50-year-old male with refractory thrombocytopenia

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1

Clinical History: OSH

- 50-year-old African American male
- Initially presents to an outside hospital (OSH) with 10-month history of worsening fatigue and new onset oral bleeding and hemoptysis
- Pancytopenia
  - Had never received a transfusion before
  - Received 2 units of RBCs and 2 unit of platelets with minimal improvement of platelets
- Bone marrow biopsy
  - 05/04
    - Suboptimal specimen
    - Without definitive diagnosis
    - Complex cytogenetics
  - 05/18 repeated
    - Again suboptimal
    - Hypercellular bone marrow with at least MDS with excess blasts, could not rule out AML
- Referred to KUMC for further workup and treatment

2

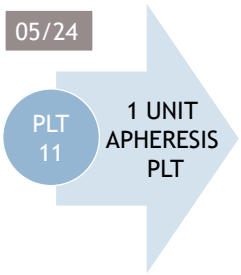
CLINICAL HISTORY: KUMC

- First CBC upon arrival showed pancytopenia
  - Mild leukopenia with neutropenia and 2% circulating blasts
    - White blood cells 4.1 K/UL
    - Absolute neutrophils 492 / UL
  - Mild normocytic anemia
    - Hemoglobin 7.4 g/dL
    - MCV 91.2 FL
  - Severe thrombocytopenia
    - Platelets 11 K/UL

3

Clinical History: KUMC

- Required platelet transfusions for his severe thrombocytopenia



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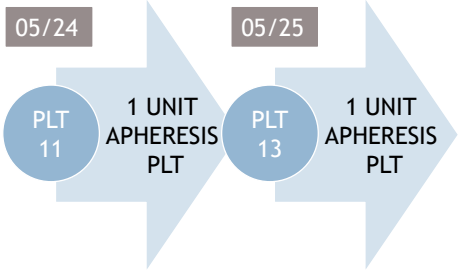
Clinical History: KUMC

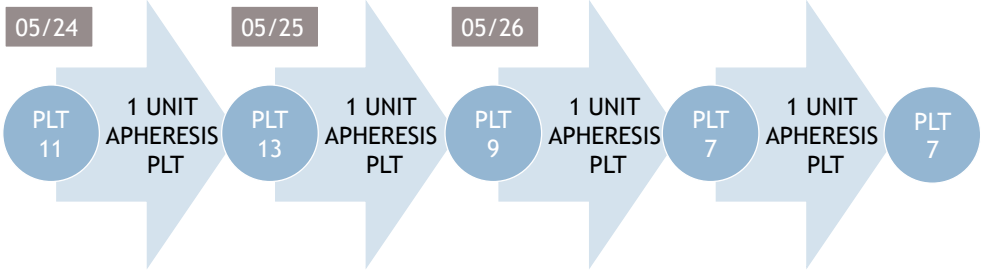
- Required platelet transfusions for his severe thrombocytopenia
- Post-transfusion platelets did not increase



5

Clinical History: KUMC

- Required platelet transfusions for his severe thrombocytopenia
- Post-transfusion platelets did not increase
- 05/26: post-transfusion platelet assessments were done around 1 hour following transfusion
  - This will better assess replacement and onset of platelet refractoriness



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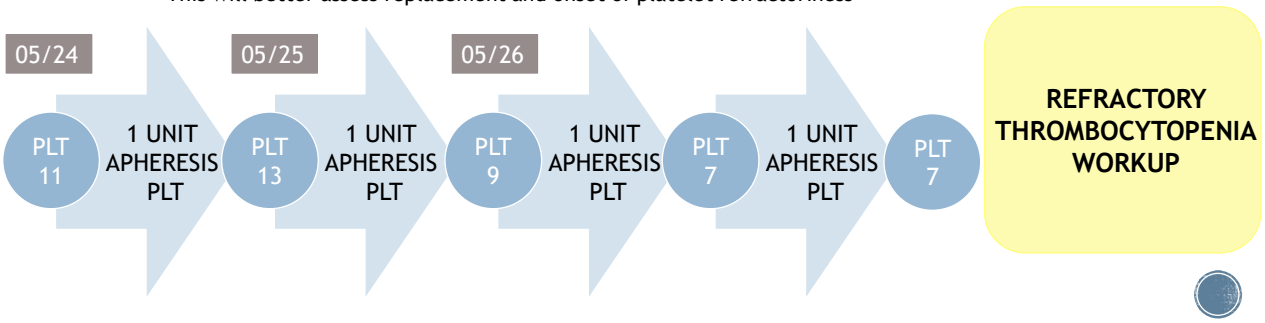


7

Refractory Thrombocytopenia

- Suboptimal response to platelet transfusion on at least two occasions
  - Platelets should be ABO compatible and completely transfused
  - Assess incremental platelet count
    - Failure to observe a post-transfusion platelet increment of at least 10,000/uL is considered suspicious for refractoriness

8

Refractory Thrombocytopenia

- Working up a patient refractory to platelet transfusions
  - Careful review of medical history
    - Current medical conditions
    - Medications
    - Events which increase likelihood of HLA and HPA alloimmunization
      - Transfusion history
      - Transplant history
      - Previous pregnancies

9

Refractory Thrombocytopenia

- Classified as either immune or nonimmune
  - Nonimmune are most common
    - Rapid consumption or sequestration of platelets from systemic circulation
      - Fever
      - Sepsis
      - **Splenomegaly**
      - Disseminated intravascular coagulation (DIC)
      - Bleeding
      - Stem cell transplant
      - Graft versus host disease
      - Veno-occlusive disease
      - Vasculitis
      - **Immune thrombocytopenia**
      - Drug induced immune thrombocytopenia
        - Heparin, amphotericin, vancomycin, ciproloxacin

10

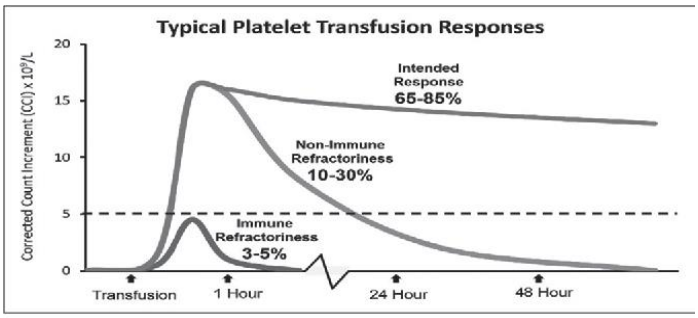
Refractory Thrombocytopenia

- Classified as either immune or nonimmune
  - Immune
    - Alloantibodies or autoantibodies reacting against antigens on donor platelets which leads to their rapid removal from circulation
    - Most common causes of alloimmune-mediated come from recipient antibodies against:
      - HLA Class I antigens to HLA-A and HLA-B
      - Human platelet antigens (HPA)
      - A and B blood group antigens on platelets

11

Refractory Thrombocytopenia

- Classified as either immune or nonimmune



12

Differential Diagnosis

- Splenic sequestration
- Immune thrombocytopenia (ITP)
- Platelet antibody

13

Differential Diagnosis

- **Splenic sequestration**
  - Platelets pool in spleen
  - Normally ~1/3 of platelets are sequestered in the spleen
  - Splenic sequestration can increase to up to 90% if spleen is enlarged
  - **Ultrasound of spleen**
    - **Normal spleen size**
- Immune thrombocytopenia (ITP)
- Platelet antibody

14

Differential Diagnosis

- ~~Splenic sequestration~~
- **Immune thrombocytopenia (ITP)**
  - Autoantibody-mediated destruction of the patient's platelets and impaired platelet production
  - Diagnosis of exclusion
  - Immature platelet fraction 13.3% (normal: 1.1 - 7.1%)
    - Indicates platelets are being produced but must be consumed in the periphery
  - Enough suspicion for ITP as contributing factor alongside MDS to **start dexamethasone** and consider IVIG if life threatening bleeding
- Platelet antibody

15

Differential Diagnosis

- ~~Splenic sequestration~~
- Immune thrombocytopenia (ITP)
  - Treated with steroids
- Platelet antibody
  - Alloantibodies for platelet antigens
  - Platelet antibody screening

16

Platelet Antibody Screening

Immunohematology Platelet Antibody Testing

Test Performed	Results	Comments	Platelet Transfusion Recommendation
Antibody Screen	Positive		Platelets compatible by crossmatch

Additional Comments

The patient's plasma was reactive with 6 of 6 (100%) of a panel of fresh random donor platelets and 8 of 13 (62%) of a panel of dried, phenotyped platelets by a solid phase red cell adherence assay. The results of these tests indicate the presence of platelet antibody.

17

Differential Diagnosis

- ~~Splenic sequestration~~
- ~~Immune thrombocytopenia (ITP)~~
- Platelet antibody
  - Alloantibodies for platelet antigens
  - Platelet antibody screening
    - **POSITIVE (highly reactive)**
    - This rules out ITP so steroids were stopped
    - Also the steroids had not improved platelet counts despite further transfusions

18

Differential Diagnosis

- ~~Splenic sequestration~~
- ~~Immune thrombocytopenia (ITP)~~
- Platelet antibody
  - Platelet antibody screening
    - **POSITIVE (highly reactive)**
  - HLA-Ab testing

19

Differential Diagnosis

Antibody Screening Report

UNOS CPRA:

Sample Date	Sample Number	Tested Date	Test	Class	Result
05/27/2023 Specificity:	905259 B:44	05/31/2023	Luminex Class I Single Antigen ID (131)	Beads I	Positive
05/27/2023	905259	05/31/2023	Luminex Class II Single Antigen ID (132)	Beads II	Negative
05/27/2023	905259	05/31/2023	Luminex Class I Antibody Screen (+/-) (129)	Beads I	Negative
05/27/2023	905259	05/31/2023	Luminex Class II Antibody Screen (+/-) (129)	Beads II	Negative

Antibody testing using Single Antigen Beads is considered the more sensitive assay. In case of positive class I and/or II Antibody Screening results and NEGATIVE Single Antigen results, please refer to Single Antigen results.

- ~~Splenic sequestration~~
- ~~Immune thrombocytopenia (ITP)~~
- Platelet antibody
  - Platelet antibody screening
    - **POSITIVE (highly reactive)**
  - HLA-Ab testing

20

Anti-CD36 Testing

Immunohematology Platelet Antibody Testing

Test Performed	Results	Comments	Platelet Transfusion Recommendation
Antibody Screen	Positive	See below	See below

Additional Comments

A solid phase ELISA assay indicated the presence of platelet antibody.The patient's plasma was reactive with 1 of 1 examples of platelet glycoprotein IV (CD36).

Mutations in the CD36 gene have been described that result in a complete lack of protein expression on both platelets and monocytes in populations of Asian and African ancestry. CD36 deficient individuals exposed to normal platelets can produce antibodies to CD36 that have been reported to cause platelet transfusion refractoriness.

Platelet antibody identification/confirmation testing as well as glycoprotein IV (CD36) typing is recommended for this patient. Please contact the reference laboratory 816-968-4053 for sample requirements and testing details if this testing is desired.

Siblings and blood relatives of the patient may be CD36 negative and should be encouraged to be tested for the CD36 antigen.

21

Anti-CD36 Testing

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23

Final Diagnosis

Platelet antibody against CD36 (glycoprotein IV)

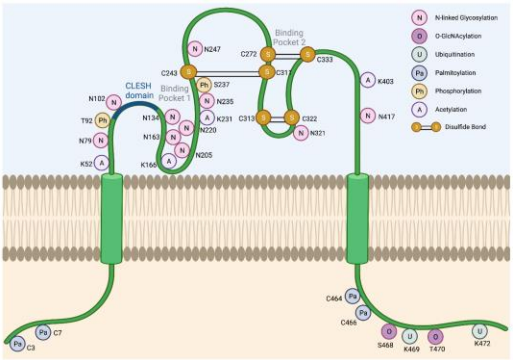
24

CD36 (glycoprotein IV)

- Found on endothelial, epithelial, and adipocytes as well as platelets and monocytes/macrophages

- Function
  - Scavenger receptor for many different processes
  - Cell adhesion molecule in platelet adhesion and aggregation, platelet-monocyte interactions, and platelet-tumor cell interactions

- Associations
  - Platelet transfusion refractoriness
  - Atherosclerosis/hyperlipidemia
  - Increased susceptibility to severe malaria versus protective against malaria (*mutation dependent)
  - Alzheimer's disease
  - Possibly aids in tumor metastasis



25

CD36 Deficiency

- Autosomal recessive inheritance caused by a homozygous or compound heterozygous mutation in the CD36 antigen gene on chromosome 7q21

- Two subgroups of CD36 deficiency
  - Type I: platelets, monocytes/macrophages, and other cell types with complete loss of CD36
  - Type II: platelets with complete loss of CD36; monocytes/macrophages with near normal expression of CD36

- CD36 deficiency is most common in Asian and African populations
  - 2 to 3% of Japanese, Thais, and Africans
    - Japanese with type I: 0.3-0.5%
    - Japanese with type II: 4.0-5.7%
  - 0.3% of Americans of European descent / 0.4% in caucasians

26

Anti-CD36

- Alloimmunization occurs when individuals with CD36 deficiency are exposed to CD36 during pregnancy, transfusion, or transplant
  - In our case, those initial platelet transfusions most likely were enough to trigger alloimmunization



27

Prior Case Reports

- First reported in a Japanese patient with AML who developed refractory thrombocytopenia
- 2 Canadian patients
  - 5-year-old female of Lebanese descent with AML on chemotherapy
  - 70-year-old male of Chinese descent with AML on induction chemotherapy
  - Both were able to receive CD36 negative platelets
- 66-year-old African American female with AML unable to complete optimal chemotherapy due to severe refractory thrombocytopenia with type I CD36 deficiency and subsequent CD36 antibody
  - Only 3 donors available in the entire US, insufficient supply to provide CD36 negative platelets
  - Survived a year with persistent disease before passing away due to *Aspergillus* pneumonia

TABLE 1. Cases of PLT refractoriness associated with anti-CD36

Case	Reference	Patient age, sex	Ethnicity	Diagnosis
1	Ikeda et al.[2]	36, female	Japanese	AML
2	Lee et al.[6]	9, female	African	Nephroblastoma
3	Fujino et al.[9]	19, male	Japanese	Testicular cancer
4	Curtis and Aster[11]	41, male	African American	AML
5	Curtis and Aster[11]	15, male	African American	Solid tumor
6	Curtis et al.[7]	26, male	Arabic	Aplastic anemia
7	Ogata et al.[13]	47, male	Japanese	Liver transplant
8	Flesch et al.[14]	36, male	Arabic	Lymphoma
9	Broderick et al.[15]	24, male	Palestinian	CML, BMT
10	Current report	5, female	Lebanese	AML
11	Current report	70, male	Chinese	AML

BMT = unrelated bone marrow transplantation; CML = chronic myelogenous leukemia.

Saw, *et al*.

28

Other Case Reported Clinical Presentations

- Post-transfusion purpura
- Transfusion-related acute lung injury (TRALI)
- Fetal/neonatal alloimmune thrombocytopenia (FNAIT)
  - Preclinical model developed in mice which replicates outcomes for FNAIT
    - Now testing different immunotherapeutic options to prevent severe clinical FNAIT outcomes
    - The polyclonal and monoclonal murine anti-CD36 antibodies used were found to work better than human IVIG treatments in preventing FNAIT
    - Still much research needed before it is moved into clinical testing for human disease

29

- Find suitable units for future transfusions which are CD36 negative
- CD36 negative units is not typically tested for and is very rare
- The best chance to get compatible platelets is from his family
  - Daughter is in Florida
  - Several cousins here and in Arkansas

30

Crossmatching for Platelets

- We used the patient's plasma and his relatives' platelets to run a crossmatch
  - Compare reactions to random donor platelets which cause a 3+ to 4+ reaction

Cousin #1	Weaker reactivity (1+ to 2+)
Cousin #2	Weaker reactivity (1+)
Cousin #3	Strongly reactive (3+ to 4+)
Cousin #4	Strongly reactive (3+ to 4+)
Cousin #5	Strongly reactive (3+ to 4+)
Cousin #6	Strongly reactive (3+ to 4+)
Cousin #7	Strongly reactive (4+)
Daughter	Strongly reactive (3+ to 4+)

31

Transfusion from cousins' platelets

Cousin #1 (1+ to 2+ reactivity)

- 34-year-old male
  - Able to donate 2 platelet units (one normal and one smaller)
    - Received first unit on 07/07 — Platelet count 5 to 15
    - Received second unit on 07/09 — Platelet count 7 to 12

Cousin #2 (1+ reactivity)

- 61-year-old female with poor vein condition which was not great for donation; however, she still wanted to donate what she could
  - Checked HLA-Ab due to age and history of pregnancy but was negative
  - Donated 1 unit which was transfused to patient on 07/14 — Platelet count <3 to 17

32

Patient's Treatment Course

- Presented to KUMC 05/24
- Began chemotherapy 05/31 for high-risk myelodysplastic syndrome which was later diagnosed as transformed to acute myeloid leukemia
  - Persistent disease on follow up bone marrows despite treatment
- Anti-CD36 platelet antibody was found on 06/01
- Waiting on crossmatching with family
  - Receiving platelets from cousins 07/07, 07/09, and 07/14

33

Patient's Treatment Course

- Difficult to maintain platelet levels with persistent disease, chemotherapy, and lack of available platelet units
- During episodes of severe acute blood loss anemia, patient was given IV solumedrol and IVIG prior to RBC and platelet transfusions
  - 06/09
    - Pretransfusion platelets: **11** (11:40) and 9 (12:09)
    - Transfused with IVIG and 2 units of apheresis platelets
    - Posttransfusion platelets the following morning: 10
  - 06/21
    - Pretransfusion platelets: 7
    - Transfused with IVIG and 1 unit of apheresis platelets
    - Posttransfusion platelets the next morning: 12
  - 06/23
    - Pretransfusion platelets: 8
    - Transfused with IVIG and 1 unit of apheresis platelets
    - Posttransfusion platelets the next morning: 7

34

Patient Follow Up

- Overall poor prognosis
  - Treatment resistant AML
  - Inability to maintain platelet levels due to lack of necessary compatible platelet units, persistent disease, and ongoing chemotherapy
- New bone marrow biopsy was performed this last week and is pending
- Ultimate goal is allogenic stem cell transplant

35

Final Overview of Refractory Thrombocytopenia

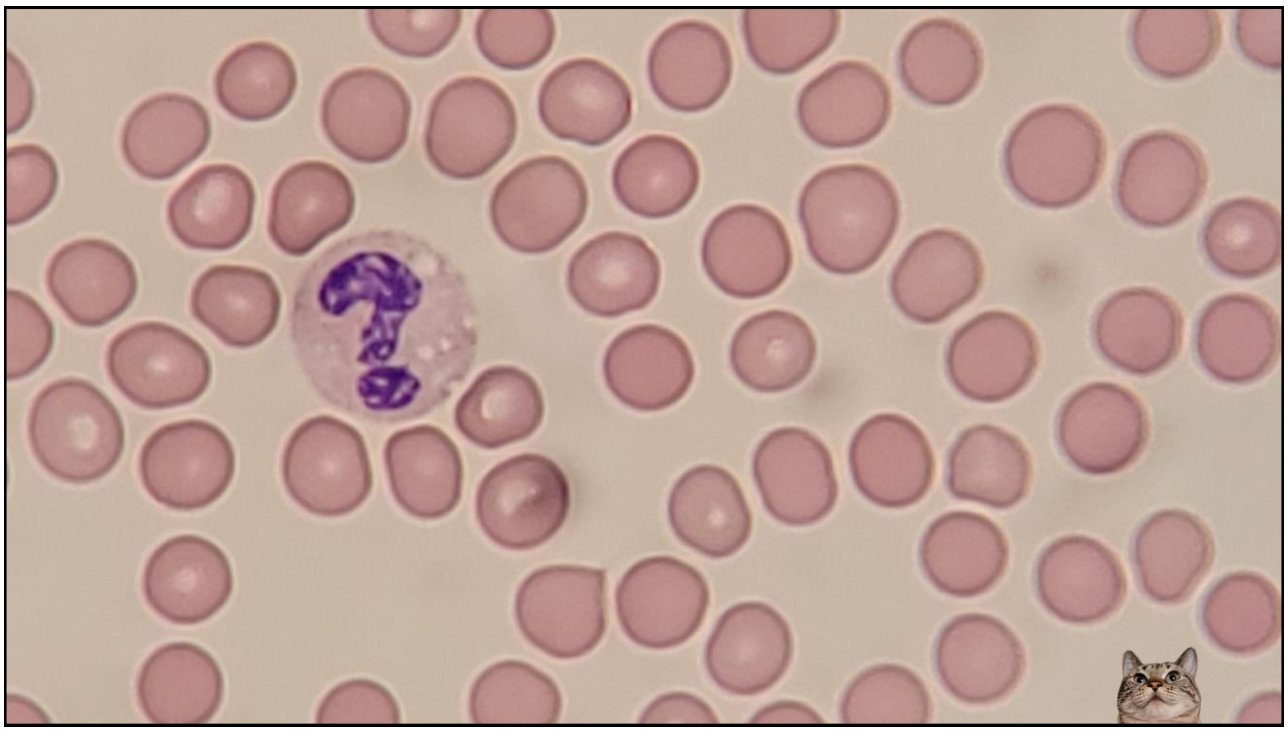
- For patients with platelet transfusion refractory thrombocytopenia, consider possible immune and nonimmune causes
- If platelet antibody screen is positive
  - Test for HLA antibodies
  - If HLA antibodies are negative, assess for the less common HPA antibodies
    - Consider anti-CD36 in those with Asian or African ancestry
- For CD36 deficient individuals with anti-CD36
  - Attempt to find CD36 negative platelet units
  - Considering family member testing and donation
  - IVIG and IV steroids before noncompatible random donor platelet units for life threatening bleeding or procedures
  - No evidence that alloimmunized patients benefit from continued prophylactic transfusion of incompatible platelets that do not increase posttransfusion platelet count

36

THANK YOU

Dr. Ye
Dr. Walkenhorst
KU Blood Bank Staff
Community Blood Center
Oklahoma Blood Institute

37



38

References

- Ahlen MT. Fighting anti-CD36-mediated FNAIT. Blood. 2021 Nov 4;138(18):1650-1652.
- Feng WW, Zuppe HT, Kurokawa M. The Role of CD36 in Cancer Progression and Its Value as a Therapeutic Target. *Cells*. 2023; 12(12):1605.
- Kaufman, RM. Refractoriness to platelet transfusion. UpToDate. 2023 Jul.
- Kniffin, CL. Platelet glycoprotien IV deficiency. Online Mendelian Inheritance in Man. 2016. Online article.
- Rajadhyaksha, B et al. "Platelet refractoriness." Global Journal of Transfusion Medicine 4 (2019): 140 - 147.
- Saw CL, Szykoluk H, Curtis BR, Zelcer S, Eckert K, Forrest D, Nickerson P, Petraszko T, Goldman M. Two cases of platelet transfusion refractoriness associated with anti-CD36. Transfusion. 2010 Dec;50(12):2638-42.
- Schmidt AE, Sahai T, Refaai MA, Sullivan M, Curtis BR. Severe Platelet Transfusion Refractoriness in Association with Antibodies Against CD36. Lab Med. 2020 Sep 1;51(5):540-544.

39