

# Positive Direct Antiglobulin Test and Autoimmune Hemolytic Anemias

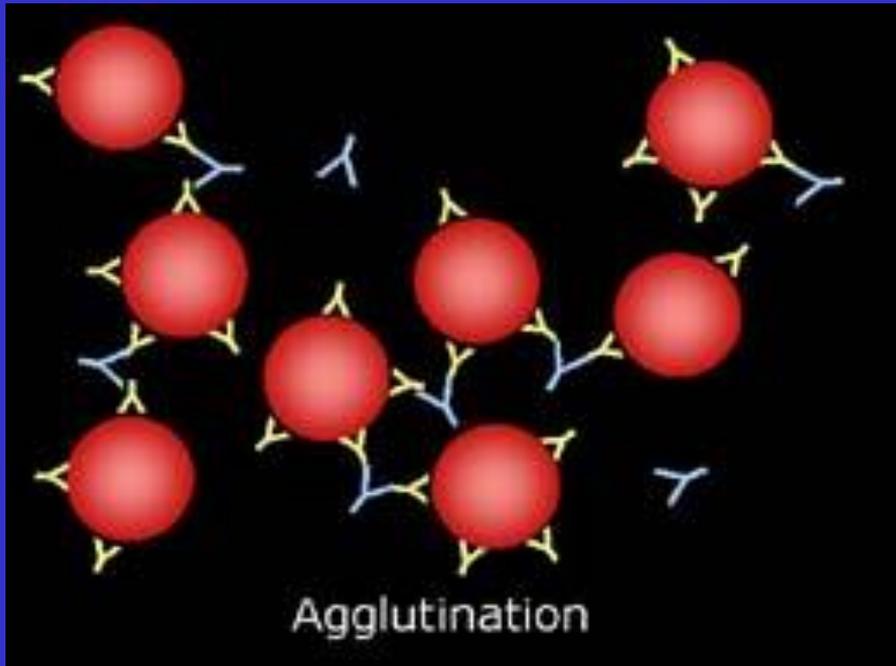
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# Direct Antiglobulin Test (DAT)

- 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?

# 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

# 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

# Classification

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

# 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

# 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

# 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 Agglutinin Disease
- Secondary forms (90%);
  - Postinfectious
    - Mycoplasma
    - CMV
    - EBV; Infectious mononucleosis
  - Lymphoproliferative disorders
    - E.G. B-cell lymphomas; sometimes intravascular

# 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

# CAIHA Serologic Investigation

- Specificity is I, IH or I (academic interest only)
  - Adult cells: I
  - Cord cells: I
- Cold Agglutinin titers and thermal amplitude studies

# 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

# Donath-Landsteiner Test (Biphasic Hemolysis)

	30' @4°C 60' @37 °C	90' @4 °C	90' @37 °C
Patient Serum	+	-	-
Patient Serum Normal fresh serum	+	-	-
Normal Fresh	-	-	-

# 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

# DIHA

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

# 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

# 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

# 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.