Platelet storage: a license to chill!

The recent National Institutes of Health (NIH) State of the Science in Transfusion Medicine meeting, held in Bethesda, Maryland, in March 2015, revealed the alarmingly weak evidence base underpinning current practices in platelet (PLT) transfusion.[1] As a scientific community, we do not know if prophylactic PLT transfusions before invasive procedures in patients with thrombocytopenia are effective. We have no high-quality data to guide PLT transfusion for patients with active bleeding, even though we understand that PLTs are absolutely vital to hemostasis. We currently base transfusion decisions on PLT counts rather than on function, despite substantial evidence of PLT dysfunction in bleeding trauma patients and the widespread use of anti-PLT drugs.[2,3] Incredibly, with six decades of PLT transfusion history behind us, we have no standards for judging the in vitro or clinical hemostatic efficacy of PLT transfusion. This situation is all the more inexcusable given that we have acknowledged the loss of hemostatic and metabolic function over storage time, the so-called "PLT storage lesion," since the very beginning of PLT transfusion therapy. Nevertheless, hard experience in cancer patients with thrombocytopenia, cardiac surgery patients with thrombocytopenia and PLT dysfunction from cardiopulmonary bypass, and more recently, military and civilian trauma patients teaches us that PLT transfusion is critical to hemostatic, lifesaving resuscitation.[4,5]

Unfortunately, as also highlighted at the NIH meeting, our collective ability to meet the needs of our bleeding patients is severely limited by the current 5-day, 22°C PLT storage paradigm. Storage at 22°C was adopted because posttransfusion PLT recovery and survival, thought to be important in prophylactic transfusion by reducing donor exposure while maximizing the probability of PLT repair of damaged endothelium, was superior to that observed after refrigerated storage at 4°C.[6] Ironically, PLTs stored at 4°C were known to be highly effective for acute hemostasis and obviously less vulnerable to bacterial contamination.[7,8] In the end, 22°C PLTs were adopted because of their perceived superiority for prophylaxis, while their hemostatic function was thought to be acceptable for bleeding patients. This decision was also driven by the logistic challenges faced by donor centers in managing two PLT inventories.

A fundamental, and apparently flawed, assumption was made by pioneers in PLT transfusion in equating recovery and survival with "viability."[9] The unfortunate conflation of vital PLT functions like adhesion, aggregation, granule release, thrombin catalysis, and clot retraction with circulation time has left us struggling with the untenable proposition that the voluminous evidence of PLT decay during 22°C storage can be ignored. Wishful thinking that PLTs miraculously recover lost function upon transfusion does not make it so.[10] There are no clinical trial data documenting that such recovery occurs or that a dose–response relationship between current PLT products and hemostasis can be demonstrated. On the other hand, it has been clearly shown that 22°C-stored PLTs, well known to lose aggregation responses to multiple agonists over storage, fail to reverse pharmacologic PLT inhibition.[7,11] By contrast, 4°C-stored PLTs, which maintain in vitro aggregation responses, are effective in restoring hemostatic function inhibited by aspirin.[7,8] Loss of aggregation response has unquestionable clinical meaning both in the setting of anti-PLT therapy and in diagnosis of genetic PLT disorders.[12]

Thus, we have applied a "one-size-fits-all" storage solution, optimized to increase circulating PLT counts, to all patient populations without regard to their specific PLT function needs, such as acute hemostasis. In doing so, we have incurred enormous costs largely driven by the increased risk of bacterial growth in 22°C PLTs: shorter shelf life means reduced availability of a lifesaving product; increased sepsis risk drives higher testing costs and reduced availability due to quarantine; a residual sepsis risk despite extensive and expensive testing undermines health system outcomes; requirements for dedicated incubators and agitators consume valuable blood bank space, power, and maintenance costs; reduced shelf life imposes greater burdens on donors and donor recruitment; reduced shelf life limits shipping of PLTs to smaller or outlying hospitals and clinics as well as the ability to resupply across state or international boundaries in times of emergency need; and reduced product efficacy due to the storage lesion drives waste. Counting PLTs is easy and following a daily PLT count simplifies inpatient rounds, but we

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

doi:10.1111/trf.13433

TRANSFUSION 2016;56;13–16

must do better. Function must become part of the discussion.

With the publication of their latest article in this issue of **TRANSFUSION,** Skripchenko and colleagues[13] provide more support to an increasingly robust body of literature that suggests viable alternatives to the current PLT storage paradigm. Their approach seeks to mitigate the obvious drawbacks of 22°C storage and acknowledges the fundamental importance of reducing storage temperature to improving PLT function and decreasing bacterial contamination risk. Automated thermocycling between 5°C for 11 hours and 37°C for 1 hour with a 6-minute agitation during warm-up yielded a product with recovery and survival variables in a mouse transfusion model that were comparable to those of PLTs stored at 22°C. On the other hand, their functional assessments comparing thermocycled to 4 to 22°C-stored PLTs yielded conflicting results such as improved aggregation responses to dual agonists in thermocycled compared to 4°C PLTs, but better responses to single agonists in 4°C PLTs. Ironically, the authors reported that neither 4°C storage nor thermocycling resulted in improved preservation of PLT hemostatic function compared to 22°C storage, in contrast to most other published studies. Whether the cost and inconvenience of the equipment required for thermocycling is justified by product performance remains an open question, but the exploration of alternatives to the current paradigm is vital to improving patient care in this arena.

In assessing future product development, it is worth keeping in mind trends in PLT use. The 2011 National Blood Collection and Utilization Survey (NBCUS) report clearly demonstrates that multiple PLT-requiring patient populations are competing for a scarce resource that is currently oriented toward one group.[14] First of all, PLT demand increased by 7.3% from 2008 to 2011. It is supposed by many that hematology-oncology patients account for 80% or more of PLT transfusions. The NBCUS reveals that these only accounted for 34% of PLT transfusions, while surgery and trauma accounted for about 25%, ICU patients for 12%, and general medicine for 17%. It is clear that the needs of a majority of patients receiving PLT transfusions are driven more by an acute requirement for hemostasis than by the more complicated concept of shoring up vascular integrity in the setting of prolonged thrombocytopenia, cytotoxic chemotherapy and radiation, immunosuppression, infection, and potentially graft versus host disease. For the majority of our patients, PLT products must stop bleeding and not introduce unnecessary risks such as bacterial contamination. For all patients, PLT product characteristics should be optimized to desired function rather than to merely produce a count increment. Even allowing the heroic assumption that somehow 22°C PLTs perform as well as or better than 4°C PLTs for bleeding patients, we must face squarely the problem of constrained inventory. With a 5-day shelf life, our current standard prevents many bleeding patients from ever being considered for PLT transfusion.

The most obvious solution to this imperative that requires the least infrastructure investment and will yield an immediate increase in PLT availability while delivering a substantial health systemwide cost reduction is PLT refrigeration. This topic has been recently reviewed and was extensively discussed at the NIH State of the Science meeting.[1,15] Abundant in vitro data as well as clinical trial results establish the hemostatic effectiveness of 4°C-stored PLTs.[16] The long historical experience with use of cold PLTs, both in PLT concentrates and in whole blood, suggests an acceptable safety profile, particularly in patients who face complications primarily resulting from uncontrolled bleeding (such as death due to exsanguination). PLT refrigeration has been a Food and Drug Administration (FDA)-approved option for decades as is clearly noted in the Code of Federal Regulations [21 CFR 640.24(d)(2)]. Although this option has traditionally been interpreted as applying only to whole blood-derived PLTs, the FDA recently clarified that apheresis PLTs could also be stored for up to 72 hours at 4°C [21 CFR 606.65(e) & 610.53(c)]. Forthcoming data, published so far in abstract form, indicate that apheresis PLTs stored in PLT additive solution (PAS) retain aggregation responses, favorable metabolic variables, and viscoelastic clotting properties to at least 15 days of storage without the need for agitation.[17] Cold storage will eliminate costs associated with detection of bacterial contamination, currently estimated to be about $20 per unit for bacterial culture and $30 per unit for point-of-release testing or close to 10% of the total cost of a PLT unit estimated at $535.[18] Safety concerns regarding the potential thrombogenicity of partially activated, cold-stored PLTs must be weighed against the following counterbalancing factors: if the indication for use is acute hemorrhage, improved clotting potential is a desirable characteristic that reduces risk of death or multiorgan failure associated with shock; cold-stored PLTs respond to physiologic antagonists of PLT hemostatic function, reducing the risk of inappropriate clotting;[19] and all cellular products lose function over storage time and since cold-stored PLTs are already approved for the first 72 hours of storage, when their hemostatic function is at its peak, loss of function over time suggests progressively lower thrombotic risk with extended storage. Finally, rapid clearance of cold-stored PLTs should reduce the risk of thrombosis in hospitalized patients after the establishment of hemostasis when the acute-phase response induces hypercoagulability. In short, it is abundantly clear that a viable and desirable alternative to room temperature storage of

PLTs is within immediate reach. Now is the time to extend storage of cold-stored PAS PLTs to reduce bacterial risks, improve function, lower costs, and improve availability for the majority of patients who require PLT transfusion. Management of dual PLT inventories will present some short-term challenges, but these will be outweighed by the rapid and dramatic improvement in our ability to supply an ever-growing need for PLTs.

Other products currently in development such as thermocycled PLTs, cryopreserved PLTs, or dried PLTs will provide products with different performance characteristics and shelf lives. The US Departments of Defense and Homeland Security have been the primary funding agencies for these efforts. Cryopreserved PLTs have been shown to be safe and effective in clinical trials and in military use in bleeding patients.[20-22] They are or have been used in the United States, the Netherlands, France, and Australia. Although requiring −65°C or lower temperature freezers, they offer the potential of long-term storage and prepositioned stockpiles for emergency use. Dried PLTs are similarly attractive, although these require further clinical evaluation.[23] Both cryopreserved and dried PLTs could also be used to bank autologous products in a variety of clinical settings. Many opportunities for additional improvements exist, such as pathogen inactivation. The essential point is that these approaches expand the armamentarium of functional PLT products available to both thrombocytopenic patients at risk for bleeding and patients experiencing acute hemorrhage and in need of hemostatic resuscitation. PLT counts as measured in corrected count increments (CCIs) or recovery and survival studies can no longer be justified as primary product acceptance criteria without proof of hemostatic function. Indeed, it requires a leap of faith to extrapolate clinical meaning from the recovery of microdoses of radiolabeled autologous PLTs infused into a healthy subject, considering that doses representing a significant fraction of the total PLT mass of an adult will be transfused into patients with profoundly altered coagulation, inflammation, endothelial function, hypoxia, tissue damage, and even uncontrolled bleeding treated with massive transfusion of stored blood products. Experience with sick patients rapidly dispels the notion that CCIs or bleeding responses are predictable, so how is it rational to expect that recovery and survival measured in healthy subjects will be informative in guiding transfusion decisions? For evaluation of hemorrhage control PLT products, it is time to replace the radiolabeled recovery and survival study with tests that more closely reflect desired function, such as in vitro poststorage and posttransfusion improvement in PLT aggregation and viscoelastic clotting properties. Clinical development pathways should thus first establish in vitro characteristics, with a focus on hemostatic function, and then determine basic in vivo safety through, for example, simple dose-escalation studies. Clinical bleeding is notoriously difficult to measure, but surrogate metrics such as reversal of measured PLT aggregation dysfunction (e.g., in normal volunteers on aspirin) or improvement in posttransfusion clotting function variables could provide an adequate basis for approval, with postapproval monitoring of population-level safety and efficacy assessed through Phase IV studies and hemovigilance programs.

In summary, the recent NIH State of the Science meeting, as well as discussions in other settings indicate that we, as a transfusion medicine community, have acknowledged that our current approach to PLT transfusion is untenable and that a sustained commitment to research funding in this field is vital to fostering improved patient outcomes. We are ready to implement alternatives for our patients with the understanding that a one-size-fits-all approach is unlikely to work and product evaluation standards need to evolve away from merely counting PLTs to assessing desired functions. Extending cold storage of PLTs, a US Army Strategic Technology Objective with obvious applications for deployed forces, represents the logical first step down this road, while continuing development of storage options such as pathogen inactivation, cryopreservation, dried PLTs, and thermocycling.

CONFLICT OF INTEREST

The author is an Active Duty U.S. Army Officer and has disclosed no conflicts of interest.

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