Review Article

Platelet transfusion therapy: hot topics in 2021

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Abstract: Platelet transfusion therapy has been the object of intense research and development programs over the last 60 years. Although several evidence-based clinical practice guidelines of good quality are currently available, more stakeholder involvement in their preparation and greater attention to their applicability in the real world are needed. This short review will focus on platelet storage and platelet refractoriness, two aspects of platelet transfusion where significant advancements have occurred during the last few years, which could promote important changes in laboratory and clinical practice. Renovated interest in liquid platelet storage at 2–6 ℃ as opposed or complementary to the current standard storage at 20–24 ℃ and improved technologies for platelet cryopreservation offer the opportunity to develop more efficient platelet inventory management systems and to improve the support of actively bleeding patients, particularly those undergoing surgical procedures. Novel algorithms for the selection of human leukocyte antigen compatible platelets for a small, but not insignificant proportion of chronic platelet recipients who still develop immunological refractoriness to random donor transfusion in spite of the routine use of blood component leukoreduction could help reducing the failure of bleeding prophylaxis in onco-hematology platelet recipients. *In vitro* laboratory studies and clinical trials with sufficient statistical power and adequate clinical relevance are needed to confirm the existing preliminary evidences and fully exploit the recent advancements in platelet storage and in the management of alloimmune platelet refractoriness.

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Introduction

Several evidence-based clinical practice guidelines (EB-CPG), narrative and systematic reviews and meta-analyses on platelet transfusion therapy in thrombocytopenic children, adults, medical and surgical recipients are available in the literature. Although they contain valuable clinically relevant information, their quality is variable, as reported in a recent systematic review of seven EB-CPG on platelet transfusion and use published during 2015–2018 (1). For each EB-CPG (2-8), the authors determined quality scores of six guideline domains on a standardized percent scale (0%, lowest quality, to 100%, highest quality) and reported the median (interquartile range) quality scores as follows: scope and purpose, 94% (8%); clarity of presentation, 94% (6%); rigor of development, 83% (14%); editorial independence, 77% (4%), stakeholder involvement, 63% (18%); applicability, 58% (20%). Moreover, the authors noted that "*Inconsistent recommendations were on prophylactic (platelet) transfusion in hypoproliferative thrombocytopenia in the presence of risk factors and dose recommendations*" and concluded that "*Inconsistencies between guidelines and variable quality highlight areas for future guideline writers to address. Areas of specific attention include issues of stakeholder involvement and applicability*".

In spite of the above inconsistencies and limitations, the current EB-CPG on platelet transfusion represent a valid support to inform the daily management of thrombocytopenic patients in need of platelet replacement therapy. A detailed list of the above recommendations and the grade of evidence is reported in the supplementary material of the systematic review by Al-Riyami *et al.* (1).

Table 1 Clinically relevant items discussed in most recent platelet transfusion guidelines (2-8)

Platelet source (whole blood versus apheresis)
Platelet dose
ABO compatibility
Prevention of anti-D alloimmunization
Pre-transfusion platelet count threshold
Prophylaxis and treatment of bleeding in medical and surgical conditions
Platelet transfusion before invasive procedures
Platelet transfusion refractoriness to random donors

Clinically relevant topics discussed in most guidelines are listed in *Table 1*.

This short review is focussed on two domains of platelet transfusion therapy where recent developments and novel information may prompt future modifications of current standard practice.

Platelet storage

During the late 1960s, when I was in medical school, blood component therapy was in its infancy and platelets were stored under refrigeration at 2–6 ℃. This condition was based on evident advantages of cold storage in reducing the risk of bacterial contamination in platelet units. In those years it appeared to be 'unfortunate' that platelets undergo significant modifications at cold temperatures, which determine their activation, prompt reactivity and early removal from the circulation (9). In fact, during the 1970s great strides were made in the pharmacological treatment of leukemia and other blood cancers, which progressively improved patient survival but also caused prolonged times at risk of severe bleeding due to profound thrombocytopenia. Accordingly, prevalent attention was paid in the early years of platelet transfusion therapy to storage conditions appropriate for the treatment of onco-hematology recipients, as they consumed—and still consume—more than two thirds of the available platelets (10,11). Therefore, it appeared quite logical to preserve platelet viability by preventing their activation and early removal from the circulation after transfusion. A change in platelet storage was prompted by the seminal studies by Murphy and Gardner, who showed that "platelet viability for transfusion purposes is best maintained at 22 ℃ rather than at 4 ℃" (12-14). The latter evidence prompted the universal adoption of storage under continuous gentle agitation at controlled room temperature (20–24 ℃), thus reducing the risk of clinically significant bleeding in stable thrombocytopenic patients by transfusing platelets with a quasi-physiological life span (12)—the so called 'bleeding prophylaxis' strategy. Although the clinical benefits of prophylactic prevention of bleeding in onco-hematology recipients have been questioned (1), very recent data continue to support its validity (15).

More recently, novel interest into cold platelet storage was raised after *in vitro* investigations (16-20), autologous recovery studies in healthy volunteers (21) and experimental clinical work performed both in emergency and non-emergency settings (22,23), which showed that patients affected by significant bleeding and high platelet consumption benefit from the transfusion of promptly reactive platelets, a condition easily achieved under cold storage. This evidence led to the US-FDA 2019 authorization of apheresis platelet storage at 1–6 ℃ for 3 days with optional agitation for the transfusion of actively bleeding patients (24,25).

An interesting administrative and regulatory protocol for the transition of 5-day old platelets stored at room temperature to cold storage for an additional 9 days was recently developed at the Mayo Clinic (Rochester, MN) with the aim of preserving the platelet inventory during shortages caused by the COVID pandemic. During the first month after the implementation of this protocol, 61 cold-stored PLT units were transfused to 40 bleeding patients, mainly during cardiovascular and other surgeries, with adequate hemostasis and no signs of patient harm (26).

The evidence of clinical benefits from the transfusion of promptly reactive platelets into bleeding patients triggered also renovated interest into platelet cryopreservation at –80 ℃. The latter procedure induces some degree of platelet activation, thus making the use of frozen/thawed platelets particularly appealing for patients in whom prompt reactivity (as in surgical specialties) is more important than prolonged circulation (as in onco-hematology). Recent advances in platelet cryopreservation have been thoroughly reviewed by Kelly *et al.* (27), who describe their current cryopreservation procedure to prepare a highly concentrated apheresis platelet dose of about 160×10^9 platelets in a reduced volume of about 25 mL, which offer the important advantage of not requiring removal of the dimethylsulfoxide (DMSO) cryoprotectant after thawing. Moreover, these authors discuss "the appeal of [cryopreserved platelets

(CPP)] as an alternative to traditional liquid-stored platelets (LP) in settings of supply shortages, mass casualty, active bleeding, rapid provision of human leukocyte antigen-compatible platelets, and remote care" (27).

Platelet transfusion refractoriness

A patient is conventionally defined as "refractory" to random platelet transfusion when the increase in the platelet count corrected for the transfused dose and for the patient's body surface area (BSA) [corrected count increment (CCI)] is less than 5,000 or 2,500, respectively 1 hour or 18–24 hours after at least two consecutive transfusions of fresh, ABO compatible platelets. CCI is determined using the following formula:

$$CCI = \frac{\text{Post - transfusion minus pre - transfusion platelet count per microliter}}{\text{Platelet dose (number of platelets} \times 10^{11})} \times BSA\left(m^2\right) \quad [1]$$

When 300×10^9 platelets (average dose in adults) are transfused into a 70 kg patient with a blood volume of 5 liters and a count of less than 10×10^9 platelets per liter, it could be theoretically expected that the patient's peripheral blood platelet count increases by $300:5=60\times10^9$ platelets per liter. This practically never happens, because (I) a proportion of platelets undergo degradation processes during storage (storage lesion) which cause the early removal from the circulation; (II) approximately 30% of transfused platelets are sequestered in the splenic compartment; (III) the patient's bloodstream into which they are introduced is generally not a 'hospitable' terrain. This can reduce the theoretically expected CCI to very low levels.

Two recent reviews of the literature describe in detail the 'inhospitable' patient environment that often hosts transfused platelets. The factors that determine this lack of 'hospitality' belong to two categories: non-immunological (28) and immunological (29), present respectively in more than 90% and in less than 10% of refractory cases. Belizaire and Makar (28) remind us that from 50% to 90% of the total platelet mass—and therefore also most of the transfused platelets—can be sequestered in the spleen of splenomegalic patients, in whom the post transfusion platelet count increase is often greatly reduced. Furthermore, these authors describe several recently identified non-immunological mechanisms of high platelet consumption, primarily associated with sepsis and inflammation, including: (I) the presence on the platelet membrane of pathogen recognition receptors or bacterial molecules (PRR); (II) the neutrophil extracellular traps—NETs, physiological defense mechanisms from bacteria that are activated by neutrophils as a response to infections and which consist in the release of DNA, histones and granule proteins, which can, when present in high concentration, bind to platelets causing their removal from the circulation; (III) accelerated platelet apoptosis promoted by humoral factors associated with inflammation; (IV) the removal of sialic acid that physiologically decorates platelet glycoproteins (desialylation), which causes hepatic sequestration of platelets by binding with the Ashwell-Morrell receptor (AMR) present on hepatocytes; (V) activation of the endothelium. At the end of this elegant review, in addressing the dilemma of how to restore adequate treatment in hemorrhagic patients refractory to platelet transfusion for non-immunological causes, the authors sadly conclude that "on this question, there is little clinical guidance" and that refractoriness associated with clinical factors is often "unavoidable and unmodifiable" (28).

The definition, epidemiology and clinical significance of platelet alloimmunization and refractoriness from immunological causes are reviewed by Saris and Pavenski (29). Immunological refractoriness is caused in the vast majority of cases by anti-HLA alloimmunization, while alloimmunization towards platelet specific antigens [human platelet antigens (HPA)] plays a minor role. The systematic use of leukoreduction of blood components has reduced the frequency of immunological refractoriness to less than 5% of transfused patients and to less than 10% of refractory cases. Despite the low frequency, immunological refractoriness represents a condition of high risk, which has stimulated the development of strategies aimed at selecting effective platelets for these patients.

Over the last few decades, two intervention strategies have been consolidated to correct immunological refractoriness that require a significant contribution from the transfusion physician: (I) selection of platelets based on HLA-A and HLA-B antigens (HLA match) or without HLA antigens reactive with antibody specificities present in the recipient's serum (antibody specificity prediction); (II) execution of a cross-matching test. There are no rigorous studies in the literature comparing these strategies, which are alternatively or simultaneously adopted on the basis of the local availability of an HLA typing laboratory and of a sufficient number of units of typed platelets available in the daily inventory or of a large register of HLA typed donors, readily accessible to undergo urgent apheresis procedures. Generally, cross-

matching is used when the pool of typed donors is not sufficient. The size of the pool depends on many factors, including the number and ethnicity of transfused patients, but several thousand typed donors are still needed. For example, Stanworth and co-workers (30) reported that 12,000 typed donors were not enough to select compatible platelets for 30% of refractory patients in the UK. Cross-matching offers the advantage of not requiring patient and donor typing, but also its use is not effective in all cases.

An important step forward in the management of immunological refractoriness has been made thanks to the HLAMatchmaker algorithm developed by Rene J. Duquesnoy, PhD, which, in its most recent version (31,32), may be used to determine which regions of the conformational epitopes (tertiary structure) of HLA molecules (HLA eplets) are accessible to alloantibodies, significantly reducing the number of typed donors needed to effectively treat refractory patients. A very recent randomized cross-over study (33) demonstrates its non-inferiority and greater efficiency compared to the classic HLA-A and HLA-B match.

An integrated management of cross-matching and HLA selection has been developed at the Mayo Clinic through the creation of a web-based software called Platelet Virtual Crossmatch-PLT VXM. This software performs 3 functions: (I) selection of compatible platelets by cross-matching; (II) selection of compatible HLA (and ABO) platelets from the locally available inventory; (III) selection of compatible donors from a pool of approximately 2,400 subjects. The validation of the PLT VXM (34) has shown an important reduction in the time spent in the execution of functions I–III and the absence of documentary errors compared to manually managed functions.

Although non-immunological refractoriness to platelet transfusion still represents a serious complication in many patients, the frequency of immunological refractoriness decreased significantly after the adoption of leukoreduction of blood components. Furthermore, the integrated use of cross-matching and modern HLA selection techniques allow refractoriness to be addressed successfully in most patients who develop alloimmunization despite leukoreduction or manifest a secondary response to sensitization caused by previous transfusions with non-leukoreduced blood components or pregnancies.

New studies are needed to determine the efficacy of some recent experimental pharmacological approaches to the treatment of refractoriness, including the use of oseltamivir (an inhibitor of neuraminidase that causes the removal of sialic acid from platelet glycoproteins and the consequent sequestration by interaction with the AMR receptor) (35) and eculizumab (an inhibitor of the C5 component of complement) (36).

Finally, encouraging perspectives on the generation of HLA-universal platelets derived from induced pluripotent stem cells (iPSCs) are discussed in a brilliant article by Norbnop and collaborators (37), although significant efforts to develop *in vitro* and *in vivo* studies will be needed before these developments can find application in clinical practice.

Conclusions

Improved technologies for platelet liquid and cryopreserved storage and better algorithms for the selection of HLA compatible platelets for patients developing alloimmune refractoriness to random donor platelet support are expected to contribute to improving platelet inventory management systems and the prevention and treatment of bleeding in medical and surgical thrombocytopenic patients. The full acquisition of these important achievements will require the development of laboratory investigations and clinical trials of adequate statistical power and clinical relevance.

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