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# BLOOD STORAGE LESIONS AND OTHER BIOCHEMICAL CHANGES ASSOCIATED WITH DONOR BLOOD

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## ABSTARCT

This paper reviewed lesions and some biochemical changes associated with stored Packed red blood cell in transfusion medicine. There are some biochemical changes and lesions that develop in stored blood.There is a decrease in 2,3-DPG levels in stored blood which maintain the integrity of red blood cell. There is also gardual decrease in ATP level which affect every energy-dependent process which the cell may want to conduct. The absence of ATP results in dysfunction of ion pumps and other membrane proteins, which in turn results in all sorts of impaired mechanisms, most notably the maintenance of red blood cell shape. There is change fom normal shape of red blood cell to abnormal shape. There is haemloysis and release of free haemoglobin. There is also accumulation of metabolic byproducts and proinflammatory molecules. Packed red blood cell should be transfused young irrespective of the anticoagulants. The viability of the transfused cells should be ensured for general well-being of the patient.

**KEYWORDS:** Lesions,2,3-DPG,ATP,Ion pumps,Membrane proteins,Free haemoglobin.

## STORAGE LESIONS AND OTHER BIOCHEMICAL CHANGES ASSOCIATED WITH DONOR BLOOD

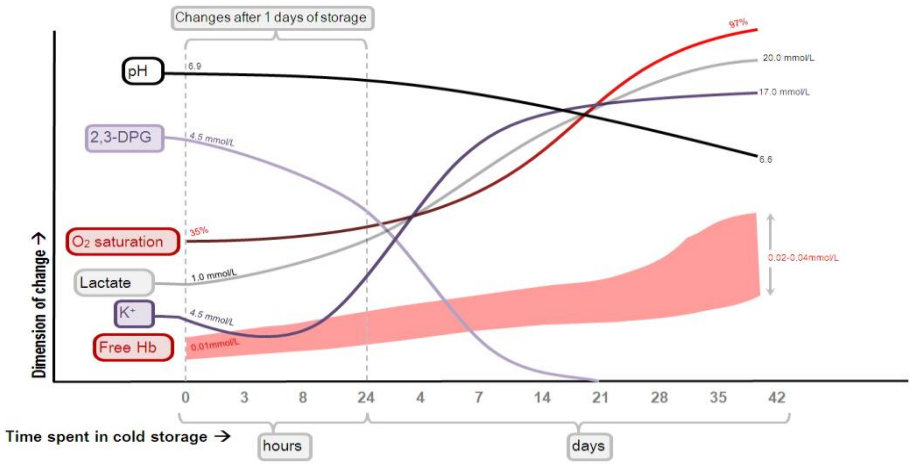
This paper discusses the changes which develop in packed red blood cells as a result of being stored in the refrigerator.

## The longevity of packed red blood cells

Blood has a use-by date. In Australia, packed red blood cells can only be stored for a maximum of 42 days (which is slightly conservative - internationally, the maximum PRBC shelf life is 49 days). These storage maximums were arrived at completely arbitrarily.

## Changes in red blood cells during storage

During the golden age of physiology, when scientists wore hats and white lab coats, a series of articles was published which addressed the problems of packed red blood cell storage in exquisite detail.




The ageing of red blood cells is a complicated tyopic. It is possible to summarise it by dividing the process by affected variables, rather than by arbitrary time stages. Time stages would also work - some lesions occur earlier, and others later. As the above graph suggests, these changes begin their evolution immediately as the blood is sucked out by the phlebotomist; however *clinically relevant* storage lesions only appear after a few days of refrigeration.

### Sudden decrease in 2,3-DPG levels

The normal value for intracellular 2,3-DPG for an erythrocyte is arounf 4.5 mmol/L. After 24 hours of refrigeration, this value drops to about 3.0-3.5 mmol/L, and the entire 2,3-DPG supply is depleted after two weeks. This has dire consequences for the oxygen-carrying capacity of these cells. 2,3-DPG is an allosteric modulator of oxy-haemoglobin binding; its absence exhibits itself as a greatly increased oxygen affinity for any given partial pressure of $O_2$ (a massive left shift of the dissociation curve).

The disadvantage of this is the utter functional uselessness of such transfused blood. Certainly, it might appear to carry oxygen, but it will not let go of it once it is in the tissues. This persists in vivo, well after the transfusion has completed - even after 8 hours 2,3-DPG levels are only 33% of their normal value.

Does this have a clinically relevant effect? perhaps not. Only one (rodent-themed) study has demonstrated a decrease in oxygenation when one uses older cells. The decrease in totally 2,3-DPG depleted cells was in the order of 15%. This finding could not be replicated, confirming that the oxygen carrying capacity of normoxic organisms is probably not affected. This makes a certain amount of sense. The top of the oxyhemoglobin concentration curve really does not move- only the middle and bottom. Given that there is an *increased* capacity for oxygen binding, one would expect that overall oxygen carrying capacity is not impaired. Rather, oxygen extraction is impaired. Thus, if one's patient had a circulating volume composed entirely of recently thawed PRBCs, their whole body oxygen extraction would be less than 25%, and their venous saturation would be high.

**Gradual decrease in ATP levels**

The levels of ATP within RBCs tend to drop slowly, and diminish to insanely low levels by the end of 5 weeks. Understandably, this has implications for pretty much every energy-dependent process which the cell may want to conduct. The absence of ATP promotes the dysfunction of ion pumps and other membrane proteins, which in turn results in all sorts of impaired cellular mechanisms, most notably the maintenance of RBC shape (which is a surprisingly active process).

**Red cell shape and rheology**

As membrane proteins are damaged and lost, and as ion pumps begin to fail, so the starving erythrocytes begin to swell and to take on a more spherical or hemispherical appearance. Then, they shapeshift into monstrous spheroechinocytes, with numerous non-deformable protrusions of cellular membrane sticking out in all directions. . Several studies of red cell morphology following cold storage have concluded that this failure of normal shape decreases the deformability of erythrocytes, and thus decreases flow through the microvasculature.

Microcirculatory changes in response to the transfusion of stored red blood cells. The rheological handicap of stored spheroechonocytes may slow the capillary flow enough to

generate a clinically significant deficit of systemic oxygen delivery, which might be felt most in those tissues which are hungriest. Brain and kidney come to mind.

**Red cell membrane vesciculation**

This phenomenon is the consequence of transmembrane protein failure, and leads to an increased inflammatory response to the red cells. Such cells are also more osmotically fragile, and (with the loss of membrane elasticity) are even less deformable. They will not find it easy to slip through the microcirculation. Furthermore, such abnormal-looking cells will not survive the passage through the reticuloendothelial system. Haemolysis may ensue.

**Haemolysis and the release of free hemoglobin**

The destruction of RBCs both in the bag of cells and in the patient's bloodstream leads to the dispersion of free haemoglobin thoughout the circulation. This has certain consequences beyond the development of a mild hyperbilirubinaemia. Free hemglobin is actually an excellent nitric oxide scavenger. This reaction is treated briefly in the section on metabolism and clearance, inside the drug monograph on inhaled NO. To summarise, excess free haemoglobin results in the loss of nitric oxide, and thus in a reversal of normal mechanisms of NO-associated hypoxic vasodilation in poorly perfused organs. The result is vasoconstriction; essentially the opposite of what happens in vasoplegic shock. In the absence of NO-mediated bloodflow autoregulation, hypoxic tissues may be unable to attract a diversion of bloodflow, and shock will persist.

**Accumulation of metabolic byproducts**

Though not strictly speaking a change of the red blood cells themselves, the waste products of their metabolism which accumulate in the bag are noxious enough to cause serious problems. For instance, one can readily identify a source of trouble in the massive amount of potassium and lactate which is sloshing wound in the extremely acidic environment of the 42-day-old blood bag. One might be tempted to conclude that hyperkalemia and lactic acidosis may result from a transfusion of too many units of old blood, but it does not seem to happen very often. A Mayo Clinic retrospective case series, spanning a term between 1988 and 2006, had unearthed only 16 cases of hyperkalemic cardiac arrest associated with PRBC transfusion. Compared to the total numbers of patients who had undergone blood trasnfusion at the Mayo Clinic over that time, this complication must be rare indeed - comparable to the risk of contracting HIV from the blood.

**Accumulation of proinflammatory molecules**

The process of slowly being pickled in your own wastes leads one to the intuitive conclusion that some of the changes will fill the environment with proinflammatory products of decay. The notion that packed red cell transfusion triggers an inflammatory response has been confirmed within the limitations of a murine model. It appears the inflammatory response requires membrane-encapsulated RBC haem, rather then the free haemoglobin which inevtiably forms. Furthermore, it seems that the transfusion of RBCs induces an acute phase response, and exacerbates inflammation induced by endotoxin. The free iron which is released after hameoglobin lysis ends up in the circulation, and this has the effect of sponsoring bacterial growth.

Of course, sceptics might point out that mice and mouse blood are not effective experimental surrogates for human blood transfusion research. This can be countered by the observations of investigators such as Guillermo A. Escobar's group, who in 2007 confirmed that stored PRBC transfusions seem to upregulate proinflammatory gene expression in the leukocytes of the transfusion recipient.The in-vivo inflammatory effects of transfusion was confirmed by McFaul et al, who in 2009 found that human packed red cell suspensions stored in standard solutions become increasingly proinflammatory as a function of storage time. The culprits seem to be lipid fragments which have been shed from disintegrating membranes of the hideously deformed stored red cells- we know this because the acellular products do not seem to have this effect. The immunoactivating effects of these lipid fragments have been recognised for some time - here is a 1997 study linking these "neutrophil-priming agents" to the pathogenesis of transfusion-associated acute lung injury (TRALI).

**The practice of using the oldest packed cells**

There has for some time existed a common blodbanking policy of using the oldest red blood cells on the shelf, in view of the fact that they were about to expire. In this fashion, stock was preserved.

Of course, this does not mean that all the PRBC units you ever get are at the borders of expiry. The mean age of transfused cells worldwide is about 16-21 days. A good article published by several luminaries of Australian ICU medicine discusses this practice and its consequences. The authors lament that *"most clinical studies in this area have been observational in nature, retrospective in design, small in size, and subject to bias, leaving this issue unresolved for more than 20 years."*

**The argument for the use of "young" packed cells in ICU**

Critically ill patients may be more susceptible to these storage lesions than the average punter. For instance, lets take TRALI. There is a good reason to believe on a theroretical basis- that the neutrophil-activating properties of the abovementioned broken lipid fragments can lead to a "second hit" in an already injured lung, rendering it even leakier, and filling it with oedema fluid to the ongoing frustration of the intensivist. This is merely one argument - consider that ICU patients have many more transfusions than other patients, that ICU patients have higher circulating proinflammatory cytokine levels already, etc etc. Lastly, the microvascular underperfusion which results from the use of stored cells is a major disadvantage to ICU patients who already have a uselessly defective microcirculation.

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4. To some extent this document has been superceded by the Australian and New Zealand Society of Blood Transfusion GUIDELINES FOR THE ADMINISTRATION OF BLOOD PRODUCTS.
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