Brrrr, It’s Cold In Here

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Patient

- 20 year old female
- Viral illness
- Presents one week later with severe hemolysis
  - Hgb 5.6
  - Increased LDH and indirect bilirubin
  - Decreased haptoglobin
Cold Autoantibodies and Cold Agglutinin Syndrome (CAS)

- Cold-reactive autoantibodies which bind optimally to red cells at 0 to 4°C
- CAS: red cell lysis or agglutination in the microvasculature of the distal extremities
- Cold autoantibodies typically are not clinically significant
Pathogenic cold autoantibodies

- IgM
- Directly agglutinate red cells at cooler temps
- Activate complement on the cellular membrane $\rightarrow$ intravascular hemolysis and anemia
- C3b deposited on red cells $\rightarrow$ C3dg
- C3dg-opsonized red cells have near normal survival in vivo
Pathogenic cold autoantibodies

• High serum concentration
• High thermal amplitude
  – Thermal amplitude: the highest temperature at which the cold autoantibody binds to red cells
  – ≥ 30 °C
  – May cause severe hemolytic anemia even at low concentrations
Clinical Presentation

- Adults after the 5\textsuperscript{th} decade of life
- Peak incidence at \(~70\) years of age
- Women
- Rare: 1 in a million
- Secondary CAS: associated with B-cell neoplasms (lymphoma, Waldenstroms) or infections (\textit{Mycoplasma pneumoniae}, EBV)
Chronic CAS

- Mild anemia
- Cold exposure exacerbates clinical signs and symptoms
  - Acrocyanosis in cold weather

- Less common: sufficient intravascular hemolysis in cold weather to cause sudden decrease in Hct and the development of dark urine
Laboratory Findings

- Mild-to-moderate anemia
- PBS: red cell agglutination!!!
- Indirect hyperbilirubinemia
- Hemoglobinuria
Immunohematologic Evaluation

- Cold agglutinins are more frequently encountered in the lab but are less problematic than warm autos.
- Warming patient samples and reagents before testing is usually sufficient to eliminate interference.
- Pathologic cold agglutinins: react up to at least 30°C, may be detectable despite prewarming techniques.
Direct Antiglobulin Test

- Positive for C3d activity
- Negative for IgG
- Eluate: Nonreactive (no immunoglobulin- only complement activity is detected on red cells)
Determination of ABO/D Blood Type

• Cold autoantibodies may cause spontaneous agglutination of the patient’s red cells at room temp and nonspecific agglutination of reagent red cells → interference with ABO and D typing reactions

• May see a typing discrepancy between the forward and reverse group reactions
Determination of ABO/D Blood Type

• The patient’s red cells should be incubated at 37 C prior to testing or washed with warm saline (37 C) before performing the forward typing reaction

• The patient’s red cells can be treated with dithiothreitol (DTT) to denature IgM autoantibodies

• Serum reactivity related to cold agglutinins can be eliminated by prewarming the sample to 37 C
Serum Evaluation for Red-Cell-Specific Antibodies

• Unlike warm autos, cold agglutinins do not usually interfere with routine antibody detection because the cold reactivity can be eliminated if reactions are performed at 37°C.

• If prewarming technique doesn’t work, adsorption methods can be performed to selectively remove the cold autoantibodies from the patient’s serum (autologous red cells).
Serum Evaluation for Red-Cell-Specific Antibodies

• Determine: serum concentration, thermal amplitude, isotype, and specificity
  – CAS: IgM titers above 1000 at 4 C
  – Cold agglutinins (asymptomatic): not more than 64 at 4 C
  – Thermal amplitude: predicts the clinical behavior of the cold autoantibody
  – Reactivity up to at least 30 C in saline or albumin are usually pathogenic
  – Specificity is for the I antigen (i = less common)
• Anti-I is detected predominantly in patients with *M. pneumoniae*
• Anti-i occurs predominantly in patients with infectious mononucleosis
Selection of Blood for Transfusion

- The RBC units selected for patients with CAS are expected to be fully compatible when crossmatching is performed at 37 C, unless the cold autoantibodies have a broad thermal range
- If red cell alloantibodies, transfused RBCs should lack the corresponding red cells antigens (duh)
Selection of Blood for Transfusion

• Transfusion of compatible units does not ensure their normal in vivo survival
• Following transfusion, patients with CAS may experience increased hemolysis until the transfused cells acquire resistance to complement-mediated lysis with the deposition of C3dg on their surface
• If I specificity, no need to transfuse (rare) i donor units
Practical Approach to Transfusion

• Transfusion is rarely needed to treat CAS because most patients can manage their symptoms by avoiding exposure to cold

• Use blood warmer

• Patient should be kept warm during infusion
Paroxysmal Cold Hemoglobinuria

- Self-limiting
- Acquired
- Children - often after a viral or bacterial infection
- Signs and symptoms of intravascular hemolysis
- IgG autoantibody
- Specificity: P antigen
Clinical Presentation

- Historical: congenital or tertiary syphilis
- Occurs within 1-2 weeks of a viral or bacterial infection (measles, mumps, chicken pox, CMV, infectious mononucleosis, influenza virus, adenovirus)
- In many instances, a specific etiologic agent is not identified
- High fever, shaking chills, back and leg pain, red-brown urine
Laboratory Findings

- Rapidly progressing anemia
- Severe hemolysis
- Initial hemoglobin may be 7 g/dL or lower
- Increased LDH, unconjugated bilirubin
- Decreased haptoglobin
- PBS: polychromasias, spherocytes, red cell agglutination and erythrophagocytosis
- Erythrophagocytosis: rarely seen in other types of autoimmune hemolytic anemia
Erythrophagocytosis
Treatment

• Usually resolves spontaneously
• Complete recovery within several weeks
• Typically does not recur
• Treatment is supportive: hydration, RBC transfusion
Immunohematologic Evaluation

• Donath-Landsteiner antibodies do not interfere in routine pretransfusion and compatibility testing because the causative autoantibody rarely causes red cell agglutination above 4 C.

• DAT is positive for C3, but negative for IgG

• Eluate: Negative
Donath-Landsteiner assay

- Looks for biphasic hemolysis
- Patient’s blood samples must be maintained at 37 C after collection
- Indirect assay: patient’s separated serum is mixed with ABO-compatible P-antigen positive RBCs
- Direct assay: whole blood from the patient
Donath-Landsteiner assay

• The starting specimen is divided into three tubes
  – One tube is incubated at 0°C (on melting ice) then at 37°C
  – The second tube is kept at 37°C
  – The third tube is kept at 0°C
Donath Landsteiner assay

• A positive test is evident by
  – 1. visible hemolysis in the first tube after the cold to warm transition
  – 2. No hemolysis in tube kept at 37°C
  – 3. No hemolysis in tube kept at 0°C
Donath Landsteiner assay
Selection of Blood for Transfusion

• Most patients with PCH who require transfusion achieve a favorable response to P-positive red cells and an adequate increment in their hemoglobin/hematocrit despite the presumed incompatibility

• P-negative blood has been used in rare cases of PCH where hemolysis was severe and prolonged

• In rare cases, plasmapheresis has been used to remove the IgG autoantibodies