Clinical Medicine Review

PLATELET TRANSFUSION REFRACTORINESS: CONTROVERSIAL ISSUES

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ABSTRACT

Platelet transfusion refractoriness (PTR) is a harmful complication of platelet transfusion that leads to increased rates of morbidity and mortality. It is a very complex process, not well known, in which there are many interplaying variables. PTR consists of an inadequate response to platelet transfusions and affects more than a third of patients with hematological malignancies who have been chronically transfused. Diagnosis of PTR is a controversial issue, since there are no standard criteria and therefore different diagnose approaches have been tried. PTR have an immune or non-immune origin. Non-immune causes involve near 80% of PTR patients, being splenomegaly, sepsis, and medications the most frequent associated conditions. Development of antibodies against human leukocyte antigens (HLA) or human platelet specific antigens (HPA) represent approximately one third of refractory episodes. Despite the clinical relevance, PTR is often underdiagnosed even in hematologic patients because of the complexity of the process. Furthermore, the management of this platelet transfusion complication is often frustating, especially for non-immune causes. Therapeutic options for PTR are limited and also controversial: There are several strategies to provide HLA or HPA matched-platelets that do not produce adequate postransfusion counts in a high percentage of patients with anti-HLA or anti-HPA antibodies.
The objective of this review is to update knowledge about PTR, focusing on controversial issues related to diagnose and management.

Keywords: platelet refractoriness, platelet transfusions, corrected count increment, alloimmunization, human leucocyte antigen antibodies

1. INTRODUCTION

Platelet transfusion is a common practice in onco-hematologic patients for preventing or treating hemorrhages. In fact, patients diagnosed of hematological malignancies undergoing chemotherapy or hematopoietic stem cell transplantation have often high platelet transfusion requirements. A challenging complication raised from multiple platelet transfusions is the platelet transfusion refractoriness (PTR). PTR is defined as the lack of adequate post-transfusion platelet count increment. PTR has a wide variety of causes, often multifactorial. Non-immune causes involve near 80% of PTR causes, the most frequently reported are infection/sepsis, fever, splenomegaly, and antibiotics. Development of antibodies against human leukocyte antigens (HLA) or human platelet specific antigens (HPA) still represent approximately one third of refractory episodes, even though the efforts made in the last decades for preventing alloimmunization. PTR is associated with adverse clinical outcomes as there is an increased risk of bleeding and decreased survival.[1] Despite the clinical relevance, PTR is often underdiagnosed even in hematologic patients because of the complexity of the process and the need for collaboration between professionals in different areas.[2]. Consequently, diagnose and treatment of this complication, are a challenge to the clinical and the blood bank teams. The objective of this review is to update knowledge about PTR, focusing on controversial issues related to diagnose and management.

2. HOW TO MAKE THE DIAGNOSE OF PLATELET TRANSFUSION REFRACTORINESS

Platelet transfusion refractoriness is defined in a general manner as an inappropriately low platelet increment after repeated platelet transfusions. There are several formulas to calculate the platelet increments of which post-transfusion platelet increment (PI) is the easiest to calculate when body surface or number of platelets transfused, are not available. PI is calculated by resting post-transfusion platelet count and pre-transfusion platelet count; an absolute platelet count increment of less than 10 x 10^9/l after administration of an aphaeresis unit is suspect for refractoriness.[3]

Another way of calculating refractoriness is the percentage platelet recovery formula (PPR) as follows:

PPR (%) = PI x total blood volume (L) x 100 / platelet dose transfused (10^{11}), being the

Total blood volume (L) = 7% of weigh (Kg)

PTR is usually considered when platelet recovery is < 30% at 1h post-transfusion and <20% at 20-24h after platelet transfusion. Other authors prefer to use the percentage platelet increment (PPI), dividing the PPR by a corrector factor of 0.62, due to the splenic pool, although this parameter can vary from 1 in splenectomized patients to 0.2 in hipersplenism.

Corrected count increment (CCI) is the most accepted formula to calculate platelet refractoriness[4]:

CCI= PI (/L) x body surface area (m^2) / platelet dose transfused (10^{11})

A 1-h CCI less than 5– 1-x 10^9/l suggests platelet refractoriness. CCI has the advantages respect PPI that doesn't take into account a fixed value of splenic pool and that body surface area is better correlated with real blood volume than using weigh alone. Both of them need to know the number of platelets transfused, which can widely vary from one product to another.[5] Nevertheless, each center should know the approximate value of its provider's products.

The Trial to Reduce Alloimmunization to Platelets (TRAP) study defined platelet refractoriness as a corrected count increment (CCI) of less than 5 ·109/L within an hour after transfusion using ABO-compatible platelets, after 2 sequential transfusions, at least 1 of which had been stored for no more than 48 hours[6]; it has been generally accepted by the American Society of Clinical Oncology.

It has been demonstrated that platelets require at least 1 hour to reach an intravascular equilibrium after transfusion[7] but in busy hospitals, results are very difficult to determine from a 1-h post-transfusion platelet count. Obtaining results 10-minutes after a transfusion platelet count provides a good correlation[8] and that is why some clinics prefer its use. Although a 10-min to 1-h determination is necessary to define platelet refractoriness, it is not usually until the following day control when suspicion may be aroused due to an inappropriate low platelet count. A 20-h CCI determination shows a good correlation with the 1-h CCI.[9] Some authors propose that the way platelets decrease can help in determining the mechanism of refractoriness; alloimmune causes should not reach a good 1-h CCI whereas non-immune causes should have a good 1-h increment and a low 24-h CCI.[10] Still, many exceptions may exist and this should be only used as an initial orientation.

3. AETIOLOGY

Platelet refractoriness could be divided into three main causal groups:

1. Non-immune causes.
2. Immune causes.
3. Factors depending on the product's characteristics

3.1 Non immune causes

Non-immune causes account approximately for two-thirds of refractory episodes.[11] Although there are many clinical factors related to reduce post-transfusion platelet increments and platelet survival, there is a small number of cases in which a direct relationship can be demonstrated. Several factors are usually present in the same patient, and on the other hand, many patients presenting these same clinical factors respond with a normal platelet increment after transfusion.

Nevertheless, there are diverse causes classically involved[12, 13]:

3.1.1 Sepsis

Although the relationship between sepsis and platelet refractoriness is well established, the mechanisms are not yet well-understood. There is a high rate of platelet consumption, a decrease in the production and sequestration by the activated endothelium.[14] During the infection, platelets express on their surface cryptic antigens; this can lead to the production of platelet auto-antibodies resulting in platelet destruction by the mononuclear phagocyte system.[15] Disseminated intravascular coagulation, often related to sepsis, also plays a role in platelet refractoriness because of the consumptive process. It's unclear if fever can negatively affect the transfusion efficiency in a direct way or it is only involved because of the underlying infection and treatment with antibiotics.[16]

3.1.2 Splenomegaly

The spleen usually holds approximately one-third of the platelets produced in the bone marrow or transfused platelets, but this rate can be increased to 90% in severe splenomegaly.[17] This condition can reduce about 15-20% the post-transfusion platelet counts.

3.1.3 Hematopoietic stem cell transplantation

Both autologous and allogeneic hematopoietic stem cell transplantations (HSCT) are related to platelet refractoriness because of diverse mechanisms. In the hematopoietic recovery phase, a temporary impairment in the immune system can lead

to the production of autoantibodies directed towards platelets. Graft versus host disease (GVHD), both acute and chronic, has been demonstrated as independent factor in PTR. It can provoke a vasculopathy or even a thrombocytopenic thrombotic purpura, increasing the platelet consumption. In addition, it's associated with the production of autoantibodies leading to an immune-mediated destruction.[18] Hepatic sinusoidal obstruction does not seem to be directly associated with refractoriness as shown in a large study with 235 patients.[19] The same study found that 80% of transplanted patients that died from any cause were refractory to platelets, suggesting that refractoriness could be a sign of clinical deterioration.

3.1.4 Medications

Many different medications can lead to thrombocytopenia and PTR, with several mechanisms implicated: bone marrow toxicity and immune or non-immune platelet destruction. Antibiotics and antifungoids are usually employed with onco-hematologic patients and have been related to PTR, penicillin, amphotericin, vancomycin being the commonest.[20]

Amphotericin can decrease the post-transfusion increment from 30% to 78%, but not all patients receiving this agent suffer from refractoriness. It probably depends on the time elapsed from administration to the transfusion, and consequently some clinical doctors choose to transfuse several hours later.

Demonstrating the presence of pharmacological induced antibodies is complex and not routinely performed.[21]

3.2. Immune causes

Platelets express on their surface antigens from the ABO, Lewis, P and I systems, human leukocyte antigen (HLA) class I and human platelet antigens (HPA). Only the ABO antigen system, HLA-A, HLA-B and HPA have an influence on platelet survival, with anti-HLA antibodies being the more commonly responsible. However, HLA-C antigens have also been reported to cause platelet refractoriness.[22] These antibodies can result from prior exposure by transfusions, pregnancy and transplantation.

Platelet transfusion itself cannot produce alloimmunization against the HLA system and needs the presence of allogenic lymphocytes.[23] The Trial to reduce Alloimmunization to Platelets (TRAP) showed a decrease in the alloimmunization rate from 45% to 17-23% when transfusing leukorreduced products and a reduction in refractoriness from 16% to 7-8%, with no difference between single-donor platelets

and pooled random-donor platelet concentrates.[6]

Alloimmunization against HPA is a less common cause of refractoriness, not generally associated with a statistically significant reduction in CCI,[24] and is usually associated with anti- HLA antibodies. There are several platelet-specific antigens that can be polymorphic, mostly due to a single amino acid change, but the most prevalent alloantibody specificity in transfused patients is HPA-5b and HPA-1b.[25] Characteristically, alloimmunization against HPA does not vary with leukoreduction.[6]

Most alloimmunized patients do not exhibit refractoriness. In the TRAP study 45% of the control group developed anti-HLA antibodies, but only 13% developed this complication. This indicates that alloimmunization is a complex process in which there is interplay between anti-HLA antibodies, B cells, and T cells. It has been recently demonstrated that immune-mediated clearance of platelets can occur independently of anti-HLA antibodies.[26]

3.2.1 Laboratory testing

There exist several laboratory tests to demonstrate alloimmunization, including lymphocytotoxicity, ELISA, and flow cytometric immunofluorescence testing.[27] However, there is no consensus as to which test is better.

In the lymphotoxicity test, serum is reacted with a panel of HLA-typed lymphocytes (known as PRA or panel-reactive antibody); a PRA >20% suggests probable HLA alloimmunization. Its main limitation is to identify antibodies that do not mediate complement-mediated lysis and can also lead to refractoriness.[28]

ELISA-based methods and flow cytometric immunofluorescence tests are simpler and more sensitive, but this can lead to the detection of weak HLA-antibodies that do not predict platelet refractoriness.

Newer tests consist of synthetic beads bearing Class I antigens that detect HLA-antibodies capable of activate the complement in vitro.[29] This method may be able to identify better clinically relevant antibodies.[30]

Identifying platelet-specific antibodies results are difficult because most patients have concurrent anti-HLA. The most widely used method is the platelet immunofluorescence test (PIFT) with chloroquine treated platelets[31] and monoclonal antibody-specific immobilization of platelet antigens (MAIPA).[32]

3.3 Factors depending on the product´s characteristics

Platelet age significantly influences the CCI. In vitro markers of platelet quality decline by day 5 of storage,[33] and platelets stored for less than 48 hours result in a better increment at both 1 hour and 18 to 24 hours following transfusion.[34] Storage induces an impressive decrease in the *in-vivo* platelet recovery and survival in patients with certain clinical conditions such as bacterial infections, treatment with amphotericin B, graft-versus-host disease, splenomegaly and veno-occlusive disease.[35]

Natural antibodies directed against the ABO system also play a role in the post-transfusion efficiency. Although ABO compatibility is not necessary when transfusing platelets, it is recommended especially in patients suffering from refractoriness, because ABO mismatched platelets can reduce by 20% the platelet increments post-transfusion.[36]

When platelet refractoriness is suspected, it is recommended to use fresh ABO-compatible platelets, in order to limit the number of confounding variables.[37] Gamma-irradiated platelets have not demonstrated a better outcome in CCI terms, so is not justified in these cases.[38]

4. HOW TO MANAGE PLATELET TRANSFUSION REFRACTORINESS

4.1. Non immune PTR

If refractoriness is suspected, fresh ABO-identical apheresis platelet products should be transfused, calculating 10 min to 1-h CCI. If the result is less than 5000/microL on at least two sequential occasions, refractoriness will probably be due to an immune cause. If not, the 24-h CCI has to be calculated, and if less than expected, refractoriness will probably be from a non-immune cause, affecting platelet survival rather than platelet recovery.[39]

When refractoriness is due to non-immune causes the following measures have to be considered, none of the which are clearly effective:

- Treating the underlying disease.
- Transfusing ABO-identical platelets of less than 48 hours. Apheresis platelets are not necessary.
- Increasing the frequency of transfusions.
- If splenomegaly is present, increasing the number (rather than frequency) of transfused platelets.
- Transfusing at least 4 hours (preferably 8-12h) after administration of amphotericin B.

4.2. Immune PTR

There are three strategies in cases of immune-mediated refractoriness, once it is determined that a patient is alloimmunized: HLA matching, crossmatching and antibody specificity prediction. It is recommended calculating the CCI after each platelet-matched transfusion, in order to reevaluate the effectiveness and necessity of use. Anti-HLA antibodies in alloimmunized patients receiving induction chemotherapy for acute leukaemia may be transient.[40] On the other hand, alloimmunized patients could develop new antibodies after new transfusions. This dynamic process requires regular reassessment.

4.2.1 HLA matching

It consists of transfusing platelets from a donor with the most similar HLA type compared with the receptor, considering only HLA-A and HLA-B loci. This measure demonstrated an improved post-transfusional CCI and survival.[41] The HLA type grading system, shown in table 1, can predict the outcome, the better the HLA match, the better results.[42]

Table 1. HLA type grading system

Grade	Description
A	**HLA identical**
BU	**3/3 detected antigens identical; 1 antigen not identified**
B2U	**2/2 detected antigens identical; 2 antigens not identified**
BX	**3/4 detected antigens identical; 1/4 cross-reactive**
BUX	**2/3 detected antigens identical; 1/3 cross-reactive: 1 antigen not identified**
B2X	**2/4 detected antigens identical; 2/4 cross-reactive**
C	**1 antigen mismatch, out of cross-reactive group**
D	**≥2 antigen mismatches**

There is no significant difference in the 1-hour to 4-hour CCIs when comparing HLA-selected units with random-donor units.[43]About 70% of HLA matched platelets give a response worse than expected, reflecting once more that there are other immune factors not well characterized. In cases where compatible donors were not found, transfusion of platelets from partially mismatched donors may provide adequate responses.[44]

The main disadvantages of this strategy are the need to type the HLA in both donors and receptors, with greater costs and delays, and the necessity of a large pool of donors. For an HLA match grade level of Bx or better, a pool of about 3000 donors is needed.[45] In addition, it is not useful against anti-HPA.

4.2.2 Crossmatching

It consists of incubating donor platelets with recipient plasma and testing for an interaction. In solid-phase red cell adherence assay (SPRCA) the serum from the patient is incubated with the donor platelet in specially treated wells, and the presence of alloantibodies is determined by using indicator red blood cells coated with anti-IgG reagents. It permits most blood centers to have a rapid and effective selection of donor platelets.[46] Other advantages compared with HLA matching, apart from its rapid availability, are its usefulness for both anti-HPA and anti-HLA, and the avoidance of HLA typing. This technique needs to be performed frequently in patients requiring long-term platelet support, with a fresh sample drawn every 72 hours.[47] Furthermore, there is a potential risk of alloimmunization against mismatched donor HLA.

Crossmatch-compatible units have demonstrated superior CCI compared with random units with a range of 50% to 90%.[48] However, there is no significant difference between the 1-hour CCI of crossmatched platelets compared with HLA-matched platelets so both approaches are appropriate.[42 (p.635)]

4.2.3 Antibody specificity prediction

This strategy determines specificities of the recipient's alloantibodies, and the patient is transfused with platelets from donors lacking those HLA antigens, similar to the approach used routinely for red blood cell alloimmunization. The main advantage is significantly increasing the donor pool: among 7247 HLA-typed donors. For each HLA alloimmunized patient a mean of six donors were HLA-A matched, 33 were HLA-BU matched, and 1426 were identified by ASP.[47 (p.1451)]

Nevertheless, it is not useful against anti-HPA and there is also a risk of

alloimmunization against mismatched donor HLA.

4.2.4 Other management strategies

Bleeding in patients presenting refractoriness is a high risk situation, even more when the previous strategies have not been successful, with high rates of morbidity and mortality.

A variety of strategies have been attempted:

- Antifibrinolytic agents could reduce bleeding with no thromboembolic complications reported.[49]
- Intravenous immune globulin: evidence supports its routine use.[50]
- Splenectomy is not recommended.[51]
- Glucocorticoids have not been useful in standard doses.
- Rituximab: two case reports and a case series involving seven patients suggest promising results, although more studies need to be conducted.[52]
- Anti Rh (D) has no effect on refractoriness and increases the requirement for red cell transfusions, and therefore is not recommended.[53]
- Massive platelet dose transfusions have anecdotal evidence, but could be considered in emergency situations.[54]
- Continuous slow platelet transfusion has no solid evidence. It could maintain vascular integrity although it indicates a lack of increase in the post transfusion platelet count.
- Recombinant human factor VIIa: anecdotal success. This could be a reasonable option as a temporary measure or last resort, taking the prothrombotic risk into consideration.

5. SUMMARY

Platelet transfusion refractoriness is a harmful complication of platelet transfusion that leads to increased rates of morbidity and mortality. It is a very complex process, not well known, in which there are many interplaying variables. In many cases, it is not possible to identify the causal factor or factors. When suspected, it is recommended to transfuse fresh ABO platelets to reduce confounding factors. After ruling out non immune causes, the diagnosis of immune refractoriness is made when CCI is less than 5 10^9/L in two sequential transfusions and anti-HLA and or anti-HPA are demonstrated. It is not yet clear which laboratory test is optimal. There are several strategies to provide matched-platelets, all

offering similar results in terms of post transfusion CCI. However, whichever strategy is chosen, this complication must be diagnosed early and there must also be an efficient communication system between hospital and blood banks.

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