HYPERHEMOLYSIS SYNDROME: CASES, CAUSES, AND SOLUTIONS

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Spring 2020

Objectives

1. Analyze case reports of hyperhemolysis syndrome in children and adults.
2. Define hyperhemolysis syndrome, including pathogenesis, detection, and treatment.
3. Discuss the importance of balancing treatment options with the need for transfusions.

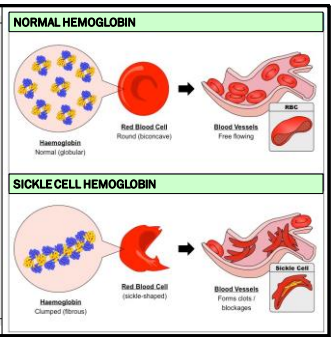
Case #1: An 8-year-old boy with Sickle Cell Disease presents in severe pain

Patient history:

- Received exchange transfusion at 4-years-old for chest crisis
- Abnormal transcranial Doppler velocities
- MRI evidence of ischemic changes in right frontal lobe
- On a regular transfusion program schedule
- Received transfusion 11 days ago; post-transfusion Hgb 11 g/dL
- Presently admitted for chest, back, RUQ abdominal pain

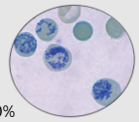
Case #1: Sickle Cell Disease

- Genetic disorder
- Hemoglobin becomes fibrous and clumped
- RBC becomes sickle shaped and rigid
- Small clots may form in vessels
- Leading to anemia, pain, ischemia, splenomegaly, infection



Case #1: Laboratory workup upon admission

- Blood type: AB positive
- Antigen type: C=, c+, E+, e+, K=
- Temp 36.4 °C (97.5 °F)
- Blood pressure 125/60 mmHg, O_2 saturation 100%
- Hgb 6.5 g/dL
- Reticulocyte count 330 x 10^9/L (normal range, <100 x 10^9/L)
- Total bilirubin 84 µmol/L (normal range, < 17 µmol/L)

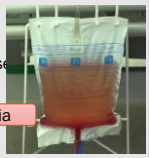


Case #1: Vaso-occlusive crisis precedes Hyperhemolysis syndrome

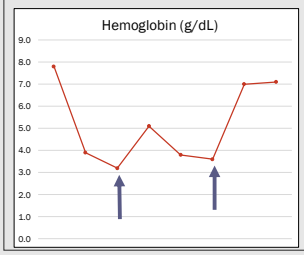
- Treated with fluids, morphine, antibiotics
- Hgb ↓ 4.1 g/dL
- Retic count ↓ 300 x 10^9/L
- Total bilirubin ↑ 107 µmol/L
- Negative DAT and antibody screens
- Given IVIG and prednisolone therapy for 1 week
- Hgb 6.2 g/dL
- Bilirubin 33 µmol/L
- Transfusion avoided
- Patient is readmitted 6 months later with more evidence of HS

Case #1: Six months later due to significant nocturnal hypoxemia

- Transfused with small volume blood type AB, C-, K- crossmatch-compatible unit Hgb 9.0 g/dL
- Readmitted 1 week later for lower back, abdominal pain Hgb 7.8 g/dL
- ↑ retic count, ↑ bilirubin, ↑ lactate dehydrogenase
- Becomes febrile within 24 hours Hgb 4.0 g/dL Hemoglobinuria



Case #1: Hyperhemolysis progression



- Persistent evidence of hemolysis
- Developed respiratory failure
- Received 2 transfusions (blue arrows, nadir Hgb 3.2, 3.6 g/dL)
- Methylprednisolone, IVIG, and EPO treatments

Case #1: Follow-up and Resolution

- IVIG-steroid therapy may shorten course of hemolysis
- EPO treatment not fully understood
- Patient was D/C after 19 days with Hgb 7.1 g/dL
- No additional transfusions; currently on hydroxyurea
- Stable Hgb level of 8.0 g/dL
- DAT remained negative
- No RBC alloantibodies were ever detected

Case #2: An 18-year-old girl with acute chest syndrome

Patient history:

- Sickle cell disease/anemia
- Admitted for acute chest syndrome
- Treated with IV fluids, analgesics, antibiotics
- Transfused 1 unit of crossmatch compatible packed RBCs

Lab Test	Pre-transfusion	Post-transfusion	Reference Range
Hemoglobin	6.10 g/dL	9.32 g/dL	12.5 g/dL
Hematocrit	19.2%	30.8%	38%
LDH	743 U/L	643 U/L	< 280 U/L
Bilirubin	5.95 mg/dL	4.29 mg/dL	<2.0 mg/dL

Case #2: One week later…

Lab Test	Pre-transfusion	Post-transfusion	7 days later	8 days later	Reference Range
Hemoglobin	6.10	9.32	4.71	3.0	12.5 g/dL
Hematocrit	19.2%	30.8%	14.7%	----	38%
LDH	743	643	3910	6680	< 280 U/L
Bilirubin	5.95	4.29	13.65	----	<2.0 mg/dL

Case #2: Was this a delayed transfusion reaction? Or something worse?

- Upon admission, blood bank studies do not reveal any RBC alloantibodies or HLA antibodies
- High dose steroid therapy was initiated
- Working diagnosis made of delayed hemolytic transfusion reaction (DHTR)/hyperhemolysis syndrome (HS)
- What else do we need to know about this patient's history?

Case #2: Suspicious prior history

- Received pRBC transfusion during hip surgery at age 15:
  - ➢Presented 7 days later with jaundice, fatigue, tachycardia

Hgb 4.08 g/dL	Hct 12.7%	LDH 3346 U/L	Bili 12.09 mg/dL	WBC 41,500/L

- History of > 16 pRBC transfusions by age 15

Case #2: Follow-up and Resolution

- Received 1 unit pRBC upon nadir Hgb 3.0 g/dL
- Also had IVIG, EPO, and folic acid
- D/C after 2 weeks with Hgb 7.96 mg/dL, Hct 24.5%
- DAT remained negative
- No RBC alloantibodies were ever detected

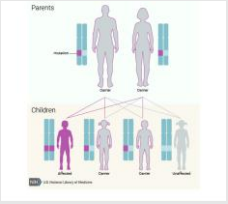
Case #3: Teenage boy needs a splenectomy

Patient history:

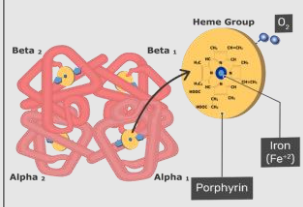
- 17-year-old Iranian male
- Diagnosed with β-thalassemia major at 6 months old
- Receives regular blood transfusions
- Started having delayed transfusion reactions 1 year ago
  - ↓ Hgb and dark urine, suggestive of hemolysis

Case #3: What is β-thalassemia?

- Genetic disorder
- Autosomal recessive
- Mutations in the *HBB* gene, which codes for the beta subunit of hemoglobin



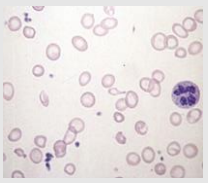
Case #3: A Closer Look at Hemoglobin



- Two α-subunits
- Two β-subunits
- Porphyrin ring inside each subunit
- Iron molecule inside each porphyrin ring
- Oxygen molecule reversibly binds to iron

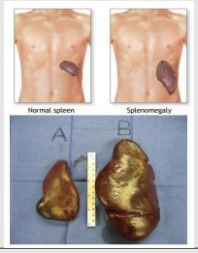
Case #3: RBC defects in β-thalassemia

- Double dose of mutated *HBB* gene results in no β-subunits
- Misshapen RBC
- Decreased O_2 carrying capacity
- Severe, profound anemia
  - ➢Microcytic, hypochromic
  - ➢↓ MCV, ↓ MCH, ↓ MCHC
  - ➢Basophilic stippling, polychromasia
  - ➢Target cells, anisopoikilocytosis

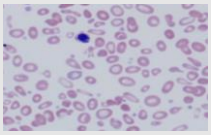


Case #3: Hemolysis, hemoglobinuria, and splenomegaly

- Patient condition worsening despite repeated transfusions
- IVIG and prednisone given before each transfusion
- Abdominal sonography: huge spleen (19 cm) and multiple gallbladder stones
- Patient underwent emergent splenectomy and cholecystectomy

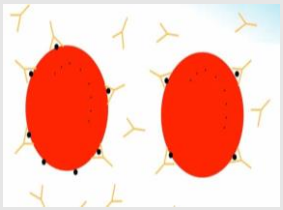


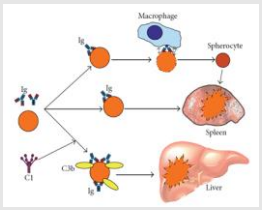
Case #3: Laboratory investigation



- Patient is blood type A positive
- Antibody panel revealed anti-Jk^a
- Positive autocontrol
- DAT positive for both IgG and C3d
- Acid elution revealed anti-Jk^a
- Hgb continued to drop despite transfusion with Jk^a-neg units, and prophylactic IVIG/prednisone
- Peripheral blood smear showed microcytic, hypochromic anemia with target cells, elliptocytes, and nRBCs

Case #3: Repeated transfusions exacerbating hyperhemolytic episode





Case #3: Follow-up and Resolution

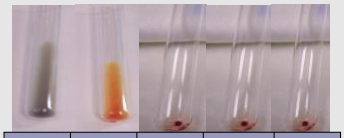
- Transfusions eventually discontinued
- Splenectomy was key to resolution for this patient
- Patient improved over 14 days following surgery
- Discharged with Hgb 8.3 g/dL
- Will require IVIG/pred prophylaxis and Jk^a-neg units
- May require plasma exchange

Case #4: Healthy 55-year-old man gets in a motorcycle crash

Patient history:

- Sustained fractures of all 4 extremities
- Massive transfusion protocol activated
- Received 10 units of pRBCs
- Patient was otherwise healthy prior to accident

Case #4: Initial evaluation



Anti-A	Anti-B	Anti-D	A1 cells	B cells
0	0	+	+	+

- Type O positive
- Negative antibody screens
- No hemoglobinopathy
- Received transfusion 30 years ago
- No other significant medical history
- D/C to rehab facility

Case #4: Ten days later…

- Presents to ED with severe dyspnea and fatigue

Lab Test	Result	Reference Range
Hemoglobin	5.4 g/dL	12.5 g/dL
Hematocrit	15%	38%
LDH	2355 U/L	< 280 U/L
Bilirubin	5.9 mg/dL	<2.0 mg/dL
Urinalysis	Dark colored urine, + hemoglobin	

Case #4: Alloanti-Jk^a sneaks again!

- Repeat IAT revealed anti-Jk^a
- DAT persistently positive
- Repeat transfusions days 1 – 4
- Poor retic response
- Elevated ferritin
- B12 and folate normal
- Coag studies normal
- G6PD normal
- Guaiac negative
- Blood cultures negative

Case #4: Hyperhemolysis progression

Lab Test	Admit	Day 1: 2 units	Day 1: PM	Day 2: AM	Day 2: 1 unit	Day 2: PM	Day 3: 1 unit	Day 4	Ref Range
Hgb	5.4	6.1	5.0	4.6	5.8	5.4	5.3	4.3	12.5 g/dL
Hct	15	16	14	13	17	15	15	12	38%

- Further transfusions withheld after Day 4
- Hematology deferred bone marrow exam
- Iron supplementation given

Case #4: Follow-up and Resolution

- By day 16, patient Hgb 8.2 g/dL, Hct 24%
- Patient was D/C back to rehab facility

- Consistent with hyperhemolysis as result of delayed hemolytic transfusion reaction
  - Continued transfusions potentiate drops in Hgb/Hct
  - Suggests autologous RBC destruction as well as donor RBC
  - Peripheral consumption and destruction via macrophages and C'

Case #5: 58-year-old woman with HIV

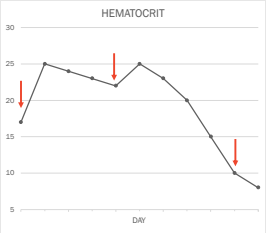
Patient history:

- HIV, Hepatitis C, COPD, Lyme disease, MRSA pneumonia
- 20-pound weight loss over past 3 months
- Travel to Dominican Republic, Mexico, Florida
- Prior transfusion history
- No hemoglobinopathy
- Baseline Hgb 10 g/dL, Hct 30%

Case #5: Laboratory work-up upon admission

- Admitted for dyspnea and cough with Hct 17.9%
- Blood type: B positive
- Alloantibodies identified: anti-Fy^a, anti-E, anti-s, anti-C^w
- EBV, cytomegalovirus, parvovirus serology = past infection
- Stains for AFB and *Pneumocystic jirovecii* = negative
- CT scan showed mild splenomegaly (14.8 cm)

Case #5: Transfusions for anemia



- Day 1: Hct 17.9%, transfused
- Day 2: Hct 25.9%
- Day 3: Hct 23.9%
- Day 4: Hct 23.1%
- Days 5-12: Hct drops despite transfusions
- Days 11-14: received EPO and methylprednisone

Case #5: Transferred to Massachusetts General Hospital

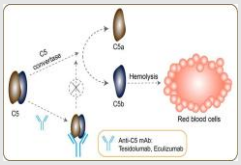
- Hct 12.1%
- Tachycardia and coarse breath sounds
- Repeat CT scan showed definite splenomegaly (17.4 cm)
- Symptomatic with fatigue and dyspnea on minimal exertion
- Grossly visible hemoglobinuria

Case #5: Further Antibody Investigation

- anti-Fy^a, anti-E, anti-s, anti-C^w
- American Red Cross National Reference Lab identified an additional alloantibody
  - ➢Directed against a high frequency antigen
  - ➢Thought to be responsible for immediate intravascular hemolysis
  - ➢Remained unresolved

Case #5: Subsequent clinical course

- DAT weakly reactive
- Antigen negative cross-matched units were still incompatible (1+ to 2+)
- Eculizumab given to no avail
- Hct ↓ 12.8%, 11.3%, 10.2%
- Further transfusion withheld



Case #5: Follow-up and Resolution

- Treated with supplemental O_2 via nasal cannula, methylprednisone, and EPO
- All antibiotics were D/C
- Blood draws were minimized
- Remained hemodynamically stable
- After 33 days in hospital, Hct 21.9%
- By day 37, Hct 28.1%

Case #5: Follow-up and Resolution

- EPO continued so patient could give autologous donations
- On day 51, Hct was 41.3%

- Developed RUQ pain
- Laparoscopic cholecystectomy
- Had pigmented gallstones
- Discharged with Hct 29%

What did all these cases have in common?

- Evidence of autologous RBC destruction as well as destruction of donor RBCs
- Continuous drops in Hgb and Hct despite repeated transfusion
  - Post-transfusion Hgb that is lower than pre-transfusion Hgb

Hyperhemolysis Syndrome (HS)

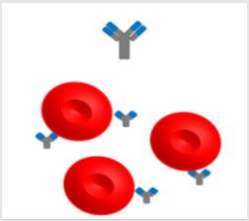
- Development of severe anemia
- Post-transfusion Hgb ↓ than pre-transfusion Hgb
- May be subdivided into ACUTE and DELAYED forms
  - ACUTE occurs within 7 days of transfusion
  - DELAYED occurs later than 7 days; alloantibody formation

Who is most at risk for developing HS?

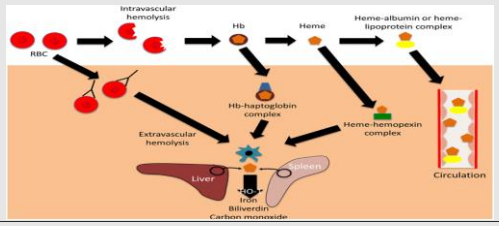
- Underlying hemoglobinopathies
  - Sickle cell disease
  - Thalassemias
- Other co-morbidities requiring frequent transfusion
  - Myelofibrosis
  - Anemia of chronic disease
  - Lymphoma

Why do RBC lyse? How are RBC removed?

- Normal senescence
- Antibody binding
- Complement activation
- Phagocytic clearance



What happens after RBC lyse?

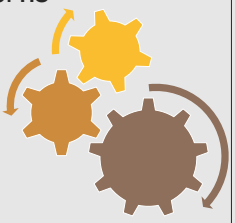


Clinical presentation of HS

- Usually includes fever, jaundice, and pain
- Elevated bilirubin
- Elevated lactate dehydrogenase
- Decrease in absolute reticulocytes
- Direct antiglobulin test (DAT) negative
- New alloantibodies may be present
- Recent history of transfusion
- Drops in Hgb and Hct despite transfusions

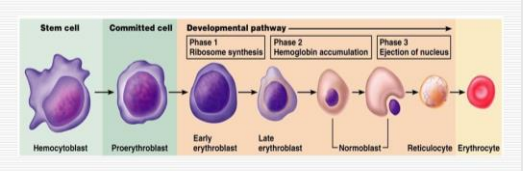
The multiple mechanisms of HS

- Suppression of erythropoiesis
- "Bystander hemolysis"
- Activated macrophages
- Increased expression of phosphatidylserine

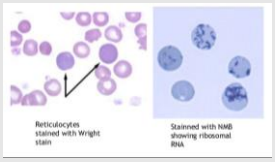


What suggests the suppression of erythropoiesis?

- Poor reticulocyte response



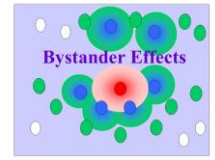
Poor reticulocyte response



- Previously believed to be secondary to multiple transfusions
- More likely due to "bystander hemolysis"

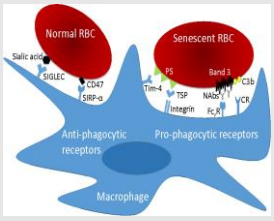
What is bystander hemolysis?

- When native and donor cells are destroyed
- Complement cascade goes to completion on healthy cells
- Peripheral consumption and destruction via activated macrophages
- Major cause of anemia in HS



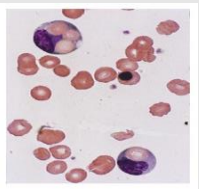
What's the deal with activated macrophages?

- Large phagocytic WBC
- Essential to immune response
- Bind to RBC surface proteins
- Receive "eat me" signals



What's the deal with activated macrophages?

- RBC expose surface protein phosphatidylserine
- Engulfed RBC are removed from circulation
- Processed in spleen and liver
- Byproducts are recycled



Corticosteroids, i.e. Prednisone

- May depress the rate of hemolysis
- Diminish titer of antibody (ies)
- Reduce complement activity
- Suppress migration of phagocytes
- Improve capillary permeability
- Allow for better reticulocyte response

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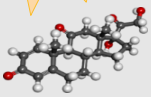
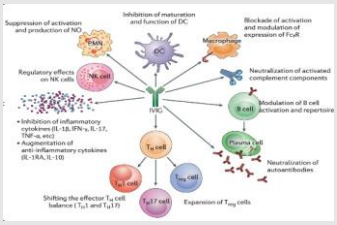
INFLAMMATORY



Intravenous Immunoglobulin (IVIG)



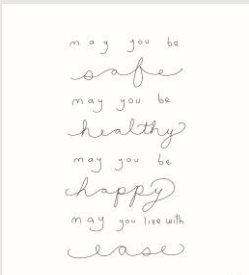
- Pooled Ab prepared from blood donors
- Anti-inflammatory
- Immunomodulation
- Neutralizing actions

Monoclonal antibody therapy (mAbs)

- Ritixumab, eculizumab
- Anti-inflammatory
- Immunomodulation
- Neutralizing actions
- Further studies are needed

When hyperhemolysis has the final say…

- Address underlying condition
- Corticosteroids and IVIG to suppress the immune response
- Some mAbs to suppress the immune response
- EPO and iron supplementation (questionable efficacy)
- Provide O2 as needed
- Restrict further transfusions unless absolutely necessary



Questions and Discussion

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