Editorial Commentary

When platelets are left in the cold

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Storage of platelets typically is at room temperature (RT) in large, gas permeable bags that allow some CO_2 and O_2 exchange. The bags are kept on an orbital shaker in a thermostable cabinet (22±2 ℃). These physical storage conditions were rationally selected following scientific breakthroughs in the previous century (1,2). RT storage was selected over storage at 4 ℃ based on the observation that cold-stored platelets are cleared significantly faster from circulation compared to RT (3). Although gold standard for decades now, RT storage is suboptimal because of the higher risk for bacterial bloom compared to 4 ℃ storage (4). Recent estimates suggest that still between 1:1,000 to 1:2,500 platelet concentrates (PC) are contaminated with bacteria (5). Transfusion of a contaminated concentrate can cause sepsis and because patients often receive multiple units, their individual clinical risk for transfusion transmitted infection is a multiplicate of the mathematical risk for contamination of a single PC (6). It should be noted however that bacterial infection caused by platelet transfusion, and resulting in a serious adverse event is very rare in the EU with only 16 cases on 2.3 million platelet transfusions and one death in 2017 (EU DG Health and Food Safety 147152—10/01/2020). Nonetheless, to limit bacterial contamination PC shelf lives are mandatorily short and typically range from 4 to 7 days. This limitation continuously strains the inventory management of blood banks worldwide. Novel ways to extend platelet shelf life without risking sterility or quality, represent a major goal in transfusion medicine.

PCs can be administered prophylactically to thrombocytopenic patients at risk for bleeding or therapeutically to actively bleeding patients. Platelet transfusion is prophylactic in the vast majority (>70%) of cases in the developed world (7). Because RT storage of platelets guarantees longer survival in circulation than 4 ℃ storage, RT is optimal for prophylactic transfusion. However, extended circulation may not be relevant for the actively bleeding patient in need of immediate hemostasis. In this case, any efficacious platelet may serve, regardless the circulation time post transfusion. Which raises the question if 4 ℃ stored platelets would be a valid alternative for RT stored platelets in treating active bleeding. This can only be answered in a well-designed, properly powered, double blinded, randomized controlled clinical trial. But in the latest issue of *Transfusion*, Stolla *et al.* started to address this question (8). In their study, 4 ℃ stored platelets were compared to RT stored platelets for (I) recovery and survival after autologous transfusion in healthy volunteers and (II) for a selection of platelet *in vitro* parameters.

In vivo platelet recovery was determined based on the fraction of ^{111}In labeled platelets found in circulation two hours after transfusion of a pre-defined platelet number. Results of 4 ℃ stored platelets were expressed relative to fresh, autologous platelets. Platelet recovery gradually declined as a function of 4 ℃ storage time. Consequently, 20 day 4 ℃ stored platelets had half the recovery of 5 day 4 ℃ stored platelets. The results thus show that for every additional day of cold storage, the number of recovered platelets decreases. This finding suggests that even for the actively bleeding patient in need for immediate hemostasis platelet storage time at 4 ℃ may have to be curtailed. Especially because clinically significant bleeding often is

not a matter of minutes, but rather hours. This finding thus raises additional questions for clinical trial designers. These must take into account storage time as a significant variable or may choose to include it as part of the study question. Another consequence of this observation is that the "priming" for clearance is a continuing process during 4 ℃ storage. This suggests that bringing platelets to low temperatures is not a single hit but instead follows a gradual course. Of note, the same experiment demonstrates that this "priming" for clearance takes place at RT as well because recovery of seven-day RT stored platelets is 30% lower compared to fresh RT platelets. Platelet "priming" thus is a substantial part of the platelet storage lesion at all temperatures. Stolla *et al.* now elegantly demonstrate that 4 ℃ storage indeed contributes to this phenomenon incessantly and significantly.

Some biochemical "priming" factors behind the 4 ℃ storage lesion have been identified, primarily in mouse platelets (9-12). Interventions based on these findings have successfully prolonged platelet circulation time in murine models of transfusion (10,12), but have not (yet) successfully been translated to humans (13). More research is needed to fully understand the biochemistry of cold stored human platelets and the reasons for their poor recoveries. As a contribution to this quest, Stolla *et al.* have investigated a number of *in vitro* platelet parameters like platelet metabolism, P-selectin expression, integrin $\alpha_{IIb}\beta_3$ activation, microparticle release and apoptosis markers. As expected (14,15), platelet metabolism significantly slows down at 4 ℃ following laws of thermodynamics. This may seem favorable to platelets because acidosis is detrimental and often a reason for discarding a regular RT stored PC (16,17). The slower metabolism does however not translate in a profoundly quiescent platelet. Integrin $\alpha_{IIb}\beta_3$ for instance, is prematurely activated in resting cold stored platelets albeit slightly. This is a confirmation of previous research that indicated that intracellular Ca^{2+} fluxes underlie premature integrin activation in cold stored platelets (18). In addition, a gradual increase in microparticles was found in correspondence to Johnson *et al.* (15). Both high microparticle content and premature integrin $\alpha_{IIb}\beta_3$ activation may indicate a destabilized platelet cytoskeleton (19,20). In line with this, platelet rounding and subsequent loss of swirl is often seen in 4 ℃ stored platelets (14).

Spontaneous platelet granule release, a hallmark of platelet storage lesion, was delayed during cold storage in comparison to seven-day RT storage in this study. This was determined based on P-selectin (CD62P) expression and has been observed before (21). Shifting platelets to storage in additive solution may further slow down this particular effect of cold storage (18).

Finally, platelet apoptosis markers were tested including mitochondrial membrane potential using the JC-1 probe, caspase 3,7 levels and phosphatidylserine expression. It is well known that platelets harbor apoptosis machinery (22) but it is yet to be established if this is relevant to transfusion. Increased caspase activity does not normally appear in RT stored platelets unless stored beyond 19 days (23). Similar observations were made in the Stolla *et al.* study for 4 ℃ stored platelets. Phosphatidylserine expression increased almost linearly during 20-day storage at 4 ℃ and was significantly higher compared to seven day RT stored platelets at all timepoints. This suggests that platelets rapidly lose control over membrane asymmetry at 4 ℃, in a continuous manner. We hypothesize that this is in line with the cytoskeletal and membrane mechanics changes that contribute to integrin activation and microparticle formation, but requires more research. It has been suggested that platelets expressing phosphatidylserine may be procoagulant and thus promote hemostasis (14,15,24). In fact, several groups have indeed demonstrated *in vitro* that thromboelastography, platelet aggregation and fibrin formation under flow are increased in cold stored platelets compared to RT storage (14,24,25). Whether this indeed translates to the clinic requires more research.

The *in vitro* data included in the Stolla *et al.* paper are interesting, but limited to a selected set of parameters commonly used in blood banking research. To understand the basic biochemical changes induced by cold storage and the hemostatic consequences of this, researchers will have to dig deeper and perform assays investigating integral platelet function. In addition, *in vitro* findings should subsequently be tested in relevant models including rodent models (26), although these should also be interpreted with caution as regards to translatability (13).

There is little scientific work assessing the clinical efficacy of cold stored platelets even though these platelets were transfused quite often historically (27). The choice of moving towards RT storage in those days was a logical one based on the genuine need for prolonged platelet circulation during prophylaxis (3). However, recent information published in the AABB 2017 congress proceedings suggests that at least in the setting of elective surgery with blood loss, cold stored platelets are efficacious (28). The increased interest of regulators, clinicians and blood institutions may eventually result in a dual inventory of both RT and

cold stored platelets, each supporting a specific group of patients based on indications and on acute need. Until then, substantially more randomized controlled clinical trials are needed in order to better understand the safety and efficacy of cold stored platelets. At the same time, basic laboratory research is still needed as well because Stolla *et al.* have elegantly demonstrated that long term cold storage of platelets significantly decreases platelet recovery and survival in healthy volunteers. We need to understand why this is in order to develop storage conditions or additives that can combine the best of both worlds: an efficacious storable platelet that circulates sufficiently long to support hemostasis in all thrombocytopenic patients.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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