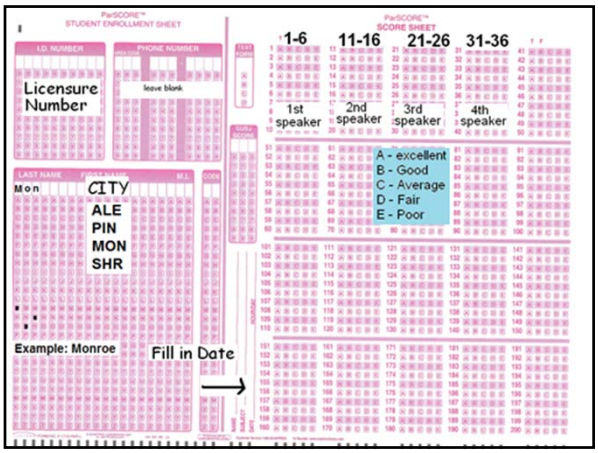


Fill out circle completely

- fill in the center completely
- if center is left open, the scanner will not read it.

Licensure Number

- leave first column blank
- fill in zero for next three columns
- licensure number from the right and the others with zeros
  - Gen – 1
  - Spec – 2
  - Lab Asst – 3
  - Tech – 4
  - Cyto – 5
  - Phleb - 6

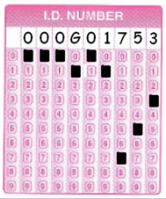
CLP.201753-PHL

The licensure number in the example to the left doesn't have a letter in front.

It is in the 200,000 series.

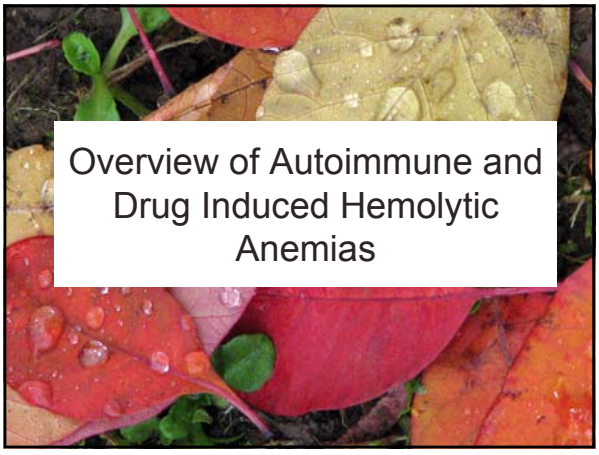
Just fill in the number.

CLP.G01753-Gen



The example on the right is the has only 5 numbers preceded with a letter.

The letter has a corresponding number that can be filled in.



Overview of Autoimmune and Drug Induced Hemolytic Anemias

Objectives

- Summarize the clinical findings associated with anemia.
- Define immune hemolytic anemia and indicate the types of antibodies involved.
- Identify and investigate potential serologic discrepancies caused by autoimmune and drug-induced hemolytic anemias.

Introduction to Anemia

- One of the most common problems encountered in clinical medicine
- Is not a disease
  - Expression of an underlying disorder or disease
    - Must determine what is causing the anemia

Introduction to Anemia

- Defined as a decrease in the competence of blood to carry oxygen to tissues.
  - Causing tissue hypoxia
- Decrease in the normal concentration of Hgb and/or RBCs
  - RBCs are destroyed prematurely
    - Normal life span ~120 days

Introduction to Anemia

- Individual may or may not become anemic
- Presence and severity of anemia depends on:
  - Severity of hemolysis
  - Ability of BM to compensate for RBC loss

Introduction to Anemia

- Anemia develops when:
  - RBC loss or destruction exceeds the maximum capacity of the BM RBC production
  - The BM RBC production is impaired

Introduction to Anemia

- Diagnosing Anemia
  - Most often is discovered through laboratory tests
  - Patient History is always important
    - Family history
    - Description and duration of symptoms
    - Medications
    - Blood loss
    - Organomegaly

Laboratory Investigation

- CBC
  - RBC count, Hgb, Hct, RBC indices, WBC count, platelet count
  - Differential with RBC morphology
- Additional tests
  - Reticulocyte count
  - Bilirubin
  - Urine and stool can be examined for blood

Functional Classification of Anemia

- Three pathophysiologic mechanisms
  - Proliferation defect
    - decreased production
  - Maturation defect
  - **Survival defect**
    - **increased destruction**

Functional Classification

- Survival defects
  - Results from loss of circulating RBCs
    - Either by hemorrhage or hemolysis
- BM maturation is increased and orderly

Functional Classification

- Survival defects
  - No anemia
    - If BM can ↑ production at same rate as cells being lost or hemolyzed
      - Compensated hemolytic anemia
  - Can rapidly develop into anemia
    - RBC destruction ↑ beyond capacity of BM (hemolytic crisis)
    - BM stops producing RBCs (aplastic crisis)

Survival defects

- Classify hemolytic anemia based on cause of shortened RBC survival
  - Extrinsic anemia
    - Antagonist in RBC's environment causes injury to RBC
      - toxic substances
      - RBC trauma in circulation
      - **Immune-mediated destruction**

Survival defects

- Common observation in circulation
  - Poikilocytes form after the RBC leaves the BM
    - Schistocytes
      - Result of intravascular mechanical trauma to RBC
    - Spherocytes
      - Indicate extravascular RBC membrane damage

Survival defects

- Usually patients demonstrate normocytic normochromic RBCs
  - May see macrocytosis if reticulocyte production is ↑ enough
  - May see microcytosis if there is a predominance of schistocytes and or spherocytes

Survival defects

- Sites of destruction in hemolytic anemia
  - Intravascular hemolysis occurs within circulation due to:
    - Activation of complement on RBC membrane
    - Physical or mechanical trauma to RBCs
    - Presence of soluble toxic substances

Sites of destruction in hemolytic anemia

- Intravascular hemolysis
  - release of cell contents into the plasma destruction of red blood cells in the circulation

Sites of destruction in hemolytic anemia

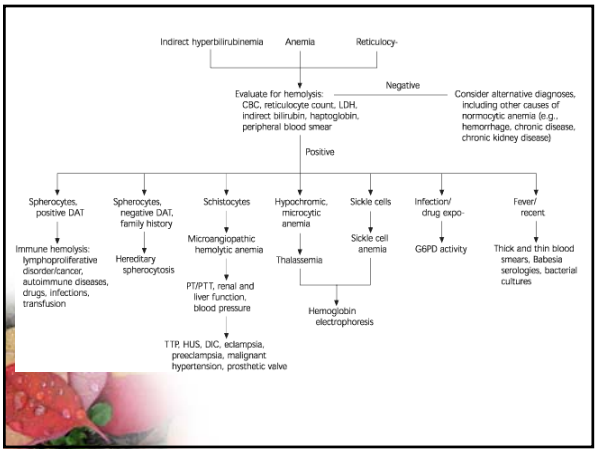
- Intravascular hemolysis
  - Laboratory findings:
    - Hemoglobinemia
    - Hemoglobinuria
    - Hemosiderinuria
      - the presence of hemosiderin in the urine
        - » "brown urine"
    - ↓ haptoglobin
    - ↑ LD (early indicator)
    - Schistocytes on RBC morphology

Sites of destruction in hemolytic anemia

- Extravascular hemolysis
  - Occurs within macrophages of the spleen, liver, and BM due to phagocytes in the tissues removing RBCs from circulation
  - Hgb NOT released directly in the plasma
    - NO Hemoglobinemia
    - NO Hemoglobinuria

Sites of destruction in hemolytic anemia

- Extravascular hemolysis
  - Measurements of the products of heme catabolism
    - ↑ exhaled CO
    - ↑ carboxyhemoglobin
    - ↑ serum bilirubin
    - ↓ haptoglobin if severe or chronic
    - ↑ urine or fecal urobilinogen
    - Spherocytes on RBC morphology



Immune hemolytic anemia (IHA)

- Underlying mechanism involves the reaction of an antibody and/or complement with RBC antigens
  - With subsequent cell destruction

Classification of IHAs

- Based on stimulus for antibody production
  - Alloimmune hemolytic anemia
  - Autoimmune hemolytic anemia
  - Drug-induced hemolytic anemia
  - Important to determine process because each type requires specific treatment

Immunity

- In a normally functioning immune system, some individuals will produce antibodies to foreign antigens.

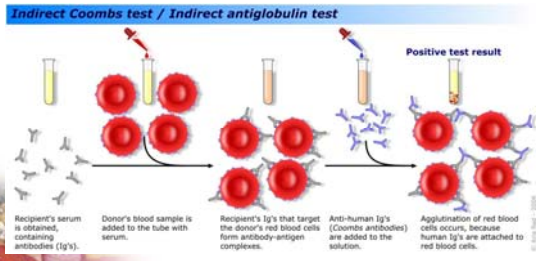
Alloimmune hemolytic anemia

- Antibody (Ab) development to RBC antigen (Ag) that the individual lacks.
  - Does not react with individual's own RBCs
    - HDFN
      - mother makes Abs against Ags on fetal RBCs
        - » Inherited from the father
    - Transfusion reactions
      - where recipient makes Abs to Ags on transfused (donor) cells

IAT

- IAT (indirect Coombs)
  - detects free antibody in the serum/plasma
  - Used for antibody screen and antibody ID panels

IAT



IAT

Donor	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P$_1$	M	N	S	s	Patient Results Gel IAT
I	+	+	0	0	+	+	+	0	+	+	0	+	0	0	+	0	+	+	3+
II	+	0	+	+	0	0	+	0	+	0	+	0	+	0	0	+	0	+	3+
III	0	0	+	0	+	0	+	+	0	+	+	0	+	+	+	0	+	0	0

Donor	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P$_1$	M	N	S	s	Patient Results Gel IAT
1	+	+	0	0	+	+	+	0	+	+	+	0	0	0	0	+	0	+	3+
2	+	+	0	0	+	0	+	0	+	0	+	+	0	0	+	0	+	0	3+
3	+	+	0	0	+	0	+	+	0	+	0	0	+	0	+	0	+	+	3+
4	+	0	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	+	3+
5	+	0	+	+	0	0	+	0	+	+	0	+	0	+	+	0	0	+	3+
6	0	+	+	0	+	0	+	0	+	+	+	+	0	+	+	+	0	+	0
7	0	0	+	+	+	0	+	+	+	+	0	0	+	+	+	+	0	+	0
8	0	0	+	0	+	+	+	+	0	+	+	+	0	+	+	0	+	0	0
9	0	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	0
10	0	0	+	0	+	0	+	+	+	0	0	+	0	+	0	+	0	+	0
																		Autocontrol	0

0 = no agglutination
+ = agglutination (with or without strength of reaction noted in front)

DAT

- DAT – (direct Coombs)
  - Means for determining whether immune-mediated destruction of RBCs is contributing to anemia
  - Positive result confirms presence of RBCs coated *in vivo* with antibodies or complement

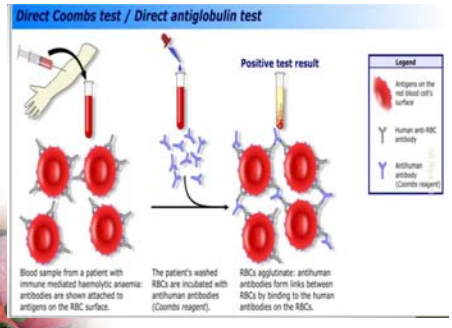
DAT

- Does not distinguish between autoantibodies and alloantibodies

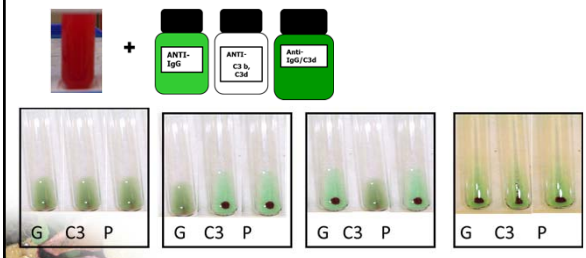
DAT

- Antihuman globulin (AHG) directed against
  - IgG alone
  - Complement components only
    - Specifically C3d and/or C3b
  - Polyspecific
    - Combination of anti-IgG and anti-Complement

DAT



DAT



DAT

- Applications of the DAT
  - Investigation of HDFN
  - Hemolytic Transfusion Rxn (HTR)
    - First immunologic evidence of HTR
    - alloantibody coating transfused cells
  - **Investigation of autoantibodies**
    - autoantibodies coating your own cells
  - **Drug Induced Antibodies**

Autoimmune hemolytic anemia (AIHA)

Autoimmunity

- Occasionally, a malfunction in the mechanism regulating immune responses occurs and antibodies against "self" (autoantibodies) are produced.

Autoimmune hemolytic anemia (AIHA)

- Shortened RBC survival
  - <120 days
  - Caused by production of autoantibodies against RBC antigens
    - Antibody attaches to RBC creating an altered RBC which is removed from circulation
      - Most hemolysis is extravascular via splenic macrophages.

AIHA

- Further classified
  - Warm-antibody autoimmune HA (WAIHA)
  - Cold-antibody AIHA
    - Cold Agglutinin Disease (CAD) or syndrome (CAS)
    - Paroxysmal Cold Hemoglobinuria (PCH)
  - Mixed-Type AIHA

Warm-antibody autoimmune (WAIHA)

Warm-antibody autoimmune (WAIHA)

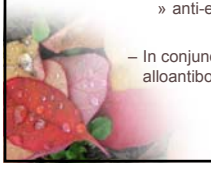
- Most common form of AIHA
  - ~70% of the cases
  - Optimal reactivity at 37° C

Warm-antibody autoimmune (WAIHA)

- Can occur at any age
  - Incidence increases after age 40
  - Childhood incidence peaks in the first 4 years of life

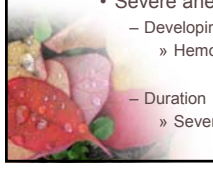
Warm-antibody autoimmune (WAIHA)

- Culprit
  - Usually IgG antibody
    - rarely IgM, IgA
    - Most Abs react with "Rh protein" complex
      - Do not react with Rh null
      - Occasionally have single specificity within Rh system
        - » anti-e
  - In conjunction with or precipitated by formation of alloantibody



Warm AIHA

- Idiopathic WAIHA
  - Accounts for about 60% of cases of warm AIHA
  - Acute idiopathic WAIHA
    - Severe anemia
      - Developing over two to three days
        - » Hemolysis is self limited
  - Duration
    - » Several weeks → several years duration

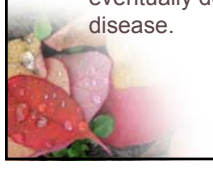


Warm AIHA

- Chronic idiopathic WAIHS
  - Hemolysis is unabating

Warm AIHA

- Secondary WAIHA associated with:
  - Lymphoproliferative disease
    - CLL , Hodgkin's
  - Many children with idiopathic WAIHA eventually develop a lymphoproliferative disease.



Warm AIHA

- Secondary WAIHA associated with:
  - Neoplastic Diseases
    - referring to both malignant and benign growths
    - Few Examples
      - Leukemia
      - Metastatic Carcinoma
      - Multiple Myeloma
      - Osteosarcoma

Warm AIHA

- Secondary WAIHA associated with:
  - Other Autoimmune disorders
    - SLE, RA, Crohn's disease, etc.

WAIHA Blood Bank Testing

- Warm Autoantibodies
  - ABO type generally not affected
    - Testing is at RT (IS)
  - Rh grouping
    - only affected if weak D testing is done
      - Weak D is not recommended

WAIHA Blood Bank Testing

- Warm Autoantibodies
  - DAT is positive (usually)
    - cells coated with IgG
  - IAT may be positive or negative

Common Blood Bank findings in AIHA

- Positive IAT if the DAT is so strongly positive that Ab is "spilling" into the serum
  - Everything may be positive
    - Including the autocontrol
- Why it matters:
  - Risk the inability to detect underlying alloantibody; must rule-out or ID alloantibody by warm auto/allo adsorption techniques

	IS	37°c	AHG (IAT)	ENZYME (FICIN IAT)
Screen Cell I	0	0	2+	4+
Screen Cell II	0	0	2+	4+
Screen Cell III	0	0	2+	4+
Autocontrol	0	0	2+	4+

- Autocontrol positive…only an autoantibody?
  - No way to know without further testing
    - The autoantibody must be removed so we can see if there is an alloantibody
      - Adsorption and Elution techniques

- Warm antibodies in the serum may show a relative anti-e specificity
  - Reacting weakest with e negative RBCs
  - May react with all RBCs of normal Rh type

							SERUM/PLASMA		
CELL		D	C	E	c	e	RT	37°C	AHG(IAT)
R1R1-39	1	+	+	0	0	+	0	0	3+
R1R1-44	2	+	+	0	0	+	0	0	3+
R2R2-23	3	+	0	+	+	0	0	0	±
rr-33	4	0	0	0	+	+	0	0	3+
rr-26	5	0	0	0	+	+	0	0	3+
r'r-4	6	0	+	0	+	+	0	0	3+
r"r-17	7	0	0	+	+	+	0	0	3+
R0r-13	8	+	0	0	+	+	0	0	3+
R1r-14	9	+	+	0	+	+	0	0	3+
R1R2-8	10	+	+	+	+	+	0	0	3+
Autocontrol Untreated RBCs	11								3+
Autocontrol EGA treated	12	EGA= EDTA glycine acid treated							3+

Common Blood Bank findings in AIHA

- Reasons for negative IAT
  - All autoantibody bound to red cells
    - no "spill over" into serum
  - Why it matters:
    - Generally better prognosis because total antibody production is lower

Common Blood Bank findings in AIHA

- Positive DAT because the antibody is already present *in vivo*
  - Patients who have not been recently transfused

- DAT and autocontrol are not equivalent

Poly AHG	Anti-IgG	Anti-C3	Control
3+	2+	0	0

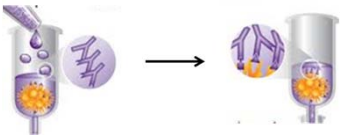
- Negative DAT
  - May not detect very small amounts of IgG antibodies on RBC
  - Result negative if complete destruction of antibody or complement-coated RBCs occurred

Laboratory Goals

- Establish that antibody on patients cell is autoantibody
- Determine if there is a specificity to the autoantibody
  - Detect and identify any clinically significant "underlying" alloantibodies

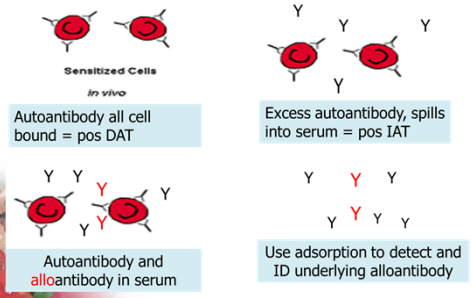
Identification Techniques

- Adsorption
  - Technique to remove an Ab from a patient's plasma
    - Removing antibody from plasma by binding to the corresponding antigen on RBCs
      - using optimal incubation conditions



- Adsorption
  - useful in differentiating autoantibody from alloantibody
    - Remove autoantibody reactivity so that alloantibody detection tests and diagnostic tests for differentiating the immune hemolytic anemias can be performed.

Auto/allo antibodies



- Adsorption
  - Autologous RBCs could be coated with antibodies (DAT+)
    - Adsorption is facilitated by dissociating autoantibody from the RBC membrane
      - Uncovering Ag sites that can bind free autoantibody to remove it from the serum
        - » Can be performed by heat elution RBCs placed at 56° C for 3-5 minutes
        - » Subsequent treatment of RBCs with enzymes enhances that adsorption process

- Treatment of RBCs with enzymes enhances that adsorption process by removing membrane structures that otherwise hinder the association between antigen and antibody
  - ZZAP reagent
    - Mixture of proteolytic enzyme and sulfhydryl reagent dithiothreitol (DTT)

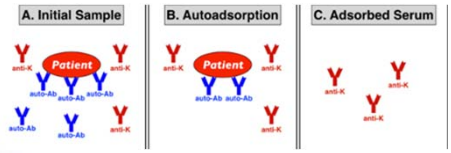
- ZZAP removes immunoglobulins and complement from the RBCs and enhances the adsorption process

- Autologous adsorption
  - Use when patient has not been transfused within last 3 months
  - Use patient's own cells for adsorption

Autoadsorption

- Patient has not been transfused within last 3 months
  - All RBCs are self
    - Only auto antibodies will be removed with the use of autologous cell for the autoadsorption because alloantibodies are made to RBC Ags the patient lacks.

Autoadsorption



Allogeneic/Homologous adsorption

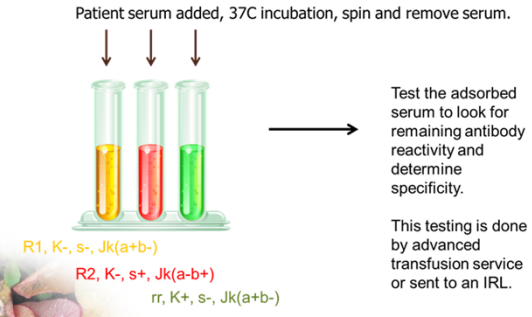
- Allogeneic/Homologous adsorption
  - Use when patient has been recently transfused
    - Transfused (donor) cells present in circulation are likely to adsorb the alloantibodies being sought.
      - Use cells with complementary phenotypes to remove the autoantibody and leave the alloantibody in the test system
        - » Alloantibodies that remain can be confirmed by testing against a panel of reagent RBCs

Allogeneic/Homologous adsorption

- Treating the adsorbing cells with enzyme or ZZAP typically enhances the adsorption process.
  - Treated cells may lack the antigens
    - Those antigens may be destroyed by DDT and/or enzymes.

Alloadsorption

- Use when the patient has been recently transfused
  - RBCs are both self and donor
    - should not be used for adsorption
  - Use cells with complementary phenotypes
    - To the patient's phenotype
    - Or common phenotypes of the population
      - R1R1, R2R2, rr, etc.



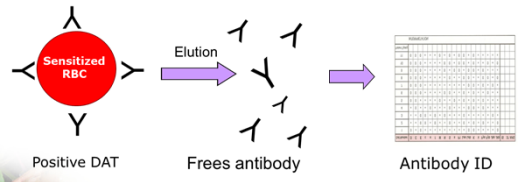
- Elution
  - Whenever the DAT is positive we can perform an elution
  - Or after adsorption techniques
    - Recovery of adsorbed antibody by treating antibody-coated RBC's to break the bonds between antigen and antibody

- Elution
  - Autoantibody in eluate will usually react with all cells tested
    - Pan-agglutination
      - Concentration of antibody removed from the patient's cells may be greater than the quantity of antibody in the serum
  - Differentiate autoantibody from alloantibody to high prevalence antigen
    - » by testing eluate against patient's cells

- Prior to an elution, cells must be thoroughly washed to remove serum containing excess antibody
  - Supernatant fluid from the final wash of RBCs to be eluted should be tested in parallel with the eluate
    - Should be non-reactive
      - Ensures antibody detected in the eluate is on RBC bound antibody and not free antibody from the plasma

- Elution
  - Ag-Ab bond must be broken
    - Releasing Ab from the Ag on the RBC into fluid medium
      - Usually saline

Positive DAT or following an Adsorption



		ELUATE						
CELL		D	C	E	c	e	Eluate IAT	Last Wash IAT
R1R1-39	1	+	+	0	0	+	4+	0✓
R1R1-44	2	+	+	0	0	+	4+	0✓
R2R2-23	3	+	0	+	+	0	4+	0✓
rr-33	4	0	0	0	+	+	4+	0✓
rr-26	5	0	0	0	+	+	4+	0✓
r'r-4	6	0	+	0	+	+	4+	0✓
r"r-17	7	0	0	+	+	+	4+	0✓
R0r-13	8	+	0	0	+	+	4	0✓
R1r-14	9	+	+	0	+	+	4+	0✓
R1R2-8	10	+	+	+	+	+	4+	0✓
Autocontrol Untreated RBCs	11						4+	3+
Autocontrol EGA treated	12						4+	0✓

		Warm Autoantibody with underlying alloanti-E																Patient IAT	Auto-ADS
																		37	IAT	Ficin IAT
cell	D	C	E	c	e	P1	M	N	S	s	Lea	Leb	K	k	Fya	Fyb	Jka	JKb			
1	+	+	0	0	+	+	+	+	0	+	0	+	0	+	+	0	+	+	0	3+	0✓
2	+	+	0	0	+	+	+	+	0	+	0	+	0	+	+	+	+	0	0	3+	0✓
3	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	2+	4+	3+
4	0	0	0	+	+	0	+	0	+	+	+	0	0	+	0	+	0	+	0	3+	0✓
5	0	0	0	+	+	+	+	+	+	+	0	+	+	+	+	+	+	0	0	3+	0✓
6	0	+	0	+	+	0	+	0	+	+	0	0	+	+	0	+	0	+	0	3+	0✓
7	0	0	+	+	+	+	+	+	+	0	0	+	0	+	+	0	0	+	1+	4+	2+
8	+	0	0	+	+	+	+	+	+	0	0	0	0	+	0	0	+	+	0	3+	0✓
9	+	+	0	+	+	+	0	+	+	+	+	0	0	+	+	0	+	+	0	3+	0✓
10	+	+	+	+	+	0	+	0	+	+	0	+	0	+	0	+	+	+	1+	4+	2+
11 AC																			0	3+	3+
12 AC treat																			0	3+	0✓

Treatment of WAIHA

- Steroids
  - suppress autoantibody production
- Splenectomy
  - Decreases antibody production
  - Removes potent site of RBC damage and destruction

Treatment of WAIHA

- Immunosuppressive drugs
- To transfusion or not to transfuse?

Treat with Transfusion?

- One Perspective:
  - Transfusion should be avoided unless life threatening anemia.
  - Suitable units can be challenging and time-consuming to find
    - due to the need to rule out alloantibody/ies

Treat with Transfusion?

- Another Perspective:
  - Do not withhold blood transfusion to patient with a justified need
  - RBC destruction of transfused cells should be no different than currently circulating cells

Cold-antibody AIHA

Benign Cold Reactive Autoantibody Characteristics

- Demonstrable in serum of many normal, healthy adults when testing at 4° C
  - Usually does not present a serological problem because routine tests are not done at 4° C
- Usually IgM, anti-I
  - **Low titer (<64 at 4° C)**

- May interfere with routine tests performed at RT
  - RBCs may be heavily coated
  - May directly agglutinate
    - Causing false positives

	Anti-A	Anti-B	Anti-D	D control
Serum suspended RBCs	1+	1+	1+	1+
Warm-washed, saline suspended RBCs	0	0	1+	N/A

	I.S	37C	IAT	Enzyme IAT
Screen cell I	I+	0	0√	3+
Screen cell II	I+	0	0√	3+
Screen cell III	I+	0	0√	3+
Auto Control	2+	0	0√	3+

Cord cell I	0
Cord cell II	0

Cord cells are I negative, i positive, confirming Anti-I in panel above

Pathologic Cold Autoagglutinins

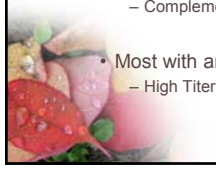
- Cold Agglutinin Syndrome (CAS) or Disease (CAD)
- Paroxysmal Cold Hemoglobinuria (PCH)

Cold AIHA

- Cold Agglutinin Disease (CAD) or syndrome (CAS)
- Accounts for ~ 16–30% of cases of AIHA
  - Less common in children than adults

Cold Agglutinin Disease (CAD)

- Optimal reactivity < 37° C
- Culprit
  - Usually IgM (rarely IgA or IgG) antibody
    - Capable of binding complement
      - Complement mediated lysis
    - Most with anti-I specificity
      - High Titer ≥ 1000

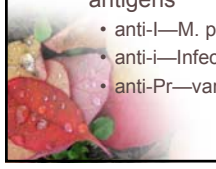


Cold Agglutinin Disease (CAD)

- Idiopathic chronic CAS
  - Usually chronic, occurring after age 50
  - Anti-I
    - Monoclonal IgM/kappa light (κ) chain

Cold Agglutinin Disease (CAD)

- Secondary CAS
  - Acute, self-limiting usually associated with infectious disease
  - Polyclonal IgM auto-Abs with specificity for Ii antigens
    - anti-I—M. pneumoniae
    - anti-i—Infectious mononucleosis
    - anti-Pr—varicella, rubella



Cold Agglutinin Disease (CAD)

- Secondary CAS
  - Chronic form is associated with Lymphoproliferative disorders
    - Lymphoma
    - Waldenstrom's Macroglobulinemia
    - Monoclonal IgM/κ light chain

Cold Agglutinin Disease (CAD)

- Most are not clinically significant
  - But can be with resultant IHA.
  - Severity of disease
    - Related to thermal range of the Ab

Cold Agglutinin Disease (CAD)

- Cold-reacting antibodies with a wide range of thermal reactivity (up to 32° C) can cause problems when the peripheral circulation cools to this temperature.
  - Chronic hemolytic or Episodic hemolysis associated with chilling
    - Areas of the body that cool to the Ab thermal range
    - Sludging of blood flow within capillaries

Cold Agglutinin Disease (CAD)

- Extremities affected
  - Nose, fingers, toes and ears
    - Vascular changes
      - Acrocyanosis
        - » Hands and/or feet turn blue
      - Raynaud's phenomenon
        - » Pain with color change patterns in skin
        - » White: spasm of the vessels
        - » Blue: cyanosis
        - » Red: indicates return of blood flow to the area

Cold Agglutinin Disease (CAD)

- Hemoglobinuria
  - Accompanies acute hemolytic attacks
- Automated blood counts
  - Must warm blood
    - Falsely ↓ RBC
    - Falsely ↑ MCV

Pathologic CAS serology

- May interfere with ABO/Rh, antibody detection and ID, and cross-match
- Enhanced reactivity with enzymes
  - Most have anti-I specificity

Pathologic CAS serology

- Positive DAT
  - Due to complement
  - Polyspecific AHG
  - anti-C3b/C3d
    - IgM with titer often >1000 at 4° C
      - May react over a wide thermal range up to 37° C

Cold Agglutinin Disease (CAD)

- Most patients require no treatment and are instructed to avoid the cold, keep warm, or move to a milder climate

Paroxysmal Cold Hemoglobinuria (PCH)

Paroxysmal Cold Hemoglobinuria (PCH)

- Least common type of AIHA
  - Can occur at any age
    - Accounts for 30–40% of all AIHA in children
      - Most frequent < age 5
      - Can be a ssociated with viral and bacterial infections
        - » measles, mumps, influenza, adenovirus, chickenpox, CMV, and Epstein-Barr virus (infectious mononucleosis)
        - » syphilis, *Haemophilus influenzae* and *Mycoplasma pneumoniae*

Paroxysmal Cold Hemoglobinuria (PCH)

- Usually transient disorder
  - Resolves spontaneously with resolution of infectious
  - Transfusions may be needed in severe cases
- Chronic was linked to syphilis

Paroxysmal Cold Hemoglobinuria (PCH)

- Culprit
  - Bi-phasic complement fixing IgG antibody
    - Donath Landsteiner antibody
      - usually anti-P specificity
      - At lower temperatures (< 20° C), IgG binds to RBCs and activates complement
      - At 37° C IgG elutes off leaving only complement coating cells
        - » Cells undergo complement-mediated intravascular lysis

Paroxysmal Cold Hemoglobinuria (PCH)

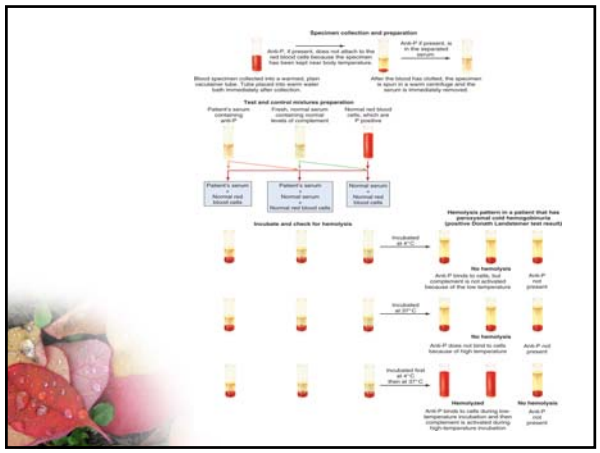
- Clinical findings
  - Hemoglobinuria, Hemoglobinemia, Jaundice
  - ↓ serum complement
  - Erythrophagocytosis
  - Anemia depends on frequency and severity of attack
    - Hgb drops sharply
      - Can ↓ as low as 5 g/dL

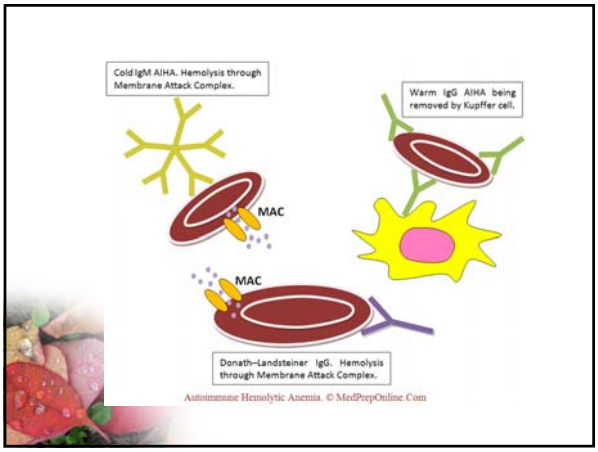
PCH serology

- DAT is positive
  - due to complement
  - Polyspecific
  - Anti-C3b/C3d

PCH serology

- Definitive test is Donath-Landsteiner that detects biphasic properties of the antibody
  - Sample must be collected and maintained during clotting at 37° C
  - Immediately separate serum from red cells to isolate the antibody for processing





Mixed Autoimmune Hemolytic Anemia (MAIHA)

MAIHA

- Simultaneously have two types of autoantibodies causing both WAIHA and CAIHA
  - Rare

MAIHA

- Presence of warm-reacting IgG autoAb and cold-reacting IgM autoAb
  - Both have high titer and ↑ thermal amplitude
    - Reacts at >30° C
  - Mixture of both intravascular (IgM) and extravascular (IgG) hemolysis

MAIHA

- Idiopathic
  - 50% of cases are idiopathic
- Remainder are secondary to:
  - lymphoproliferative disorder
  - SLE
  - HIV

MAIHA

- Treatment may lead to one form of AIHA predominating over the other
  - Most patients respond to corticosteroids without transfusions.

Let's "do" drugs

Drug-Induced HA

- Acquired cause of hemolytic anemia
  - Not all individuals taking the same drug develop HA
- > 125 drugs identified
  - The drug itself does not cause RBC injury
    - Immune response to drug-induced alteration of the RBC

Drug-Induced HA

- Drugs implicated
  - Nonsteroidal anti-inflammatory drugs
  - Diuretics
  - Antineoplastic drugs
  - Antimicrobials
    - 3rd generation cephalosporins
      - Cefotetan and ceftriaxone with the majority of cases
      - Most fatalities
        - » >50% with ceftriaxone
    - Piperacillin

Drug-Induced HA

- Suspected when there is no other explanation for the serologic or hematologic findings and if the patient has a history of taking the drug
- Resolution is withdrawal of the offending drug

Drug-Induced HA

- Mechanisms are controversial and not well defined

Drug-Induced HA

- New "unifying" hypothesis
  - Drug binds to RBC membrane
    - Once bound, antibodies can be produce to react with:
      - Ag specific to the drug
      - Ag that resemble a combination of drug and RBC proteins
      - Ag primarily on the RBC membrane
  - Explains how patients develop more than one type of drug-induced Ab

Drug-Induced HA

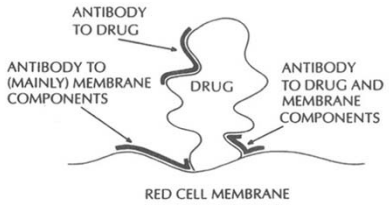


Figure 20-1. Proposed unifying theory of drug-induced antibody reactions (based on a cartoon by Habibi as cited by Garratty[24]). The thicker darker lines represent antigen-binding sites on the F(ab) region of the drug-induced antibody. Drugs (haptens) bind loosely, or firmly, to cell membranes and antibodies may be made to: a) the drug (producing in-vitro reactions typical of a drug adsorption (penicillin-type) reaction); b) membrane components, or mainly membrane components (producing in-vitro reactions typical of autoantibody); or c) part-drug, part-membrane components (producing an in-vitro reaction typical of the so-called immune complex mechanism).[24(p55)]

Drug-Induced HA

- Two major types of drug induced antibodies have been described
  - Drug dependent antibodies
    - requires presence of drug during testing
  - Drug independent antibodies
    - reacts without presence of drug

Drug-Induced HA

- Drug dependent antibodies
  - Drug adsorption mechanism
    - Drug binds covalently to the RBC
      - Ab produces against the drug Ag
      - Ab attaches to the drug-coated RBC which is removed from circulation
        - » Destruction is usually extravascular, complement is seldom involved
    - Drugs implicated
      - Piperacillin and cefotetan
      - IV penicillin
        - » high dose

Drug-Induced HA

- Drug dependent antibodies
  - Drug binds weakly to the RBCS
    - Ab is formed to Ag that consist of both drug and erythrocyte membrane
      - Ab fixes complement
        - » Intravascular hemolysis
    - Drug implicated
      - Ceftriaxone

Drug-Induced HA

- Drug independent antibodies
  - Drug binds to the RBC
    - Ab is produced against primarily RBC Ag
  - Can be due to:
    - Alteration of the RBC membrane by the drug
    - Molecular mimicry

Drug-Induced HA

- Drug independent antibodies
  - Serological reactions indistinguishable from warm AIHA
  - Thought that the RBC membrane is altered by the drug, thus the immune system sees the alteration as a foreign antigen.
    - Historically linked to anti-hypersensitive drug Methyldopa (Aldomet)
    - Today Fludarabine (CLL cancer drug)

Drug-Induced HA

- Drug independent antibodies
  - NIPA, non-immunologic protein adsorption
    - Modification of the RBC membrane that results in noimmunologically absorbed IgA, IgM, IgG, C3
      - No drug antibody is involved
      - β-lactamase inhibitors
        - » Clavulanic acid
        - » Sulbactam
        - » Tazobactam
      - Cefotetan
      - platinum-based chemotherapeutic drugs

Drug Autoantibody serologic and clinical observations

- Positive DAT –
  - Usually due to IgG
  - Polyspecific
  - Anti-IgG

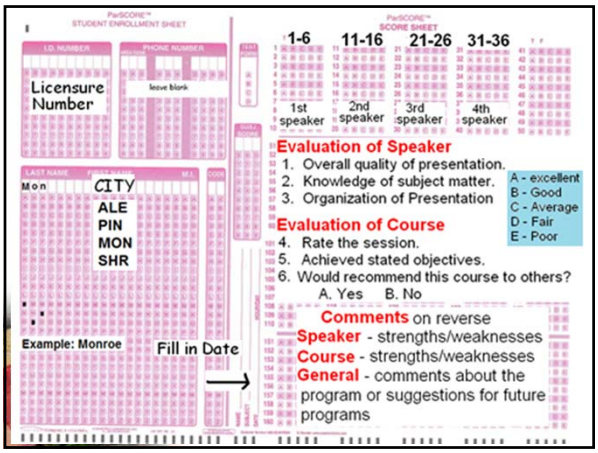
Eluate reacts with all normal cells tested, occasionally showing Rh specificity

Drug Autoantibody serologic and clinical observations

- Autoantibody may “spill over” into serum, causing routine test to be positive
- Alloantibodies are difficult to identify or rule out due to pan-agglutination

Not routine testing

- Most blood banks do not do extensive investigation if patient has positive DAT suspected to be drug related unless there is a hematologic complication
  - Drugs may be continued unless a hemolytic anemia results
    - **If the drug is implicated, STOP giving it immediately**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1st speaker
2nd speaker
3rd speaker
4th speaker
Evaluation of Speaker
1. Overall quality of presentation.
2. Knowledge of subject matter.
3. Organization of Presentation
A - excellent
B - Good
C - Average
D - Fair
E - Poor
Evaluation of Course
4. Rate the session.
5. Achieved stated objectives.
6. Would recommend this course to others?
A. Yes B. No
Comments on reverse
Speaker - strengths/weaknesses
Course - strengths/weaknesses
General - comments about the program or suggestions for future programs