
بسم الله الرحمن الرحیم

A 15minute review on Platelet refractoriness : diagnosis,prevention and management

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Tehran-1397

- 15 years old girl
- Known case of refractory aplastic anemia on IST,waiting for HSCT
- Comes to clinic suffering from Sever GI bleeding
- Hx of repeated PC and PRBC transfusion(both random and LR products)
- Plt=15000 Hb=6
- PR=100 BP=90/60

- Q-1:

Which products do you order for her?

Platelet Components

Random Donor Platelet [Whole Blood Drived Platelet Concenterate(WBDPC)]

- At least 5.5 x 10^{10} platelets/unit
- Store at 20 - 24^{o}C with continuous agitation
- 50-65 ml plasma , Shelf life of 5 days

Single Donor Platelet – [Apheresis Drived Platelet Concenterate(ADPC)]

- At least 3.0 x 10^{11} platelets/unit (6 times)
- Store at 20- 24^{o}C with agitation ,Shelf life of 5 d.
- Suspended in 300 ml plasma (equivalent to 4-8 random donor platelets)

Prevention of alloimmune platelet refractoriness

- Avoiding platelet transfusions as much as possible
- Prophylactic transfusions are not recommended
- Leukocyte depletion of blood products by filtration and the experimental approach of ultraviolet B irradiation of theplatelet product can decrease sensitization significantly.

The TRAP Study Group *New Engl J Med* 1997; **337**: 1861–1869.

- No difference in quality between:standard platelets from platelet rich plasma or platelet buffy coat or between apheresis platelets and standard platelets
- Cryopreservation of autologous platelets harvested during remission from patients with autologous retransfusion during episodes of subsequent thrombocytopenia

WHO bleeding grades

- WHO bleeding grades
  - **grade 0**, none;
  - **grade 1**, petechiae, ecchymosis, occult blood in body secretions, and mild vaginal spotting;
  - **grade 2,** evidence of gross hemorrhage **not requiring red cell transfusions** over routine transfusion needs (e.g., epistaxis, hematuria, hematemesis);
  - **grade 3,** hemorrhage **requiring transfusion** of 1 or more units of red cells/day;
  - **grade 4**, **life-threatening hemorrhage**, defined as massive bleeding causing **hemodynamic compromise** or bleeding into a vital organ (e.g., intracranial, pericardial, or pulmonary hemorrhage)
- WHO bleeding grades 1 and 2 are usually considered directly attributable to the degree of a patient's thrombocytopenia, while more severe bleeding— WHO grades 3 and 4—is more often associated with contributing factors
- Therapeutic plt transfusions in patients with chronic thrombocytopenia are usually indicated when bleeding is ≥ WHO grade 2.

Factors affecting refractoriness

- **The time required for antibody formation:**
  - The mean time to the development of antibodies was 2–3weeks
- **The number of transfusion**
- **Platelet age** significantly affects CCI:
  - platelets stored for less than 48 hours resulting in a significantly improved platelet increment at both 1 hour and 18 to 24 hours following transfusion
- **White cell load in blood components**
- **Immune status of recipient :**
  - more sever and frequent in AA than leukemia
- **Underlying disease:**
  - in AML
    - there was no relationship between the number of transfusions or donor exposures, and the development of new anti-HLA antibody
    - The majority had persistence of the antibody, but 20% had disappearance of antibody over time
    - Immune tolerance to histocompatibility antigens in most of the patients(60%) following marrow recovery

*Dutcher JP. et al.Blood 1981; **58**: 1007–1011*

- After 2 times of LR-PC and PRBC she still bleeds severely:

Q-2 :

- Does she have platelet refractoriness Or there is a local problem?
- Which kind of refractoriness do you assume for her?

Defenition

- Refractoriness to platelet transfusions is defined as a repeated failure to achieve the **expected increment** in platelet count after **2 or more** platelet transfusions using **ABO-compatible platelets**, at least 1 of which had been stored for **no more than 48 hours**;
  - **10`- 1hr** Increment is <**5000**-7500

  OR
  - 4h-24h increment <4500 at 24 hrs

Schiffer CA, Anderson KC, Bennett CL, et al. Platelet transfusion for patients withcancer: clinical practice guidelines of the American Society of Clinical Oncology.J Clin Oncol 2001;19(5):1519–38.

Hod E, Schwartz J. Platelet transfusion refractoriness. Br J Haematol 2008; 142(3):348–60.

Box 1
CCI calculation

$$CCI^{a} = \frac{\text{posttransfusion platelet count} - \text{pretransfusion platelet count } (/L) \times \text{BSA } (m^2)^{b}}{\text{platelets transfused } (10^{11})^{c}}$$

Abbreviation: BSA, body surface area.

[a] For example, using a BSA of 2.0 m^2, an absolute platelet increment of less than 10×10^9/L after administration of an apheresis unit of platelets is suspect for refractoriness (CCI<5.0×10^9/L).

[b] Average adult BSA = 2.0 m^2.

[c] Platelets transfused = approximately 4×10^{11} platelets in apheresis unit, 0.7×10^{11} for each random donor platelet concentrate.

the incidence of refractoriness in hematology/oncology patients varies from 7% to 34%.

Immune mediated (20%)

1 Hour CCI<5000 :

1. Alloimmunization to :
   - HLA Class I antigen;
   - Platelet specific antibody
   - ABO incompatibility
2. Autoimmunization
3. Drug induced Immunization

Non-Immune mediated (80%)

24 Hour CCI<4,500 :

1. Sepsis
2. Fever
3. DIC
4. Drugs
5. Slenomegaly
6. TTP
7. Platelet age (>3 d) and poorly stored platelet concentrates

Alloimmunization against platelets (HLA class I antigens)

- Mainly HLA-A &B (Bw4/ Bw6)
- Caused by prior exposure from pregnancy, transfusions, and/or transplantation
- Reducing contaminating leukocytes in blood products by filtration or ultraviolet B irradiation reduces the development of lymphocytotoxic antibodies
- 3 main types:
  - **Refractoriness to platelet transfusion** (an increase in the platelet count after platelet transfusion that is significantly lower than expected
  - **Post-transfusion purpura(PTP)** (thrombocytopenia after transfusion of red cells or other platelet-containing products, associated with the presence of platelet alloantibodies)
  - **Neonatal alloimmune thrombocytopenia(NATP)** (mother's alloimmunization against fetal platelet antigens, most often resulting from previous pregnancies but can be seen in a first pregnancy)

Alloimmunization against platelets (Platelet-specific Antigens)

- only 5 of them are known to be polymorphic, leading to alloimmunization and platelet refractorinessGPIa, GPIb, GPIIb, GPIIIA, and CD109 ***Kopko PM, Transfusion 2015;55(2):235–44***
- Incidence: varies from 2% to 11%.
- There are significant differences in the prevalence of the HPA polymorphisms in various populations
- Platelet-specific antibodies are generally not associated with a statistically significant reduction in CCI
- the presence of antiplatelet antibodies does not mean PR, since in approximately 30% of cases, they occur in the absence of clinically detected PR.

Antiplatelet Antibodies (ABO incompatibility)

- can also sometimes be responsible for platelet refractoriness **due to high titer (« hemolytic ») Anti-A or Anti-B**

  (LEE, Transfusion 1989)

- ABO incompatibility **can trigger platelet refractoriness due to anti-HLA or platelet antibodies appearance**

- Her 1st Hr CCI is 2000/UL

Q-3:

How is recommended to assesse her alloimmunization status?

Antiplatelet antibodies detection:

1. Screening for HLA and HPA antibodies as well as specific identification of the most commonly involved HPA antigens :
   - Platelet immunofluorescence test (PIFT) either by microscopy or flow cytometry (FC-PIFT)
   - Immunoassays such as , Solid-Phase Modified Antigen Capture Elisa (MACE) ,the solid-phase RBC adherence assay
   - monoclonal antibody immobilization of platelet antigens (MAIPA) : golden standard, but very laborious and time-consuming

NOTE:

- there is no consensus regarding which test is ideal for diagnosing refractoriness
- May be too sensitive:
  - Positive: may be identifying weak HLA antibodies that do not predict platelet refractoriness
  - A negative result: strongly suggests nonimmune causes of refractoriness.

2. Microcytotoxicity assays against a panel of 30-60 different lymphocyte cells can demonstrate lymphocytotoxic HLA antibodies:
   - The percentage of cells to which the patient's serum reacts is referred to as the **panel-reactive antibody (PRA) level**. PRA values **greater than 20%** indicate significant alloimmunization to HLA antigens and correlate with **an increased risk for PR**

- NOTE:
  - may better predict platelet refractoriness; however, these tests are more cumbersome than the more automated techniques

DIAGNOSIS OF IMMUNE-MEDIATED PLATELET REFRACTORINESS

- The results of platelet refractoriness testing should be interpreted in conjunction with the clinical picture
- No gold standard test

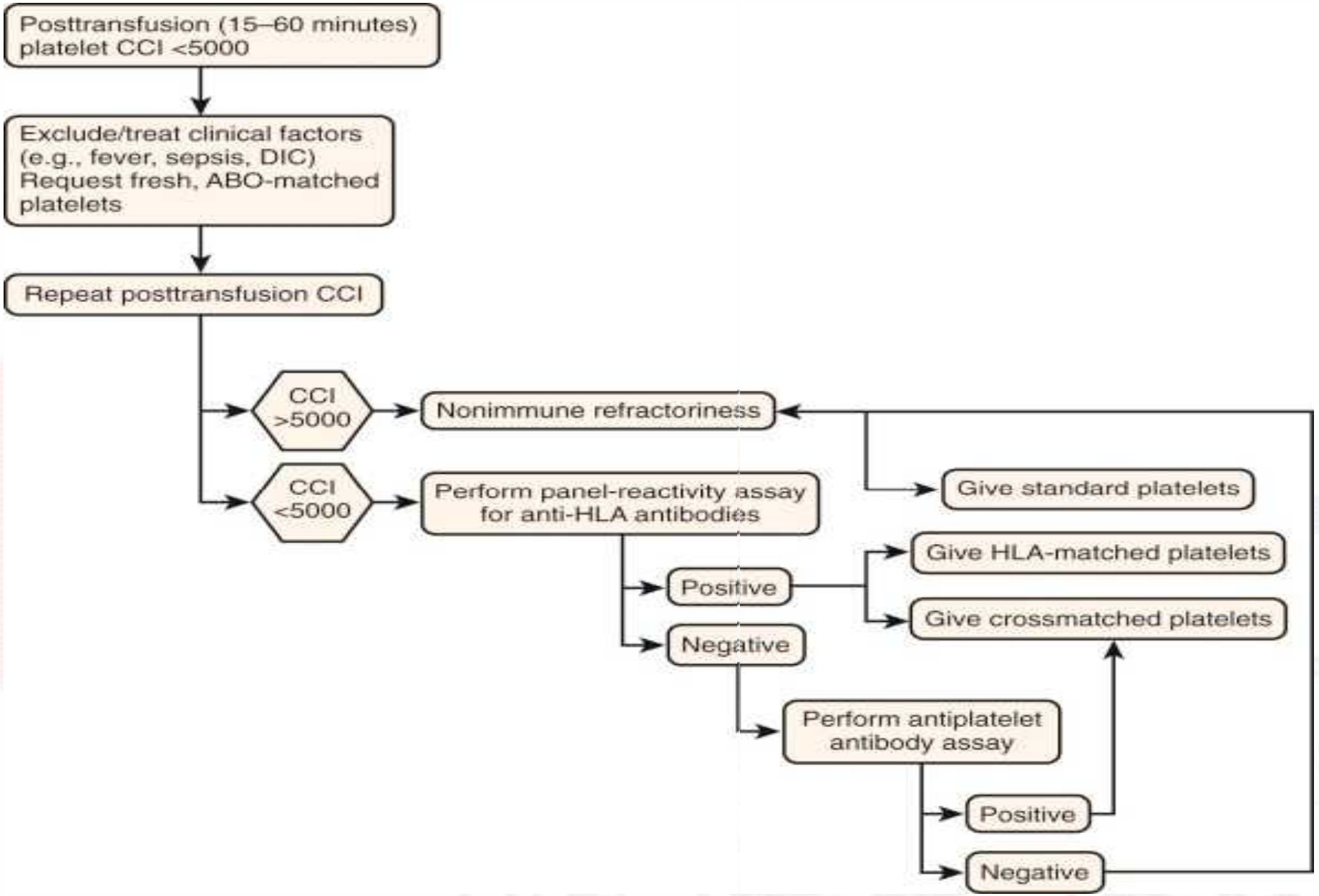
Fontao-Wendel R,et al. Vox Sang 2007; 93(3):241–9.

- Transfusion management should only be pursued if both clinical and laboratory evidence suggest the presence of true immune-mediated platelet refractoriness.

Kopko PM, et al. Methods for the selection of platelet products for alloimmune-refractory patients. Transfusion 2015;55(2):235–44.

- Q-4
- How do you manage her based on evidences according to literature?
- What do you order for her in IRAN?

After diagnosing alloimmune platelet refractoriness



usion Medicine * Sloan, Steven R., Nathan and Oski's Hematology and Oncology of Infancy and Childhood, Chapter 36, 1127-1164.e10

Table 1
Methods for managing immune-mediated platelet refractoriness

	HLA Matched	Crossmatched	Antibody Specificity Prediction
Method	HLA type the patient and provide platelets collected from an HLA-matched donor	Combine donor platelets with patient's serum to determine crossmatch compatibility	Identify HLA antibodies in patient and then provide platelets without those specific HLAs
Pros	Prevents future alloimmunization if high-grade match	• Useful for anti-HPA and anti-HLA • Rapid availability • HLA typing not required	• Larger donor pool • Patient HLA typing not required
Cons	• Not useful for anti-HPA • Patient and donor HLA typing required • Must recruit HLA-matched donors • Limited donor pool for rare HLA types	• Difficult to find suitable crossmatch in highly sensitized patients • Risk of alloimmunization against mismatched donor HLAs • Frequent crossmatching necessary	• Not useful for anti-HPA • Potential risk of alloimmunization against mismatched donor HLAs • Must type donor HLA

They seem to offer similar results in terms of posttransfusion CCI. However, there are no randomized clinical trials comparing the effectiveness of these methods on clinical outcomes.

HLA MATCHING

Comparing HLA-selected units with random-donor units:

- there is good correlation between the match grade and the CCI after transfusion:
  - **30%** of HLA-selected platelet transfusions (A, B1U, or B1X match grade) compared with **12%** of random-donor transfusions
- no significant difference in the 1-hour to 4-hour CCIs
- other clinical factors may contribute to PR

A pool of up to 3000 donors is needed to meet the transfusion needs at an HLA match grade level of Bx or better

Table 3
HLA match grade

Grade	Degree of Matching
A	All 4 HLA-A and HLA-B loci are identical
BU	Only 3 antigens detected in donor; all present in recipient Recipient cells do not possess HLA-A or HLA-B antigens that differ fr donor because of homozygosity at HLA-A or HLA-B loci
BX	Three donor antigens identical to recipient One HLA-A or HLA-B incompatibility that is cross reactive
C	Three donor antigens identical to recipient One noncrossreactive antigen difference
D	Two or more noncrossreactive mismatches

Masse B, Cohn C, Lindgren B, et al. Utilization of cross-matched or HLAmatched platelets for patients refractory to platelet transfusion. Transfusion 2014;54(12):3

CROSSMATCHING

- Rapid ,feasible and effective selection :Can be performed in a few hours
- superior CCIs with a success rate ranging from 50% to 90%.
- Frequent crossmatches for patients requiring long-term platelet support because of the possibility of a change in alloantibody reactivity: crossmatches should be performed on a fresh sample drawn from the recipient every 72 hours

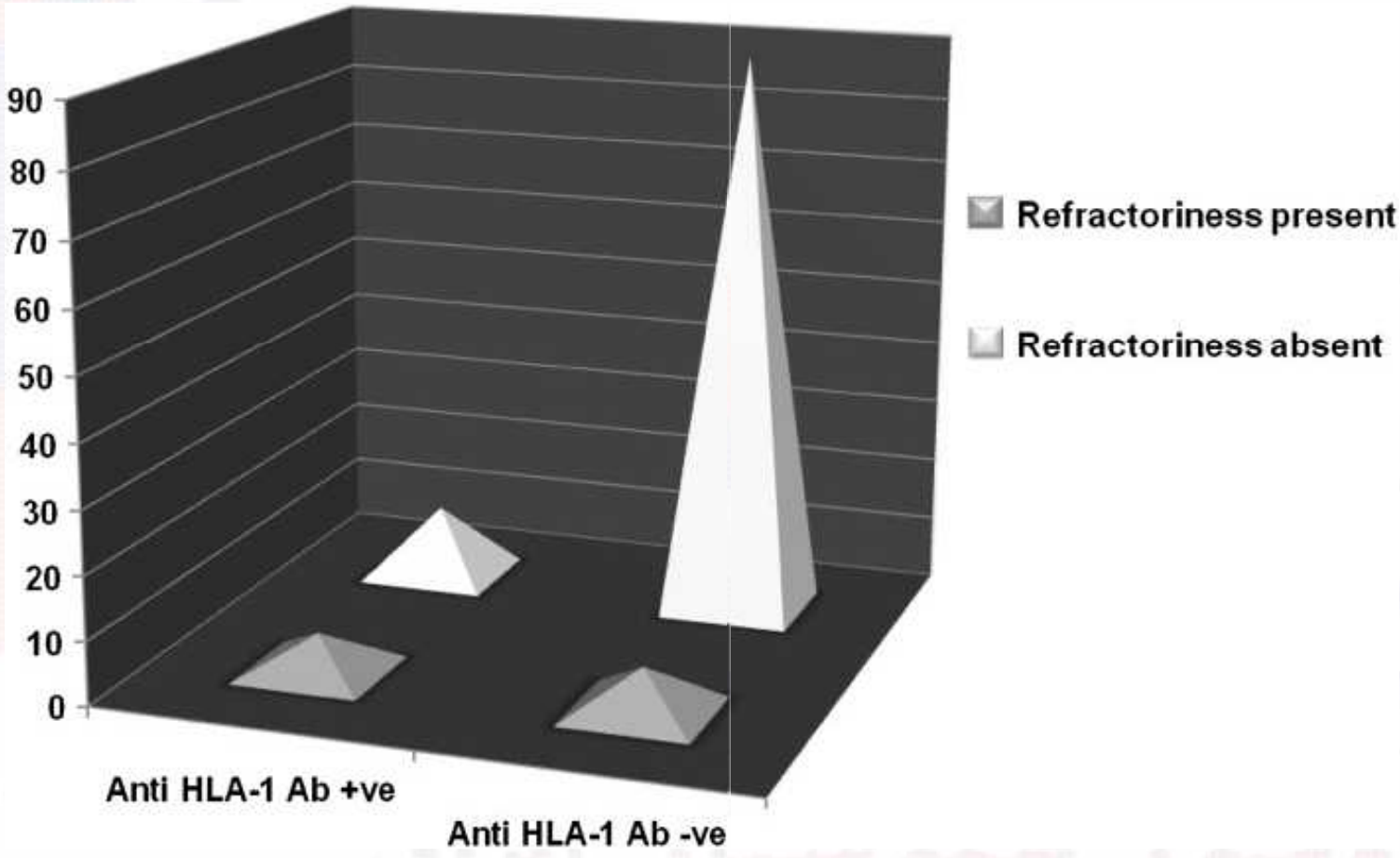
ANTIBODY SPECIFICITY PREDICTION

the ASP method does not require a full match; only the antigens to which the patient has alloantibodies are matched

This method increases the donor pool significantly:

- For example, among 7247 HLA-typed donors, for each HLA-alloimmunized patient a mean of 6 donors were HLA-A matched, 33 were HLA-BU matched, and 1426 were identified by ASP.

Petz LD, Garratty G, Calhoun L, et al. Selecting donors of platelets for refractory patients on the basis of HLA antibody specificity. Transfusion 2000;40(12): 1446–56.


90
80
70
60
50
40
30
20
10
0
Anti HLA-1 Ab +ve
Anti HLA-1 Ab -ve
Refractoriness present
Refractoriness absent

HOWEVER:

- Most patients who have HLA antibodies do not develop platelet refractoriness.
  - In the TRAP study, 45% of the control group developed anti-HLA antibodies, but only 13% developed platelet refractoriness.
- A dose-response relationship between the number of platelets transfused and the incidence of alloimmunization is also not observed
- A significant fraction of HLA ‘matched’ transfusions do not produce satisfactory increments (due to of serologic cross-reactivity), however, while some ‘mismatched’ transfusions are successful(because of weak expression of common HLA Ags such as HLA B44 and 45)

SUMMARY OF RECOMMENDATIONS

Rule out nonimmune, autoimmune, and drug-related causes of platelet efractoriness and treat accordingly: Providing immune-compatible platelet s unlikely to be effective in the presence of nonimmune causes of efractorines

Perform HLA Ab detection and Retest serum every month during transfusio

f HLA Ab is not yet detected & PR is assumed:

- Transfuse **ABO-compatible fresh (aged < 48 h)** platelet concentrates.
- Re-assess non-immune causes.
- Contact the Blood Service to discuss options (e.g. repeat HLA testing, HPA testing, other)

The use of **HPA1a/5b-negative platelets** has been successful in cases of **posttransfusion purpura** and **neonatal platelet alloimmunization**. These ntigens are involved in most (95%) of these cases

Human Platelet-Specific Antigen Systems

Platelet Antigen System	Protein Antigen	Synonyms	Alleles	Antigen Frequency
HPA-1	GPIIIa	Pl^A,Zw	HPA-1a = Pl^{A1} HPA-1b = Pl^{A2}	97% 26%
HPA-2	GPIb	Ko, Sib	HPA-2A HPA-2b	99% 14%
HPA-3	GPIIb	Bak, Lek	HPA-3a HPA-3b	85% 66%
HPA-4	GPIIa	Pen, Yuk	HPA-4a HPA-4b	>99% < 1%
HPA-5	GPIa	Br, Hc, Zav	HPA-5a HPA-5b	99% 20%

If Anti-HLA/HPA has been detected:

- Select HLA-matched platelets:
  - **Perform HLA typing of patients** who will receive multiple transfusions **before** they become pancytopenic (eg, bone marrow transplant recipients).
  - **Perform HLA typing of donors:**
    - Matching for both private (ie, HLA-A, HLA-B) and public (ie, cross-reacting groups) antigens is best achieved by **computerized selection of donors**, based on the results of the **PRA assay**.
- Select cross matched platelets:
  - significantly improve platelet recovery in approximately **50% of patients** who are refractory to random-donor platelets
  - is especially indicated for patients with **high PRA levels** or those who **do not respond to HLA-matched platelets**.
  - A significant fraction of HLA 'matched' transfusions do not produce satisfactory increments (due to of serologic cross-reactivity), however, while some 'mismatched' transfusions are successful(because of weak expression of common HLA Ags such as HLA B44 and 45)

f matched/compatible Platelet is not possible OR available:

1. Consider **alternatives to platelet transfusion** :antifibrinolytics,LHA
2. Transfuse with **Apheresis derived & Irradiated** platelets from blood **relatives :**Obtaining platelets from blood relatives is worthwhile because the chance of matching 2 or more HLA and platelet antigens is high
3. 24-h (British Journal of Haematology, 2018, 181, 386–417) OR 6-h American Journal of Hematology 79:79–82 (2005) slow continuous infusion of platelets for patients with platelet transfusion refractoriness

4. Empirical Use of **high-dose platelet transfusion** (eg, 1 apheresis unit tid or 2-3 apheresis units before invasive procedures): may result in a lower overall titer of the effecting antibody(ies), overwhelming the mononuclear-phagocyte system, and increasing the survival of transfused platelets.

f matched/compatible Platelet is not possible OR available:

Pretreat with IVIG before transfusion:

- Success rates vary and depend on the degree of alloimmunization.
- Decrease and possibly interferes with platelet destruction by platelet-associated immunoglobulins .
- IVIG is more effective in improving short-term (1-6 h) recovery of platelets than longer term platelet survival (>24 h).

Consider administering immunosuppressive drugs. While steroids are not effective, isolated reports suggest that immunosuppressive therapy may be beneficial. As examples, the use of vincristine and cyclosporin A has been successful but require 2-3 weeks to take effect.

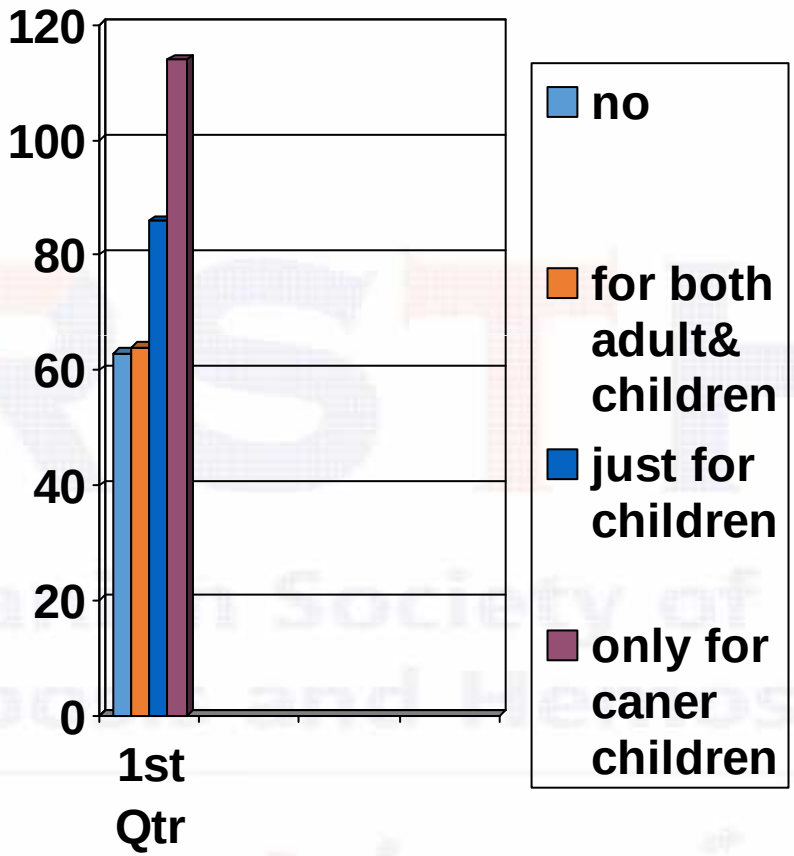
Attempt large-volume plasmapheresis. Plasmapheresis (eg, 2 plasma volumes for 1-3 d) before bone marrow transplantation OR invasive procedures

use of nonviable platelet substitutes in contrast to the traditional transfusion of intact platelets: *(reviewed by Alving et al.*):

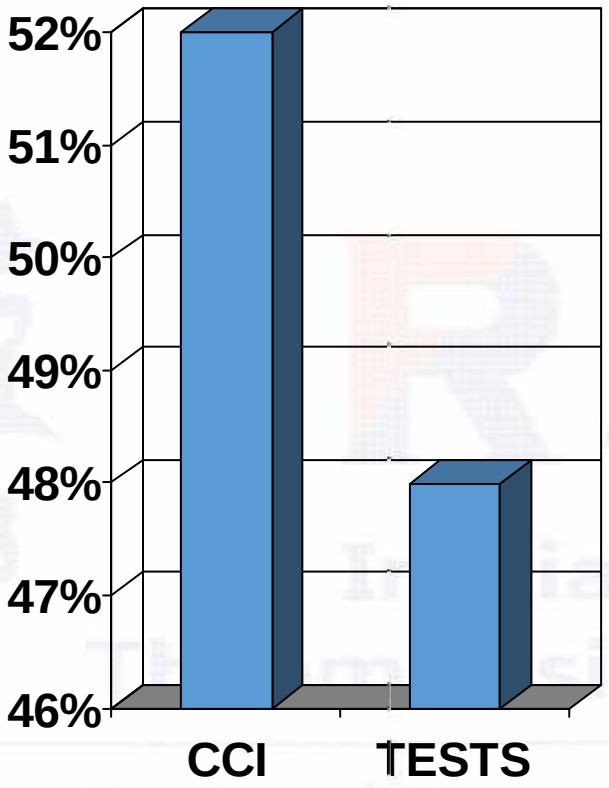
- Lyophilized and freeze dried platelets, as well as platelet membranes and even erythrocytes to which subendothelial binding proteins have been attached (‘thromboerythrocytes’)

A.S.C.O study on current practice in USA:

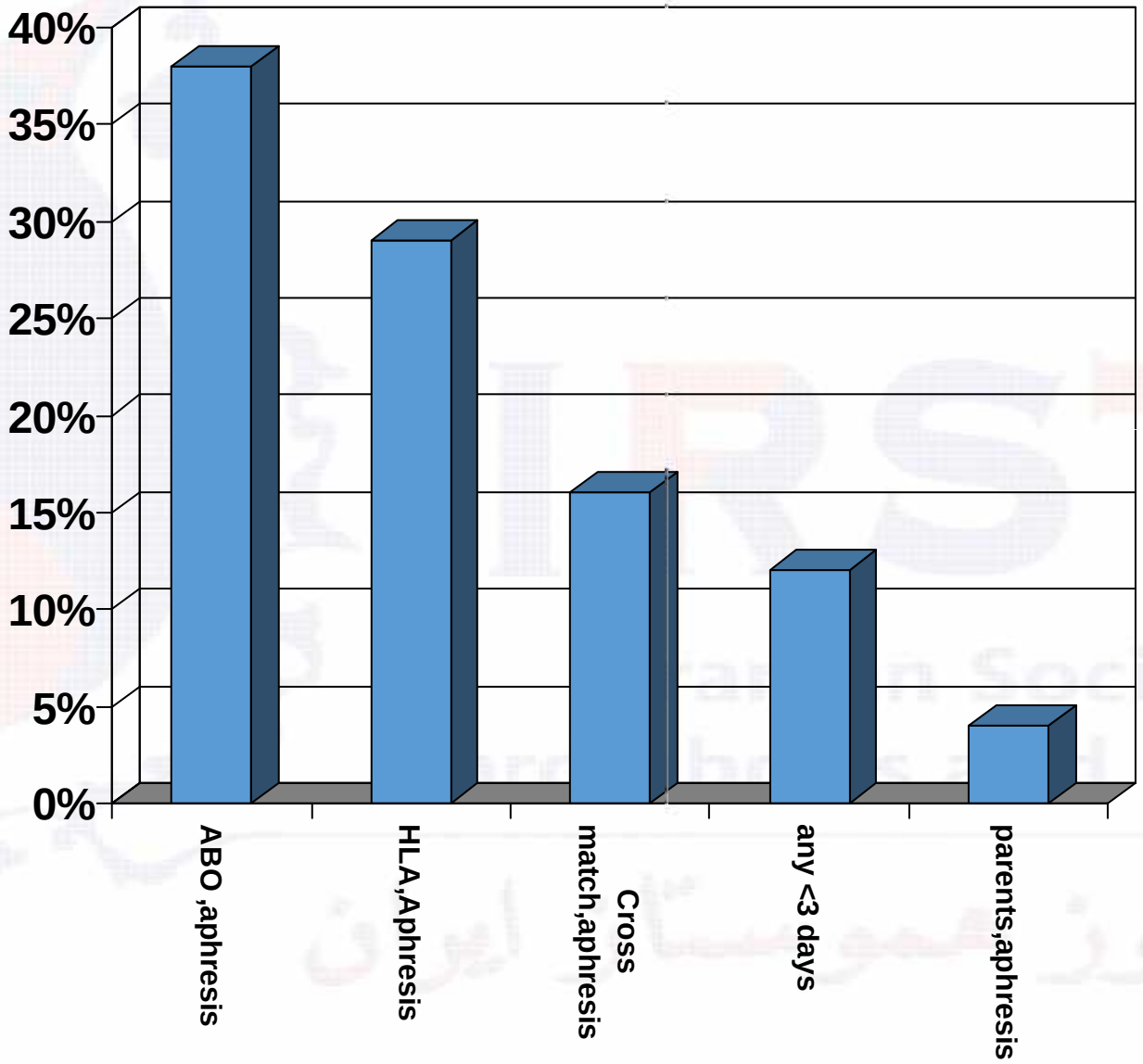
- 841 Members of ASPHO inside USA were asked
- 264 Members responded



Diagnosis of Refractoriness



Treatment of *suspected* refractory



KEY POINTS

- Platelet refractoriness is defined as an inadequate response to platelet transfusions and is diagnosed by a corrected count increment of less than 5 109/L after 2 sequential transfusions.
- Nonimmune causes are the most likely and the first that should be explored in the diagnosis of platelet refractoriness.
- Immune-mediated platelet refractoriness is cause by antibodies to human leukocyte antigens (HLAs) and/or human platelet antigens.
- If antibodies are identified, there are 3 strategies for identifying compatible platelet units: HLA matching, crossmatching, and antibody specificity prediction

SLIDE WILL BE AVAILABLE AT THE THE BELOW SITES NEXT WEEK:

PCHD.SBMU.AC.IR

IRSTH.IR