When Selfies Go Bad!

Decoding Autoantibodies and Autoimmune Hemolytic Anemia

D. Joe Chaffin, MD
April 17, 2017

Outline

- Background
- **Warm autoantibodies and WAIHA**
- Cold autoantibodies and CAD
- Paroxysmal Cold Hemoglobinuria
- Mixed Warm and Cold AIHA

Note

Autoimmune:

Antibodies produced against substances present in the person's own body

- Hemolytic anemia categories:
  - Autoimmune Hemolytic Anemia
  - ~~Alloimmune Hemolytic Anemia~~
  - ~~Drug-induced Hemolytic Anemia~~
  - ~~Mechanical (non-immune) hemolysis~~
  - ~~Congenital (structural) hemolysis~~

Case

- 72 year old female admitted with weakness and anemia

Initial Labs:

WBC	**62.5/µL**
Hemoglobin	**6.9 g/dL**
Smear	Spherocytes
Total bilirubin	8.5 g/dL

- Transfusion of 1 unit RBCs requested

Case

Antibody Screen:

		Rh							Kell						Kidd		Duffy		Lewis		MNS				P	Luth.		Results	
Cell	Rh-hr	D	C	E	c	e	f	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	Le^a	Le^b	M	N	S	s	P1	Lu^a	Lu^b		Gel	
1	R1R1	+	+	0	0	+	0	0	+	+	0	+	0	+	0	+	+	0	0	+	+	+	0	+	+	0	+	1	4+	
2	R2R2	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	+	+	0	0	+	2	4+	

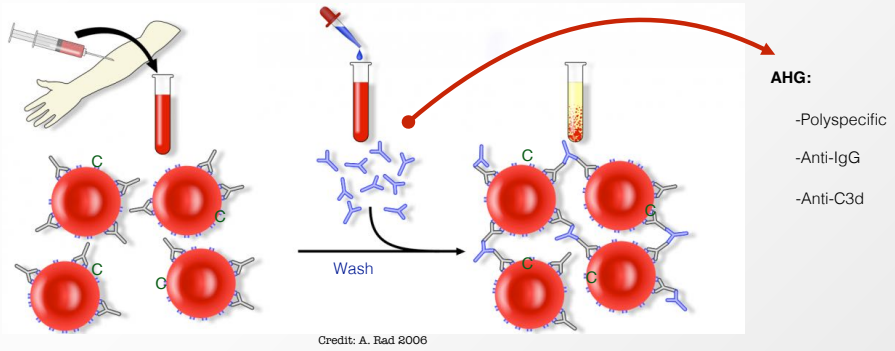
Case

		Rh							Kell						Kidd		Duffy		Lewis		MNS				P	Luth.		Results	
Cell	Rh-hr	D	C	E	c	e	f	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	Le^a	Le^b	M	N	S	s	P1	Lu^a	Lu^b		Gel	
1	R1R1	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	0	0	+	+	0	+	1	3+	
2	R1R1	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	2	4+	
3	R2R2	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	3	4+	
4	R0r	+	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	+	+	0	+	+	0	+	4	3+	
5	r'r	0	+	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	0	+	0	+	+	0	+	5	3+	
6	r''r	0	0	+	+	+	+	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	0	+	0	+	6	4+	
7	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	+	0	+	7	4+	
8	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	8	4+	
9	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	9	4+	
10	rr	0	0	0	+																							10	3+	
11	R1R1	+	+	0	0																							11	4+	
																												AC	4+	

DAT	Polyspecific	Anti-IgG	Anti-C3
	4+	3+	1+

Modified from Dr. Dan Ambruso	WAIHA	CAD	PCH	Mixed
Frequency	70-80%	18%	<2%	Rare
Peak Age	60's	60's	Children	Older
DAT (poly)	Positive	Positive	Us. Positive	Positive
DAT (IgG)	Positive (90%)	Negative	Negative	Positive
DAT (C3)	+/–	Positive (90%)	Positive	Positive
Antibody	IgG	IgM	IgG	IgG & IgM
Temp.	37°C	4°C	4°C → 37°C	4-37°C
Target	Rh-related	I (rarely i)	P	Rh and I
Transfusion	Auto/allo adsorp, Matching	Autoadsorb, prewarm	P-neg not necessary	Avoid if possible; As for WAIHA
Cause	Malignancy, Autoimmune, HIV	Lymphoprolif. d/o, Infx Mono, Mycoplasma	Viral infx, syphilis	SLE, drugs
Treatment	Block spleen (steroids, drugs, surgery)	Symptomatic	Supportive	Steroids

Direct Antiglobulin Test



Credit: A. Rad 2006

DAT: *In-vivo* coating of RBCs

DAT Pos ≠ AIHA

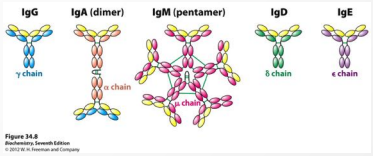
- VERY nonspecific
- Up to 15+% of hospital patients are DAT+
- Only important with hemolysis

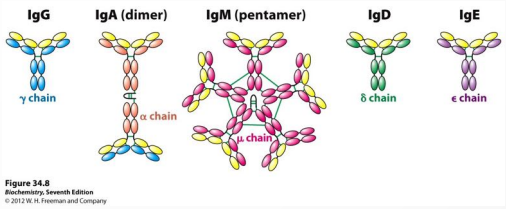
Antibodies ≠ AIHA

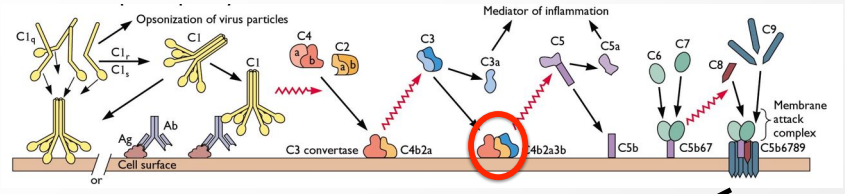
- WAAs not uncommon
- WAA + hemolysis IS uncommon!
- With evidence and +DAT, may mean AIHA

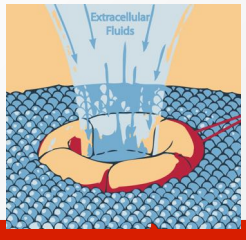
Warm Autoantibodies

- React best at 37°C
- Almost always IgG
  - Uncommonly IgA or IgM
- Ab binds but doesn't fully fix complement

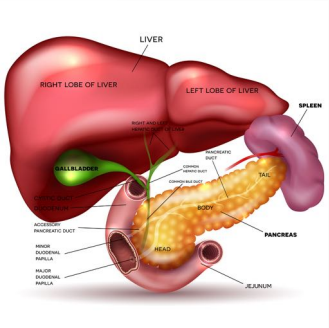


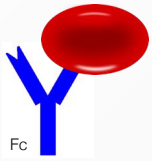


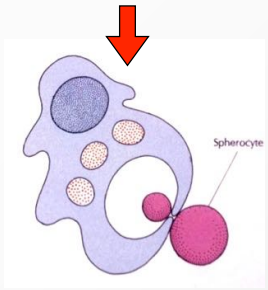


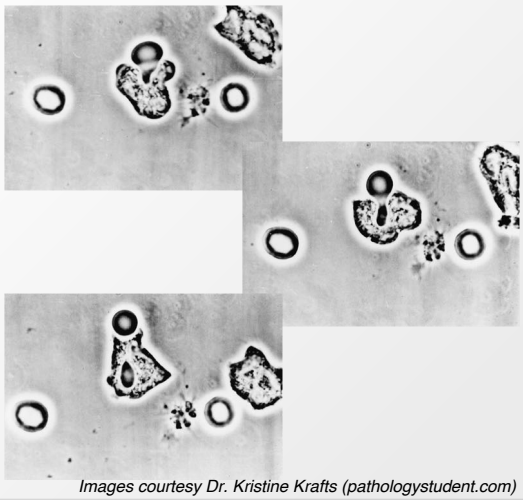


Extravascular Hemolysis









Images courtesy Dr. Kristine Krafts (pathologystudent.com)

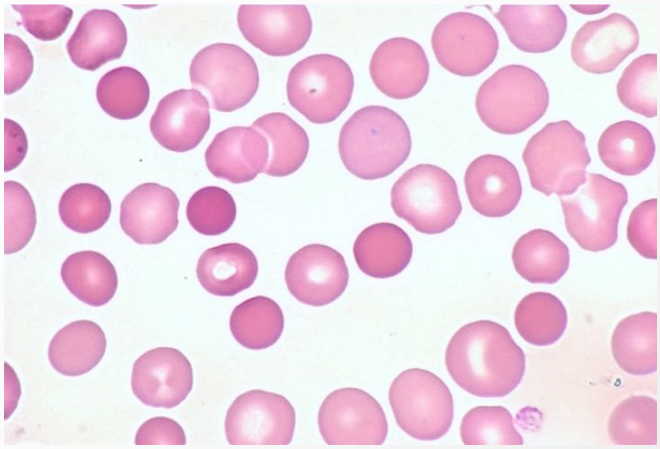


Image courtesy Dr. Kristine Krafts (pathologystudent.com)

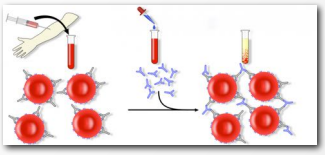
WAIHA

- Older patients (7th-8th decade)
  - 50% idiopathic / 50% secondary
    - ✓Malignancies (especially lymphoproliferative, like CLL)
    - ✓Autoimmune disorders (Lupus, RA)
    - ✓HIV
  - May precede diagnosis (esp. Lupus, CLL)

Why?

- Theories abound:
  - Altered inflammatory pathways (decreased tolerance)
  - Pathogens induce cross-reacting Abs
  - Immune complexes; RBCs just bystanders
- NOT malignant clone from CLL/lymphoma

DAT in WAIHA



- Pattern:
  - Positive with polyspecific AHG: 90+%
    - Anti-IgG + anti-C3: 67%
    - Anti-IgG only: 20%
    - Anti-C3 only: 13%

DAT	Polyspecific	Anti-IgG	Anti-C3
WAIHA	POS	POS	NEG

Most common DAT result in WAIHA

DAT in WAIHA

- DAT **negative**: Up to 10%
  - Low antibody coating (<100-300/cell)
  - Other antibodies (IgA, IgM)

DAT	Polyspecific	Anti-IgG	Anti-C3
WAIHA	NEG	NEG	NEG

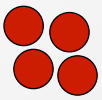
DAT-negative result in WAIHA

WAIHA Findings

- Pallor, weakness, fatigue, dyspnea
- Lab evidence of hemolysis:

Bilirubin
LDH

Hemoglobin
Haptoglobin



- IgG antibody with broad reactivity
  - May show "relative specificity"
    - ✓Commonly e, c, or E

Things to Know

- Get on the phone!!!
  - Has patient been transfused?
    - ✓If yes, how long ago?
  - Is transfusion necessary?

How Does it Look?

Generally, ABO/RhD testing are fine, but…

Antibody screen (liquid):

		Rh							Kell						Kidd		Duffy		Lewis		MNS				P	Luth.		Results	
Cell	Rh-hr	D	C	E	c	e	f	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	Le^a	Le^b	M	N	S	s	P1	Lu^a	Lu^b		IS	AHG
1	R1R1	+	+	0	0	+	0	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	+	0	+	1	0	3+
2	R2R2	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	+	0	0	+	+	0	+	+	+	0	+	2	0	3+
3	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	0	+	3	0	3+

Antibody Screen (gel):

		Rh							Kell						Kidd		Duffy		Lewis		MNS				P	Luth.		Results	
Cell	Rh-hr	D	C	E	c	e	f	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	Le^a	Le^b	M	N	S	s	P1	Lu^a	Lu^b		Gel	
1	R1R1	+	+	0	0	+	0	0	+	+	0	+	0	+	0	+	+	0	0	+	+	+	0	+	+	0	+	1	4+	
2	R2R2	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	+	0	+	+	+	+	+	0	0	2	4+	

How Does it Look?

		Rh							Kell						Kidd		Duffy		Lewis		MNS				P	Luth.		Results		
Cell	Rh-hr	D	C	E	c	e	f	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	Le^a	Le^b	M	N	S	s	P1	Lu^a	Lu^b		Gel	IS	AHG
1	R1R1	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	0	0	+	+	0	+	1	4+	0	3+
2	R1R1	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	2	4+	0	3+
3	R2R2	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	3	4+	0	3+
4	R0r	+	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	+	+	0	+	+	0	+	4	4+	0	3+
5	r'r	0	+	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	0	+	0	+	+	0	+	5	4+	0	3+
6	r''r	0	0	+	+	+	+	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	0	+	0	+	6	4+	0	3+
7	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	+	0	+	7	4+	0	3+
8	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	8	4+	0	3+
9	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	9	4+	0	3+
10	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	10	4+	0	3+
11	R1R1	+	+	0	0	+	0	0	+	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	11	4+	0	3+
																												AC	4+	0	0✓

How Does it Look?

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Cell	Rh-hr	D	C	E	c	e	f	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	Le^a	Le^b	M	N	S	s	P1	Lu^a	Lu^b		Gel		IS	AHG
1	R1R1	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	0	0	+	+	0	+	1	4+		0	3+
2	R1R1	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	2	4+		0	3+
3	R2R2	+	0	+	+	0	+	0	+	+	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	3	0		0	0
4	R0r	+	0	0	+	+	+	0	+	+	0	+	0	+	+	0	0	0	0	0	+	+	0	+	+	0	+	4	4+		0	3+
5	r'r	0	+	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	0	+	0	+	+	0	+	5	4+		0	3+
6	r"r	0	0	+	+	+	+	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	0	+	0	+	6	4+		0	3+
7	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	+	0	+	7	4+		0	3+
8	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	8	4+		0	3+
9	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	9	4+		0	3+
10	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	10	4+		0	3+
11	R1R1	+	+	0	0	+	0	0	+	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	11	4+		0	3+
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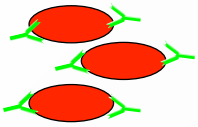
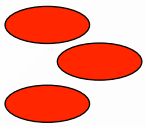
1	R1R1	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	0	0	+	+	0	+	1	1+			
2	R1R1	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	2	w+			
3	R2R2	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	3	2+			
4	R0r	+	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	+	+	0	+	+	0	+	4	1+			
5	r'r	0	+	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	0	+	0	+	+	0	+	5	w+			
6	r"r	0	0	+	+	+	+	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	0	+	0	+	6	w+			
7	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	+	0	+	7	0			
8	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	8	1+			
9	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	9	1+			
10	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	10	w+			
11	R1R1	+	+	0	0	+	0	0	+	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	11	1+			
																												AC	1+			



Elution

		Rh							Kell						Kidd		Duffy		Lewis		MNS				P	Luth.		Results				
Cell	Rh-hr	D	C	E	c	e	f	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	Le^a	Le^b	M	N	S	s	P1	Lu^a	Lu^b		Gel		Eluate	
1	R1R1	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	0	0	+	+	0	+	1	3+		4+	
2	R1R1	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	2	3+		4+	
3	R2R2	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	3	3+		4+	
4	R0r	+	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	+	+	0	+	+	0	+	4	3+		4+	
5	r'r	0	+	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	0	+	0	+	+	0	+	5	3+		4+	
6	r''r	0	0	+	+	+	+	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	0	+	0	+	6	3+		4+	
7	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	+	0	+	7	3+		4+	
8	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	8	3+		4+	
9	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	9	3+		4+	
10	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	10	3+		4+	
11	R1R1	+	+	0	0	+	0	0	+	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	11	3+		4+	
																												AC	3+			

Not terribly helpful



Elution

		Rh							Kell						Kidd		Duffy		Lewis		MNS				P	Luth.		Results				
Cell	Rh-hr	D	C	E	c	e	f	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	Le^a	Le^b	M	N	S	s	P1	Lu^a	Lu^b		Gel		Eluate	
1	R1R1	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	0	0	+	+	0	+	1	1+		3+	
2	R1R1	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	2	w+		3+	
3	R2R2	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	3	2+		3+	
4	R0r	+	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	+	+	0	+	+	0	+	4	1+		4+	
5	r'r	0	+	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	0	+	0	+	+	0	+	5	w+		3+	
6	r''r	0	0	+	+	+	+	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	0	+	0	+	6	w+		3+	
7	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	+	0	+	7	0		4+	
8	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	8	1+		3+	
9	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	9	1+		3+	
10	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	10	w+		3+	
11	R1R1	+	+	0	0	+	0	0	+	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	11	1+		3+	
																												AC	1+			

THIS one is helpful!

The Big Question:

Will we see THIS?

		Rh							Kell						Kidd		Duffy		Lewis		MNS				P	Luth.		Result				
Cell	Rh-hr	D	C	E	c	e	f	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	Le^a	Le^b	M	N	S	s	P1	Lu^a	Lu^b		Gel				
1	R1R1	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	0	0	+	+	0	+	1	4+	2+			
2	R1R1	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	2	4+	2+			
3	R2R2	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	3	4+	3+			
4	R0r	+	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	+	+	0	+	+	0	+	4	4+	3+			
5	r'r	0	+	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	0	+	0	+	+	0	+	5	4+	0			
6	r"r	0	0	+	+	+	+	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	0	+	0	+	6	4+	0			
7	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	+	0	+	7	4+	0			
0	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	8	4+	0			
0	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	9	4+	0			
10	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	10	4+	0			
11	R1R1	+	+	0	0	+	0	0	+	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	11	4+	3+			
																												AC	4+				

If we get rid of THIS

Alloantibody Evaluation

- Dilution approach (Yikes!)
- Warm autoadsorption
- Allogeneic adsorption
- Phenotypically matched

Dilution Approach (1:5)

- Dilute the auto-, leave the alloantibody
  - Dangerous assumption!
- Unreliable (Leger/Garratty, *Transf.* 1999)
  - 27% of alloabs missed
- Emergency strategy

Alloantibody Evaluation

- Dilution approach (unreliable)
- Warm autoadsorption
- Allogeneic adsorption
- Phenotypically matched

		Rh							Kell						Kidd		Duffy		Lewis		MNS				P	Luth.		Results		
Cell	Rh-hr	D	C	E	c	e	f	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	Le^a	Le^b	M	N	S	s	P1	Lu^a	Lu^b		Gel	
1	R1R1	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	0	0	+	+	0	+	1	4+	
2	R1R1	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	2	4+	
3	R2R2	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	3	4+	
4	R0r	+	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	+	+	0	+	+	0	+	4	4+	
5	r'r	0	+	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	0	+	0	+	+	0	+	5	4+	
6	r"r	0	0	+	+	+	+	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	0	+	0	+	6	4+	
7	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	+	0	+	7	4+	
8	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	8	4+	
9	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	9	4+	
10	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	10	4+	
11	R1R1	+	+	0	0	+	0	0	+	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	11	4+	
																												AC	4+	

DAT 3+ Poly/IgG, No recent transfusions

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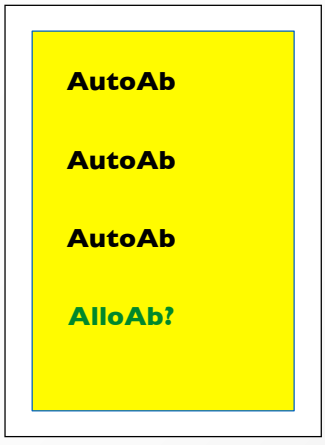
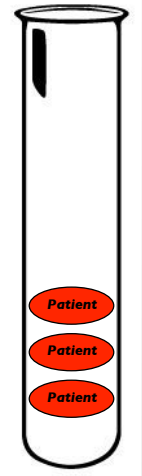
Warm Autoadsorption



"Adsorbed Serum"



Patient RBCs

1. Present?
2. What is it?

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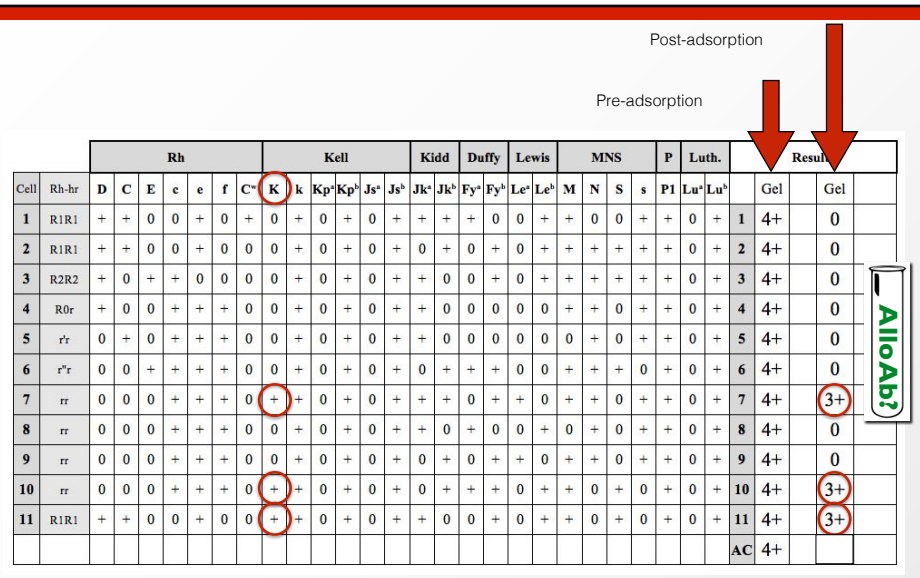


		Rh							Kell						Kidd		Duffy		Lewis		MNS				P	Luth.		Result			
Cell	Rh-hr	D	C	E	c	e	f	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	Le^a	Le^b	M	N	S	s	P1	Lu^a	Lu^b		Gel		Gel
1	R1R1	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	0	0	+	+	0	+	1	4+		0
2	R1R1	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	2	4+		0
3	R2R2	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	3	4+		0
4	R0r	+	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	+	+	0	+	+	0	+	4	4+		0
5	r'r	0	+	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	0	+	0	+	+	0	+	5	4+		0
6	r"r	0	0	+	+	+	+	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	0	+	0	+	6	4+		0
7	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	+	0	+	7	4+		3+
8	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	8	4+		0
9	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	9	4+		0
10	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	0	+	10	4+		3+
11	R1R1	+	+	0	0	+	0	0	+	+	0	+	0	+	+	0	0	+	0	+	+	+	0	+	+	0	+	11	4+		3+
																											AC	4+		

1. **Warm Autoantibody**
2. **Allo-anti-K**

Ruling Out Alloantibodies

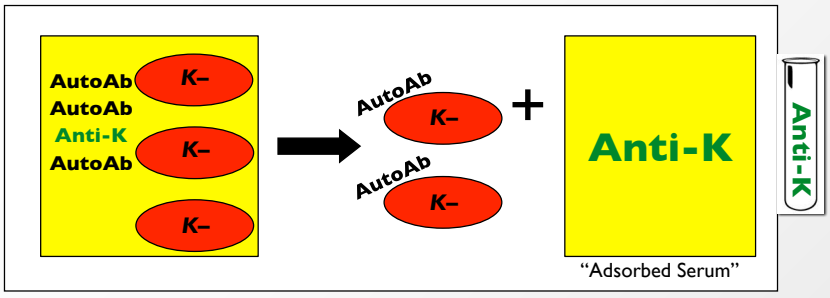
- Dilution approach (unreliable)
- Warm autoadsorption
- Allogeneic adsorption
- Phenotypically matched

		Rh							Kell						Kidd		Duffy		Lewis		MNS				P	Luth.		Results	
Cell	Rh-hr	D	C	E	c	e	f	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Jk^a	Jk^b	Fy^a	Fy^b	Le^a	Le^b	M	N	S	s	P1	Lu^a	Lu^b		Gel	
1	R1R1	+	+	0	0	+	0	+	0	+	0	+	0	+	+	+	+	0	0	+	+	0	0	+	+	0	+	1	4+	
2	R1R1	+	+	0	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	+	+	+	+	+	0	+	2	4+	
3	R2R2	+	0	+	+	0	0	0	0	+	0	+	0	+	+	0	0	+	0	+	+	+	+	+	+	0	+	3	4+	
4	R0r	+	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	+	+	0	+	+	0	+	4	4+	
5	r'r	0	+	0	+	+	+	0	0	+	0	+	0	+	+	0	0	0	0	0	0	+	0	+	+	0	+	5	4+	
6	r''r	0	0	+	+	+	+	0	0	+	0	+	0	+	0	+	+	+	0	0	+	+	+	0	+	0	+	6	4+	
7	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	+	0	+	7	4+	
8	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	+	0	+	+	0	+	8	4+	
9	rr	0	0	0	+	+	+	0	0	+	0	+	0	+	0	+	0	+	+	0	+	+	0	+	+	0	+	9	4+	
10	rr	0	0	0	+	+	+	0	+	+	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	10	4+	
11	R1R1	+	+	0	0	+	0	0	+	+	0	+	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	11	4+	
																												AC	4+	

DAT 3+ Poly/IgG, Transfused 2 U RBC elsewhere last week

Allogeneic Adsorption

- Non-self RBCs of various phenotypes



Classic series: R_1R_1, R_2R_2, rr ("triple adsorption")

Alloadsorption Issues

- Time and energy-intensive
- Choosing RBCs can be tough
- With high frequency antibody, may be impossible to get antigen-neg RBCs

Ruling Out Alloantibodies

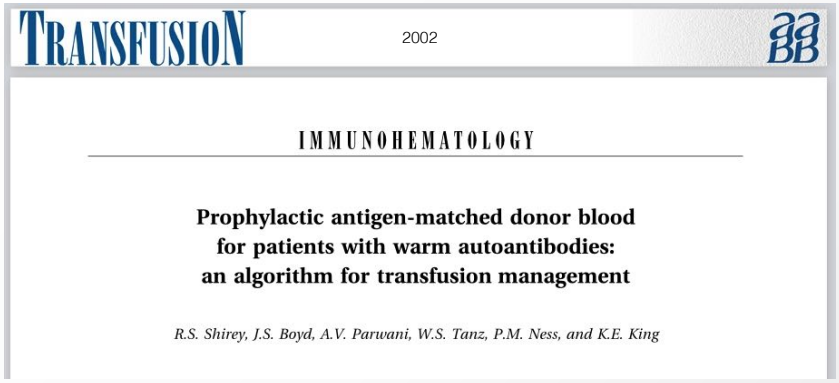
- Dilution approach (unreliable)
- Warm autoadsorption
- Allogeneic adsorption
- Phenotypically matched

Phenotype Matching

- "What alloantibodies COULD this patient make?"
- Preventative and prophylactic
- Serologic (meh) vs molecular (yay)
  - More on that shortly…

Matching

- How far to go?
- WAA? 32% chance of alloimmunization
- Comparable to risk in sickle cell!
- These patients deserve special attention



TRANSFUSION 2002

IMMUNOHEMATOLOGY

Prophylactic antigen-matched donor blood for patients with warm autoantibodies: an algorithm for transfusion management

R.S. Shirey, J.S. Boyd, A.V. Parwani, W.S. Tanz, P.M. Ness, and K.E. King

-Match all WAA for C, c, E, e, K, Jk^a, Jk^b, Fy^a, Fy^b, S, and s
-Accompanying editorial by Dr. Garratty (nice idea)

"Specificities"
-Ignore relatives
-Honor absolutes
-Avoid silly risk…
-Auto-anti-e in D neg

My Beliefs

Urgent patient need "trumps" all rules

Appropriate test for alloantibodies

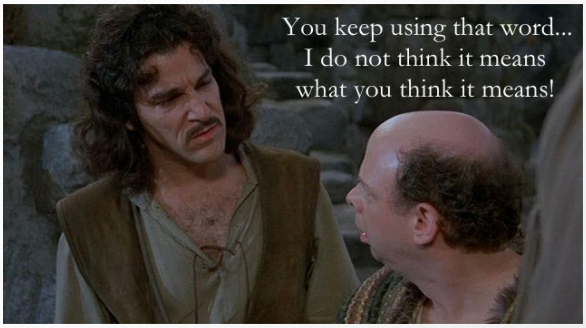
Full Phenotype or Genotype before Tx

No Alloantibodies → Match for **Rh** and **K**

Alloantibodies → Match for **Rh, K, Jk, Fy, Ss**

Let's Talk About…

Least Incompatible!



Even if you do it right…

1	4+
2	3+
3	3+
4	2+

NO evidence number 4 is REALLY safer!!

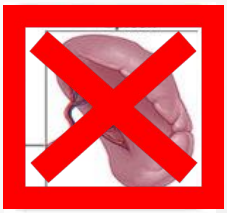
"And, doctor, we will choose the LEAST INCOMPATIBLE unit!"

Talk to your Providers

- CALM THEM DOWN!
- HUGE knowledge gap in non-Heme-Onc providers
- Risk vs. benefit decision
- "Appropriately tested blood"
- Questions:
  - "Will my patient hemolyze the blood?"
  - "Can I really give incompatible blood?"

WAIHA Treatment

- "Block the Spleen"
  - Corticosteroids
  - Stronger immunosuppressants (rituximab, IVIG)
  - Splenectomy if necessary



Case

- 48 year old male recently diagnosed with pneumonia due to *Mycoplasma*.
- Felt great pain in fingers and toes while walking in the snow; now has fatigue and pallor

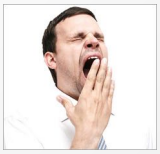
Initial Labs (re-run after first sample "clotted"):

Hemoglobin	**6.3 g/dL**
Smear	Spherocytes
LDH	500 U/L
Total bilirubin	1.9 g/dL

Cold Autoantibodies

- Antibodies against self antigens that react best at 4°C (generally < 30°C when benign)
- MUCH more common than WAA!
- Appearances matter!

	N	2	4	8	16	32	64	128	256	512	1024	2048	4096
37C	0	0	0	0	0	0	0	0	0	0	0	0	0
30C	0	0	0	0	0	0	0	0	0	0	0	0	0
20C	1+	0	0	0	0	0	0	0	0	0	0	0	0
4C	4+	4+	3+	1+	0	0	0	0	0	0	0	0	0



Low Titer, Low Thermal Amplitude = Benign Cold Autoantibody

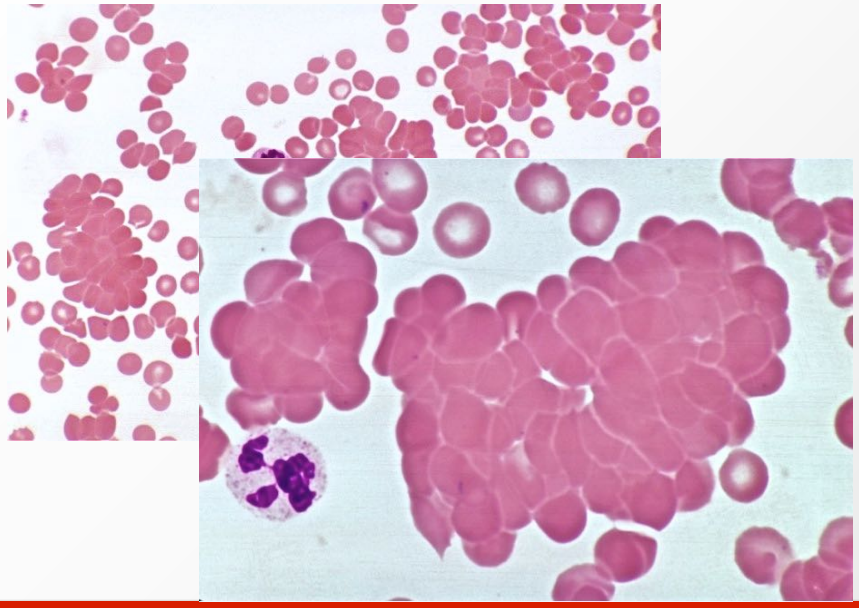
	N	2	4	8	16	32	64	128	256	512	1024	2048	4096
37C	1+	1+	0	0	0	0	0	0	0	0	0	0	0
30C	2+	2+	2+	1+	1+	0	0	0	0	0	0	0	0
20C	2+	2+	2+	2+	2+	1+	1+	1+	0	0	0	0	0
4C	4+	4+	4+	4+	4+	4+	3+	3+	3+	2+	1+	1+	0

High Titer, High Thermal Amplitude = Pathologic Cold Autoantibody

Cold Autoantibodies

- A real nuisance
- Cold "agglutinin" due to in-vitro effect
  - Routine lab testing
  - ABO discrepancies
- DAT+ with anti-C3 not anti-IgG

DAT	Polyspecific	Anti-IgG	Anti-C3
	POS	NEG	POS



BloodBankGuy

Images courtesy Dr. Kristine Krafts (pathologystudent.com)

BBGuy.org

Dealing with CAAs

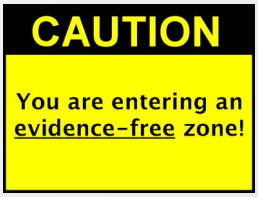
- Unhappy cold often happier warm
- Cold adsorption may be needed with stronger antibodies
  - RESt
  - Cold autoadsorption

BloodBankGuy

BBGuy.org

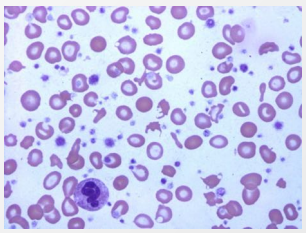
"What About Pre-Warm?"

- Reduces or eliminates reactions of benign cold autoantibodies
  - Can then detect alloantibodies
- BUT, it CAN make significant antibodies undetectable (e.g., anti-Vel)
- Only use if workup is thorough



Cold Agglutinin Disease

- Hemolysis with cold-reactive autoantibody
- Similar lab findings to WAIHA
  - May see schistocytes
- Less common than WAIHA (18%)
- Types:
  - Idiopathic
  - Secondary



Idiopathic CAIHA

- Older, female patients
- Chronic hemolysis worse in cold
  - Extremity temp drops to 28°C!
- Antibody is IgM vs. I
- Transfusion: r/o allos; blood warmer
- Treat supportively (avoid cold, move to Phoenix)

Secondary AIHA

- *Mycoplasma pneumonia* infection
  - IgM auto-anti-I (high titer)
  - Intravascular hemolysis, substantial
  - Resolves after infection
- Infectious mononucleosis
  - IgM auto-anti-i (may be high titer)

Case

- 5 year old female admitted with fever, malaise, and "cola-colored" urine.
- 2 weeks ago: Diagnosed with viral URI, but had recovered

Initial Labs:

Hemoglobin	**5.8 g/dL**
Smear	Spherocytes, rare PMN erythrophagocytosis
LDH	**985 U/L**
Total bilirubin	2.8 g/dL

Case

- Antibody screen (tubes LISS): 1+ at IS, 0 at 37/AHG)
- Antibody panel: Similar appearance

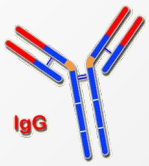
DAT	Polyspecific	Anti-IgG	Anti-C3
	4+	**0**	**3+**

Paroxysmal Cold Hemoglobinuria

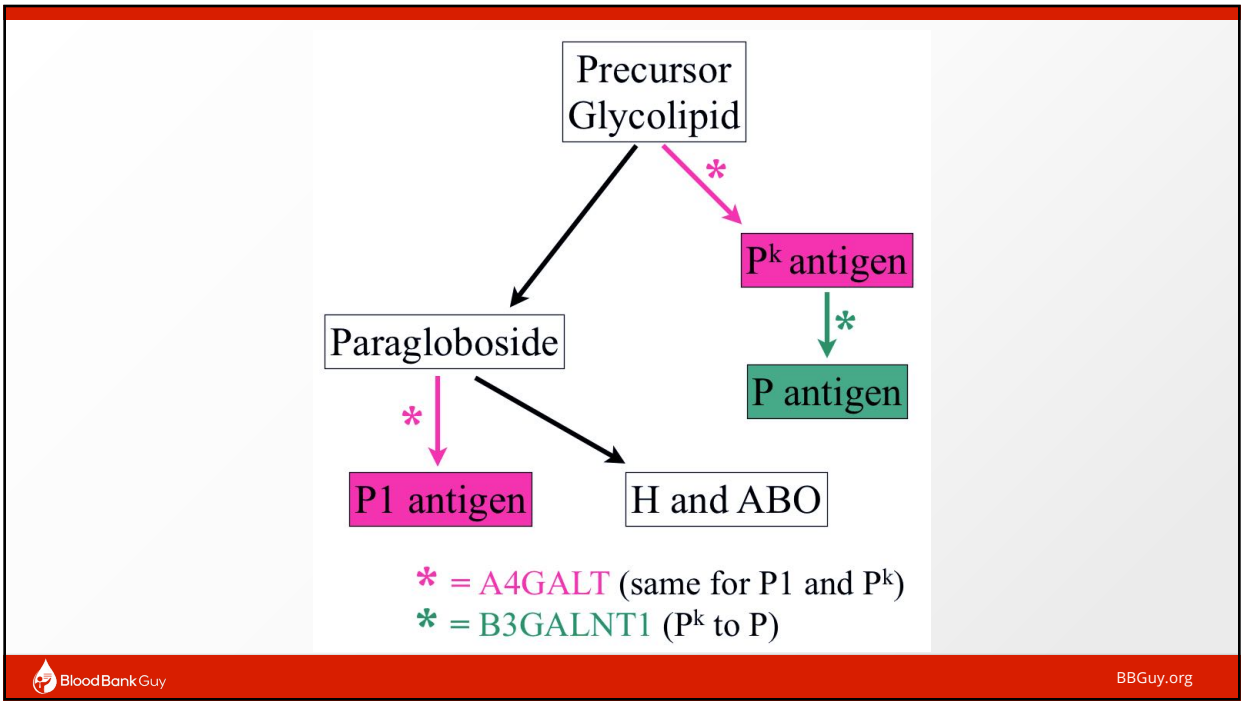
- Very uncommon (~2% of AIHA)
- Children after upper respiratory infection
  - Also viral exanthems, infectious mononucleosis
- Acute severe intravascular hemolysis, with sudden onset hemoglobinuria

Image source: e-learning.studmed.unibe.ch

PCH Antibody



- Biphasic autoantibody (IgG) vs. P
  - P antigen is very high frequency
  - Biphasic:
    - ✓Binds in cold
    - ✓Hemolyzes in warm
- "Donath-Landsteiner biphasic hemolysin"



P1PK/GLOB Systems

Phenotype	P1	P	Pk	Caucasians	African-Americans
P_1	+	+	–	79%	94%
P_2	–	+	–	21%	6%

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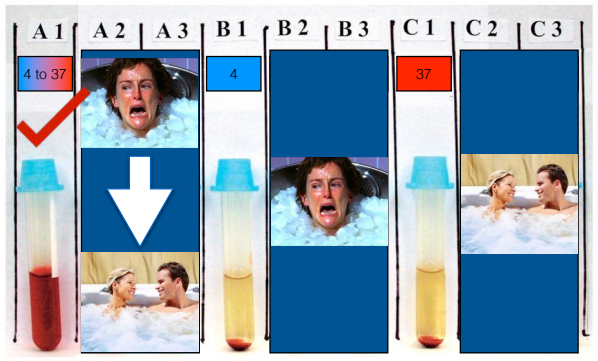
Donath-Landsteiner Test



Sanford KW and Roseff SD_Detection and significance of Donath-Landsteiner antibodies in a 5 year old female presenting with hemolytic anemia_Lab Medicine_0410

A: 4C to 37C
B: 4C only
C: 37C only

1: 10 ml patient serum
2: 5 ml pt/5 ml fresh donor serum
3: 10 ml fresh donor serum

PCH

- Supportive treatment; usually self-limited
- Transfusion often IS necessary at diagnosis…
- Uh-oh! What about P?
  - P-neg blood extremely rare!
  - P-pos RBCs usually survive well
  - Consider plasma exchange if not

Case

- 62 year old female being treated for CLL came to ER with chest pain and dyspnea; appeared pale
- 3 weeks ago: Given new (at that time) drug called "fludarabine"

Initial Labs:

Hemoglobin/Hematocrit	**3.8 g/dL / 6.1%**
Smear	Clumped RBCs
LDH	**1064 U/L**
Indirect bilirubin	4.1 g/dL

Mixed AIHA

- May be confused with the rare IgM WAIHA (both are severe and life-threatening)
- Presence of pathologic IgM and IgG
- Severe hemolysis, with IgM mediated intravascular hemolysis
- May present with VERY low HGB
- DAT typically + for both anti-IgG and anti-C3

Vox Sanguinis

Case Report

Vox Sang 1998;74:122–126

Received: April 16, 1997
Accepted: June 18, 1997

Dan J. Vick [a]
John C. Byrd [b]
Connie L. Beal [a]
Donald J. Chaffin [a]

[a] Department of Pathology and Area Laboratory Services and
[b] Hematology/Oncology Service, Department of Medicine, Walter Reed Army Medical Center, Washington, D.C., USA

Mixed-Type Autoimmune Hemolytic Anemia following Fludarabine Treatment in a Patient with Chronic Lymphocytic Leukemia/Small Cell Lymphoma

Abstract

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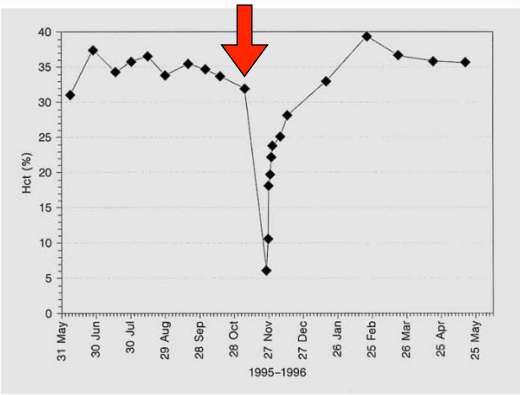

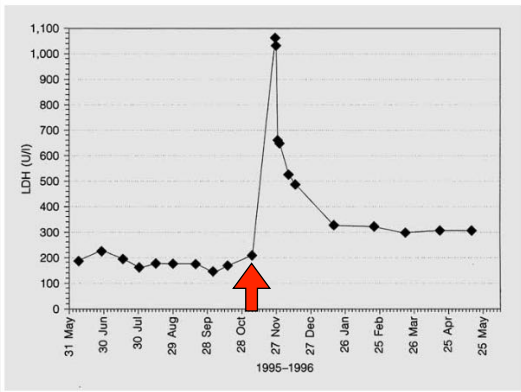

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Mixed AIHA

- Thankfully, often responds dramatically to corticosteroids
- Avoid transfusion if possible
  - If needed, must use special techniques to r/o alloantibodies

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Modified from Dr. Dan Ambruso	WAIHA	CAD	PCH	Mixed
Frequency	70-80%	18%	<2%	Rare
Peak Age	60's	60's	Children	Older
DAT (poly)	Positive	Positive	Us. Positive	Positive
DAT (IgG)	Positive (90%)	Negative	Negative	Positive
DAT (C3)	+/–	Positive (90%)	Positive	Positive
Antibody	IgG	IgM	IgG	IgG & IgM
Temp.	37°C	4°C	4°C → 37°C	4-37°C
Target	Rh-related	I (rarely i)	P	Rh and I
Transfusion	Auto/allo adsorp, Matching	Autoadsorb, prewarm	P-neg not necessary	Avoid if possible; As for WAIHA
Cause	Malignancy, Autoimmune, HIV	Lymphoprolif. d/o, Infx Mono, Mycoplasma	Viral infx, syphilis	SLE, drugs
Treatment	Block spleen (steroids, drugs, surgery)	Symptomatic	Supportive	Steroids

Take Homes

- Cold AutoAbs (IgM) >> Warm AutoAbs (IgG)
- WAIHA >> CAIHA
- Transfusion is generally safe in WAIHA
- Always rule out alloantibodies
- "Least incompatible" is not a strategy
- Block spleen for WAIHA, not CAIHA
- PCH: Biphasic IgG auto-anti-P