype. Therefore, shall we make more complex the selection of the "right" donor for the "right" patient at the "right" moment by controlling the storage lesions? The answer is obvious; we want to offer the best product ever produced, as close as possible of the blood. However, because of technical, scientific, logistic and economic reasons, the answer may not be so clear.

Role of funding source

Disclosure of interest

The authors declare that they have no competing interest.

References

[1] Hoffmeister KM, Josefsson EC, Isaac NA, Clausen H, Hartwig JH, et al. Glycosylation restores survival of chilled blood platelets. Science 2003;301:1531–4.

[2] Hoffmeister KM, Falet H. Platelet clearance by the hepatic Ashwell-Morrell receptor: mechanisms and biological significance. Thromb Res 2016;141:S68–72.

[3] Lee-Sundlov MM, Ashline DJ, Hanneman AJ, Grozovsky R, Reinhold VN, et al. Circulating blood and platelets supply glycosyltransferases that enable extrinsic extracellular glycosylation. Glycobiology 2017;27:188–98.

[4] Lefrancais E, Ortiz-Munoz G, Caudrillier A, Mallavia B, Liu F, et al. The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. Nature 2017;544:105–9.

[5] Howell WH, Donahue DD. The production of blood platelets in the lungs. J Exp Med 1937;65:177–203.

[6] Garraud O. Platelet components: is there need or room for quality control assays of storage lesions? Blood Transfus 2017, http://dx.doi.org/10.2450/2016.0319-16.

[7] Garraud O, Cognasse F, Tissot JD, Chavarin P, Laperche S, et al. Improving platelet transfusion safety: biomedical and technical considerations. Blood Transfus 2016;14:109–22.

[8] Delobel J, Garraud O, Barelli S, Lefrère JJ, Prudent M, et al. Storage lesion: history and perspectives. Worl J Hematol 2015;4:54–68.

[9] Gibson 2nd JG, Gregory CB, Button LN. Citrate-phosphate-dextrose solution for preservation of human blood: a further report. Transfusion 1961;1:280–7.

[10] Bicalho B, Serrano K, Dos Santos Pereira A, Devine DV, Acker JP. Blood bag plasticizers influence red blood cell vesiculation rate without altering the lipid composition of the vesicles. Transfus Med Hemother 2016;43:19–26.

[11] Hogman CF, Hedlund K, Zetterstrom H. Clinical usefulness of red cells preserved in protein-poor mediums. N Engl J Med 1978;299:1377–82.

[12] D'Alessandro A. Leucoreduction of blood components: clinical and molecular evidence. Blood Transfus 2016;14:212–3.

[13] Abonnenc M, Sonego G, Kaiser-Guignard J, Crettaz D, Prudent M, et al. In vitro evaluation of pathogen-inactivated buffy coat-derived platelet concentrates during storage: psoralen-based photochemical treatment step-by-step. Blood Transfus 2015;13:255–64.

[14] Kaiser-Guignard J, Canellini G, Lion N, Abonnenc M, Osselaer JC, et al. The clinical and biological impact of new pathogen inactivation technologies on platelet concentrates. Blood Rev 2014;28:235–41.

[15] Prowse CV. component pathogen inactivation: a critical review. Vox Sang 2013;104:183–99.

[16] Holcomb JB, Tilley BC, Baraniuk S, Fox EE, Wade CE, et al. Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma: the PROPPR randomized clinical trial. JAMA 2015;313:471–82.

[17] Bardyn M, Rappaz B, Jaferzadeh K, Crettaz D, Tissot JD, et al. Red blood cells ageing markers: a multi-parametric analysis. Blood Transfus 2017;15:239–48.

[18] Blasi B, D'Alessandro A, Ramundo N, Zolla L. Red blood cell storage and cell morphology. Transfus Med 2012;22:90–6.

[19] D'Alessandro A, Kriebardis AG, Rinalducci S, Antonelou MH, Hansen KC, et al. An update on red blood cell storage lesions, as gleaned through biochemistry and omics technologies. Transfusion 2015;55:205–19.

[20] Hess JR. Red cell changes during storage. Transfus Apher Sci 2010;43:51–9.

[21] D'Alessandro A, Liumbruno G, Grazzini G, Zolla L. Red blood cell storage: the story so far. Blood Transfus 2010;8:82–8.

[22] D'Alessandro A, Nemkov T, Hansen KC, Szczepiorkowski ZM, Dumont LJ. Red blood cell storage in additive solution-7 preserves energy and redox metabolism: a metabolomics approach. Transfusion 2015;55:2955–66.

[23] Prudent M, Tissot JD, Lion N. The 3-phase evolution of stored red blood cells and the clinical trials: an obvious relationship. Blood Transfus 2017;15:188.

[24] Rinalducci S, Zolla L. Biochemistry of storage lesions of red cell and platelet concentrates: a continuous fight implying oxidative/nitrosative/phosphorylative stress and signaling. Transfus Apher Sci 2015;52:262–9.

[25] Kriebardis AG, Antonelou MH, Stamoulis KE, Economou-Petersen E, Margaritis LH, et al. Progressive oxidation of cytoskeletal proteins and accumulation of denatured hemoglobin in stored red cells. J Cell Mol Med 2007;11:148–55.

[26] Kriebardis AG, Antonelou MH, Stamoulis KE, Economou-Petersen E, Margaritis LH, et al. Membrane protein carbonylation in non-leukodepleted CPDA-preserved red blood cells. Blood Cells Mol Dis 2006;36:279–82.

[27] Prudent M, Sonego G, Abonnenc M, Tissot JD, Lion N. LC-MS/MS analysis and comparison of oxidative damages on peptides induced by pathogen reduction technologies for platelets. J Am Soc Mass Spectrom 2014;25:651–61.

[28] Roussel C, Dussiot M, Marin M, Morel A, Ndour PA, Duez J, et al. Spherocytic shift of red blood cells during storage provides a quantitative whole cell–based marker of the storage lesion. Transfusion 2017;57:1007–18, http://dx.doi.org/10.1111/trf.14015.

[29] Delobel J, Prudent M, Crettaz D, ElHajj Z, Riederer BM, et al. Cysteine redox proteomics of the hemoglobin-depleted cytosolic fraction of stored red blood cells. Proteomics Clin Appl 2016;10:883–93.

[30] Delobel J, Prudent M, Rubin O, Crettaz D, Tissot JD, et al. Subcellular fractionation of stored red blood cells reveals a compartment-based protein carbonylation evolution. J Proteomics 2012;76:181–93.

[31] Prudent M, Crettaz D, Delobel J, Seghatchian J, et al. Differences between calcium-stimulated and storage-induced erythrocytes-derived microvesicles. Transfus. Apher. Sci 2015;53:153–8.

[32] Rubin O, Delobel J, Prudent M, Lion N, Kohl K, et al. Red blood cell-derived microparticles isolated from blood units initiate and propagate thrombin generation. Transfusion 2013;53:1744–54.

[33] Rubin O, Crettaz D, Tissot JD, Lion N. Pre-analytical and methodological challenges in red blood cell microparticle proteomics. Talanta 2010;82:1–8.

[34] Rubin O, Crettaz D, Tissot JD, Lion N. Microparticles in stored red blood cells: submicron clotting bombs? Blood Transfus 2010;8:s31–8.

[35] Rubin O, Crettaz D, Canellini G, Tissot JD, Lion N. Microparticles in stored red blood cells: an approach using flow cytometry and proteomic tools. Vox Sang 2008;95:288–97.

[36] Tissot JD, Rubin O, Canellini G. Analysis and clinical relevance of microparticles from red blood cells. Curr Opin Hematol 2010;17:571–7.

[37] Jy W, Johansen ME, Bidot C, Horstman LL, Ahn YS. Red cell-derived microparticles (RMP) as haemostatic agent. Thromb. Haemost 2013;110:751–60.

[38] Prudent M, D'Alessandro A, Cazenave JP, Devine DV, Gachet C, et al. Proteome changes in platelets after pathogen inactivation–an interlaboratory consensus. Transfus Med Rev 2014;28:72–83.

[39] Nguyen KA, Chavarin P, Arthaud CA, Cognasse F, Garraud O. Do manual and automated processes with distinct additive solutions affect whole blood-derived platelet components differently? Blood Transfus 2013;11:152–3.

[40] Abonnenc M, Crettaz D, Tacchini P, Di Vincenzo L, Sonego G, et al. Antioxidant power as a quality control marker for completeness of amotosalen and ultraviolet A photochemical treatments in platelet concentrates and plasma units. Transfusion 2016;56:1819–27.

[41] Abonnenc M, Sonego G, Crettaz D, Aliotta A, Prudent M, et al. In vitro study of platelet function confirms the contribution of the ultraviolet B (UVB) radiation in the lesions observed in riboflavin/UVB-treated platelet concentrates. Transfusion 2015;55:2219–30.

[42] Abonnenc M, Crettaz D, Marvin L, Grund B, Sonego G, et al. Metabolomic profiling highlights oxidative damages in platelet concentrates treated for pathogen inactivation and shows protective role of urate. Metabolomics 2016;12:188, http://dx.doi.org/10.1007/s11306-016-1136-0.

[43] Prudent M, Tissot JD, Lion N. In vitro assays and clinical trials in red blood cell aging: Lost in translation. Transfus Apher Sci 2015;52:270–6.

[44] Zimring JC. Established and theoretical factors to consider in assessing the red cell storage lesion. Blood 2015;125:2185–90.

[45] Jordan A, Chen D, Yi QL, Kanias T, Gladwin MT, et al. Assessing the influence of component processing and donor characteristics on quality of red cell concentrates using quality control data. Vox Sang 2016;111: 8–15.

[46] Dern RJ, Gwinn RP, Wiorkowski JJ. Studies on the preservation of human blood. I. Variability in erythrocyte storage characteristics among healthy donors. J Lab Clin Med 1966;67:955–65.

[47] Lacroix J, Hebert PC, Fergusson DA, Tinmouth A, Cook DJ, et al. Age of transfused blood in critically ill adults. N Engl J Med 2015;372:1410–8.

[48] Garraud O, Tissot JD, Vlaar AP. Short-term versus long-term blood storage. N Engl J Med 2017;376:1091.

[49] Heddle NM, Cook RJ, Eikelboom JW. Short-term versus long-term blood storage. N Engl J Med 2017;376:1092–3.

[50] Jairath V, Kahan BC, Gray A, Dore CJ, Mora A, et al. Restrictive versus liberal blood transfusion for acute upper gastrointestinal bleeding (TRIGGER): a pragmatic, open-label, cluster randomised feasibility trial. Lancet 2015;386:137–44.

[51] Spahn DR, Vamvakas EC. Is best transfusion practice alone best clinical practice? Blood Transfus 2013;11:172–4.

[52] Spahn DR, Shander A, Hofmann A. The chiasm: transfusion practice versus patient blood management. Best Pract Res Clin Anaesthesiol 2013;27:37–42.

[53] Spahn DR, Goodnough LT. Alternatives to blood transfusion. Lancet 2013;381:1855–65.

[54] Zacharowski K, Spahn DR. Patient blood management equals patient safety. Best Pract Res Clin Anaesthesiol 2016;30:159–69.

[55] Spahn DR. Patient blood management: success and potential in the future. Ann Surg 2016;264:212–3.

[56] Meybohm P, Richards T, Isbister J, Hofmann A, Shander A, et al. Patient blood management bundles to facilitate implementation. Transfus Med Rev 2017;31:62–71.

[57] Meybohm P, Froessler B, Goodnough LT, Klein AA, Munoz M, et al. Simplified international recommendations for the implementation of patient blood management (SIR4PBM). Perioper Med (Lond) 2017;6:5.

[58] Levy JH, Steiner ME, Transfusion M, Hemostasis Clinical Trials N. Clinical studies of erythrocyte outcomes and mortality: size really counts. Anesthesiology 2013;118:10–2.

[59] Rahbar MH, Fox EE, del Junco DJ, Cotton BA, Podbielski JM, et al. Coordination and management of multicenter clinical studies in trauma: experience from the Prospective Observational Multicenter Major Trauma Transfusion (PROMMTT) study. Resuscitation 2012;83:459–64.

[60] Triulzi DJ, Yazer MH. Clinical studies of the effect of blood storage on patient outcomes. Transfus Apher Sci 2010;43:95–106.

[61] Vamvakas EC. Meta-analysis of clinical studies of the purported deleterious effects of "old" (versus "fresh") red blood cells: are we at equipoise? Transfusion 2010;50:600–10.

[62] Heddle NM, Ness PM. CONSORT and clinical trial reporting: room for improvement. Transfusion 2016;56:781–3.

[63] Cook RJ, Heddle NM. Clinical trials evaluating pathogen-reduced platelet products: methodologic issues and recommendations. Transfusion 2013;53:1843–55.

[64] Sonego G, Abonnenc M, Tissot JD, Prudent M, Lion N. Redox proteomics and platelet activation: understanding the redox proteome to improve platelet quality for transfusion. Int J Mol Sci 2017:18.

[65] Tudisco C, Jett BW, Byrne K, Oblitas J, Leitman SF, et al. The value of pH as a quality control indicator for apheresis platelets. Transfusion 2005;45:773–8.

[66] Germain M, Gregoire Y, Vassallo RR, Acker JP, Cardigan R, et al. Quality control of apheresis platelets: a multicentre study to evaluate factors that can influence pH measurement. Vox Sang 2017;112:318–25.

[67] Prudent M, Crettaz D, Delobel J, Tissot JD, Lion N. Proteomic analysis of Intercept-treated platelets. J Proteomics 2012;76:316–28.

[68] Osman A, Hitzler WE, Ameur A, Provost P. Differential expression analysis by RNA-Seq reveals perturbations in the platelet mRNA transcriptome triggered by pathogen reduction systems. PLoS One 2015;10:e0133070.

[69] Osman A, Hitzler WE, Meyer CU, Landry P, Corduan A, et al. Effects of pathogen reduction systems on platelet microRNAs, mRNAs, activation, and function. Platelets 2015;26:154–63.

[70] Osman A, Hitzler WE, Provost P. Peculiarities of studying the effects of pathogen reduction technologies on platelets. Proteomics Clin Appl 2016;10:805–15.

[71] Dahiya N, Sarachana T, Kulkarni S, Wood Iii WH, Zhang Y, et al. miR-570 interacts with mitochondrial ATPase subunit g (ATP5L) encoding mRNA in stored platelets. Platelets 2017;28:74–81.

[72] Prudent M, Tissot JD, Lion N. Proteomics of blood and derived products: what's next? Expert Rev Proteomics 2011;8:717–37.

[73] Lion N, Crettaz D, Rubin O, Tissot JD. Stored red blood cells: a changing universe waiting for its map(s). J Proteomics 2010;73:374–85.

[74] Lion N, Tissot JD. Application of proteomics to hematology: the revolution is starting. Expert Rev Proteomics 2008;5:375–9.

[75] Thadikkaran L, Siegenthaler MA, Crettaz D, Queloz PA, Schneider P, et al. Recent advances in blood-related proteomics. Proteomics 2005;5:3019–34.

[76] Tissot JD. Blood proteomics. J Proteomics 2010;73:466–7.

[77] Tissot JD, Lion N. The picture of Dorian Gray: news from the red blood cell storage lesion. Blood Transfus 2014;12:293–5.

[78] Garraud O, Tissot JD, Osselaer JC, Follea G, Vernant JP, et al. Transfusion safety from the viewpoint of a musical quintet. Blood Transfus 2015;13:687.

[79] Moeremans K, Warie H, Annemans L. Assessment of the economic value of the INTERCEPT blood system in Belgium. Transfus Med 2006;16:17–30.

[80] Simon MS, Leff JA, Pandya A, Cushing M, Shaz BH, et al. Cost-effectiveness of blood donor screening for Babesia microti in endemic regions of the United States. Transfusion 2014;54:889–99.

[81] McCullough J, Goldfinger D, Gorlin J, Riley WJ, Sandhu H, et al. Cost implications of implementation of pathogen-inactivated platelets. Transfusion 2015;55:2312–20.

[82] Borkent-Raven BA, Janssen MP, van der Poel CL, Bonsel GJ, van Hout BA. Cost-effectiveness of additional blood screening tests in the Netherlands. Transfusion 2012;52:478–88.

[83] Marshall DA, Kleinman SH, Wong JB, AuBuchon JP, Grima DT, et al. Cost-effectiveness of nucleic acid test screening of volunteer blood donations for hepatitis B, hepatitis C and human immunodeficiency virus in the United States. Vox Sang 2004;86:28–40.

[84] Rappaz B, Barbul A, Emery Y, Korenstein R, et al. Comparative study of human erythrocytes by digital holographic microscopy, confocal microscopy, and impedance volume analyzer. Cytometry Part A 2008;73A:895–903.

[85] Prudent M, Rochat B, Goffaux G, Marvin L, et al. Dynamic Metabolic Flux Analysis of Stored Red Blood Cells. Transfusion 2016;56 [25A–25A].