Cold Antibody Blues
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American Red Cross
Objectives

- Define Cold Antibodies
- Differentiate Cold alloantibodies vs Cold Autoantibodies
- Removing Cold Antibody Interference
- Risks associated with removing
- Case Studies
- Conclusion
What is a Cold Antibody

- An antibody that binds its target antigen best at levels below 37C
  - Thermal Range: 0C - 30C
  - Most don’t react at 37C (reducing clinical significance)
- IgM in nature
- Often naturally occurring
- Most are benign
  - No HDFN, most don’t cause HTRs
- Common Allos: Anti-Le^a, -Le^b, -I, -i, -M, -N, and –P_1
- Some are clinically significant
  - ABO antibodies (RT reactive)
  - Reaction at 37C may associate with disease
IgM at 4°C  
IgM at 37°C
Clinical Significance

- How close to body temperature are the antibodies going to react?
  - If cold antibodies demonstrate a high thermal range (ex: 30°C) discuss treatment options with Medical Director such as a blood warmer
- Cold Antibodies that react closer to body temperature and demonstrate high titer (>1000) are more likely to be clinically significant
Cold Alloantibodies

- Made against foreign red cells
- Autocontrol negative
- Common: Anti-Le\(^a\), -Le\(^b\), -M, -N, and –P\(_1\)
- Because they only react below body temperature:
  - Generally benign
  - Rarely cause RBC destruction
- Enjoy causing ABO discrepancies
  - Anti-M
- Panel:
  - Positive at IS
  - Negative or weaker at 37C / AHG
Cold Alloantibody Panel

- Reactions only at IS, 37C incubation and AHG are negative
- Fits anti-M pattern perfectly, other colds are ruled out
- Anti-M identified
What is a Cold Autoantibody

- Antibody that targets against self antigens on RBCs
- Reacts best below body temperature
- DAT usually positive with C3, negative with IgG
- Auto-Anti-I very common
  - Allo-anti-I rare
- Panel:
  - All Panel cells equally positive at IS
  - Negative or weaker at 37C / AHG
  - AC most likely positive
Benign Cold Autoantibodies

- Most CAs are not clinically significant
- Mask underlying clinical significant IgG antibodies, ABO discrepancies
- React at 4C, low thermal range
  - Pathologic Cold autos typically can bind to RBCs at 30-32C
- Low titer (<1:64)
- Usually polyclonal
  - Monoclonal associated with pathogenic cold auto-antibodies
- Common benign autoantibodies: anti-I, anti-IH
Cold Autoantibodies due to Disease State

- **Primary:**
  - Cold agglutinin disease (CAD)

- **Secondary:**
  - Infections
    - *Mycoplasma* pneumonia, CMV, EBV
  - Lymphoma
    - IM, Waldenstroms
  - Drugs
    - Lenalidomide (Treats myelodysplastic syndrome, multiple myeloma)
Cold Agglutinin Disease (CAD)

- Complement binding IgM antibody
  - IgM + RT/Cold exposure = Agglutination
- Avid complement fixation caused by the IgM
  - C3 is bound and fixed causes Intravascular Hemolysis
  - High titer: >1000 @ 4C, reactive @ 30C
  - RT / IS reactive
  - Pos DAT, C3 detected on RBC surface (not IgM)
  - Eluate nonreactive (only C3 coating red cells)
  - Most often anti-I
- Either Acute or Chronic
  - Antibody is monoclonal: Chronic Disease
  - Antibody is polyclonal: Acute Disease
Cold Agglutinin Disease (CAD)

- Commonly seen in elderly patients
- RBCs agglutinate in blood vessels of extremities in cold conditions
- Common complaint is Acrocyanosis
  - Cold exposure = painful fingers and toes, purplish color
  - More symptomatic in colder months
- **Gold standard** Diagnostic Test: Positive DAT
  - C3 will be pos, IgG will be neg, C3 has been fixed by IgM
  - Others indicators: 1. LDH/Bili ↑ 2. Haptoglobin ↓(Consumed) 3. Hgb/Hct ↓
- Look for other diseases after Hemolytic Anemia diagnosis
  - Ex: Mycoplasma, Lymphoma
Cold Agglutinin Disease (CAD)

- **Prevention**
  - **Avoid Cold Exposure**

- Affects 15% of population with Autoimmune Hemolytic Anemia

- **Treatment:**
  - Blood transfusion via a blood warmer
    - Can have HTR symptoms without
  - Keep warm
Cold Agglutinin Disease (CAD)
Cold Autoantibody Panel

- All cells pos at IS, including AC
- Cells negative when warmed at 37C, AHG added
- Cold Autoantibody suspected in this case, further investigation required to confirm
Cold Autoantibodies due to Disease State

- May cause Autoimmune Hemolytic Anemia (AIHA), as a result of Cold Autoantibodies, caused by disease
- Secondary:
  - Infections
    - *Mycoplasma* pneumonia (Auto-anti-I), CMV, EBV
  - Lymphoma
    - IM (Auto-anti-i), Waldenstroms
  - Drugs
    - Lenalidomide (Treats myelodysplastic syndrome, multiple myeloma)
- Paroxysmal Cold Hemoglobinuria
Cold Autoantibodies due to Disease State

- Paroxysmal Cold Hemoglobinuria (PCH)
  - Exception to the Cold Autoantibody Rule
  - Auto-anti-P seen most frequent
  - DAT positive, \( C_3 \) bound to RBCs (like in CAD)
  - "Biphasic" IgG that reacts against P antigens
    - IgG binds to RBC at cold temp, complement is attached,
    - When RBCs are warmed they lyse with complement
    - Antibody then dissociates, complement remains
    - Result is Intravascular hemolysis
  - Formerly associated with syphilis
  - Now associated with viral infections in children
    - Measles, mumps, influenza
Cold Autoantibody Workup Options

- **Unmasking the Cold antibody**
  - Prewarm
  - RESt adsorption
  - Cold Auto-Screen
  - Cold Autoadsorption or allogeneic adsorption
    - Cannot perform Autoadsorption if transfused within past 3 months

- **Crossmatching**
  - Prewarm technique, adsorptions ok
    - RESt used only for ABID, Not used for compatibility testing
Prewarming

- **Purpose:** to disable cold antibodies (allo and auto) in the potential presence of clinically significant antibodies
- Prewarming incubates the sample at 37°C
- **Pros:**
  - Eliminates unwanted/insignificant reactions in workup
- **Cons:**
  - Can weaken significant antibodies or be missed completely
  - Ab Enhancement (PEG/LISS) does not ensure detection when prewarmed
  - May be inappropriately used
- **Use only to confirm cold antibodies**
  - Do not use solely to make reactions go away
Rabbit Erythrocyte Stroma “RESt”

- Cold autoantibodies mask significant alloantibodies
- Treated rabbit cells adsorb cold antibodies from pt plasma
- Goal is to remove interference by cold antibodies, to make rule-outs possible
  - Auto-anti-I most common
- Like Prewarm, can adsorb clinically significant antibodies as well
  - Anti-D, -E, -Vel and -M reportedly adsorbed by RESt
- Risk of performing technique must be considered
- Do not use for ABO testing, anti-B can be partially or completely adsorbed
**RESt Panel**

- Cold antibodies adsorbed from plasma
- Cells negative at all phases, all antibodies ruled out
Cold-Auto Screen

- Performed if cold antibody is suspected without history
- Incubate patient plasma and red cells at 4C 15-30 min
- Positive result indicates cold antibodies activated onto all red cells
- Always use auto control
  - Will be positive in Cold Auto-antibody cases
- Used as a tool for workup, does not confirm Cold Antibody
Cold-Auto Screen

• Screen cell set incubated at 4C, creating cold environment “suspected” Autoantibody likes
• Autoantibody is activated, All cells including Auto Control are positive
• Test used as a tool in suspecting Cold Autoantibody cases, further investigation required to confirm
Cold-Autoadsorption

• “Gold Standard” for confirming Cold Autoantibodies
• Procedure binds Autoantibodies currently in plasma to patients own red cells
• Purpose is to have plasma to be free of Cold Autoantibodies and test plasma for what is left behind
  • Eliminates Autoantibody Interference
  • Minimal risk for clinically significant antibodies being missed
• Can take up to 6 adsorptions to fully remove Cold Autoantibody depending on strength & panel reactions
  • Each case is unique
Cold-Autoadsorption

A. Initial Sample
- Patient
- Y anti-K
- auto-Ab
- auto-Ab
- auto-Ab
- Y anti-K
- Y anti-K
- Y anti-K

Treat Pt RBCs to remove autoantibody

B. Autoadsorption
- Patient
- Y anti-K
- auto-Ab
- auto-Ab
- Y anti-K
- Y anti-K

Incubate treated Pt RBCs with serum

C. Adsorbed Serum
- Y anti-K
- Y anti-K
- Y anti-K
- Y anti-K

Remove coated RBCs, anti-K visible
ABO RH Interference

- RBCs may be heavily coated with cold antibody, may react at RT
- Causes *false positive* reaction on front type (anti-A and anti-B)
- If suspected, Group O rbc's can test vs patient plasma
  - Both tubes likely to be positive if cold autoantibody is present
- Resolutions
  - Wash red cells with warm saline
  - Treat plasma with DTT (reduces disulfide bonds on IgM molecules, cannot agglutinate red cells)
Crossmatching

- Prewarm technique, adsorptions ok
  - RESt used only for ABID, Not used for compatibility testing
- Neat and adsorbed plasma both tested
  - Testing plasma with and without Cold Autoantibody present
- Report Compatible/Incompatible Crossmatches according to Lab Director Discretion and Lab specific procedures
Cardioplegia

- Dextrose and potassium solution
- Inhibits myocardial muscle contraction during surgery (ex: CABG)
- May affect patients with cold antibodies if solution creates low enough environment for antibodies to react
- Depends on antibody strength, what temp they prefer to react at
  - Thermic range will vary (0C-30C)
Cold Case Study 1

- 73 year old woman
- Coronary artery bypass surgery
  - Cardioplegia to be used during surgery
- Anti-M identified, nonreactive at 37C prewarm
- Compatible AHG Crossmatch
- Compatible units issued
- No recommendation on management of cardioplegia
Cold Case Study 1

- Surgery begins, cold blood cardioplegia initiated
- RBC agglutination immediately observed in cardioplegia line
- Cardioplegia stopped, patient warmed, RBC agglutinates intercepted before reach patient, no clinical reaction noted
- Warm cardioplegia used, surgery complete at 7 hours, no further incident
- TR Workup:
  - Neg DAT, no hemolysis, no history of cold agglutinin disease
- Ref Lab Workup:
  - Titer of 512 at 4C with M-neg RBCs, non reactive at 30C
  - Findings consistent with nonpathogenic Cold Antibody (CA)
Cold Case Study 1 Takeaways

- 512 titer at 4C explains the agglutination with the cold cardioplegia during surgery
- Warming the patient during surgery disables the cold antibodies ability to agglutinate red cells (same as with CAD)
- Necessary to inform Clinical team of cold antibody presence
- Patients with history of cold antibodies should avoid hypothermic conditions during surgery
- Cardioplegia line should always be monitored during surgery
Cold Case Study 2

- 73 year old man
- Admitted for CABG
- Antibody Screen Positive
- Pos panels at IS and 37C, Negative @ AHG with PEG & LISS
- Pos DAT (Poly & C3)
- Neg 2 hour settle @ 37C
- 2 XMs performed: compatible in PEG
Cold Case Study 2

- Cold Autoantibody ID’d
- Undergoing Plasmapheresis in Femoral Vein to mitigate the cold-auto
  - Warm albumin used
    - Via waterbath
- Plasmapheresis did not remove the cold-auto reactivity
- Communication to clinical team occurred
  - Blood warmer used
  - Heated room and patient
- No issued noted in OR during the cardiac surgery
Final Cold Case

- 20 year old female
- Right upper quadrant pain, throat irritation, 1 min loss of consciousness
- Febrile at 38.8°C, jaundiced and right upper quadrant tenderness
- Initial Lab Results:
  - Increased: ESR, MCV (Macrocytic), Retics (35%), total bilirubin
  - Decreased: Hgb (9.7 g/dl), haptoglobin
  - Positive DAT, Monospot Test
  - Cold Autoantibody ID’d, EBV diagnosed in high titers
- Clinical team notes progressively anemic
- Patient admitted, treated, released after hematocrit stabilized
Final Cold Case Takeaways

- Infectious Mononucleosis diagnosed
- Cold reactive autoantibodies formed secondary to disease
- Patient developed Autoimmune Hemolytic Anemia due to Cold autoantibodies produced due to IM
- Jaundice experienced due to the AIHA caused by Cold Autoantibodies
  - increased bilirubin and decreased haptoglobin
- 0.5-3% of IM patients experience AIHA (due to Cold Autoantibodies)
  - Usually anti-I
Conclusion

- IgM in nature, wide thermal range
- Cold agglutinins are rarely clinically significant
- Can be result of disease state (CAD, PCH)
  - Pos Poly DAT, pos C3, IgG neg
- Cause Interference in ABO, Screens and Panels
- Workup Options
  - Prewarm, RESSt, Cold Agglutinin Screen, Autoadsorption
- Susceptible patients require special needs during procedures
Questions?
Thank you!