



 **Community Blood Center**

Donath Landsteiner Antibody and Paroxysmal Cold Hemoglobinuria

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Objectives

- List the laboratory findings associated with Paroxysmal Cold Hemoglobinuria.
- Describe what the Donath-Landsteiner antibody is.
- Describe how to perform the Donath-Landsteiner test.

Paroxysmal Cold Hemoglobinuria (PCH)

- First described as distinct disorder in medical literature in 1872
- Specific antibody associated with PCH described by Julius Donath and Karl Landsteiner in 1904
 - Donath-Landsteiner autoantibody (D-L antibody)
- Early 1900s > 90% patients with chronic PCH tested positive for syphilis
 - ~30% showed clinical evidence of syphilis

Paroxysmal Cold Hemoglobinuria

- Named for recurrent complication in late stage of congenital syphilis
 - Paroxysms of severe anemia and hemoglobinuria upon exposure to low temperatures due to massive intravascular hemolysis.



Paroxysmal Cold Hemoglobinuria

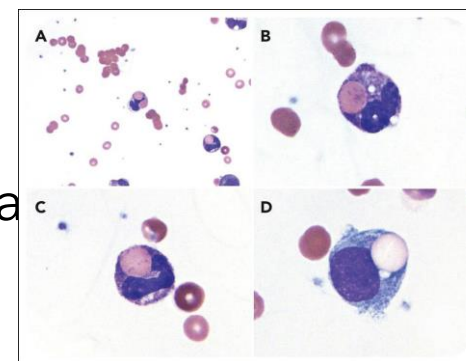
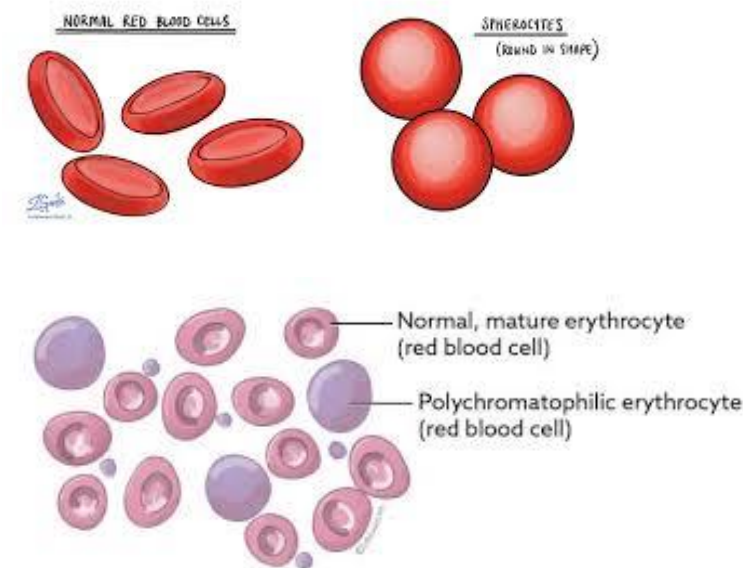
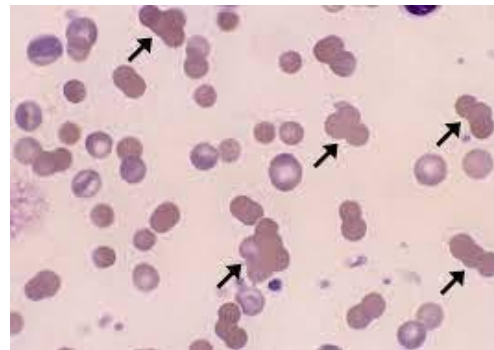
- Now most cases present in pediatric population secondary to viral respiratory infections, vaccinations, infections to common pathogens and autoimmune disorders
- Some studies report 30% - 40% of pediatric patients presenting with autoimmune hemolytic anemia demonstrate complement binding at cold temperature that defines D-L antibody

Paroxysmal Cold Hemoglobinuria

- Clinical presentation:
 - Weakness/dizziness
 - Fever
 - Chills
 - Abdominal, back and or leg pain
 - Pallor
 - Dyspnea
 - Jaundice
 - Hemoglobinuria

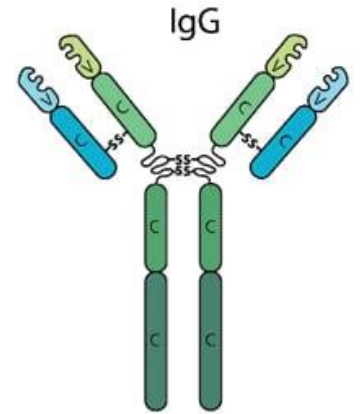
Paroxysmal Cold Hemoglobinuria

- Laboratory findings:
 - Low hemoglobin
 - Increased reticulocyte count
 - Low haptoglobin
 - Elevated LDH
 - Free hemoglobin in serum and urine
- Blood smear:
 - RBC agglutination, spherocytosis, polychromasia
 - Erythrophagocytosis mediated by neutrophils
- DAT positive for C3



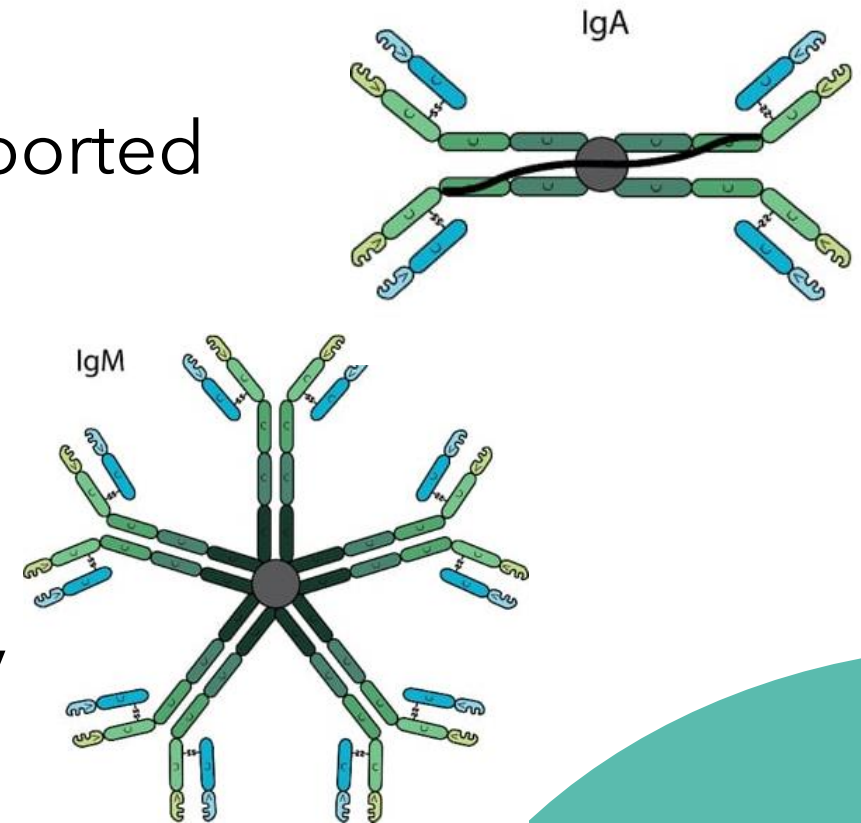
Donath-Landsteiner Antibody

- Classically a polyclonal biphasic IgG autoantibody with specificity for P antigen
 - P antigen is high incidence antigen in Globoside blood group system
- Aby binds to RBC surface antigens at temperatures below core body temperature and fixes early complement components. When blood warms to 37C, the aby dissociates from RBCs and the terminal complement sequence is activated causing RBC lysis



Donath-Landsteiner Antibody

- IgA and IgM antibodies have been reported
- May agglutinate red cells at 4C but rarely to titers > 64
- Rarely reacts in vitro above 4C
 - Hard to detect with normal antibody screening methods; negative antibody screen



The Patient

- **Patient Information:**
 - 3 year old
 - Caucasian
 - Female
- **Diagnosis:**
 - Anemia
- **History:**
 - No history
- **Hospital Reports:**
 - Positive DAT (Poly and complement, negative IgG)
 - Antibody Screen: Negative



11/3 Sample

- Sample is icteric
- Testing:
 - ABO/RH: A, Rh+



Icteric serum

Normal serum

Direct Antiglobulin Test			
Poly	IgG	C'	Saline
2+	(+)	3+	(0)

Cold Antibody Screen						
	I	II	III	Auto	A ₁	A ₂
30' RT	0	0	0	0	0	0
30' 4C	3+ ^s	3+ ^s	3+	3+ ^s	2+ ^s	2+ ^s

() indicates microscopic reading

Acid Eluate

	Rh					Kell		Duffy		Kidd		Lewis		MNS				Eluate
	D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	PEG IAT
1	+	0	+	+	0	0	+	0	+	+	0	0	+	+	+	0	+	(+)
2	+	+	0	0	+	0	+	+	+	+	0	+	0	+	0	+	0	(+)
3	0	0	0	+	+	+	+	0	+	0	+	0	+	0	+	0	+	(+)
4	+	+	0	0	+	0	+	+	0	+	0	0	+	+	0	+	0	(+)
5	+	0	+	+	0	0	+	+	0	0	+	0	+	+	+	0	+	(+)
6	0	0	0	+	+	+	+	0	+	+	+	+	0	0	+	+	+	(+)
AC																		(+)*

*EGA treated AC cells

Antibody Panel

	Rh					Kell		Duffy		Kidd		Lewis		MNS				Plasma Results			
	D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	M	N	S	s	5' RT	PEG IAT	N-HANCE 37C	N-HANCE IAT
1	+	0	+	+	0	0	+	0	+	+	0	0	+	+	+	0	+	0	(+)	0	(0) [✓]
2	+	+	0	0	+	0	+	+	+	+	0	+	0	+	0	+	0	0	(+)	0	(0) [✓]
3	0	0	0	+	+	+	+	0	+	0	+	0	+	0	+	0	+	0	(+)	0	(0) [✓]
4	+	+	0	0	+	0	+	+	0	+	0	0	+	+	0	+	0	0	(+)	0	(0) [✓]
5	+	0	+	+	0	0	+	+	0	0	+	0	+	+	+	0	+	0	(+)	0	(0) [✓]
6	0	0	0	+	+	+	+	0	+	+	+	+	0	0	+	+	+	0	(+)	0	(0) [✓]
AC																		0	(+)	0	(+)

Plasma tested with ficin treated RBCs, negative reactions

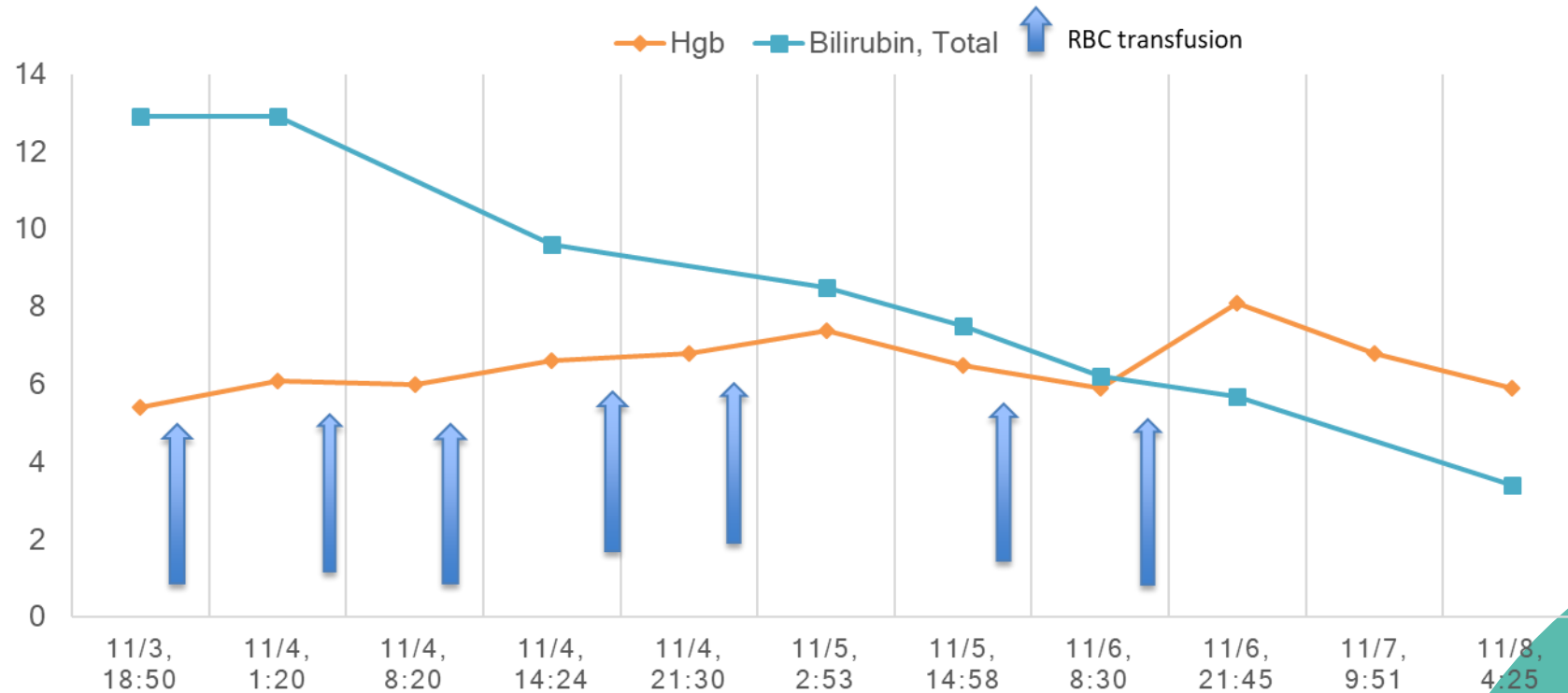
11/3 Sample Conclusions

- Group A, Rh positive patient
- DAT positive Poly, IgG and C
 - C' (3+) was much stronger than IgG (+)
- Serum contained cold autoantibody reactive at 4C
- Eluate contained very weak warm autoantibody
- No antibodies to common red cell antigens detected in plasma - tube tests N-HANCE (LISS) IAT

11/7

- Hospital worried about intravascular hemolysis
- Requests cold antibody titration and thermal amplitude study
 - Tests to determine clinical significance of cold autoantibody
- Transfusion History: 7 transfusions
- Current Hgb 6.8 g/dL
- Drug list: Prednisone, famotidine
- Diagnosis: Upper respiratory infection, mycoplasma infection, autoimmune hemolytic anemia

Lab Values and Transfusions



11/7 Sample

- Sample is warm separated before use
 - Collected and kept warm till separated; placed in 37C waterbath for 2 hours
- Sample is again icteric
- Testing:
 - IRL types patient as A, Rh positive
 - DAT is again positive

Direct Antiglobulin Test			
Poly	IgG	C'	Saline
1+	(+)	3+	(0)

Cold Autoantibody Significance

- Cold Titer
 - Indicates strength of antibody reactivity at 4C
 - Titer of 4 against I-positive cells
- Thermal Amplitude
 - Indicates the thermal range of antibody reactivity
 - No reactivity at 30C or 37C
 - No hemolysis or agglutination detected
- Cold autoantibody not clinically significant

Patient Continues to Hemolyze

- Physician is very confused why patient still hemolyzing
- Transfusion History: additional unit given since workup on 11/7
- Current Hgb 5.9 g/dL
- Drug list: Methylprednisolone, famotidine, acetaminophen, lidocaine, ondansetron, polyethylene glycol
- Hospital DAT getting positive results with poly and complement only.
 - Poly 3+, IS complement 3+, Gel IgG 0
 - Reference lab getting microscopic reactivity with IgG in tube

Donath-Landsteiner Test

- Dr. orders Donath-Landsteiner test 11-8
- Per John Judd, Methods of Immunohematology, the test is not really indicated as "patient should not have detectable autoantibody in eluate or plasma"
 - Patient has weak warm auto in eluate
 - DAT weakly positive with IgG
- Recent infection, 3-year-old child, strongly positive DAT with complement

Donath-Landsteiner Test Results

A Group (30' 4C → 60' 37C)

B Group (90' 4C)

C Group (90' 37C)

Tube 1:

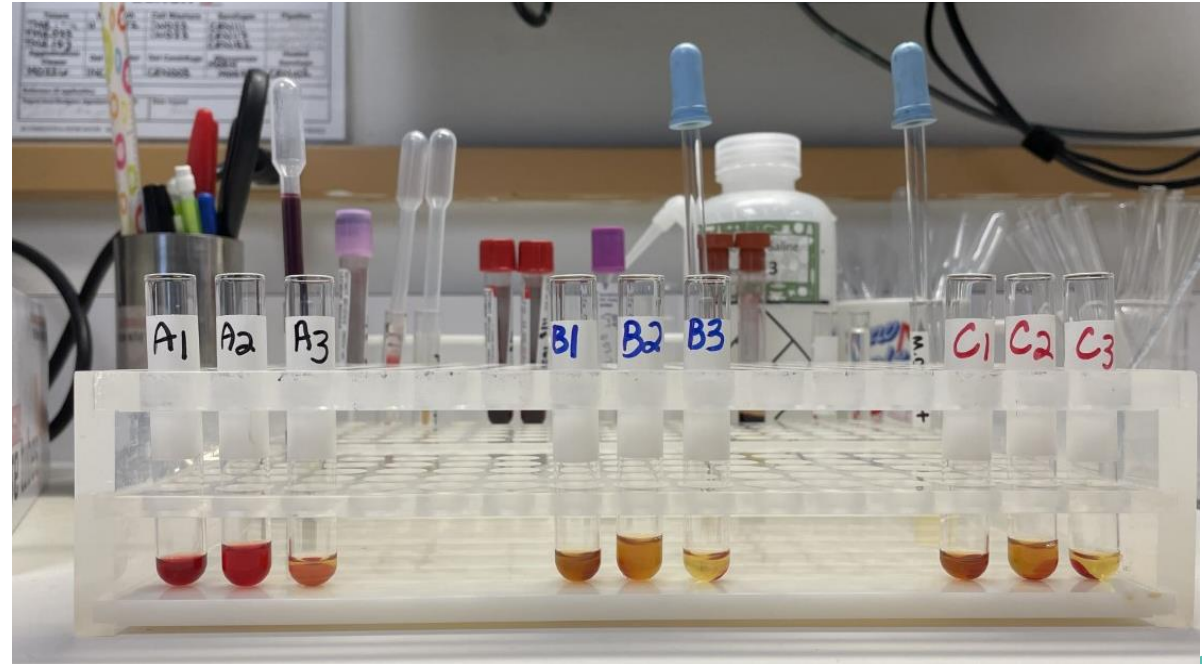
- Patient serum
- P pos cells

Tube 2:

- Patient serum
- Fresh source of complement
- P pos cells

Tube 3:

- Fresh source of complement
- P pos cells



Positive Donath-Landsteiner

Tubes A1 and A2 are positive (hemolysis noted)

Paroxysmal Cold Hemoglobinuria Treatment

- Most cases PCH are transient and self-limited and resolve spontaneously
- Largely supportive and directed toward treating anemia and prevention of complications due to intravascular hemolysis
- Treatment of relevant underlying causes/infections
- Patient and environment should be kept warm
- Corticosteroids in severe or refractory disease
- Folic acid

Types of Donath-Landsteiner Tests

- Direct D-L test:
 - Use 2 tubes of whole blood from patient
 - One tube incubated in ice bath for 1 hour and then at 37C for 30 minutes
 - 2nd tube incubated at 37C for 1.5 hours
 - Samples centrifuged and observed for hemolysis
- **Indirect D-L test:**
 - Previously discussed
- Enzyme-treated indirect D-L test:
 - Use enzyme treated O RBCs and test by indirect D-L test

Types of Donath-Landsteiner Tests

- Two stage indirect D-L test:
 - Patient's serum incubated with O red blood cells at 0C for 30 min. then centrifuged and replaced with freshly collected pooled normal sera and incubated at 37C for 1 hour
- Indirect antiglobulin test:
 - IAT performed with anti-IgG after low temp incubation
- Indirect D-L test is used by most IRLs that do D-L testing
- Direct D-L, enzyme treated indirect D-L, two stage indirect D-L and indirect antiglobulin test are more sensitive and each has its own limitations

Donath-Landsteiner Test Limitations

- Samples need to be kept at 37C until serum is separated from cells.
- False positive results:
 - Patient has cold autoantibody
 - Monophasic IgM autoantibodies with broad thermal amplitude

Donath-Landsteiner Test Limitations

- False negative results:
 - Autoadsorption of anti-P at low temps if sample not kept at 37C
 - Fresh donor serum is not added as source of complement
 - Low D-L antibody titer
 - Inhibition of D-L antibody by presence of globoside and glycosphingolipids in fresh donor serum
 - Resistance of RBC lysis due to C3dg coating RBC membrane and blocking of C3b binding which prevents activation of complement complex and lysis of RBCs
 - Transience of autoantibody

Objectives

- List the laboratory findings associated with Paroxysmal Cold Hemoglobinuria.
- Describe what the Donath-Landsteiner antibody is.
- Describe how to perform the Donath-Landsteiner test.

Test Your Knowledge

- What are the typical laboratory findings in patients with intravascular hemolysis?
 - A. Raised bilirubin, raised haptoglobin, low hemoglobin (Hb), raised lactate dehydrogenase (LDH).
 - B. Raised bilirubin, low haptoglobin, low Hb, raised LDH, hemoglobinuria
 - C. Low bilirubin, low haptoglobin, low Hb, raised LDH, hemoglobinuria
 - D. Low bilirubin, raised haptoglobin, low Hb, raised LDH

Test Your Knowledge

- Direct antiglobulin test (DAT) testing in paroxysmal cold hemoglobinuria (PCH) is likely to reveal the following:
 - A. DAT testing: IgG positive, C3 negative
 - B. DAT testing: IgG negative, C3 negative
 - C. DAT testing: IgG negative or positive, C3 positive

Test Your Knowledge

- Donath-Landsteiner (D-L) antibody is classically a biphasic hemolysin. What does this mean with regards to PCH?
 - A. D-L antibody fixes complement to red blood (RBCs) at low temperatures (<37C) and hemolysis is triggered when the blood is warmed to 37C
 - B. D-L antibody fixes complement to RBCs at 37C and triggers hemolysis.
 - C. D-L antibody fixes complement to RBCs at low temperatures (<37C) and triggers hemolysis.
 - D. D-L antibody fixes complement of RBCs at 37C and hemolysis is triggered when the blood is cooled to 4C.

Test Your Knowledge

- There is little evidence to suggest that steroids are effective in the management of PCH.

True

False

Test Your Knowledge

- Performing the D-L test is difficult. What must clinicians remember to do if they want the test to be successful?
 - A. Maintain temperature of the serum sample at 37C until the test is carried out.
 - B. Take the blood test in the evening.
 - C. Maintain temperature of the serum sample at 4C until the test can be carried out.
 - D. Doesn't matter what temperature the serum sample is kept at on transfer to the lab

References

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Special Thanks



Thank You