

Promising platelets: potential benefits and extended life-span of cold-stored and cryopreserved platelets

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The advent of component therapy and evolution of Apheresis technology has led to widespread usage of platelet transfusions. It is estimated that over 2.9 million platelet products are used annually in Europe alone [1]. The storage temperature of platelets has been the subject of much controversy and scrutiny within transfusion science. The current storage temperature of platelets prepared by both pooled buffy coats and by apheresis is 22±2°C.

A storage temperature of 22°C increases the risk of bacterial growth by 50 to 250 times the combined risk of viral transmissions, namely HIV, HBV, HCV and HTLV-1/2 [2]. Despite optimal storage, platelets undergo metabolic, functional and morphologic derangements called as storage lesions. Storage of platelet concentrates have raised serious concerns over bacterial contamination and storage lesions. Specialised gas –permeable blood bags and additive solutions have partially improved storage of platelets. However, the recent FDA (Food and Drug Administration) approval for using cold stored (4°C) apheresis platelets for up to 3 days in cases with active haemorrhage has renewed interest in platelet storage among transfusion medicine scientist [3].

Previously, some authors have reported a decrease of *in-vivo* bleeding by 40% with transfusion of cold-stored platelets than room temperature (RT)-stored platelets in cases of active bleeding [4]. Cold-stored platelets have a shelf life of 21 days and cryopreserved platelets could be stored at -80°C with cryoprotective agent DMSO for up to 2 years [5]. Cold storage and extension of shelf-life of platelets

concentrates is believed to boost transfusion practises in rural and military areas. Studies have stated that cold-stored platelets have better adhesion and aggregatory responses to agonist and physiological inhibitors forming stronger clots, when compared to RT-stored platelets. Cold-stored platelets also have shorter half-life in circulation (1-8days), and due to its rapid removal, it also decreases the chances of late thrombosis.

Johnson *et al* (2016) [6] conducted a three-arm study, where buffy coat-derived pooled platelet concentrates were stored at RT, 4°C (cold-stored) and -80°C (cryopreserved). Each arm comprised of eight platelet concentrates stored in 30% plasma and 70% SSP+ (Macropharma).

Extreme size variations in cold-stored and cryopreserved platelets were seen from the forward scatter (FSC) of the flow cytometer. At cold temperatures, platelets tend to become more spheroidal having a raised mean platelet volume (MPV) and FSC. The platelet quality test system is used to evaluate the role of microparticles and calculate platelet response to temperature stress by dynamic light scattering (DLS).

High number of microparticles decrease the time taken for clot formation by cold-stored and cryopreserved platelets because of pronounced expression of phosphatidylserine [6]. Studies have also highlighted, that the cell metabolism is reduced at low temperatures as seen by low glucose consumption and decreased lactate production, however in

cryopreserved platelets the consumption of glucose had increased by four times (6). Interestingly, the ATP levels in cold-stored platelets are lesser at the start of storage and is almost the same as in RT stored platelets at the end of storage. It is suggested that low temperatures cause less mitochondrial dysfunction, yet lower ATP levels in cold-stored platelets need further experiments. Mitochondrial membrane depolarization (measured by red-orange dye tetramethylrhodamine) showed loss of polarization due to hampered oxidative phosphorylation in cold temperatures.

We cannot rule out the impact of storage medium (T-sol/plasma/ Intersol/ SSP+) on these metabolic indicators as different compositions of storage media affect the metabolic variables significantly. For instance, the acetate in SSP+ (used in cryopreservation could act as a buffer on being metabolized, yet it could not negate the accelerated glycolytic pathways in the post-thaw period, indicating enormous 'stress' that extreme cold temperatures could induce on the cells. The pH in different platelet storage temperatures was within acceptable limits, yet the RT-stored platelets had a higher pH of 7.4 due to accumulation of bicarbonate ions, as a result of acetate oxidation and fall in hydrogen ion levels.

Nair *et al* (2017) has demonstrated that the cold-stored platelets form clots that are 2.5 times stiffer (resistance to reversible deformation) and strong (to bear large loads before irreversible deformation). RT- platelets form clots of less dense and thin fibres. Structural properties (like curvature, diameter, branch points, crosslink) on fibrin fibres play a very crucial role in clot strength and stability. Cold stored platelets have a higher platelet-bound Factor XIII, which ensures clot stiffness. Thromboelastography (TEG) and rotational thromboelastometry (ROTEM) are considered superior test for platelet function than rheology vis a vis clot stiffness and strength (expressed in maximum amplitude, MA). Cold-stored platelets demonstrate higher MA than RT-platelets [7]. However, some studies did not find any significant difference between cold-stored and RT-stored platelets. But cryopreserved platelets showed 20% lower values due to changes in functional platelet receptors (6). It is

imperative to bear in mind that the TEG values get influenced by use of autologous plasma or Fresh frozen plasma. Overall, it has been seen that cold-stored platelets have good ability to aggregate in response to ADP stimulation compared to RT platelets.

However, clinical outcomes could best analyse the platelet functions *in-vivo*. The rapid generation of thrombin in cold-stored platelets lead to quick initiation and propagation of thrombus because of high concentrations of fibrinogen and factor XIII on platelet surfaces. In all probability, it is quite likely that cold-stored platelets would also have a high tendency of forming clumps even during storage due to mechanical stimulation by rough handling[7]. However, it is of great utility in acute bleeding conditions, since it forms the clot faster and even has a rapid clearance, reducing the risk of thrombus.

The clinical use of cold-stored platelets is limited, yet transfusion of cold-stored platelets seems very promising in the Indian scenario, where acute haemorrhage has potentially fatal outcomes. However, it is imperative to bear in mind that in cases of chronic thrombocytopenia, repeated transfusions may be required because of rapid clearance. For the same reason, even in prophylactic platelet transfusions, cold-stored platelets cannot be advocated. In a resource deficient country like India, cold storage of platelets could reduce the financial burden on blood banks to procure dedicated specialized platelet agitators. The potential of cold-stored platelets could be further explored by undertaking clinical trials aimed at having clearly defined primary and secondary outcomes. Several variables are involved in calculating the desired platelet doses and transfusion intervals of differently stored platelets.

In conclusion, it is worth considering the promising results of cold-stored platelets in acute bleeding and potential use of cryopreserved platelets in military and rural areas, where availability of fresh platelets has logistical constrains.

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