Reviewer ‘s comments:
Many thanks to the two reviewers. They comments have been useful for the revision of the paper: all have been integrated.

Reviewer #1 :

The authors performed a really interesting review of red blood cell storage lesion. It's well written and complete and detailed. The introduction, with history and general informations about the topic, is really useful. Figures and tables should be added, in order to fix the essential informations. Moreover, a paragraph regarding the international guidelines should be added. However, the review could be useful and clear for the readers and, after some revisions, it could be accepted for publication.

One figure and one table have been added. A paragraph presenting the situation with international guidelines has been added.

Several parameters have contributed to the decrease of the demand: i) introduction of patient blood management (PBM), ii) progress in surgical measures, iii) better anesthesiology, iv) use of cell severs, and v) implementation of guidelines aiming to clearly describe transfusional indications notably those published by the AABB (http://www.aabb.org/sa/clinical-practice-guidelines/Pages/default.aspx), the ISBT (http://www.isbtweb.org/working-parties/clinical-transfusion/7-red-cell-transfusion-triggers/) of the American Society of Hematology (www.hematology.org/.../Guidelines-Quality/.../527). However, the number of published guidelines is correlated to the degree of uncertainty of the practices. A PubMed search (08.21.2015) yielded 559 items using ((guidelines) AND (red blood cell OR erythrocytes) AND transfusion) and with a search performed using (strategies) AND (red blood cell OR erythrocytes) AND transfusion.

Table 1: Overview of the storage lesions
*either in vivo or in vitro

	Lesions	**Reversible or Irreversibe***
Biochemical	Loss of metabolic modulation	Reversible
	Accumulation of lactate and pH drop	Reversible
	Ion leakage (K+)	Irreversible
	Decrease of antioxidant defenses	Reversible
	ATP-dependent protein function	Reversible
	Protein oxidation (sulfenic acid)	Reversible
	Protein oxidation (carbonylation) / degradation	Irreversible
	Membrame proteins (band3 dimerization / accumulation of oxidized proteins)	Irreversible
	Hemolysis	Irreversible
	Lipid oxidation	Irreversible
Morphological	Exposure of senescence markers (phosphatidylserine)	Irreversible
	Shape change	Reversible or irreversible
	Reduced deformability	Irreversible
	Microvesiculation	Irreversble

Figure 1: Representation of oxidative pathways involved in RBC storage. The model shows an RBC, divided in the middle (dashed line) to show features of early (left half) and late stages (right half) of oxidative damage. Membrane-bound and cytosolic components are indicated in the legend. A microvesicle is shown outside the RBC (right). Progressive oxidation steps are indicated as follows: Step 1: (Left of midline) RBC antioxidant defenses prevent most oxidative damage by reducing ROS, which converts ROS to less-reactive intermediates, or by becoming oxidized by ROS, and then recycling through a restorative mechanism. After prolonged storage, these defenses are overwhelmed by oxidative stress and protein oxidation, which occurs in both the cytosol and the plasma membrane. Step 2: As oxidized proteins unfold, they expose hydrophobic moieties that are recognized by 20S proteasome complexes, which perform proteolysis. Antioxidant defense proteins are also oxidized, and they become susceptible to proteolysis; the lack of defense leads to the overoxidation of RBC content (right of midline). Step 3: At around 4 weeks of storage, overoxidized proteins undergo crosslinking, which prevents further degradation; thus, partially-oxidized proteins and damaged proteins accumulate. Step 4: Oxidized hemoglobin aggregates into hemichromes, which bind to the membrane-bound band 3 protein; this binding modifies band 3 conformation, which potentially alters its association with the cytoskeleton, and the hemichromes displace the glycolytic enzymes bound to band 3. Step 5: Hemichrome autoxidation produces ROS, which oxidize cytoskeletal and membrane proteins. Step 6: The release of microvesicles enters an exponential phase, which allows the elimination of RBC aging markers, such as altered band 3 and externalized phosphatidylserine. Reprinted from [65], with permission from Elsevier.
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Reviewer #2 :

The manuscript is well written and easy to read, only minor suggestions: 1) page 18, Pre-transfusion washing: it should be mentioned and discussed the use of this approach in Haemetological disorders and polytransfused patients. 2) Page 22, "Interestingly, this decreae in RBCC transfusion was closely correlated to a decrease of mortality". It should be mentioned and discussed the issue related to transfusion indications. Guidelines have been published, authors should mention the guidelines indications. 3) It is not clear to me the meaning of the last sentence. It could be better to delete it. 4) A table summarizing the different storage lesions could help readers.

1) A few lines have added on washing :

Washing of RBCCs has been prescribed by Sir John Dacie for patients suffering from paroxysmal nocturnal hemaglobinuria (Marchiafava–Micheli syndrome). The procedure has been also used in patients with IgA defiencency, and/or in patients presenting with recurrent severe allergic transfusion reactions such as anaphylaxis or severe urticarial reactions not prevented by pre-transfusion antihistamine and corticosteroid administration.

2) Indication and guidelines have bene described (see above)
3) The sentence has been deleted
4) A table was added (see above)

Finally, the paper was edited by SanFrancisco Edit, and submitted to a Cross-check: no plagiarism was detected.