Cold Agglutinin Syndrome (CAS) and Mixed AIHA
Shan Yuan, MD
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I. Autoantibody characteristics
   A. Thermal amplitude and clinical significance
      o Typically IgM, can directly activate complement when bound to cell
        membrane, causes intravascular hemolysis
      o Cold reactive, bind optimally to RBCs at 0-4 C, but may react at temp
        >30C.
      o Thermal amplitude: range of temperature in which reactivity is seen
      o Thermal amplitude an important determinant of clinical severity in CAS
        along with titer.
      o Cold autoantibodies in most healthy individual only reacts at 0-4 C. Such
        antibodies are common and have no clinical significance.
   B. Antibody specificity and associations
      o usually for the I antigen, less common for the i. Also anti-Pr, Gd Lud,
        have been reported.
      o Remember: Big people Big I, Little people little i- I antigen found on
        adult RBCs and little-i antigen found on fetal or cord blood RBCs
      o Anti-I: associated with Mycoplasma Pneumoniae infection
      o Anti-i: associated with EBV infection.
      o Polyclonal when associated with infections. Monoclonal when associated
        with lymphoproliferative disorders.

II. Clinical Aspects of CAS
   A. Incidences and associations
      o Idiopathic CAS: Chronic, predominantly affects adults > 50 years of age,
        peak ~ 70 yo, Women slightly more than men
      o Secondary CAS: about 40% of all CAS, associated with B cell neoplasms,
        infections (Mycoplasma pneumoniae, EBV)
      o Chronic CAS extremely uncommon in children, responsible for less than
        10% if AIHA in childhood. Most pediatric CAS cases are transient, occur
        in older children/teens with infections.
      o Infection associated-CAS: abrupt onset, mild to moderate anemia 2-3
        weeks following infection, resolves within weeks.
   B. Clinical presentation
      o Usually mild anemia, exacerbated by cold exposure.
      o May have acrocyanosis in cold weather, caused by local vascular stasis
        and impaired circulation due to autoagglutination
      o Anemia is usually stable
      o Less commonly produce significant intravascular hemolysis in cold
        weather, leading to sudden drop in hematocrit, dark urine and other signs
        of intravascular hemolysis
      o Peripheral smear may reveal obvious agglutination
      o In CAS associated with lymphoproliferative disorders, monoclonal IgM
        may be seen on UPEP or SPEP
Cold agglutinins interfere with routine lab tests such as automated CBC (cause spuriously high MCVs, and lower RBC count), and pretransfusion testing (ABO typing discrepancies for e.g.) due to RBC clumping. Patient sample can be prewarmed to reduce such interferences.

C. Treatment

- Avoidance of cold may be sufficient in many cases
- Corticosteroids not indicated except in 1) patients with IgG or mixed IgG and IgM cold agglutinins, or 2) low titer, high thermal amplitude IgM
- Splenectomy not effective unless there is a IgG component
- In refractory cases: immunosuppression (rituximab, cyclophosphamide), plasma exchange

III. Serological Evaluation of CAS

A. General

- DAT: positive for C3d, negative for IgG. Eluate is non-reactive
- Spontaneous agglutination may cause ABO discrepancies and interfere with antibody screen, crossmatch
- Ways to get rid of the interference:
  - Pre-warm
  - Treat serum/plasma with DTT (denatures IgM)
  - Adsorption techniques:
    - 1. Autologous if pt has not been recently transfused
    - 2. REST (rabbit erythrocyte stroma)
    - 3. Allogeneic adsorption as in WAIHA (rarely necessary)

B. Determination of Thermal Amplitude, Titer and Specificity

- Sample requirement: serum collected, maintained and separated at 37C to avoid loss of cold agglutinins
- Patient sample should be tested over a range of temperatures (typically 4 degrees, 22 (room temperature), 30 and 37 degrees Celcius) to establish thermal amplitude and determine its clinical significance
- If reactive at 30-37, potentially clinically significant
- In general, potential of hemolysis increases as titer increases. Usually if titer is <40 then the cold autoantibody is not clinically significant. If titer is >640 then immune mediated hemolysis is likely
- Determination of specificity is usually not necessary from a practical point of view and not required to establish diagnosis of CAS
- However, the specificity may correlate with etiology. Furthermore, undiluted cold reactive autoantibodies often do not display specificity. Specificity determination is usually done with titration studies

IV. Selection of Blood For Transfusion in CAS

- Transfusion is rarely needed if patients can manage their symptoms by avoiding cold exposure.
- If crossmatch performed at 37C (pre-warmed), RBC units should be fully compatible unless the cold autoantibodies have broad thermal range
Transfusion of crossmatch compatible units does not ensure normal survival of transfused cells. Cold antiantobody will fix complement on transfused cells in peripheral vasculature where temperature is lower. Not necessary to provide antigen-negative RBC units.

### Cold Autoantibodies and Cardiopulmonary Bypass

Patients with known history of CAS should avoid cold-induced hemolysis, which may occur during CPB during cold cardioplegia. It is debatable whether all patients should be screened for CAS prior to surgery. Cold agglutinins are not uncommon, but clinically significant ones with high thermal amplitude or titers are uncommon. Also there is no clear relationship between the serologic findings and clinical risks. (E.g: no established threshold for the titer above which hemolysis is likely). Thus a screening test would have relatively low positive predictive value. However, it is still advisable to carefully consider patient’s history for signs or symptoms of CAS, and vigilantly observe of the bypass circuit.

If cold agglutination noted intraoperatively:
- Keep temperature warm, avoid systemic hypothermia and cold blood cardioplegia
- Alternative strategies: warm ischemic arrest (simple aortic cross clamping), continuous warm blood cardioplegia, plasma exchange pre-op

### V. Mixed AIHA: AIHA with both Warm and Cold Autoantibodies

A. Term is reserved for patients with both warm and cold autoantibodies, and BOTH are pathogenic
B. Mixed AIHA accounts for less than 10% of all AIHAs
C. Clinical features of WAIHA usually predominate
D. Onset can be abrupt, hemolysis can be severe
E. IgM component often low titer, but demonstrates reactivity up to 30-37°C
F. DAT is positive for both IgG and C3d, eluate contains the IgG warm auto
G. Special techniques may be required to eliminate interference from the autoantibodies in pretransfusion tests.