Hemolytic Anemias

Pavan K. Bendapudi, M.D.
Division of Hematology
Blood Transfusion Service

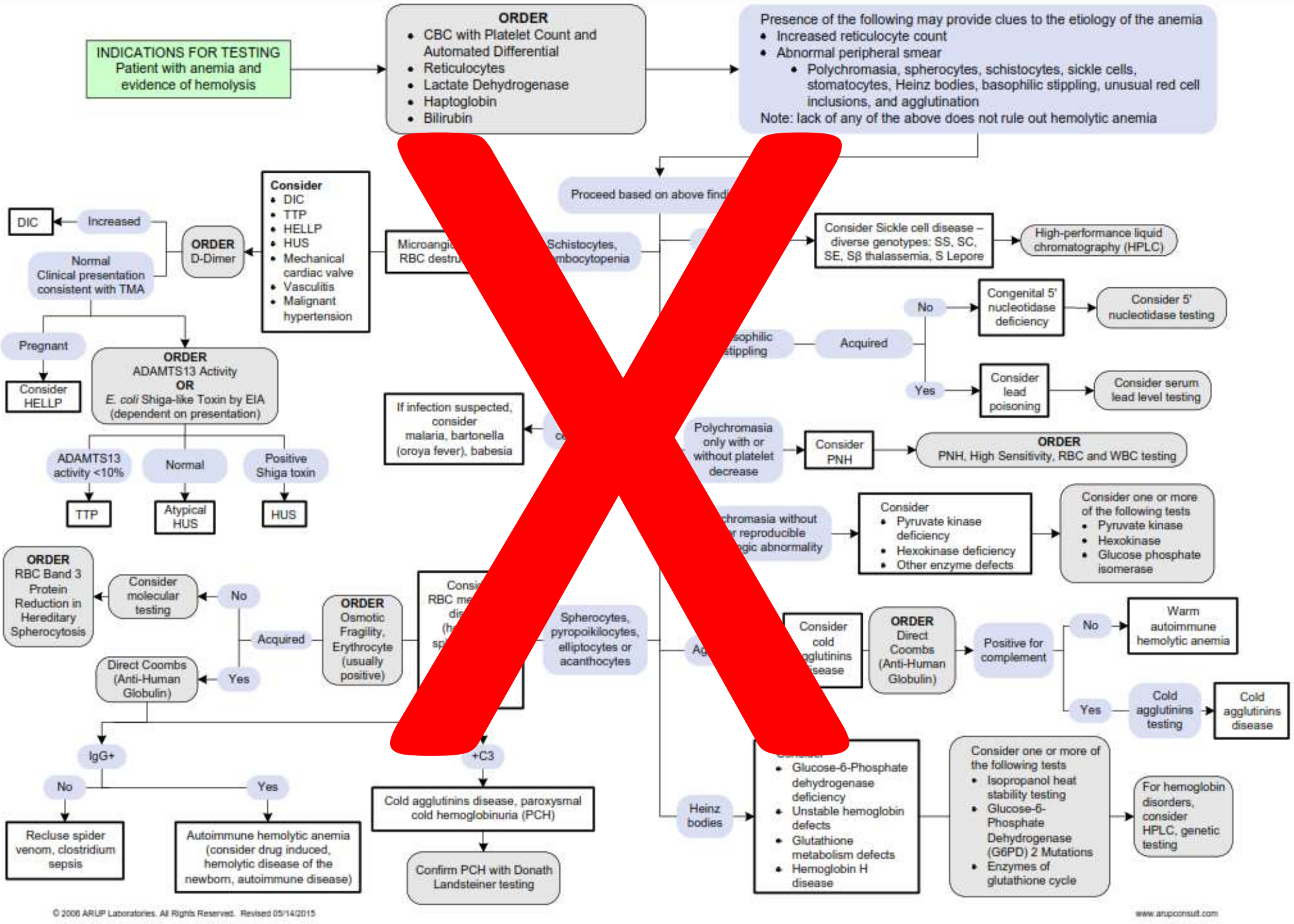
Transfusion Medicine Topics

1. Basics of transfusion medicine and blood banking
2. TMA/MAHA and therapeutic apheresis
3. Hemolytic anemias

ARUP Consult

Hemolytic Anemias Testing

Click here for topics associated with this algorithm

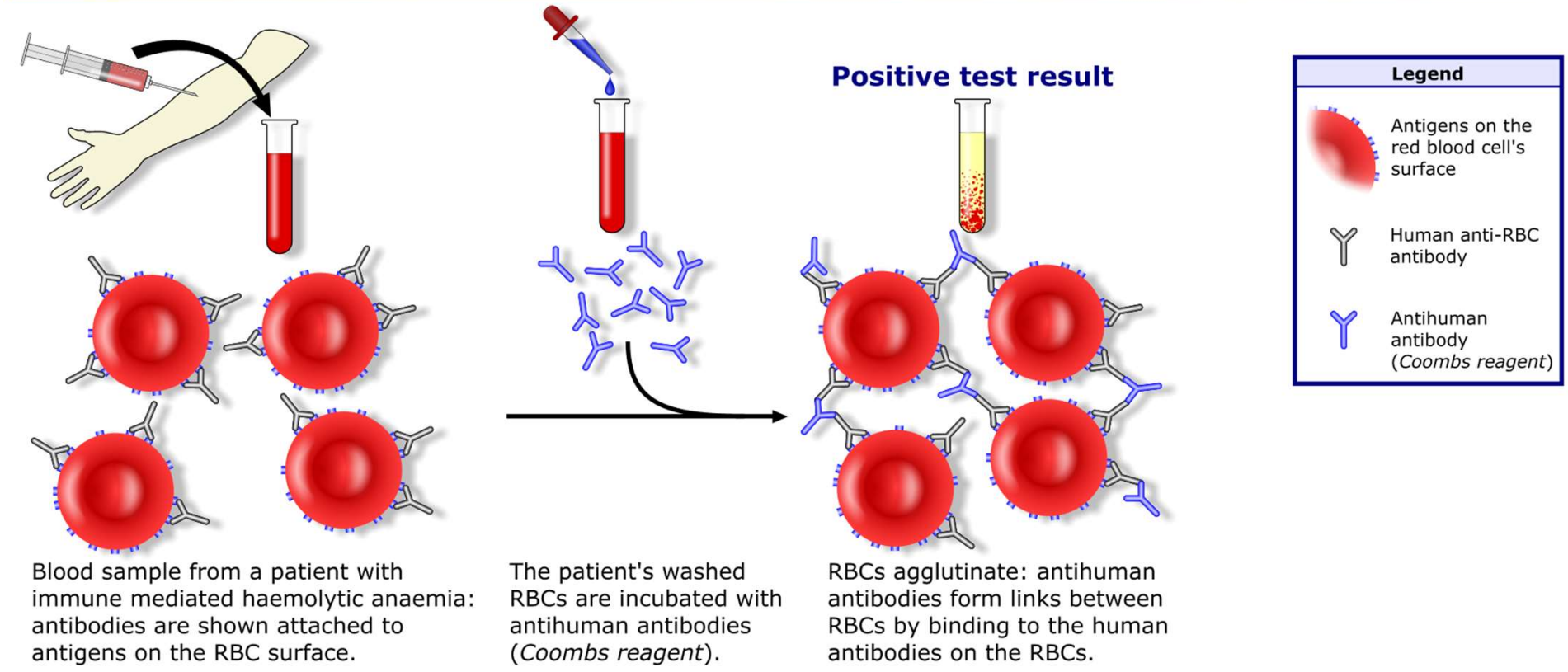


HEMOLYSIS

DAT

(Direct Antiglobin Test/Direct Coombs)

WAIHA: Direct Antiglobin Test (DAT)



Components of the DAT: Screen → IgG, C3d

Part 1: Warm Autoimmune Hemolysis

Part 2: Cold Agglutinin Disease

Part 3: Drug-associated Hemolysis

Part 4: Paroxysmal Cold Hemoglobinuria

Part 5: Case

Warm Autoimmune Hemolysis

- ~70% of autoimmune HA + 10% mixed warm/cold
- Mortality up to 20%
- Rare overall, but common with immune dysregulation (2° disease in ~50% of cases)
  - Lymphoma and CLL
  - Lupus
  - Chronic Inflammatory disorders
  - Following BM or solid organ transplant
- Evan's Syndrome: "combined WAIHA + ITP"

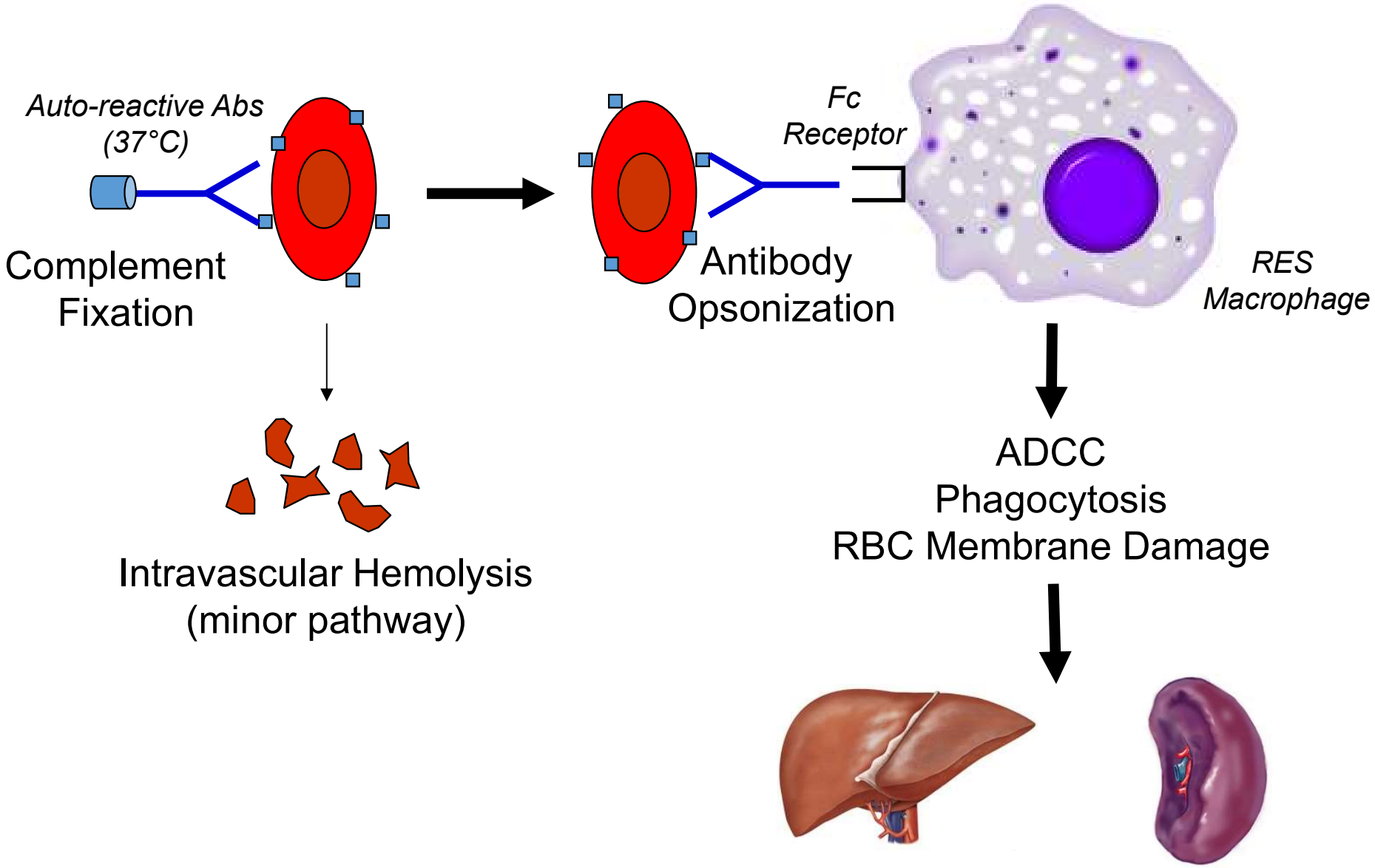
Zanella, et al. *Hematologica* 2014
Lechner, et al. *Blood* 2010

WAIHA: Presentation

- Can occur rapidly or over the course of weeks
- Common: DOE/SOB, high output heart failure, jaundice, dark urine, abd and/or flank pain
- Labs
  - **DAT:** IgG positive +/- C3 positive
  - **Haptoglobin:** very sensitive marker of intravascular hemolysis, low to absent
  - **LDH:** elevated but not sky high as in TMAs
  - **Indirect Hyperbilirubinemia**
  - **Reticulocytosis:** measure of RBC lifespan and/or severity of hemolysis
- Peripheral smear: microspherocytes

Zanella, et al. *Hematologica* 2014
Lechner, et al. *Blood* 2010

WAIHA: Pathophysiology

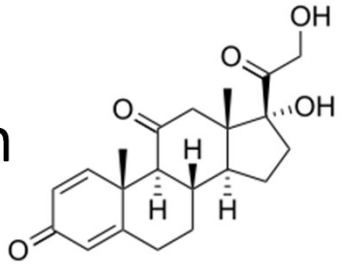


Expected DAT Results in Autoimmune Hemolysis

	DAT		
Condition	**DAT Poly**	**IgG**	**C3d**
DAT Negative Hemolysis	–	–	–
WAIHA	+	+	+/–
CAD	+	–	+
Drug-associated	+/–	+/–	+/–
PCH	+	–	+

WAIHA: Treatment

First Line: Prednisone 1 mg/kg/d



- Suppresses RES, little effect on auto Ab production
- 70-85% response rate
- Response usually within 2 wks
- 30% cured, 50% require maintenance, 20% need 2nd line Rx

Second Line: Rituximab 1g IV x 1-2 doses

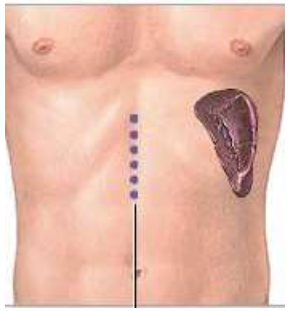
- Suppresses auto antibody production
- Response rate ~85%, ~60% DFS at 2 years
- Response in ~1 month (maybe longer)
- Consider upfront rituximab
- Retreatment commonly required
- Infectious complications very rare, but check HBV

Zanella, et al. *Hematologica* 2014
Lechner, et al. *Blood* 2010

First Line: Prednisone 1 mg/kg/d

Second Line: Rituximab 1g IV x 1 or 2 doses

Third Line: Splenectomy



- Removes a major site of RBC destruction and auto Ab production
- RES in liver remains
- Consider in refractory pts or those who need >10 mg/d prednisone maintenance
- 60% partial or complete response, ~20% cured
- Possible (but rare) infectious/operative complications

Fourth Line: MMF, Azathioprine, Cytoxan, Cyclosporin A, IVIG, Danazol, velcade, daratumumab (?)

Zanella, et al. *Hematologica* 2014
Lechner, et al. *Blood* 2010

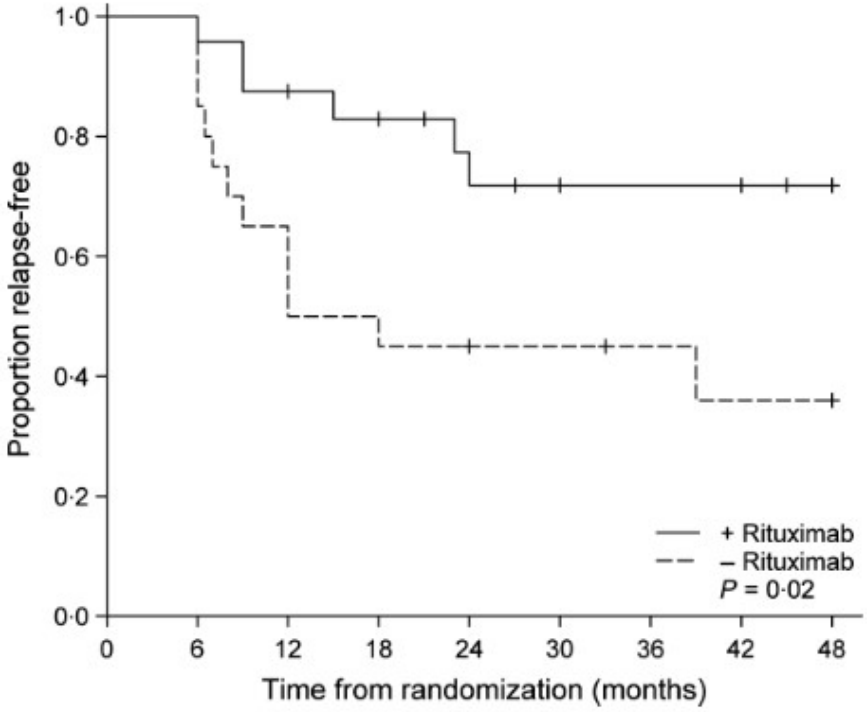
Rituximab in WAIHA

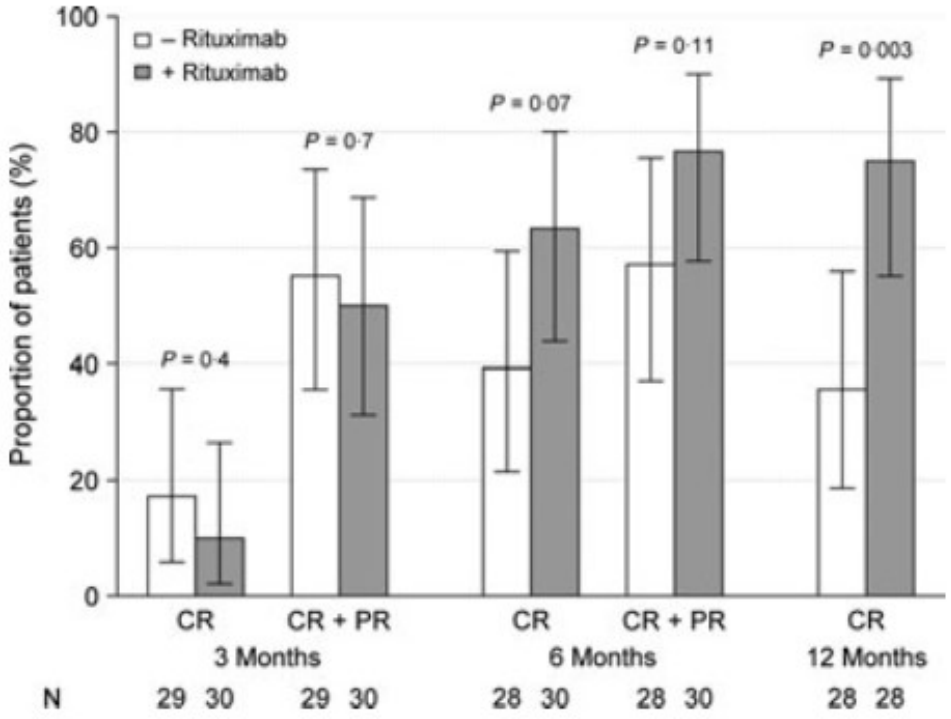
bjh research paper

A phase III randomized trial comparing glucocorticoid monotherapy *versus* glucocorticoid and rituximab in patients with autoimmune haemolytic anaemia

- 64 pts with *new* WAIHA randomized to get prednisolone alone or prednisolone + RTX
- 1° and 2° cases of WAIHA included
- Primary outcome: CR or PR
- Drug-induced hemolysis excluded

Birgens, et al. *BJH* 2013





Birgens, et al. *BJH* 2013

WAIHA: Transfusion Support

- No units will be crossmatch compatible
- Use ABO/Rh matched units
- Reserve transfusion for cases of symptomatic or life-threatening anemia
- Caution with infusion rate (TACO and high-output HF)
- Transfused RBCs will be destroyed at the same rate as endogenous RBCs. Use retic count as a marker
- Get pre-transfusion specimen to blood bank

For severe/life-threatening anemia: emergency release

Rossi's Principles of Transfusion Medicine, 4th Ed.

Part 1: Warm Autoimmune Hemolysis

Part 2: Cold Agglutinin Disease

Part 3: Drug-associated Hemolysis

Part 4: Paroxysmal Cold Hemoglobinuria

Part 5: Case

Cold Agglutinin Disease

- ~10-15% of autoimmune HA + 10% mixed warm/cold
- Usually mild-moderate hemolysis but can be serious
- 70% of cases are 2° to underlying dz
  - Infections, mycoplasma (anti-I) and EBV (anti-i)
  - Autoimmune disease
  - Waldenstrom's Macroglobulinemia (LPL) and MGUS
  - Other Malignancies
- Can be a/w cold-induced circulatory sx, e.g. livedo reticularis and acrocyanosis

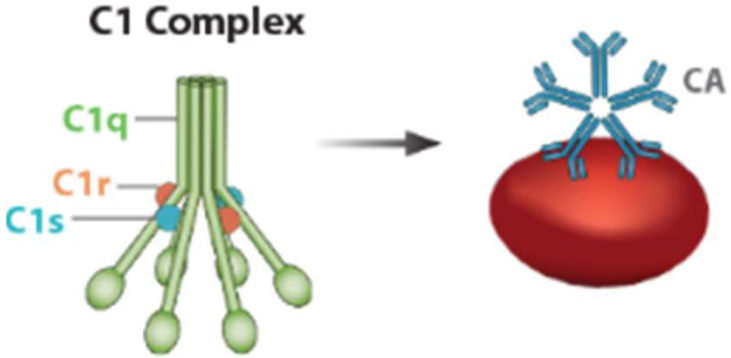
Zanella, et al. *Hematologica* 2014
Swiecicki, et al. *Blood* 2013

CAD: Pathophysiology

PERIPHERY
(<37°C)

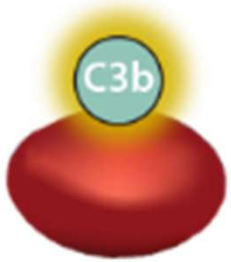
CORE
(37°C)

Cold auto-Ab IgM binds in periphery (<37°C)



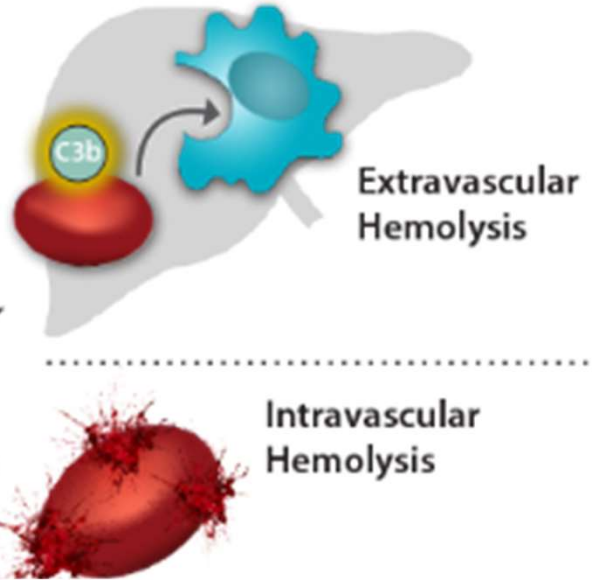
IgM fixes complement

Cold auto-Ab disassociates in central circulation (37°C)



Complement complex remains

RES macrophage-mediated RBC destruction



Complement-mediated RBC destruction

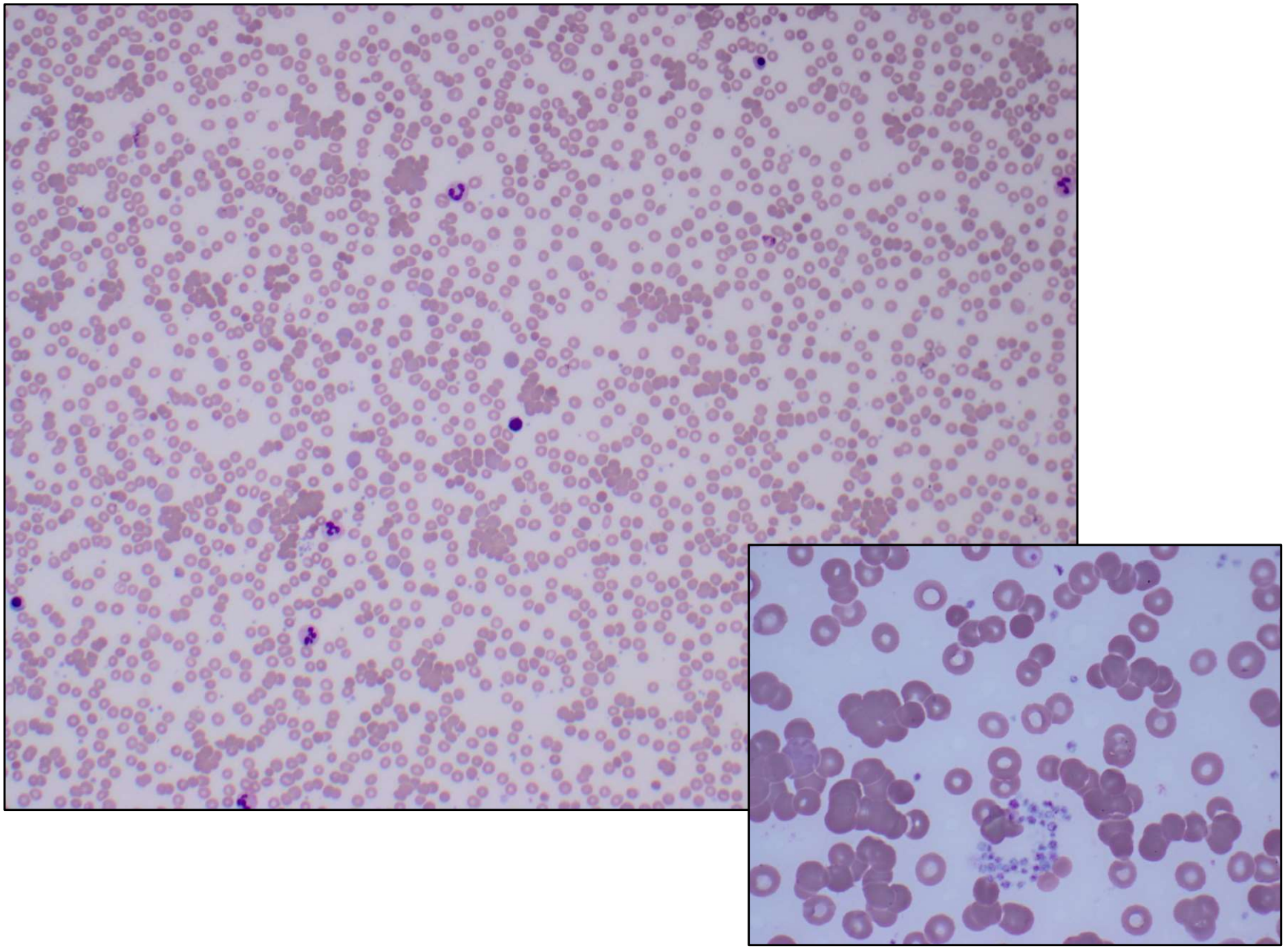
Expected DAT Results in Autoimmune Hemolysis

	DAT		
Condition	**DAT Poly**	**IgG**	**C3d**
DAT Negative Hemolysis	–	–	–
WAIHA	+	+	+/–
CAD	+	–	+
Drug-associated	+/–	+/–	+/–
PCH	+	–	+

WAIHA: Uniformly IgG

CAD: Uniformly IgM

CAD Peripheral Smear:



CAD: Treatment

First Line: Rituximab 1g IV x 1-2 doses

- Use in severe/recurrent cases with unresolvable underlying cause
- Response rate ~50%, median response ~1 yr
- Response in 1-2 month
- Retreatment is effective

Adjunctive Measures

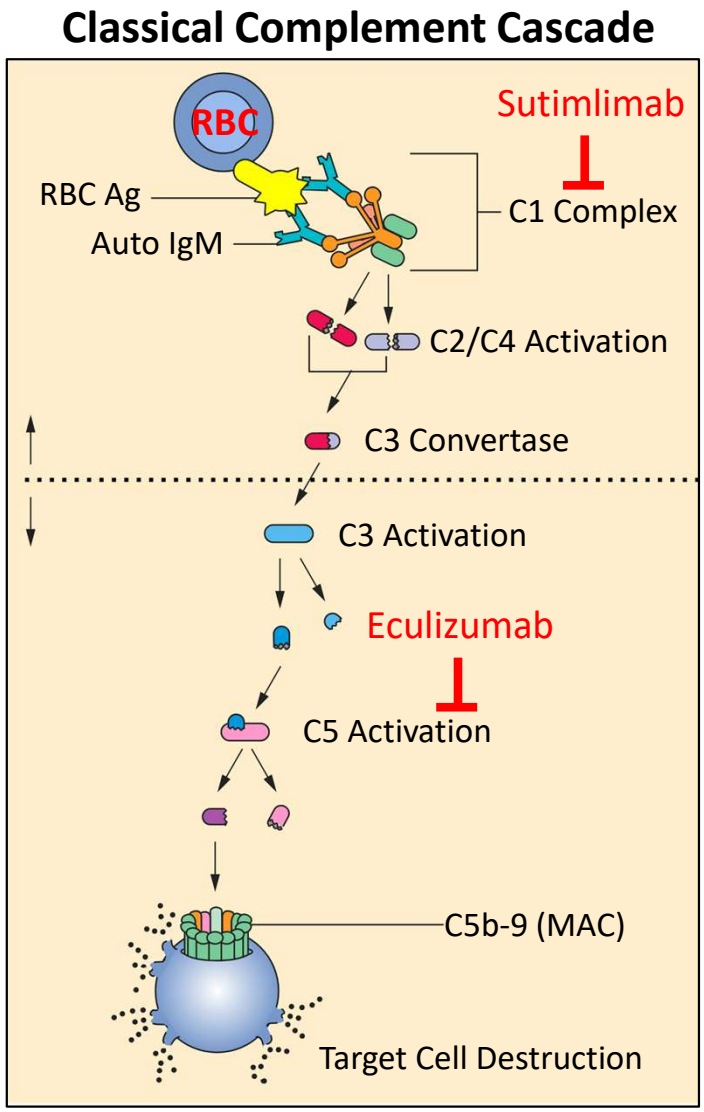
- Eculizumab or plasmapheresis for hemolytic crisis
- (daratumumab?)
- Treat underlying cause!

Poor Efficacy

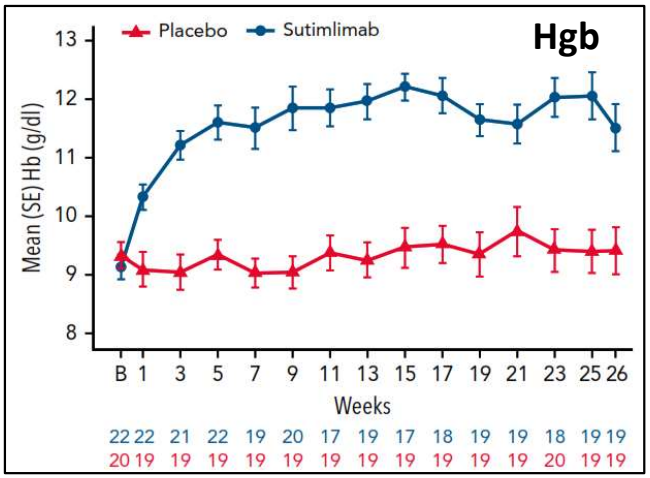
- Steroids, Splenectomy
- Chlorambucil, azathioprine, cyclophosphamide, cladribine, etc.

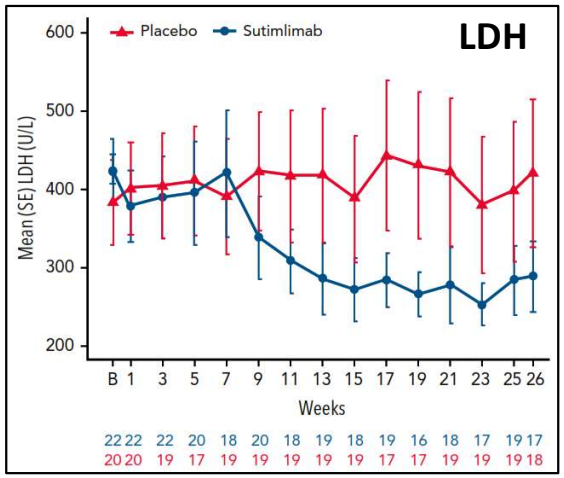
Zanella, et al. *Hematologica* 2014
Swiecicki, et al. *Blood* 2013

Sutimlimab



- Inhibitory mAb against C1s (C1qrs)
- Patients vaccinated for encapsulated organisms but no abx prophylaxis
- Significantly improves hemolysis and fatigue
- Likely provides more symptom relief than equivalent transfusion
- Approved in 2022, exact role not yet defined





Röth, et al. CADENZA Trial *Blood* 2022
Röth, et al. CARDINAL Trial *NEJM* 2021

Part 1: Warm Autoimmune Hemolysis

Part 2: Cold Agglutinin Disease

Part 3: Drug-associated Hemolysis

Part 4: Paroxysmal Cold Hemoglobinuria

Part 5: Case

Drug-associated Hemolysis

- ~10-15% of DAT+ hemolytic anemia
- Due to:
  - Antimicrobials (42%)
  - Anti-inflammatory (15%)
  - Anti-neoplastics (11%)
- Variable presentation, but some drugs cause acute, severe hemolysis (e.g. ceftriaxone, cefotetan)
- Some drugs (e.g. PCN) can cause positive DAT with or without hemolysis

Garratty, G. *Hematology* 2009

DA Hemolysis: Causes

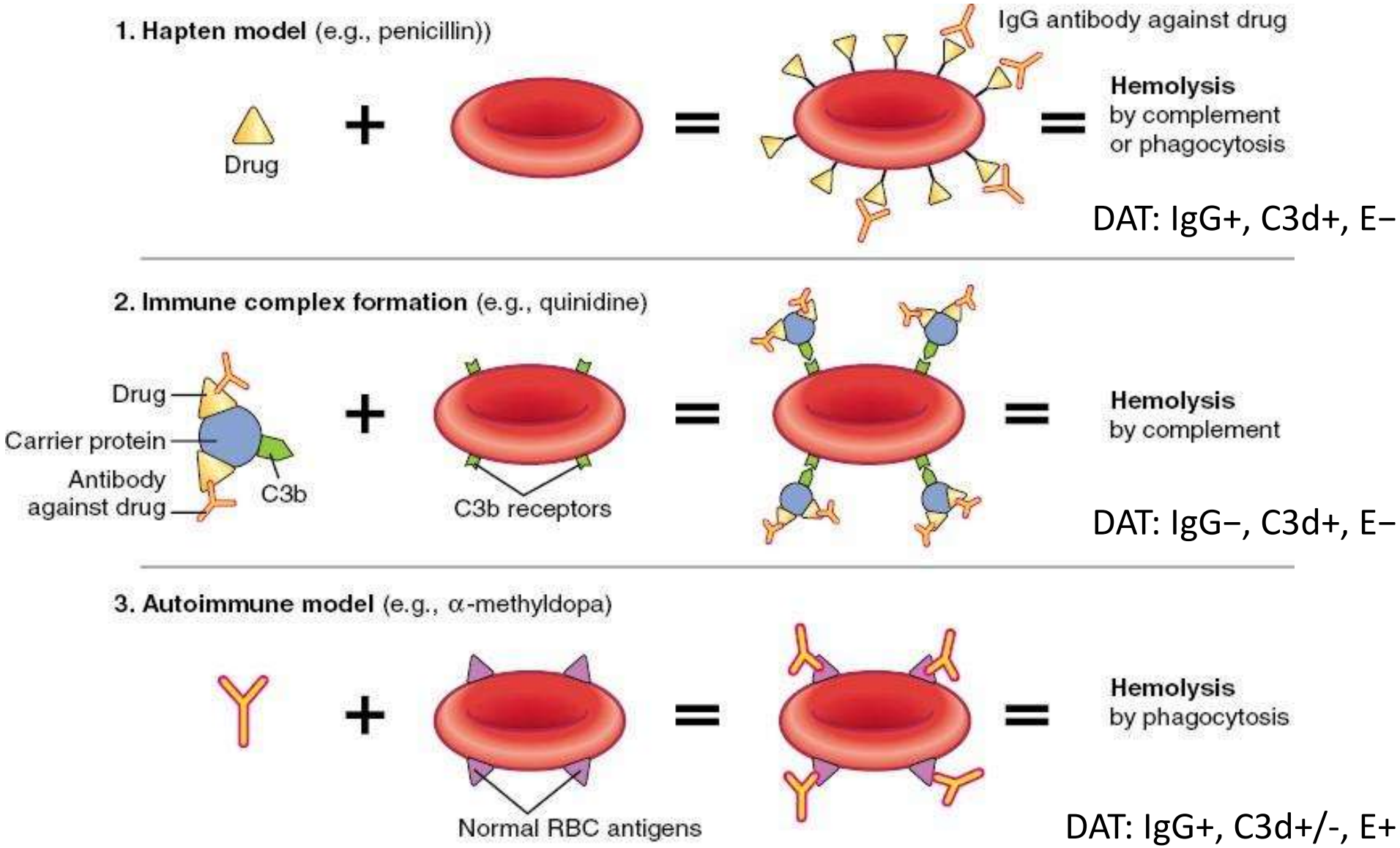
Drug*	San Francisco (Garratty and Petz) 1969-1978 (10 yrs)	Southern California (Garratty, Arndt, and Leger) 1979-1988 (10 yrs)	1989-1998 (10 yrs)	1999-2008 (10 yrs)	1979-2008 (30 yrs)
Methyldopa	29 (67%)	0	0	0	0 (0%)
Penicillin	10 (23%)	2 (15%)	0	0	2 (1.3%)
Cefotetan	0	0	36 (69%)	45 (53%)	81 (54%)
Ceftriaxone	0	1 (8%)	5 (10%)	14 (17%)	20 (13%)
Other cephalosporins	0	2 (15%)	0	0	2 (1.3%)
β-lactamase inhibitors	0	0	4 (8%)	6 (7%)	10 (7%)
Piperacillin	0	0	1 (2%)	12 (14%)	13 (9%)
Others	4** (9.3%)	8† (62%)	6‡ (12%)	8§ (9%)	22 (15%)
TOTAL	43	13	52	85	150

*Columns contain number (percentage) of cases associated with each drug
**Quinine (2), hydrochlorothiazide, rifampin
†Probenicid (2), chlorpropamide, phenacetin, nafcillin, rifampin, procainamide, erythromycin
‡Fludarabine (2), probenecid, tolectin, mefloquine, ticarcillin
§Oxaliplatin (2), carboplatin, rifampin, diclofenac, cimetidine, trimethoprim, sulfamethoxazole

Drug	Number of cases (fatalities)
Cefotetan	47 (5)
Ceftriaxone	29 (10)
Cephalothin	5 (1)
Ceftizoxime	4 (2)
Cefotaxime	3
Ceftazidime	3
Cefoxitin	2 (1)
Cefazolin	2
Cephalexin	1
Cefamandole	1
Cefuroxime	1
Cefixime	1
Total	99 (19)

Garratty, G. *Hematology* 2009

DA Hemolysis: Mechanisms



Treatment: Discontinue offending drug

Part 1: Warm Autoimmune Hemolysis

Part 2: Cold Agglutinin Disease

Part 3: Drug-associated Hemolysis

Part 4: Paroxysmal Cold Hemoglobinuria

Part 5: Case

Paroxysmal Cold Hemoglobinuria

- Hemolysis caused by an IgG cold auto Ab
  - Donath-Landsteiner Ab
  - Anti-P IgG
- D-L Ab binds RBCs and fixes early C' factors at <37°C → Ab disassociates at 37°C → Term C' factors and MAC lyses cells at 37°C
- Classically associated with syphilis, now post pediatric URIs
- Attacks can be severe, but overall PCH is almost always self-limiting

Sanford, et al. *Lab Medicine* 2010
Rossi's Principles of Transfusion Medicine, 4th Ed.

Expected DAT Results in Autoimmune Hemolysis

	DAT		
Condition	**DAT Poly**	**IgG**	**C3d**
DAT Negative Hemolysis	–	–	–
WAIHA	+	+	+/–
CAD	+	–	+
Drug-associated	+/–	+/–	+/–
PCH	+	–	+

Confirm dx of PCH with Donath Landsteiner test for biphasic hemolysins

Part 1: Warm Autoimmune Hemolysis

Part 2: Cold Agglutinin Disease

Part 3: Drug-associated Hemolysis

Part 4: Paroxysmal Cold Hemoglobinuria

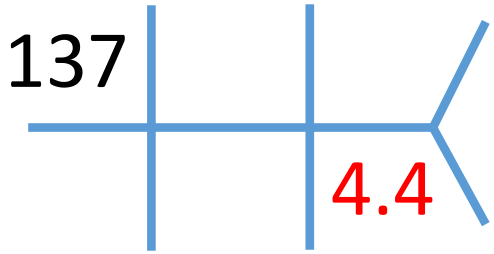
Part 5: Case

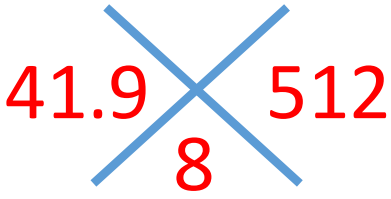
Case

- 43 year-old male with history of ITP status post splenectomy and WAIHA 11 years PTA.
- USOH until 2 days PTA: develops red urine, epigastric pain, and diarrhea. Presents to OSH ED with jaundice and found to have a Hct of 21. Sent home on prednisone 80mg for presumed flare of WAIHA.
- Subsequently develops nausea/vomiting, unable to take prednisone. Syncopized at home and taken to OSH ED.

OSH Course

Initial Labs:





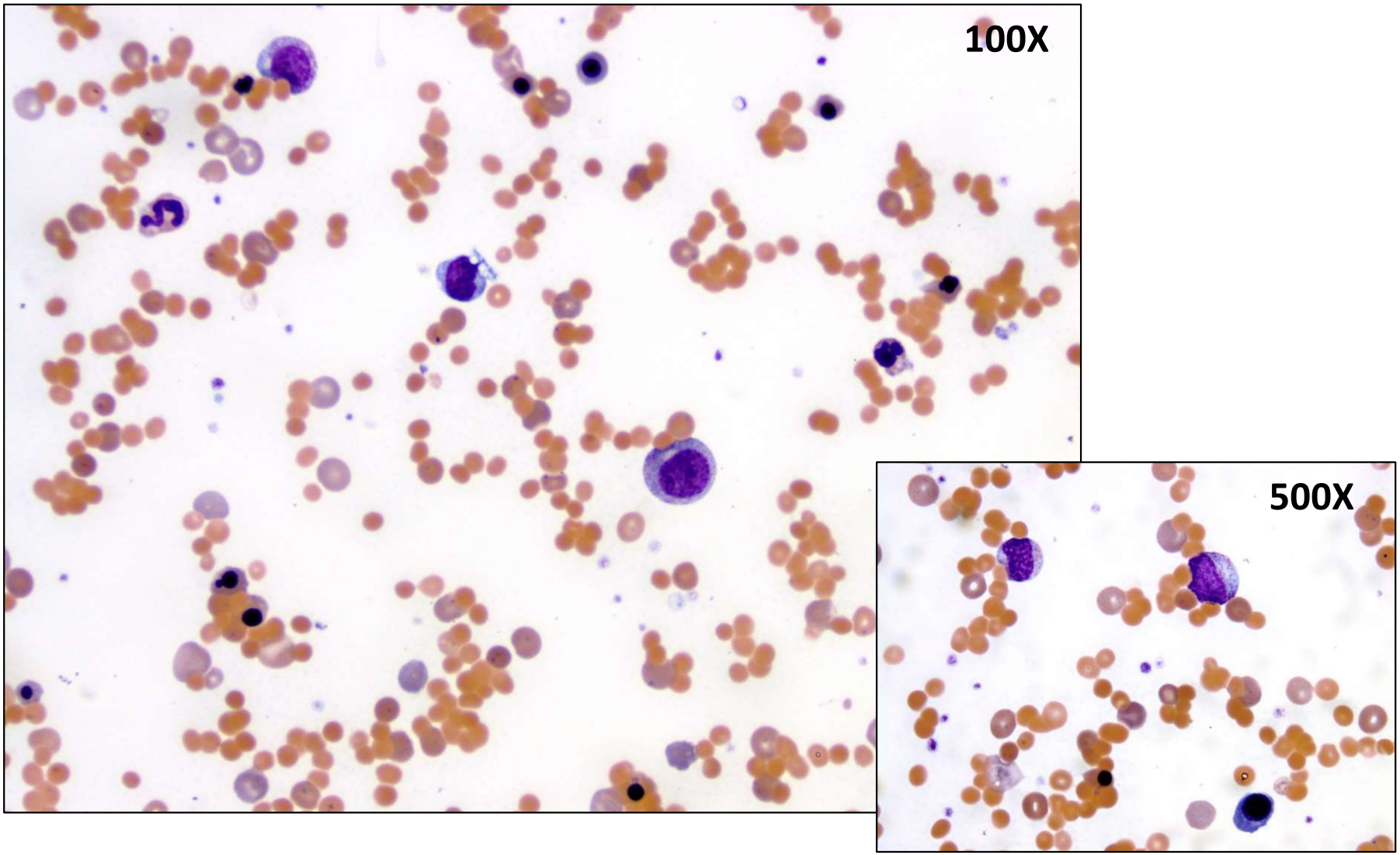
Tbili 6.2
LDH > 2500

Retic 11%

UA: protein 3+, blood 3+, RBCs 5-10, turbid, red
Coombs: positive (IgG+, C3D-)
Smear: micro-spherocytes, immature RBCs, normal granulocytes

- Treated with high-dose steroids and transfusions for 7 days (10 units pRBCs for Hct >18)
- On HD 8, transferred to MGH per family's request

Hemodynamically stable on arrival to MICU. Fatigued and confused, endorsed severe epigastric pain with PO intake.



Initial Blood Bank Workup

TYPE **DAT** **SCREEN**

TEST RESULTS

ANTI-			CELLS		DAT	IS	5'	CC	AB SCREEN /LOT#: 0.8% V		
A	B	D	AI	B	POLY	3+	2+	/		GEL IGG	REPEAT GEL
(-)	(-)	4+	4+	4+	IgG	3+		/	I	3+	
					C_3	(-)	(-)	2+	II	3+	
Du___ CC___ CtI___ CC___									III	3+	

O POS **DAT IgG 3+, C3-** **Pan-positive Antibody Screen**

Warm autoimmune hemolytic anemia

Does this explain the patient's presentation?

Workup: CBC

	46 9/26/2016 0435	47 9/25/2016 1827	48 9/25/2016 0445	49 9/24/2016 2149
COMPLETE BLOOD COUNT				
WBC	22.70 ▲	25.18 ▲	33.18 ▲	34.62 * ▲
RBC	1.63 ▼	1.89 ▼	1.79 ▼	Not Done
Hgb	6.1 ▼	6.9 ▼	6.7 ▼	Not Done
HCT	17.5 !!▼	19.6 !!▼	18.3 * !!▼	Manual Method
HCT (manual)				16.0 * !!▼
MCV	107.4 ▲	103.7 ▲	102.2 ▲	Not Done
MCH	37.4 ▲	36.5 ▲	37.4 ▲	Not Done
MCHC	34.9	35.2	36.6	Not Done
PLT	132 ▼	149 ▼	164	166 *
MPV	11.3	11.4	11.6	11.3
RDW	34.3 ▲	30.4 ▲	29.3 ▲	Not Done
BLOOD DIFFERENTIAL %				
				Manual
				100
				71.0 ▲
				4.0 ▼
				11.0
				1.0

HCT

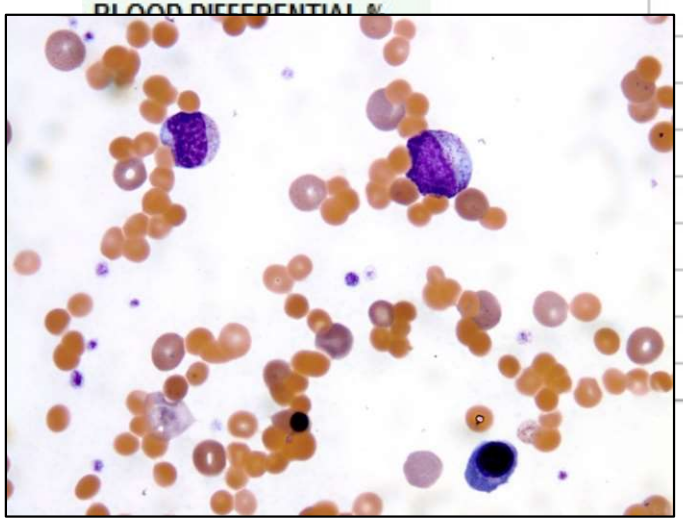
CBC

Collected:	09/25/16 0445
Result status:	Final
Resulting lab:	MASSACHUSETTS GENERAL HOSPITAL
Reference range:	41.0 - 53.0 %
Value:	18.3 ⩔
Comment:	RBC parameters calculated after warming sample. Possible reasons include but are not limited to: lipemia, hemolysis, cold agglutinins or, if present, high nucleated RBCs.

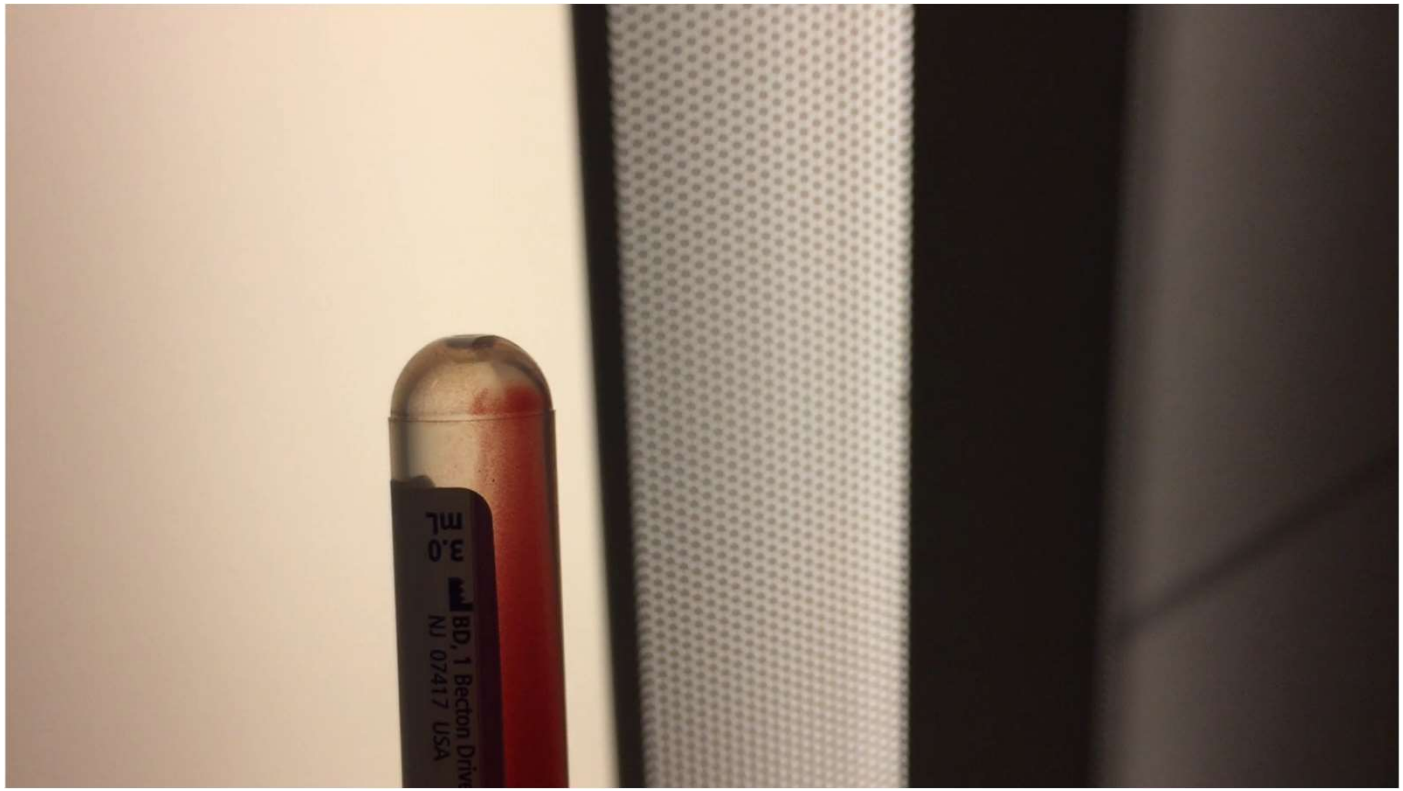
*Additional information available - comment

Workup: CBC

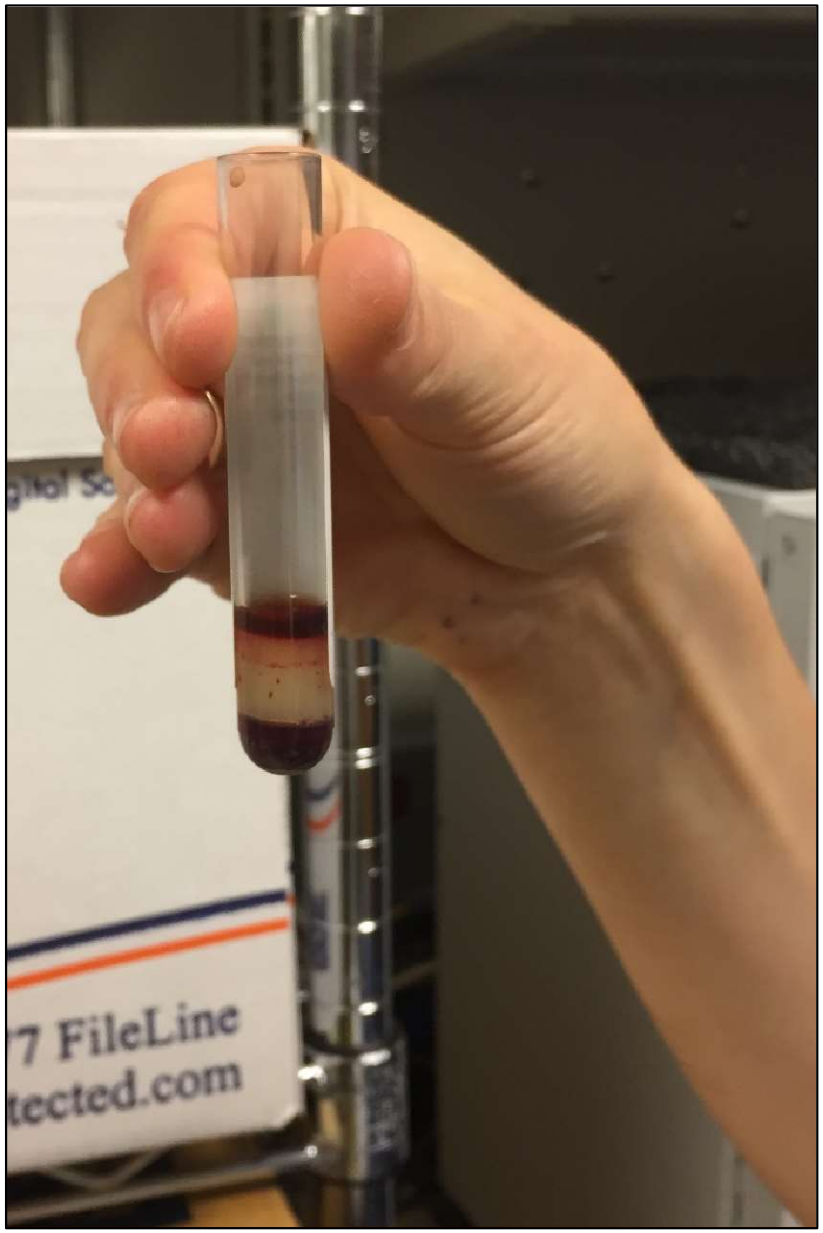
	46	47	48	49
	0435	1827	0445	2149
COMPLETE BLOOD COUNT				
WBC	22.70 ▲	25.18 ▲	33.18 ▲	34.62 * ▲
RBC	1.63 ▼	1.89 ▼	1.79 ▼	Not Done
Hgb	6.1 ▼	6.9 ▼	6.7 ▼	Not Done
HCT	17.5 !!▼	19.6 !!▼	18.3 * !!▼	Manual Method
HCT (manual)				16.0 * !!▼
MCV	107.4 ▲	103.7 ▲	102.2 ▲	Not Done
MCH	37.4 ▲	36.5 ▲	37.4 ▲	Not Done
MCHC	34.9	35.2	36.6	Not Done
PLT	132 ▼	149 ▼	164	166 *
MPV	11.3	11.4	11.6	11.3
RDW	34.3 ▲	30.4 ▲	29.3 ▲	Not Done
[illegible]				
				Manual
				100
				71.0 ▲
				4.0 ▼
				11.0
				1.0



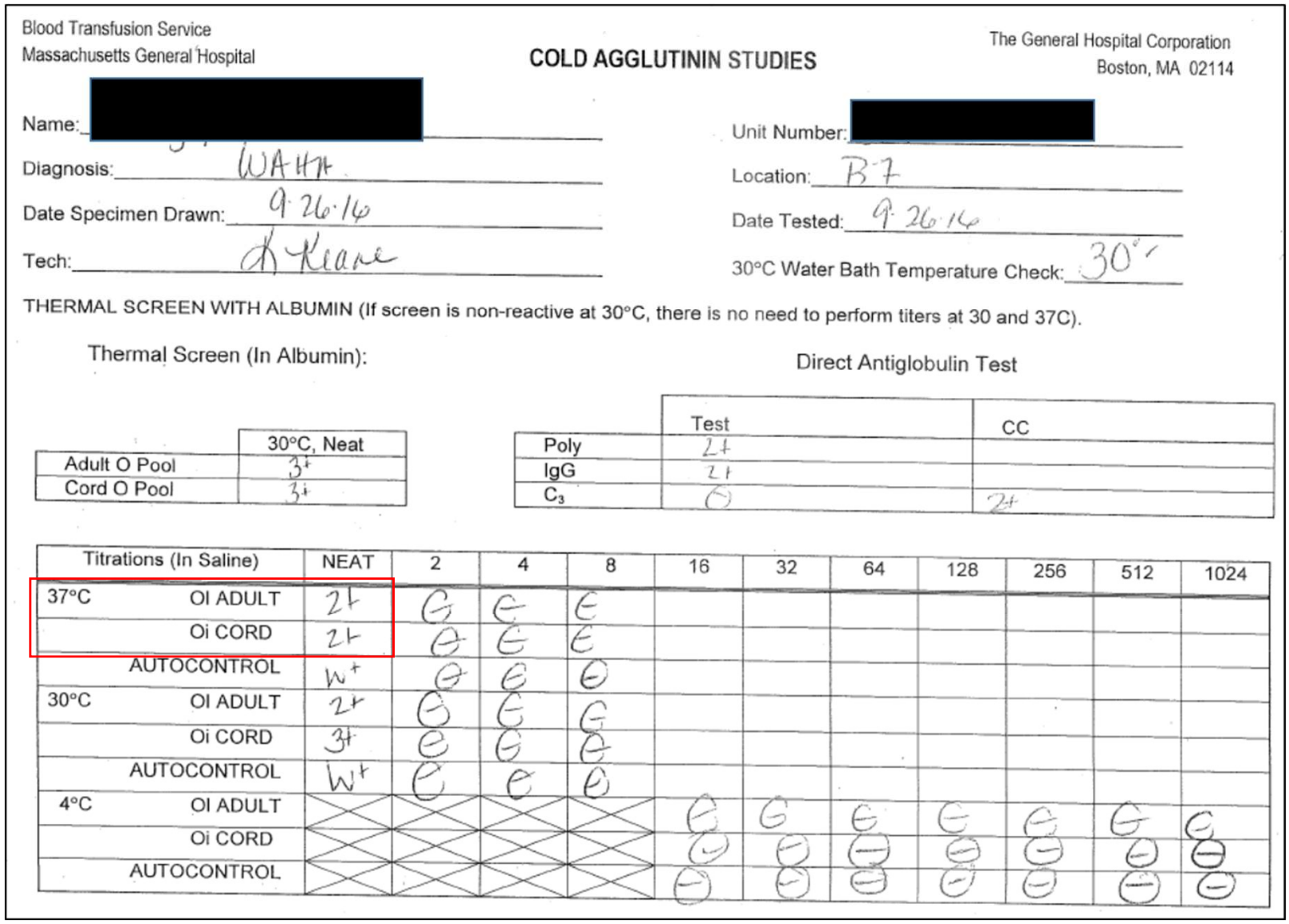
Purple top tube drawn at patient's bedside (22°C)



Spun Plasma



Cold Titer and Thermal Amplitude Testing



Blood Transfusion Service
Massachusetts General Hospital

COLD AGGLUTININ STUDIES

The General Hospital Corporation
Boston, MA 02114

Name: [redacted]

Unit Number: [redacted]

Diagnosis: WAHA

Location: B7

Date Specimen Drawn: 9.26.16

Date Tested: 9.26.16

Tech: K Keane

30°C Water Bath Temperature Check: 30°

THERMAL SCREEN WITH ALBUMIN (If screen is non-reactive at 30°C, there is no need to perform titers at 30 and 37C).

Thermal Screen (In Albumin):

	30°C, Neat
Adult O Pool	3+
Cord O Pool	3+

Direct Antiglobulin Test

	Test	CC
Poly	2+	
IgG	2+	
C_3	0	2+

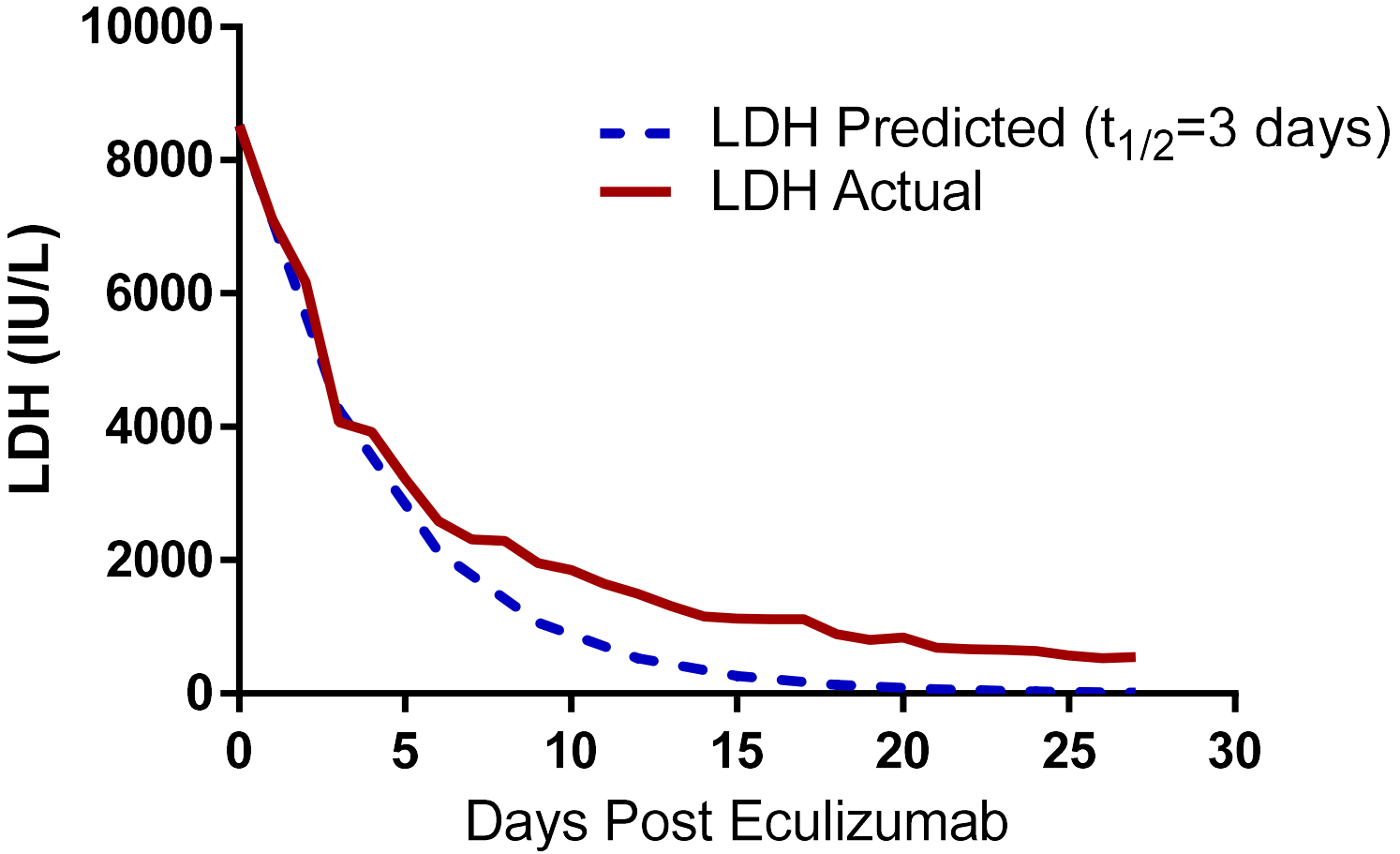
Titrations (In Saline)		NEAT	2	4	8	16	32	64	128	256	512	1024
37°C	OI ADULT	2+	G	G	G							
	Oi CORD	2+	0	G	G							
	AUTOCONTROL	W+	0	0	0							
30°C	OI ADULT	2+	0	0	G							
	Oi CORD	3+	0	0	0							
	AUTOCONTROL	W+	0	0	0							
4°C	OI ADULT					0	G	G	G	G	G	G
	Oi CORD					0	0	0	0	0	0	0
	AUTOCONTROL					0	0	0	0	0	0	0

Why was the DAT negative for C'?

Management

- Reduced transfusion threshold to Hgb >6
- All transfusions through blood warmer
- Discontinue unnecessary phlebotomies
- Continue IV solumedrol
- Rituximab 375 mg/m^2 x 4
- Eculizumab 900 mg IV x 1
  - Complement C5 inhibitor
  - Vaccinations for N. meningitidis A, B, C, Y, W135
  - Consider vaccinating against other encapsulated organisms
  - Penicillin VK 500 mg PO bid for ≥2 weeks

Impact of Eculizumab on Hemolytic Burden



Why did he have abdominal pain?

AIHA: the GIMEMA Study

Table 1. Clinical characteristics of primary AIHA patients

	N*
Patients, N	308
Male/female	111 (36)/197 (64)
Median age at onset, y (range)	58 (0-95)
<18 y	10 (3)
18-45 y	73 (24)
45-65 y	104 (34)
>65 y	121 (39)
AIHA serological type**	
Warm, DAT positive for IgG	131 (43)
Warm, DAT positive for IgG+C	52 (17)
CAD	84 (27)
Mixed	24 (8)
Atypical	16 (5)
Median follow-up, mo (range)	33 (6-372)
Alive/dead at time of study†	221 (72)/63 (21)
Died of AIHA	11/63 (17)

Table 2. Relationship between AIHA serological type and clinical severity at onset

	Hb at onset (g/dL)			
AIHA serological type	**<6**	**6.1-8**	**8.1-10**	**>10**
Warm (n = 183)				
IgG (n = 131)	38 (29%)	46 (35%)	33 (25%)	14 (11%)
IgG+C (n = 52)	16 (31%)	23 (44%)	10 (19%)	3 (16%)
Cold (n = 84)	8 (9.5%)	29 (34.5%)	27 (32%)	20 (24%)
Mixed (n = 24)	15 (63%)	6 (25%)	2 (8%)	1 (4%)
Atypical (n = 16)	8 (50%)	5 (31%)	3 (19%)	0

Risk factors associated with death: acute renal failure (OR 17.99), infections (HR 11.47), multi-treatment (HR 9.1), Evans syndrome (HR 6.8), previous splenectomy (HR 3.21)

Barcellini, et al. *Blood* 2014

Take Home Points

- The DAT is used to separate different types of hemolysis
- Hemolysis labs and peripheral smear will guide the diagnosis
- Always look for an underlying cause of DAT positive hemolysis
- Immunosuppressants are useful in the management of DAT + hemolysis
- Specialized transfusion support may be needed

Thank You