Autoimmune and Drug-Induced Immune Hemolytic Anemias
Autoimmune hemolytic anemia (AIHA)

- Warm autoimmune hemolytic anemia (WAIHA)
- Cold Agglutinin Syndrome (CAS) and Paroxysmal Cold Hemoglobinuria (PCH)
- Mixed-type autoimmune hemolytic anemia
- Drug-induced immune hemolytic anemias
Overview

- Autoantibodies can be produced to a variety of self-antigens
- May be responsible for decreased red cell survival
- A positive DAT usually occurs
- Questions to ask:
  - Is there hemolysis? Positive DAT does not always mean hemolysis will occur; no hemolysis is not as critical
  - What is the patient’s history? Pregnancy, transfusion
  - What is the patient’s diagnosis? SLE, mono, etc
  - What medications are the patient taking? Antibiotics, etc
Overview

- Decreased RBC survival usually occurs because antibodies are coating the RBC (pos DAT)
- Cells become less pliable and fit through small sequestrations of the spleen (splenomegaly)
- As a result, they are removed from the circulation sooner than 120 days
## Incidence of Autoimmune Hemolytic Anemias

<table>
<thead>
<tr>
<th>Type of Autoimmune Hemolytic Anemia</th>
<th>Incidence (%)</th>
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<tbody>
<tr>
<td>Warm Autoimmune Hemolytic Anemia</td>
<td>60-70%</td>
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<tr>
<td>Cold Agglutinin Syndrome</td>
<td>16-32%</td>
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<tr>
<td>Mixed-type Autoimmune Hemolytic Anemia</td>
<td>7-8%</td>
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<tr>
<td>Paroxysmal Cold Hemoglobinuria</td>
<td>Up to 2%</td>
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<tr>
<td>Drug-induced Immune Hemolytic Anemia</td>
<td>12-18%</td>
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</tbody>
</table>
Classification

- Warm Autoimmune (WAIHA)
  - 70-80%
- Cold Autoimmune (CAIHA)
  - 20-30%
- Mixed
  - 7-8%
- Paroxysmal Cold Hemoglobinuria
  - rare in adults
- Drug Induced Hemolytic Anemia
Clinical & Laboratory Findings

- **WAIHA**
  - Fatigue
  - Palpitations
  - Mild jaundice
  - Shortness of breath
  - Moderate splenomegaly
  - Positive DAT
  - Microspherocytes
  - **Polychromasia**
  - NRBCs (↑ retic count)
  - ↑ bilirubin (unconjugated)

- **Chronic CAS**
  - Clinical manifestations similar to WAIHA
  - Raynaud’s phenomena sometimes reported
    - Pallor and cyanosis of extremities

- **Acute CAS**
  - 2° to infection (*Mycoplasma pneumoniae*)
  - Severe hemolysis
  - Positive DAT
  - Polychromasia
  - Autoagglutination of RBCs interferes with automated cell counters
Warm vs. Cold Auto

**WARM**
- Reacts at 37 degC
- Insidious to acute
- Anemia severe
- Fever, jaundice frequent
- Intravascular not common
- Splenomegaly
- Hematomegaly
- Adenopathy
- None of these

**COLD**
- Reacts at room temperature
- Often chronic anemia
- 9-12 g/dL (less severe)
- Autoagglutination
- Hemoglobinuria, acrocyanosis and raynaud’s with cold exposure
- No organomegaly
Clinical & Laboratory Findings

- **PCH**
  - Hemoglobinuria
  - Similar to CAS

- **Drug-induced**
  - Similar to chronic or acute anemias
  - DAT almost always positive
Direct Antiglobulin Test (DAT)

- Have red cells been coated in-vivo with Ig, complement or both?

DAT can detect 100-500 molecules of IgG and 400-1100 molecules of C’

Polyspecific reagent
If positive, then IgG and C3d specific reagents

DAT may be positive without evidence of hemolysis; Therefore clinical info important
Serologic Investigation of a positive DAT

- Previous slide→ what proteins are coating the cell: IgG only, complement, or both
- Test an eluate: remove the coating antibodies and test them against panel cells
- Test the patient serum to identify alloantibodies that may exist to red cell antigens
Positive DAT may result from:

- Autoantibodies to intrinsic red cell antigens
- Circulating Alloantibodies bound to transfused donor cells
- Alloantibodies in donor plasma containing products reacting with transfused recipient’s cells
- Maternal Alloantibodies that cross the placenta and bind to fetal red cells
- Antibodies against drugs on red cells
- Non-red cell immunoglobulins bound to red cell (e.g. IVIG)
- A positive DAT does not mean decreased red cell lifespan and therefore a history and physical is needed to determine the significance of a positive DAT
If there is no evidence of increased red cell destruction (anemia, ↑ reticulocytes, ↑ LDH, ↓ haptoglobin, hemoglobinemia, hemoglobinuria, etc), no further work-up of a positive DAT is necessary.
Questions to ask...

- Decreased red cell survival?
- Has the patient been recently transfused?
  - Red cells, plasma containing products
- Is the patient on any medications that can cause a positive DAT and hemolysis (e.g. penicillin, aldomet, cephalosporins)?
- Has the patient received a transplant?
- Is the patient receiving IVIG?
- Is the patient pregnant? Is the patient a newborn infant?
Intravascular vs. Extravascular

**Intravascular**
- red cells lyse in the circulation and release their products into the plasma fraction; obvious and rare
- Anemia
- Decreased Haptoglobin
- Hemoglobinemia
- Hemoglobinuria
- Urine hemosiderin
- Increased LDH

**Extravascular**
- ingestion of red cells by macrophages in the liver, spleen and bone marrow
- Little or no hemoglobin escapes into the circulation
- Anemia
- Decreased Haptoglobin
- Normal plasma hemoglobin
- Increased LDH
Hemolysis

- Def’n: Premature destruction of red blood cells that may be due to the intravascular environment or defective red cells
- Normal red cell life span is 120 days; decreased red cell survival studies
- Def’n Immune Hemolysis: shortening of red cell survival due to the products of an immune response
Warm Autoimmune Hemolytic Anemia

- **IgG** (sometimes occurs along with IgA and IgM)

- **Primary or idiopathic**

- **Secondary** to patients with lymphoma, SLE, and chronic lymphocytic leukemia (CLL)

**DAT**

- Positive with **polyspecific AHG** (anti-IgG and anti-C3d); variable reactions with monospecific

- Rarely is the DAT negative (low levels of IgG, etc)
WAIHA

- ABO and Rh typings may show false positive results if excessive amounts of agglutination are occurring

- Chemicals like **chloroquine diphosphate** can disassociate the IgG so that antigen typing can occur
ANTIBODY SCREENING STUDIES

POSITIVE

NEGATIVE
No further studies are indicated

ANTIBODY IDENTIFICATION STUDIES

ALLOANTIBODY
No further studies are indicated
Provide antigen-negative red cells should transfusion therapy be indicated

AUTOANTIBODY
Apparent autoantibody, but unable to assess the simultaneous presence of alloantibody

ANTIBODY ADSORPTION

AUTOLOGOUS ADSORPTION
Autologous red cells are modified to remove bound immunoglobulin
Patient's serum incubated with autologous red cells

ALLOGENEIC ADSORPTION
May be useful for patients who have been transfused or have very low hematocrits
Select Rh, R, and r cells that among them also lack Jk, Jkb, and s
Other allogeneic red cells may be selected if patient's own phenotype is known
Enzyme premodification ensures that they are Fy(a2b2) and S2
Patient's serum is incubated with separate aliquots of all cells

REPEAT ANTIBODY SCREENING STUDIES

POSITIVE

NEGATIVE
No further studies are indicated

PERFORM ANTIBODY IDENTIFICATION STUDIES
1. Identify alloantibody
   If identification studies are inconclusive:
2. Evaluate possibility of incomplete adsorption of autoantibody;
   Continue to adsorb to completion.
3. Evaluate possibility that antigen to which autoantibody is directed was denatured as the adsorbing cells were prepared. Antigen denaturation could occur for EnTS and EnFS, and Kell and LW antigens. Repeat adsorption studies, using red cells prepared by a different method, would be required.
Adsorption

- If all cells are positive on the antibody panel at AHG phase, adsorption is performed to determine what type of antibodies are present (allo-, auto- or both) in the serum.

- Adsorption is the removal of antibodies in serum onto cells positive for the antigens:
  - Allogeneic adsorption (for the recently transfused)
  - Autologous adsorption (not recently transfused)
    - ZZAP (common)
    - Chloroquine diphosphate
    - Citric acid
    - PEG (common)
Warm Auto

- Most are idiopathic (30%)
- Older patients
- Secondary (acute or chronic) (70%)
  - Malignancy esp. lymphoproliferative disorder
    - predominantly B-cell lymphomas
  - Rarely carcinoma
  - Autoimmune disorders (e.g. SLE)
WAIHA Serologic Investigation

- **DAT+**
  - Anti-IgG only 20-60%
  - Anti-C3d only 7-14%
  - Both 24-63%
- **Antibody screen+**
- **All panel cells+**
- **Autocontrol+**
- 50% of patients will have autoimmune antibody left over in the serum (DAT should be 4+)
WAIHA Serologic Investigation

- **Eluate:** Remove antibody coating the patient’s red cells and react them with test cells
- **Panagglutinin:** $>90\%$
- **Defined Specificity:** $<10\%$ (e.g. broad or narrow anti-Rh; anti-e, anti-LW)
- **Rarely other specificities such as Kell**
WAIHA Underlying Alloantibodies

- Remove antibodies coating the patient’s red cells
- Incubate these uncoated cells with the patient plasma to adsorb autoantibodies
- Repeat as many times as necessary to get autoantibodies out of plasma
- React patient plasma, which should have all autoantibodies removed, with panel cells
- Rule out underlying alloantibodies
Treatment

- **WAIHA**
  - **Prednisone**: corticosteroid used as antiinflammatory
  - Splenectomy may be required if no response to steroids
  - **Azothioprine**: when previous treatments fail; immunosuppressive antimetabolite
Don’t wait to transfuse

- Transfusion can be life saving in the setting of WAIHA and severe anemia or unstable clinical/cardiac status
- Do not wait for “compatible blood”
- Do not wait for underlying alloantibodies to be worked up (several hours) when the anemia is severe and life threatening
- “Least incompatible”? 
Therapy

- B12, folate
- Steroids
  - Prednisone 1-2mg/kg/day then taper when Hgb>10
- Splenectomy
  - If non-responder to steroids
- Rituxan
- Plasmapheresis is not effective (IgG is extravascular; feedback may increase IgG)
Selection of Blood

- ABO compatible
- Negative for alloantibody and autoantibody specificity
- Phenotype identical
- All units will be incompatible → least incompatible
Cold Auto

- 16-32% of all Immune Hemolysis
- Idiopathic (10%) Cold Agglutininin Disease
- Secondary forms (90%);
  - Postinfectious
    - Mycoplasma
    - CMV
    - EBV; Infectious mononucleosis
  - Lymphoproliferative disorders
    - E.G. B-cell lymphomas; sometimes intravascular
Cold Agglutinin Syndrome & Paroxysmal Cold Hemoglobinuria

- Pathologic cold autoantibodies may cause cold agglutinin syndrome (CAS)

- PCH occurs less frequently (more in children)

- CAS is usually associated with *Mycoplasma pneumoniae* infection and infectious mononucleosis

- PCH associated with more transient infections and is also known as **Donath-Landsteiner** hemolytic anemia
CAIHA Serologic Investigation

- Spontaneous agglutination in EDTA tube; difficulties with ABO typing
- DAT+
  - >90% positive for C3d only
  - Antibody is usually IgM, binds in cold (periphery), then dissociates in warm
  - C3d may or may not shorten red cell survival
- Antibody Screen+
- Determine underlying alloantibodies using autoabsorption techniques
Cold autoantibodies

- Bind best at temperatures **less than 4°C**

- Most frequently **IgM**, rarely IgA or IgG

- Antibodies are usually:
  - CAS - **anti-I**, followed by anti-i and anti-IH
  - PCH - **anti-P** is more common
    - there may also be an IgG biphasic hemolysin in PCH

- As previously stated, strong antibodies may interfere with ABO and Rh typings
- Thiol reagents (dithiothreitol or 2-mercaptoethanol) can be used to treat the cells and abolish autoagglutination
CAIHA Serologic Investigation

- Specificity is I, IH or I (academic interest only)
  - Adult cells: I
  - Cord cells: I

- Cold Agglutininin titers and thermal amplitude studies
Treatment

- CAS or PCH
  - Chlorambucil or cyclophosphamide (cytotoxic)
  - Transfusion (using blood warming devices)
Cold Auto Treatment

- Again, with severe anemia or unstable disease, transfusion can be life threatening
- Keep the patient warm
- Transfuse through a blood warmer
- Folate and B12
- Treat underlying disease
- Steroids usually poor response
Cold Auto Transfuse

- ABO/Rh compatible units
- Rule-out underlying alloantibodies and give antigen negative units
- Crossmatch in warm
- Again, transfuse through a blood warmer while keeping the patient warm
Paroxysmal Cold Hemoglobinuria

- Idiopathic (rare)
- Post-infectious (more common)
- Occasionally seen in syphilis
- Biphasic Hemolysin
  - IgG antibody that binds in the cold and fixes complement
  - At warm temperatures, IgG dissociates and complement remains
PCH Serologic Investigation

- DAT+ (>50%)
  - Usually IgG; sometimes C3d
- Eluate often negative
- Antibody screen w+
- Antibody is panagglutinin with P or IH specificity
- Donath-Landsteiner Test positive
Pathophysiology of paroxysmal cold hemoglobinuria

Julius Donath and Karl Landsteiner discovered a unique "biphasic hemolysin" in blood that could be demonstrated in the laboratory. This antibody attaches to red blood cells (RBCs) in the cold and induces hemolysis when the RBCs are warmed due to complement activity.

Together these investigators devised and published in 1904 what was to be the first immunohematologic test, referred to as the Donath-Landsteiner test.

In children as many as 40% of postviral IHA due to the Donath-Landsteiner (D-L) antibody
In adult population, infections and neoplasms have been associated with the development of D-L antibody.

Reported neoplasms include solid organ carcinomas as seen with:

- Pulmonary small cell carcinoma
- Hematopoietic disorders such as:
  - non-Hodgkin lymphoma (NHL), chronic lymphocytic lymphoma (CLL), primary
  - myelofibrosis with myeloid metaplasia, and in the presence of a monoclonal protein with Bence Jones proteinuria
D-L antibody is not classified as a monophasic immunoglobulin M (IgM), but rather a biphasic, usually polyclonal, IgG.

The D-L antibody is known to bind to various antigens such as I-, i-, p-, Pr-, on the RBC surface; yet, the glycosphingolipid P antigen is considered its primary target.

Unlike cold hemagglutinin disease in which the IgM-complement interaction results in the cells' removal (via extravascular phagocytosis), paroxysmal cold hemoglobinuria occurs upon completion of complement lysis within the vascular circulation.

Interestingly, the P antigen has been found on lymphocytes and skin fibroblasts; the latter is thought to be the reason for the development of urticaria in persons with paroxysmal cold hemoglobinuria.
Testing

- Adsorption studies can be performed as in WAIHA
- Elutions are **not** indicated for CAS or PCH
- Why? Only C3d is present on the red cells
- Elutions are **only** indicated if there is a possible delayed transfusion reaction in addition to cold antibodies

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<th>DAT</th>
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<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>C3</td>
<td></td>
</tr>
<tr>
<td>CAS</td>
<td>0</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>PCH</td>
<td>0</td>
<td>+</td>
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## Donath-Landsteiner Test (Biphasic Hemolysis)

<table>
<thead>
<tr>
<th></th>
<th>30’@4°C</th>
<th>90’@4°C</th>
<th>90’@37°C</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>60’@37°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient Serum</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Patient Serum Normal fresh serum</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Normal Fresh</td>
<td>-</td>
<td>-</td>
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PCH

- Transfusion can be life threatening in the setting of severe anemia or clinical instability
- Support with transfusions; B12 and folate
- Corticosteroids not helpful
- Treat underlying disorder
- ABO/Rh compatible units
Mixed-Type Autoimmune Hemolytic Anemia

- Features similar to both WAIHA and CAS

- DAT
  - Both IgG and C3d are detected
  - IgG – warm autoantibody
  - C3d – activated by IgM cold autoantibody

- Serum and Elution
  - Reacts because of the IgG warm autoantibody
Drug-Induced Hemolytic Anemias

- Antibodies directed against drug or one of its metabolites

- **All** may involve IgG and C3

- **4 mechanisms:**
  - Penicillin-type (drug adsorption)
  - Immune complex
  - Membrane modification
  - Drug-independent
DIHA

- Three types:
  - Haptenic (e.g. penicillin)
  - Immune Complex
  - Induction of Autoimmunity (e.g. aldomet, L-dopa, procainamide)
1. Penicillin-Type

- Penicillin or its metabolites are adsorbed onto the RBC surface

- Antibodies (if produced) will attach to the drug, causing a positive DAT and may increase RBC destruction
  - Positive DAT occurs more frequently
  - Immune hemolysis is less frequent
  - If an elution is performed, it should be tested with red cells sensitized with penicillin (or metabolite)
Haptenic (e.g. Penicillin, Cephalosporins)

- Drug Coats cell; antibody directed against drug/red cell membrane
- DAT+ for IgG and possibly complement
- Eluate negative
- Nonreactive for unexpected antibodies
- Antibody eluted off red cells reacts with cells+drug but not cells alone
- Hemolysis develops gradually
- Discontinue the drug and red cell survival increases
Drug

RBC

Drug-coated RBC

RBC coated with drug and anti-drug, hemolysis primarily extravascular

Anti-drug

Drugs

- Penicillin
- Methicillin
- Nafcillin
- Tetracycline
- Cephalothin
- Erythromycin
- Carbromal
- Cefazolin
- Cefamandole
2. Immune Complex

- After the patient receives drug, a drug-antidrug complex may form.

- The complex adsorbs loosely to the RBCs.

- Complement is activated, and the RBCs are considered “innocent bystanders”, resulting in hemolysis.
Immune Complex (e.g. ceftriaxone)

- Acute intravascular hemolysis; renal failure common
- IgG or IgM antibody
- Hemolysis due to drug/anti-drug immune complexes that associate with the cell membrane
- Drug must be present for demonstration of this antibody
Drugs that cause immune complexes

- Classic drugs:
  - Quinine
  - Quinidine
  - Cephalosporins

- Others:
  - Acetaminophen
  - Hydralazine
  - Methotrexate
3. Membrane Modification

- Drugs that **modify the cell membrane** of RBCs
  - Cephalothin
  - Cisplatin
  - Diglycoaldehyde
  - Suramin

- Rarely associated with RBC destruction

- The modification causes protein to adsorb to RBCs; the positive DAT may demonstrate IgG, IgM, IgA, C3, C4, and albumin and fibrinogen

- Eluates and antibody screening are nonreactive because there is **no drug antibody present**
4. Drug-Independent

- Very similar to WAIHA
- Drugs include:
  - **Methyldopa** – anti-hypertensive; interferes with suppressor T cell function, leading to elaboration of autoantibodies by B cells
  - Levodopa – anti-hypertensive
  - Procainamide – cardiac antiarrhythmic
  - Mefenamic acid – nonsteroidal antiinflammatory

- Positive DAT – IgG after 3-6 months of use

- Eluate will react with normal cells due to IgG; type of antibody is of little importance
- Hemolysis rarely occurs (1% of Methyldopa patients)
Drug-independent AIHA (e.g. alpha-methyldopa)

- Drug on membrane alters the tertiary structure of the membrane
- Antibodies are generated against the neoantigen induced by the drug
- The drug does not need to be present for antibody detection if the membrane has already been altered.
Treatment

- **Drug-Induced**
  - Discontinue drug
  - Transfuse only if insufficient oxygen delivery occurs
Summary

- Distinguishing AIHAs (cold, warm, drug, etc) is of utmost importance
- Workups of autoimmune antibodies is usually unnecessary
- It is more important to identify underlying clinically significant alloantibodies