Acquire Hemolytic Anemia

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Hemolytic anemia

- Anemia due to increased rate of RBC destruction
- Anemia occurs when rate of destruction exceeds production
Normal red cell turnover

- Normal RBC survival of ~ 120 days
- Macrophages of the Reticuloendothelial (RE) system removes RBC’s
  - Unclear what marks a red cell for removal
  - Spleen is major site of RBC clearance
- RE system is Extravascular
  - 90% of normal RBC destruction occurs without release of hemoglobin into circulation.
Is there hemolysis?

• Look for 3 lines of evidence:
  – 1. Damaged red cells on the blood film
     – Spherocytes (Immune hemolysis, HS)
     – Red cell fragments (Microangiopathic anemias)
  – 2. Marrow response to Hemolysis
     – Polychromasia on blood film
     – Reticulocytosis
     – Erythroid hyperplasia in marrow
  – 3. Biochemical evidence of RBC destruction
     – Increased unconjugated bilirubin
     – Increased lactate dehydrogenase
     – Decreased/absent haptoglobin
An approach to hemolytic anemia

Hemolytic anemia (Acquired)

Immune
- Autoimmune
- Alloimmune
- Drug-induced

(Other causes of immune hemolysis are rare)

Defects of:
- RBC membrane/skeleton
  (eg. Hereditary spherocytosis)
- RBC enzymes
  (eg. G6PD deficiency)

Non-immune

Congenital

Acquired

- Infections
  Sepsis
  Malaria
- Mechanical
  Prosthetic heart valve
  Microangiopathic HA
Acquired hemolytic anemia
Immune mediated hemolytic anemia (Direct Coombs test is positive)

1- Autoimmune hemolytic anemia

Warm antibody autoimmune hemolytic anemia
Idiopathic
Systemic lupus erythematosus (SLE)
Evans' syndrome (antiplatelet antibodies and hemolytic antibodies)

Cold antibody autoimmune hemolytic anemia
Idiopathic cold hemagglutinin syndrome
Infectious mononucleosis and mycoplasma (atypical) pneumonia
Paroxysmal cold hemoglobinuria (rare)

2-Alloimmune hemolytic anemia

Haemolytic disease of the newborn (HDN)
Rh disease (Rh D)
ABO hemolytic disease of the newborn
Anti-Kell hemolytic disease of the newborn
Rhesus c hemolytic disease of the newborn
Rhesus E hemolytic disease of the newborn
Other blood group incompatibility (RhC, Rhe, Kidd, Duffy, MN, P and others)
Alloimmune hemolytic blood transfusion reactions (ie from a non-compatible blood type)

Drug-induced autoimmune hemolytic anemia Penicillin in high doses via the Hapten mechanism
Spherocytes. One arrow points to a spherocyte; the other, to a normal RBC with a central pallor.
Acquired hemolytic anemia

Non-immune mediated hemolytic anemia (Direct Coombs test is negative)

Drugs (i.e., some drugs and other ingested substances lead to haemolysis by direct action on RBCs, e.g. Ribavirin)

Toxins (e.g., Snake venom; plant poisons such as Aesculin)

Trauma

  Mechanical (from Heart valves, extensive vascular surgery, microvascular disease, repeated mechanical vascular trauma)

Microangiopathic hemolytic anemia

Infections (Note: Direct Coombs test is sometimes positive in hemolytic anemia due to infection)

  Malaria
  Babesiosis
  Septicaemia

Membrane disorders

  Paroxysmal nocturnal hemoglobinuria (rare acquired clonal disorder of red blood cell surface proteins)

  Liver disease
Diagnosis of immune hemolytic anemia

- 1. Direct Antiglobulin Test (DAT or direct Coomb’s test)
  - Detects IgG or complement on patient’s red cells
  - the vast majority of patients with active immune hemolysis will have a positive DAT.

- 2. Indirect Antiglobulin Test (IAT, indirect Coomb’s test)
  - Detects antibody in patient’s serum against red cell antigens
  - A positive IAT does not necessarily mean hemolysis is occurring - It may simply mean allo-immunization due to previous exposure to “foreign” red cell antigens (past pregnancy or transfusion).

- 3. Peripheral Blood Film: spherocytes
IMMUNE HEMOLYTIC ANEMIA

General Principles

- All require antigen-antibody reactions
- Types of reactions dependent on:
  - Class of Antibody
  - Number & Spacing of antigenic sites on cell
  - Availability of complement
  - Environmental Temperature
  - Functional status of reticuloendothelial system

Manifestations

- Intravascular hemolysis
- Extravascular hemolysis
<table>
<thead>
<tr>
<th>Laboratory feature</th>
<th>Intravascular hemolysis</th>
<th>Extravascular hemolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td>Grossly elevated</td>
<td>Elevated</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>Reduced or absent</td>
<td>Normal or reduced</td>
</tr>
<tr>
<td>Methemalbumin</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Hemosidinuria</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Blood film</td>
<td>Schistocytes, helmet cells, fragmented red cells</td>
<td>Spherocytes, erythrophagocytosis</td>
</tr>
<tr>
<td>Hemoglobinemia</td>
<td>Present</td>
<td>Absent/present in severe cases</td>
</tr>
<tr>
<td>Hemoglobinuria</td>
<td>Present</td>
<td>Absent/present in severe cases</td>
</tr>
</tbody>
</table>
Antibodies combine with RBC, & either
1. Activate complement cascade, &/or
2. Opsonize RBC for immune system

If 1, if all of complement cascade is fixed to red cell, intravascular cell lysis occurs

If 2, &/or if complement is only partially fixed, macrophages recognize Fc receptor of Ig &/or C3b of complement & phagocytize RBC, causing extravascular RBC destruction
IMMUNE HEMOLYTIC ANEMIA

Coombs Test - Direct

- Looks for immunoglobulin &/or complement of surface of red blood cell (normally neither found on RBC surface)
- Coombs reagent - combination of anti-human immunoglobulin & anti-human complement
- Mixed with patient’s red cells; if immunoglobulin or complement are on surface, Coombs reagent will link cells together and cause agglutination of RBCs
IMMUNE HEMOLYTIC ANEMIA

*Coombs Test - Indirect*

- Looks for anti-red blood cell antibodies in the patient’s serum, using a panel of red cells with known surface antigens
- Combine patient’s serum with cells from a panel of RBC’s with known antigens
- Add Coombs’ reagent to this mixture
- If anti-RBC antigens are in serum, agglutination occurs
IMMUNE HEMOLYSIS

Drug-Related

- Immune Complex Mechanism
  - Quinidine, Quinine, Isoniazid
- “Haptenic” Immune Mechanism
  - Penicillins, Cephalosporins
- True Autoimmune Mechanism
  - Methyldopa, L-DOPA, Procaineamide, Ibuprofen
Drug & antibody bind in the plasma

Immune complexes either
- Activate complement in the plasma, or
- Sit on red blood cell

Antigen-antibody complex recognized by RE system

Red cells lysed as “innocent bystander” of destruction of immune complex
DRUG-INDUCED HEMOLYSIS

Haptenic Mechanism

• Drug binds to & reacts with red cell surface proteins
• Antibodies recognize altered protein, ± drug, as foreign
• Antibodies bind to altered protein & initiate process leading to hemolysis
• Certain drugs appear to cause antibodies that react with antigens normally found on RBC surface, and do so even in the absence of the drug.
Transfusion reactions

- Antibodies to the A, B, and O antigens on red blood cells are usually IgM class.
- An individual with blood group A has antibodies against B in their blood.
- If a type A individual is accidentally transfused with blood containing type B cells, the anti-B antibodies will bind to the B blood cells and mediate their destruction by means of complement-mediated lysis.
(a) Galactose – Lipid or protein
  - Fucose
  - N-Acetylglucosamine
  - O antigen
  - N-Acetylgalactosamine
    - A antigen
    - B antigen

(b) | Genotype | Blood-group phenotype | Antigens on erythrocytes (agglutinins) | Serum antibodies (isoagglutinins) |
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>AA or AO</td>
<td>A</td>
<td>A</td>
<td>Anti-B</td>
</tr>
<tr>
<td>BB or BO</td>
<td>B</td>
<td>B</td>
<td>Anti-A</td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
<td>A and B</td>
<td>None</td>
</tr>
<tr>
<td>OO</td>
<td>O</td>
<td>None</td>
<td>Anti-A and anti-B</td>
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</tbody>
</table>
ALLOIMUNE HEMOLYSIS

Hemolytic Transfusion Reaction

- Caused by recognition of foreign antigens on transfused blood cells
- Several types
  - Immediate Intravascular Hemolysis (Minutes) - Due to preformed antibodies; life-threatening
  - Slow extravascular hemolysis (Days) - Usually due to repeat exposure to a foreign antigen to which there was a previous exposure; usually only mild symptoms
  - Delayed sensitization - (Weeks) - Usually due to 1st exposure to foreign antigen; asymptomatic
Delayed hemolytic reaction

- Occurs in individuals who have received repeated transfusions of ABO-compatible blood that is incompatible for other blood groups.
- The reaction develops within 2-6 days after transfusion.
- The transfused blood induces clonal selection and production of IgG against a variety of receptors.
- Blood group antigens that cause this: Rh, Kidd, Kell, and Duffy.
- Symptoms: fever, low hemoglobin, increased bilirubin, jaundice and anemia.
INCOMPATIBLE RBC TRANSFUSION

Rate of Hemolysis

Weeks Post-Transfusion

Surviving Cells (%)
Type II hypersensitivity
(Antibody-mediated cytotoxic)

- Involves antibody-mediated destruction of cells
- This type is exemplified by blood transfusion reactions
- Host antibodies react with foreign antigens on the incompatible transfused blood cells and mediate destruction of those cells
- Antibodies mediate cell destruction by activating the complement system or though antibody-dependent cell-mediated cytotoxicity (ADCC) (cytotoxic cells bind to the Fc region of antibodies on target cells)
Patient died after getting wrong blood transfusion

BY PAUL WILKINSON

A HEART surgery patient died after he was given a pint of blood intended for the person in the next bed, an inquest jury was told yesterday.

Two nurses were suspended after the death of Norman Lynes but were later exonerated by a hospital inquiry. Relatives of Mr Lynes, 73, a retired stock controller, have taken legal advice over action against Castle Hill Hospital at Cottingham, near Hull.

Mr Lynes died from kidney and bowel failure last June seven days after undergoing heart bypass surgery. The inquest in Hull was told that he had been given a pint of the A-positive blood group instead of O-positive during a post-operation transfusion as he lay in intensive care on a ventilator.

Staff nurse Nicola Wild discovered the error three hours later when she opened the bedside fridge for a second pint of blood. Inside, she found all of Mr Lynes's blood units still there.

Police carried out an investigation into possible criminal negligence but the Crown Prosecution Service decided to take no action. Despite suffering two heart attacks the previous year, Mr Lynes had been given a 95 per cent chance of surviving the operation.

The inquest continues today.
HAZARDS REPORTED

• Incorrect blood/component transfused (IBCT)
• Acute transfusion reaction (including anaphylaxis) (ATR)
• Delayed transfusion reaction (DHTR)
• Transfusion-associated graft-versus-host-disease (TA-GVHD)
• Transfusion-related acute lung injury (TRALI)
• Post-transfusion purpura (PTP)
• Transfusion transmitted infection, including bacterial contamination (TTI)
• Autologous pre-donation incidents

<table>
<thead>
<tr>
<th>Blood Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Cells</td>
<td>2,737,572</td>
</tr>
<tr>
<td>Platelets</td>
<td>249,622</td>
</tr>
<tr>
<td>Fresh Frozen Plasma</td>
<td>365,547</td>
</tr>
<tr>
<td>Cryoprecipitate</td>
<td>94,114</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>3,446,855</strong></td>
</tr>
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INCORRECT BLOOD COMPONENT TRANSFUSED

Episodes where a patient was transfused with a blood component or plasma product which did not meet appropriate requirements or which was intended for another patient.
QUESTIONNAIRES BY INCIDENT
1996/97 - 1999/00 (n=862)

- IBT: 13%
- ATR: 12%
- DTR: 7%
- PTP: 4%
- TRALI: 3%
- TA-GVHD: 1%
- TTI: 1%
- Unclassified: 59%
### IBCT Outcome of Cases 1996/97 - 1999/00

(n=509)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>No. of cases</th>
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<tbody>
<tr>
<td>Death definitely related to transfusion</td>
<td>5</td>
</tr>
<tr>
<td>Death probably related to transfusion</td>
<td>1</td>
</tr>
<tr>
<td>Death possibly related to transfusion</td>
<td>2</td>
</tr>
<tr>
<td>Death unrelated to transfusion</td>
<td>37</td>
</tr>
<tr>
<td>Major morbidity</td>
<td>54</td>
</tr>
<tr>
<td>Survived – no ill effects</td>
<td>406</td>
</tr>
<tr>
<td>Unknown</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>509</strong></td>
</tr>
</tbody>
</table>

- 36% possibility that a random unit will be incompatible
- Likelihood of fatal outcome of ABO incompatible unit is <10%
<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>ABO-incompatible</td>
<td>237</td>
<td>1/38,000</td>
</tr>
<tr>
<td>ABO-compatible</td>
<td>221</td>
<td>1/41,000</td>
</tr>
<tr>
<td>Total†</td>
<td>462</td>
<td>1/19,000</td>
</tr>
<tr>
<td>Adjusted total‡</td>
<td>659</td>
<td>1/14,000</td>
</tr>
<tr>
<td>Fatal reaction</td>
<td>5</td>
<td>1/1,800,000</td>
</tr>
</tbody>
</table>

* 9,000,000 transfusions were performed during this period.
† Includes 4 cases in which ABO compatibility was not reported.
‡ Adjusted to correct for estimated underreported/undetected ABO-compatible erroneous transfusions. A compatible-to-incompatible ratio of 1.78 was used.
ALLOIMMUNE HEMOLYSIS

Testing Pre-transfusion

- ABO & Rh Type of both donor & recipient
- Antibody Screen of Donor & Recipient, including indirect Coombs
- Major cross-match by same procedure (recipient serum & donor red cells)

• Error Management
In vitro model of the hemolytic transfusion reactions
"Davenport"

Group O blood + Group A blood  Complement mediated lysis

IgG-coated RBCs + Mononuclear cells from fresh whole blood
IgG mediated HTRS
<table>
<thead>
<tr>
<th></th>
<th>INF-a (pg/ml)</th>
<th>Hb (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incompatible RBC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2hr</td>
<td>388</td>
<td>54</td>
</tr>
<tr>
<td>24hr</td>
<td>144</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>±</td>
</tr>
<tr>
<td>Compatible RBC</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>2hr</td>
<td>3</td>
<td>±</td>
</tr>
<tr>
<td>24hr</td>
<td>3</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>±</td>
<td>±</td>
</tr>
</tbody>
</table>
Leukocyte phenotypic changes in an in vitro model of ABO HTRs

Percoll gradient separated MN cells from group O whole blood resuspended in autologous plasma (5 x 10^6 cell/ml) in 1 ml of this suspension + 6 ul of group A RBCs plasma. Studies: TNF, IL8 cellular studies: phenotypic markers
<table>
<thead>
<tr>
<th>Specificity</th>
<th>Antigen/protein</th>
<th>Cellular expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD25</td>
<td>Tac antigen (interleukin-2 receptor)</td>
<td>Activated B- and T-lymphocytes; macrophages</td>
</tr>
<tr>
<td>CDw108</td>
<td>JMH protein</td>
<td>Activated polymorphonuclear cells; T-, B-, and natural killer-lymphocytes</td>
</tr>
<tr>
<td>CD109</td>
<td>Glycosylphosphatidylinositol-linked protein</td>
<td>Activated T-lymphocytes</td>
</tr>
<tr>
<td>CD14</td>
<td>Lipopolysaccharide receptor</td>
<td>Monocytes (decreased with activation by lipopolysaccharide)</td>
</tr>
<tr>
<td>CD44</td>
<td>Hyaluronic acid receptor</td>
<td>All WBCs</td>
</tr>
<tr>
<td>Mediator</td>
<td>Hemodynamic effects</td>
<td>Coagulation effects</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------------</td>
<td>----------------------------------------------------------</td>
</tr>
<tr>
<td>TNF</td>
<td>Hypotension</td>
<td>Increase tissue factor activity and decrease thrombomodulin expression by endothelial cells</td>
</tr>
<tr>
<td>IL-1</td>
<td>Hypotension</td>
<td>Increase tissue factor activity and decrease thrombomodulin expression by endothelial cells</td>
</tr>
<tr>
<td>IL-6</td>
<td>Endothelin release</td>
<td></td>
</tr>
<tr>
<td>IL-8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complement</td>
<td></td>
<td>Thromboplastin release by WBCs</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>Vasodilation</td>
<td></td>
</tr>
<tr>
<td>Endothelin</td>
<td>Vasoconstriction</td>
<td></td>
</tr>
<tr>
<td>Leukocyte adhesion molecules</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2. Coagulation abnormalities.
Fig. 1. Hemodynamic alterations and inflammatory response.
Stop Transfusion Immediately

General Considerations
- 1. Establish Large-bore Venous Access
- 2. Monitor Vital Signs
- 3. Transfer to ICU
- 4. Consider Placement of Swan-Ganz Catheter

Prevention of Renal Complications
- 1. Low-dose Dopamine
- 2. Maintain Blood Pressure and Urine Output
- 3. Diuretics

Treatment of Pulmonary Complications
- 1. Monitor Pulmonary Function
- 2. Oxygen Therapy
- 3. Intubation and Ventilator Support

Prevention and Management of DIC
- 1. Consider Early Heparinization
- 2. Treat Bleeding Diatheses with Blood Components (Platelet Concentrates, Fresh-frozen Plasma, etc.)

Fig. 3. Treatment strategies; ICU = intensive care unit.
Future Treatment Strategies

Inhibition of IL-1
- Soluble IL-1 Receptors
- IL-1 Receptor Antagonist
- Antibodies to IL-1 Receptors
- Corticosteroids

Neutralization of Circulating TNF
- Monoclonal Antibodies to TNF
- Soluble TNF Receptors

Interpretation of DIC
- Tissue Factor Pathway Inhibitor
- Protein C Concentrates

Fig. 4. Future treatment strategies.
ALLOIMMUNE HEMOLYSIS
Hemolytic Disease of the Newborn

• Due to incompatibility between mother negative for an antigen & fetus/father positive for that antigen. Rh incompatibility, ABO incompatibility most common causes

• Usually occurs with 2nd or later pregnancies

• Requires maternal IgG antibodies vs. RBC antigens in fetus
ALLOIMMUNE HEMOLYSIS

Hemolytic Disease of the Newborn - #2

- Can cause severe anemia in fetus, with erythroblastosis and heart failure
- Hyperbilirubinemia can lead to severe brain damage (kernicterus) if not promptly treated
- HDN due to Rh incompatibility can be almost totally prevented by administration of anti-Rh D to Rh negative mothers after each pregnancy
Prevention

• Hemolytic disease of the newborn caused by Rh incompatibility can be almost entirely prevented by administering antibodies against the Rh antigen to the mother within 24-48 hours after the first delivery.

• These antibodies are called Rhogam.

• They bind to fetal red blood cells that have entered the mother’s circulation and facilitate their clearance before B-cell activation.
DEVELOPMENT OF ERYTHROBLASTOSIS FETALIS (WITHOUT RHOGAM)

1st Pregnancy
- Placenta
- Maternal circulation
- RBCs with Rh antigen

Delivery
- Mother
- Plasma cells
- Rh-specific B cell
- Anti-Rh IgM
- Memory cell

2nd Pregnancy
- Memory cell
- Plasma cells
- IgG
- IgG anti-Rh Ab crosses placenta and attacks fetal RBCs causing erythroblastosis fetalis

PREVENTION (WITH RHOGAM)

- Mother (treated with Rhogam)
  - B cell
  - Rhogam

Prevents B-cell activation and memory cell formation
The End