

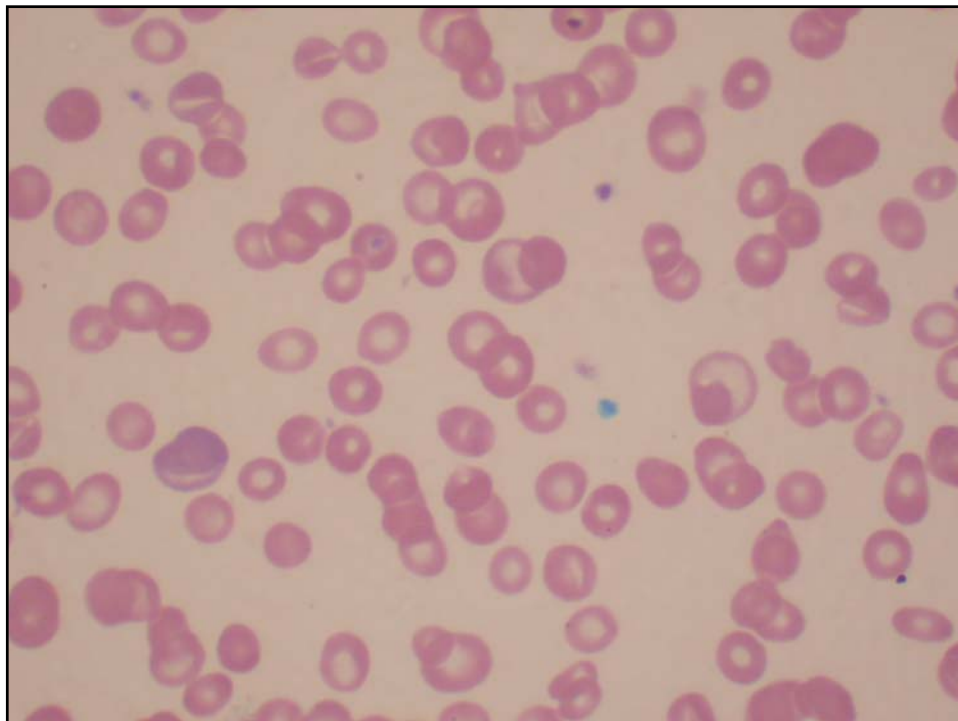
RBC abnormalities

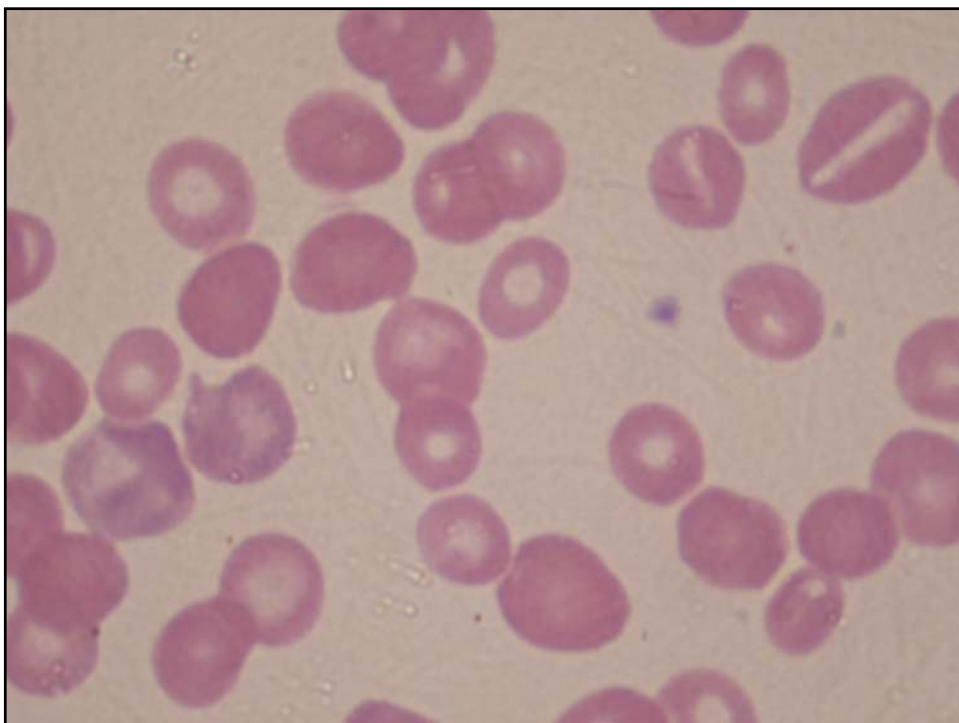
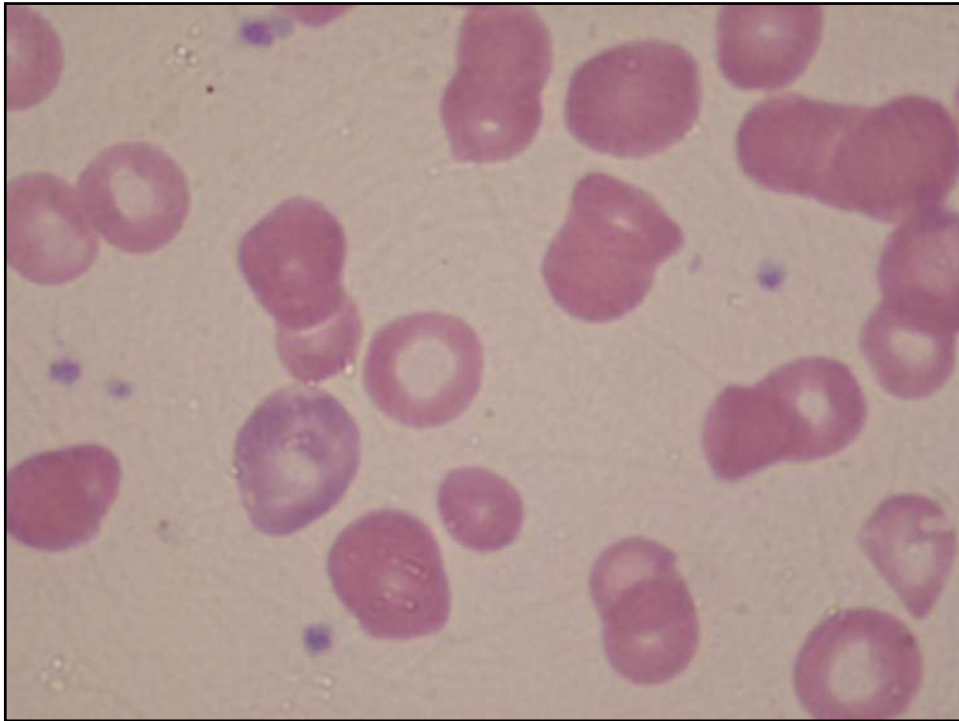
Winona State University CLS
Program

November 6, 2010

18 month old male
seen in the ED for fever

CBC W/DIFF			STAT
WBC	H 20.0	(6.0-17.5)	THOUS/CMM
RBC	L 2.16	(3.7-5.3)	MILL/CMM
HGB	C 2.5	(10.5-14.0)	GM/DL
HCT	C 11.3	(33.0-42.0)	%
MCV	L 52.3	(70-88)	FL
MCH	C 11.6	(25.0-35.0)	PG/CELL
MCHC	L 22.1	(32.0-36.0)	G/DL
RDW	H 24.2	(<15.0)	%
PLATELET	H 885	(140-440)	THOUS/CMM
MPV	8.0	(7.4-10.4)	FL
POLYNUC NEUTROPHIL	28		%
IMMATURE NEUT.	1		%
LYMPHOCYTE	48		%
MONOCYTE	11		%
EOSINOPHIL	8		%
BASOPHIL	1		%
ABS POLYNUC NEUT	5.7	(1.5-8.0)	THOUS/CMM
ABS IMMATURE NEUT	0.2	(0.0-0.3)	THOUS/CMM





Diagnosis of Iron Deficiency Anemia

- MCV
- Serum iron
- TIBC
- Iron saturation
- Ferritin
- Bone marrow tests

Testing for Iron Deficiency

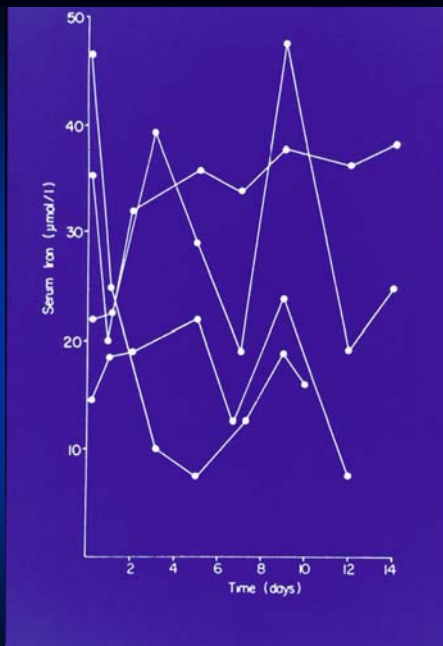
- “Classic” tests only helpful in few patients
- Tests affected by concurrent illness and age
- Fe: Highly variable, diurnal variation, diet
- MCV: lacks sensitivity and specificity
- RDW: not very useful
- Saturation: low in both ACD and iron deficiency

MCV

- Decreased due to lack of rbc iron
- Can be decreased in anemia of chronic disease
- $MCV < 70$ seen only in iron deficiency and thalassemia
- Can be normal in iron deficiency

Serum Iron

- Decreased in any stressful situation
- Marked variation in levels



Total Iron Binding Capacity

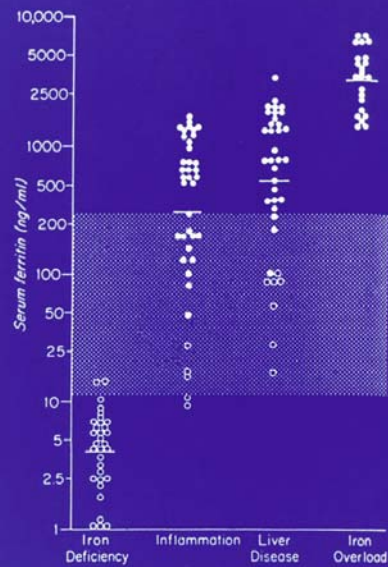
- Surrogate for transferrin
- Increased in iron deficiency
- Decreased in inflammation
- Specific but not sensitive

Iron Saturation

- Iron/TIBC
- <16% seen in both iron deficiency and anemia of chronic disease
- Does not add to information provided by TIBC

Serum Ferritin

- Serum ferritin proportional to iron stores
- Needs iron to be produced
- Acute phase reactant only in presence of iron
- Most accurate non-invasive test of iron stores

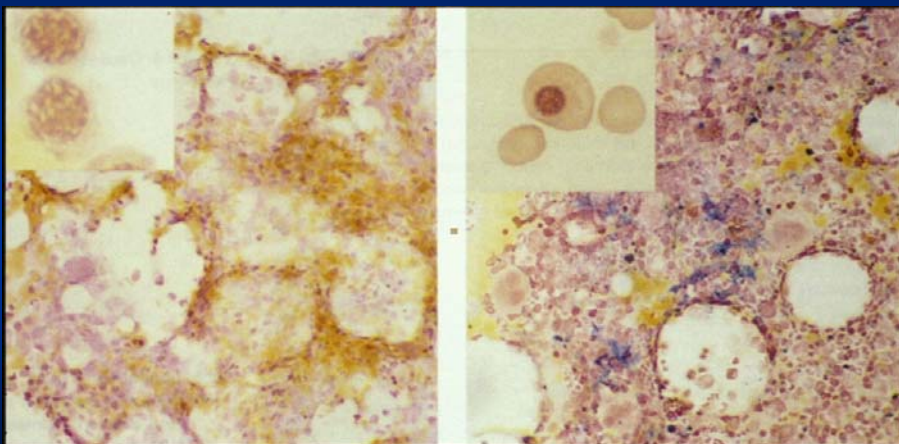


Iron Deficiency

- Serum ferritin is best non-invasive test of iron status
 - > 100 ng/dl rules out iron deficiency
 - Lower limit changes with age and condition
 - Patient over 65 with ferritin < 50 all iron deficient
 - Iron deficiency predictor of colon cancer

Bone Marrow

- Direct measure of iron stores
- “Gold standard”
- Invasive and expensive



ACD – Anemia of Chronic Disease

- Defective iron utilization
- Acts like iron deficiency – but plenty of iron in marrow

Summary

- RDW, serum iron, saturation: little value
- TIBC: specific but not sensitive
- Ferritin: best non-invasive test
- Bone marrow: gold standard

Response to Oral Iron

- Increased retic 7-10 days
- Increased hct 2-3 weeks
- Normalized 2 months

Lesions Identified in Patients With Iron Deficiency

- Esophageal: 12%
- Stomach: 25%
 - Cancer: 3%
- Ulcer: 6%
- Large intestine: 22%
 - Cancer: 9%
- Nothing: 35%

10 year old boy
Developmental delay
Mother noted jaundice

COMP METABOLIC PANEL

GLUCOSE	L	66	(70-99)	MG/DL
UREA NITROGEN		12	(5-18)	MG/DL
CREATININE		0.6	(0.2-0.7)	MG/DL
BUN:CREAT RATIO	H	21	(6-20)	
EST GLOM FILT RATE		<<DO NOT REPORT>>		
			(>60)	ML/MIN
SODIUM		139	(135-145)	MMOL/L
POTASSIUM		4.3	(3.5-5.3)	MMOL/L
CHLORIDE		104	(98-108)	MMOL/L
CO2		25	(23-32)	MMOL/L
ANION GAP		14	(10-18)	MMOL/L
CALCIUM		9.5	(8.6-10.3)	MG/DL
TOTAL PROTEIN		7.4	(6.0-8.0)	G/DL
ALBUMIN		4.8	(3.5-5.0)	G/DL
GLOBULIN		2.6	(2.0-3.9)	G/DL
A:G RATIO		1.8	(1.0-2.7)	
SGOT/AST	H	50	(0-37)	U/L
SGPT/ALT		26	(6-37)	U/L
ALK PHOSPHATASE		186	(117-390)	U/L
BILIRUBIN, TOTAL	H	1.6	(0.0-1.2)	MG/DL

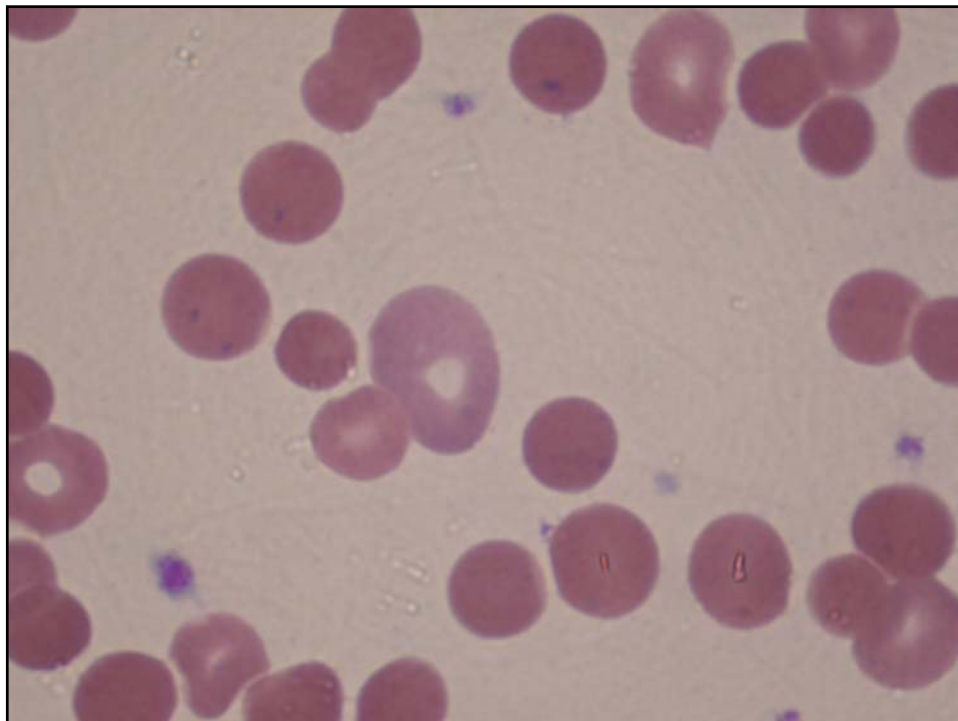
HEPATIC PANEL

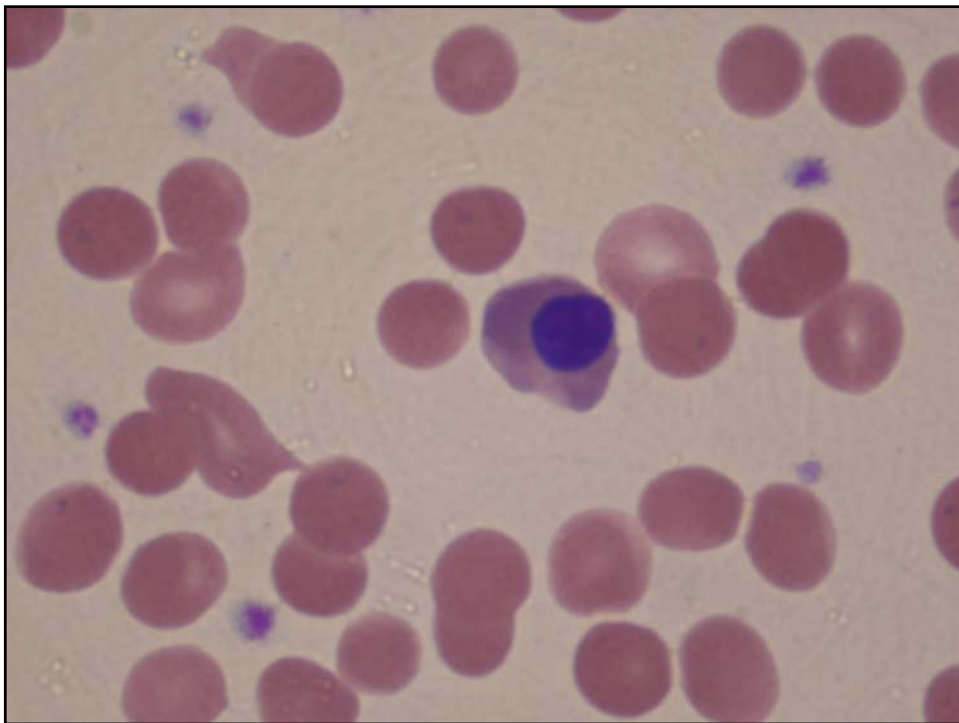
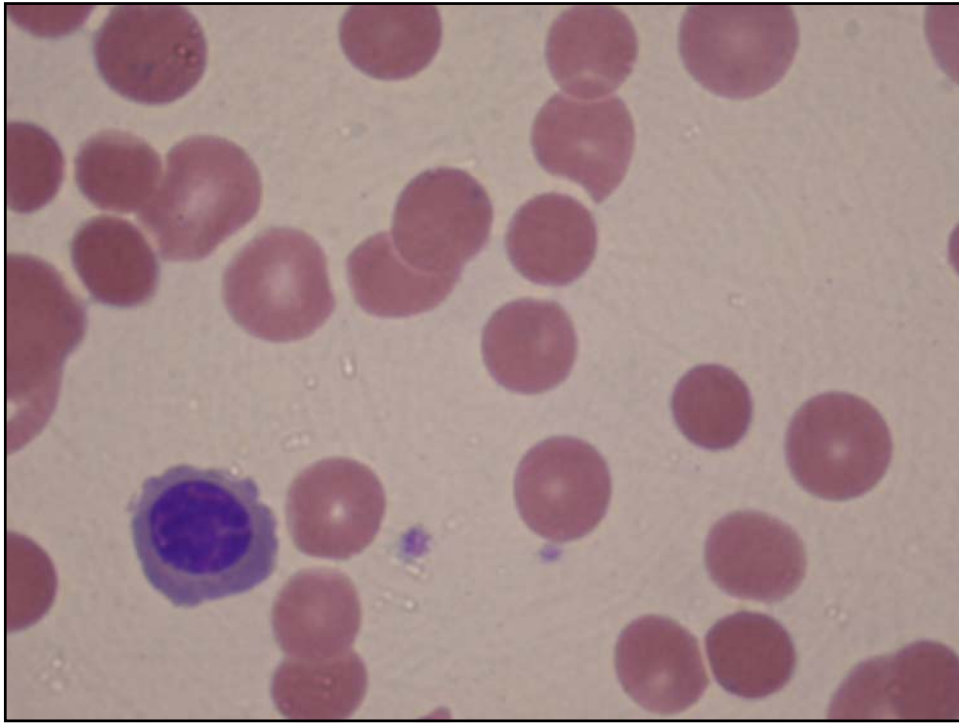
ALBUMIN		4.7	(3.5-5.0)	G/DL
ALK PHOSPHATASE		179	(117-390)	U/L
SGOT/AST	H	61	(0-37)	U/L
SGPT/ALT		26	(6-37)	U/L
BILIRUBIN, TOTAL	H	1.5	(0.0-1.2)	MG/DL
BILIRUBIN, DIRECT	H	0.4	(<0.3)	MG/DL
TOTAL PROTEIN		7.4	(6.0-8.0)	G/DL

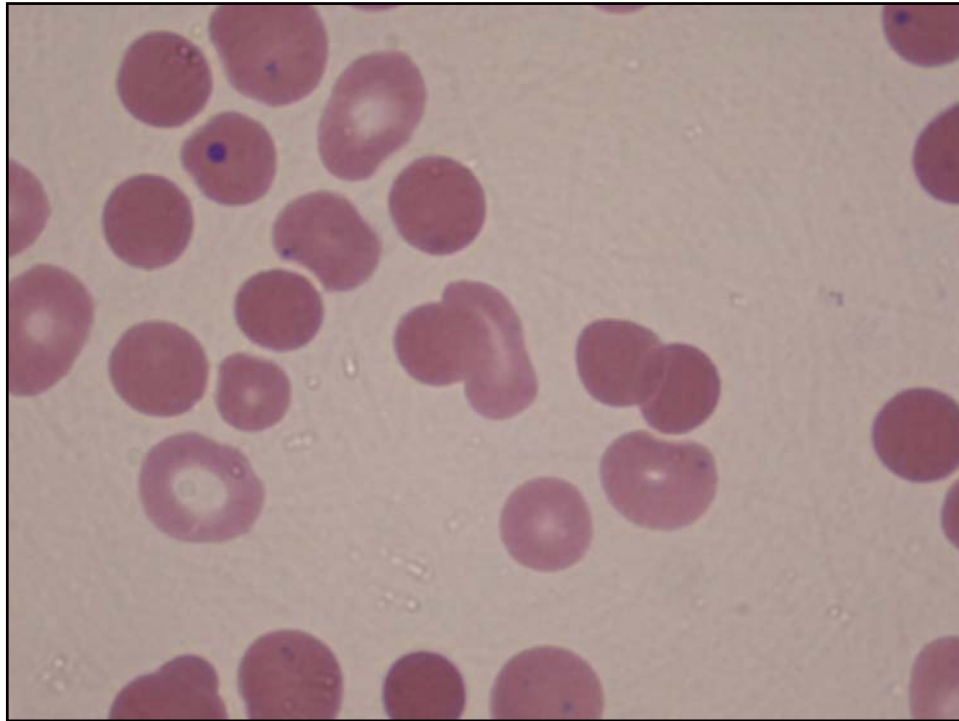
CBC W/DIFF

WBC		9.3	(4.0-14.5)	THOUS/CMM
RBC		4.78	(4.0-5.4)	MILL/CMM
HGB		12.5	(11.5-15.0)	GM/DL
HCT		37.1	(35.0-45.0)	%
MCV		77.6	(77-94)	FL
MCH		26.2	(25.0-35.0)	PG/CELL
MCHC		33.7	(32.0-36.0)	G/DL
RDW	H	18.5	(<15.0)	%
PLATELET		404	(140-440)	THOUS/CMM

CBC W/DIFF				
WBC		9.3	(4.0-14.5)	THOUS/CMM
RBC		4.78	(4.0-5.4)	MILL/CMM
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MCH		26.2	(25.0-35.0)	PG/CELL
MCHC		33.7	(32.0-36.0)	G/DL
RDW	H	18.5	(<15.0)	%
PLATELET		404	(140-440)	THOUS/CMM
RETICULOCYTES				
ABSOLUTE RETIC	H	293.0	(23-93)	THOUS/CMM
RETIC PERCENT	H	6.1	(0.5-1.5)	%
LDH				
	H	385	(94-250)	U/L

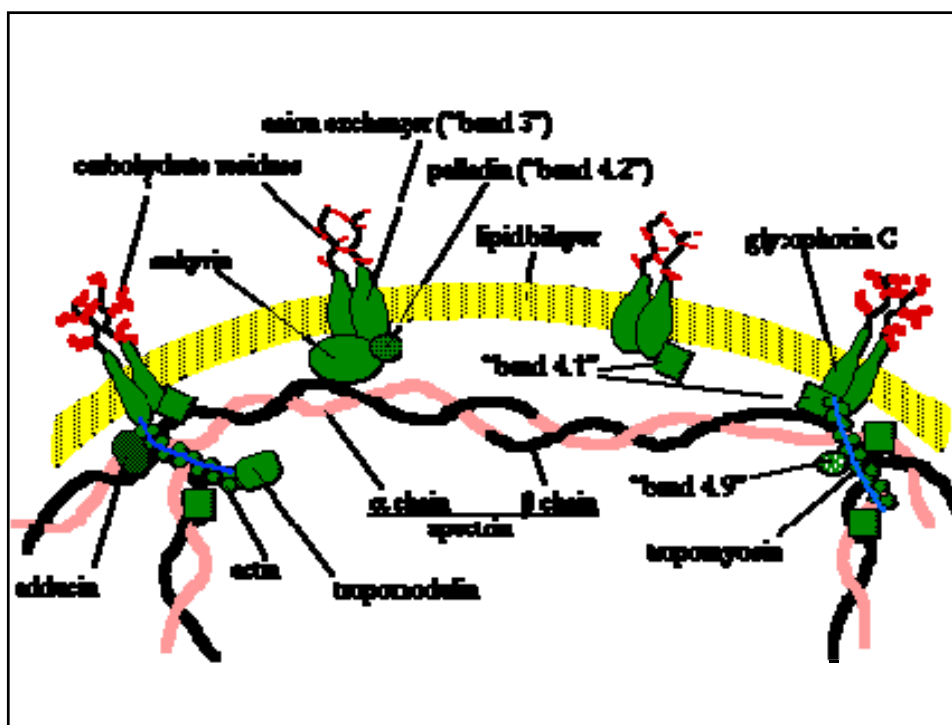


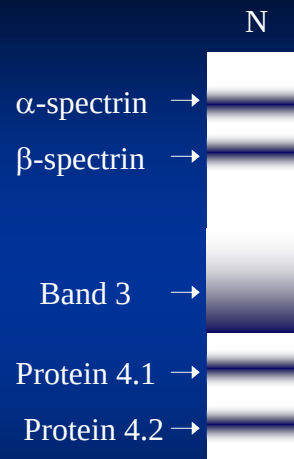




Test	Result	HI LO	Units	Expected Values
Hereditary Spherocytosis Evaluation				
Osmotic Fragility, 0.50 g/dL NaCl	93.1	H	% hemol	0.0-47.8
Osmotic Fragility, 0.60 g/dL NaCl	98.5	H	% hemol	18.7-67.4
Osmotic Fragility, 0.65 g/dL NaCl	99.6	H	% hemol	4.4-36.6
Osmotic Fragility, 0.75 g/dL NaCl	79.2	H	% hemol	0.8-9.1

Test	Result	HI LO	Units	Expected Values
Hereditary Spherocytosis Evaluation				
Osmotic Fragility, 0.50 g/dL NaCl	93.1	H	% hemol	0.0-47.8
Osmotic Fragility, 0.60 g/dL NaCl	98.5	H	% hemol	18.7-67.4
Osmotic Fragility, 0.65 g/dL NaCl	99.6	H	% hemol	4.4-36.6
Osmotic Fragility, 0.75 g/dL NaCl	79.2	H	% hemol	0.8-9.1
Spherocytosis Interpretation				
Reviewed by Dr. J.D. Hoyer				
Osmotic fragility testing shows increased red blood cell lysis. Furthermore, flow cytometry studies using a fluorescent dye which binds to band 3 show a decreased amount of fluorescence. Although correlation with patient's clinical history, family history, and the appearance of the peripheral blood smear is necessary, the combination of these two results is supportive of a diagnosis of hereditary spherocytosis.				





Iolascon, Achille, et.al., Hæmatologica 1996, p548.

Chart Review

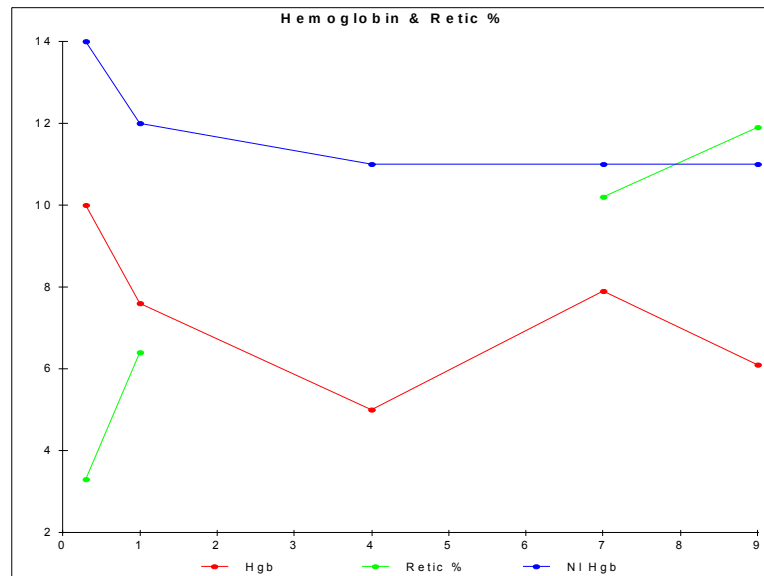
- Patient developmentally delayed
- Abnormal chromosomes – translocation involving chromosome 14q24
- Gene for spectrin is located at 14q23

Infant with hereditary spherocytosis

Patient B.B.

Age	10 days	1 month	4 months	7 months	9 months
Hgb	10	7.6	5	7.9	6.1
Retics %	3.3	6.4		10.2	11.9
MCV	92.5	88.4	81.7	82.8	81
MCHC	36.9	35.5	32.9	33.5	32.4

B.B.



Hereditary spherocytosis

- Consider dx in normocytic anemia with RDW>14
- Only disease with increased MCHC (>35)
- 1/5000 Europeans

44 y.o. WM

- Presented to Hospital 1
- 1 week history of weakness, fatigue, dark urine.
- No other symptoms

Outside Hospital #1

- Positive DAT (Coomb's)
- Negative antibody screen
- Specimen sent to Red Cross Reference Laboratory for workup of + DAT
- 10 units pRBCs given
- Transferred to Hospital 2

Hospital 2 - Labs

- H/H 8.2/23.3
- Tbil 13, mostly indirect
- + Mycoplasma IgM

Hospital 2

- Blood Type O+
- DAT negative (*no antibodies coating the red cells*)
- Antibody screen negative (*no antibodies in the serum*)
- All crossmatches compatible (*no reaction with any donor units*)
- 14 units pRBCs given
- Transferred to referral hospital

ARC Report – Hospital 1

SEROLOGICAL TEST RESULTS

ABO Group: O

Rh Type: Positive Probable Rh Genotype: R₀r (DcE/dce)

Direct Antiglobulin (Coombs) Test: 1+^w Monospecific IgG: 1+^w C-3: Positive Microscopically

Antigen Typing

M	N	S	s	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁				

ANTIBODY IDENTIFICATION

Anti- _____ Reactive: _____

Anti- _____ Reactive: _____

Anti- _____ Reactive: _____

ELUTION STUDIES

Method: Rapid Acid Specificity: No Reactivity Detected

INTERPRETATION - ADDITIONAL COMMENTS

No atypical antibody reactivity was detectable in the _____ serum at this time.

To investigate the positive direct coombs, a rapid acid elution was performed on the _____ cells. The eluate obtained was tested against a panel of select red blood cells and no reactivity was observed. The protein coating the cells could not be identified at this time.

Recommended blood for transfusion is that which is crossmatch compatible.

Referral Hosp Labs

- H/H: 5.3/14.9 after 24 units RBCs
- Decreased retics
- WBC 14,000; 66%P
- Platelets 61,000
- Fe studies normal
- Tbil 4.2, Dbil 0.8

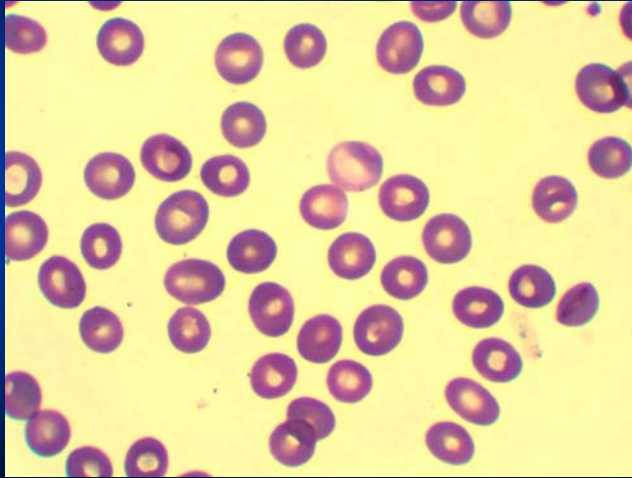
Serum Chemistries

- Alb 2.1 (SPE, IFE normal)
- K 3.4, Ca 6.7, Mg 2.4, BUN 21, Creat 0.6
- AST 66; ALT 104; Alk phos 72

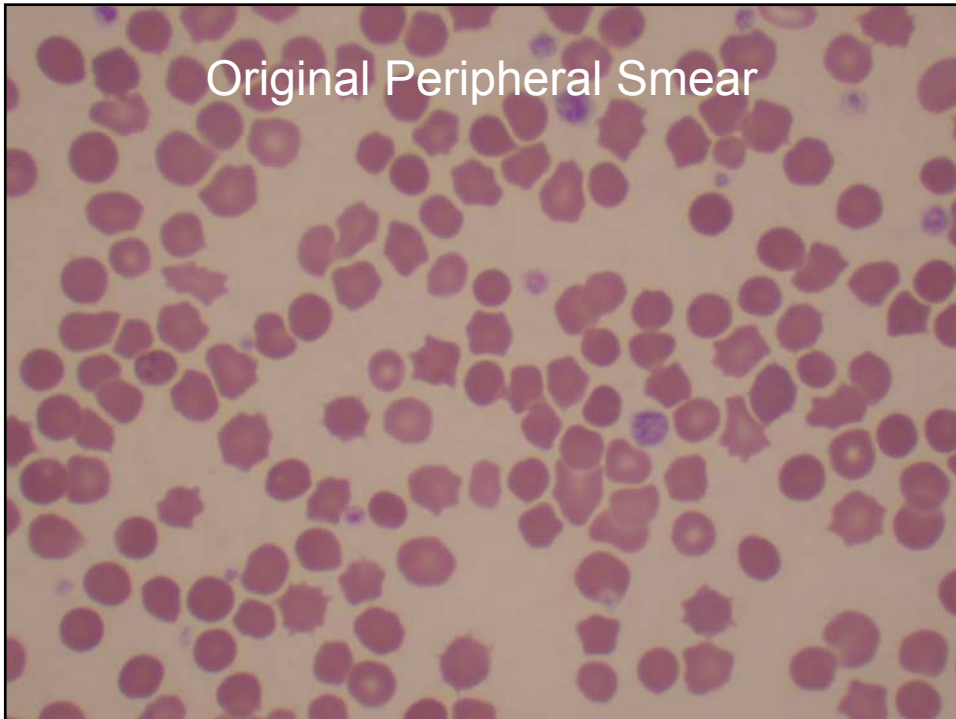
Referral Hospital Imaging

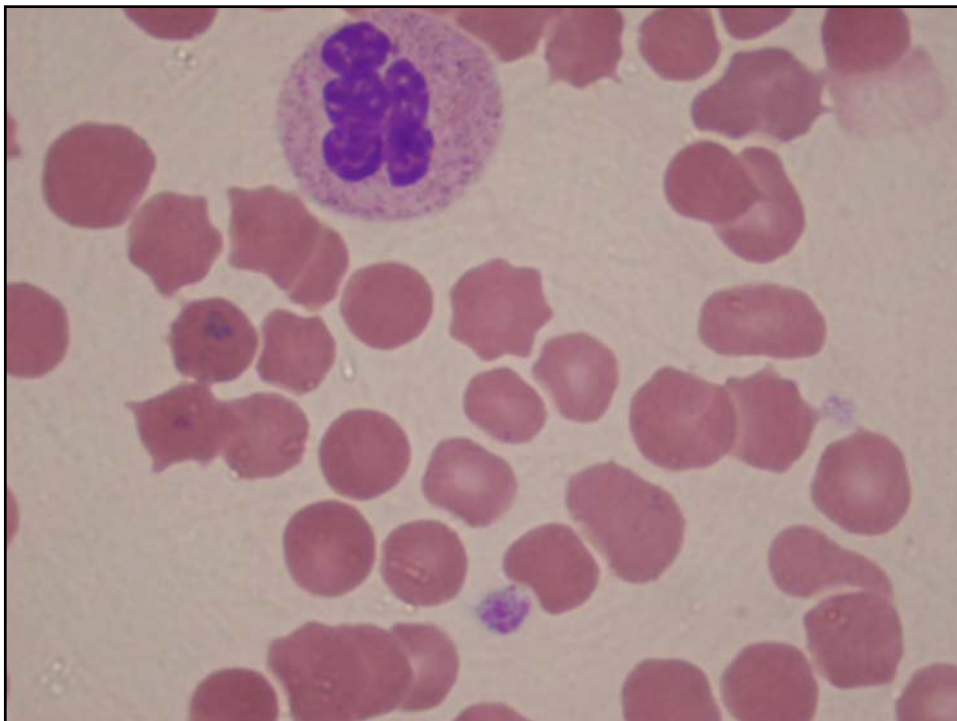
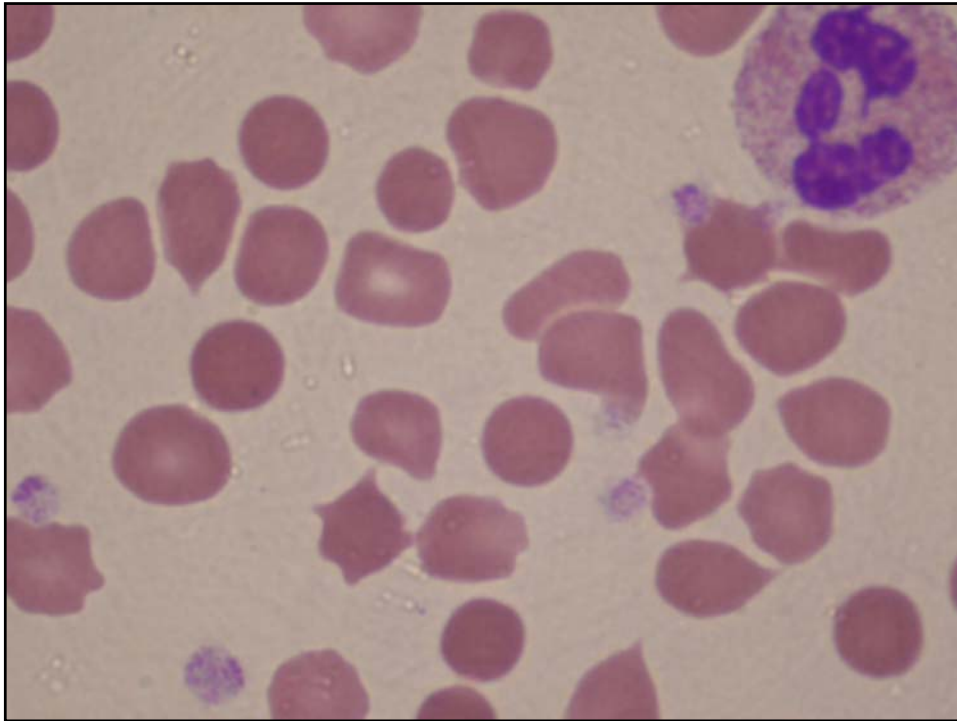
- CXR – normal
- GB U/S – normal
- CT - enlarged spleen with peripheral inhomogeneities suggestive of infarcts

Peripheral Smear
(mostly transfused cells)



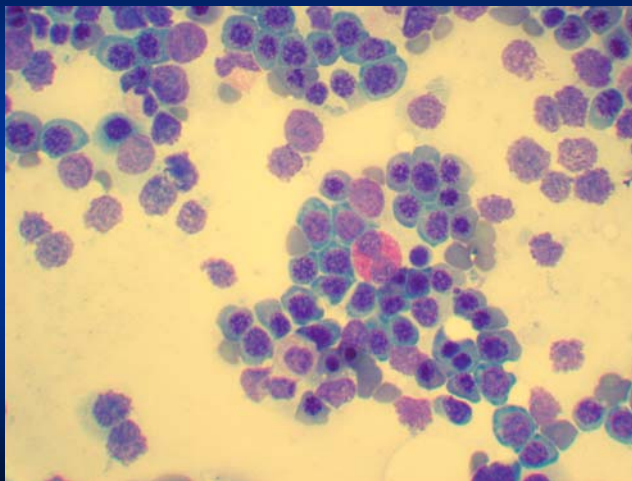
Original Peripheral Smear



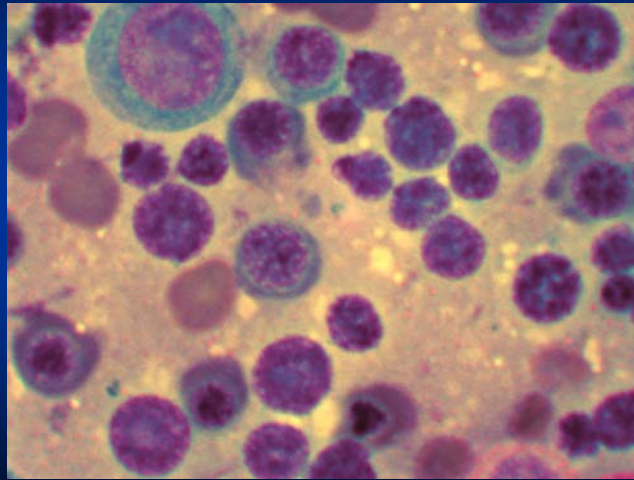


Bone Marrow Workup

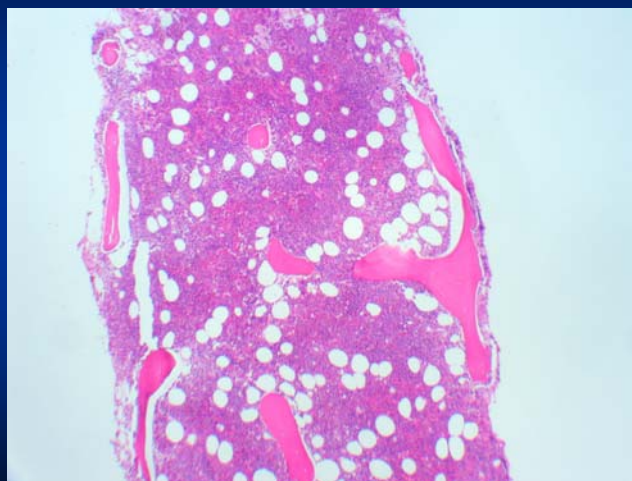
Bone Marrow Aspirate



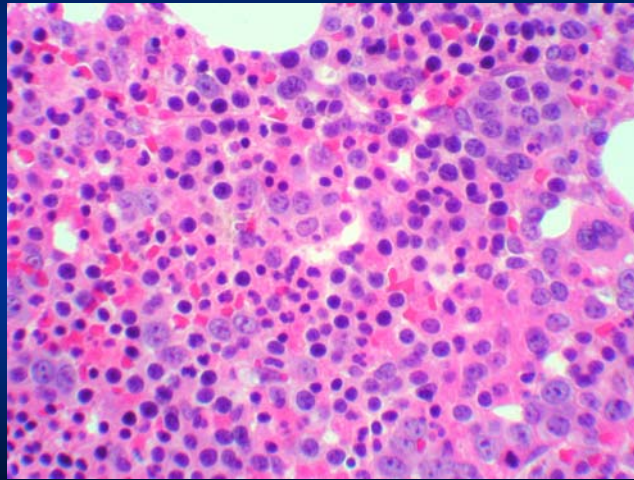
Bone Marrow Aspirate - Oil



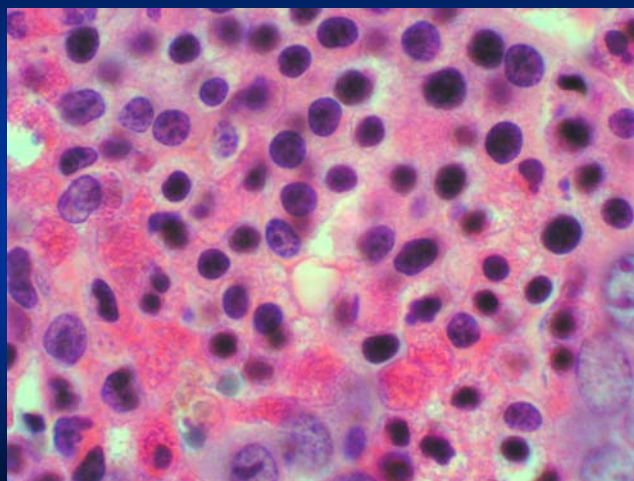
Bone Marrow Biopsy



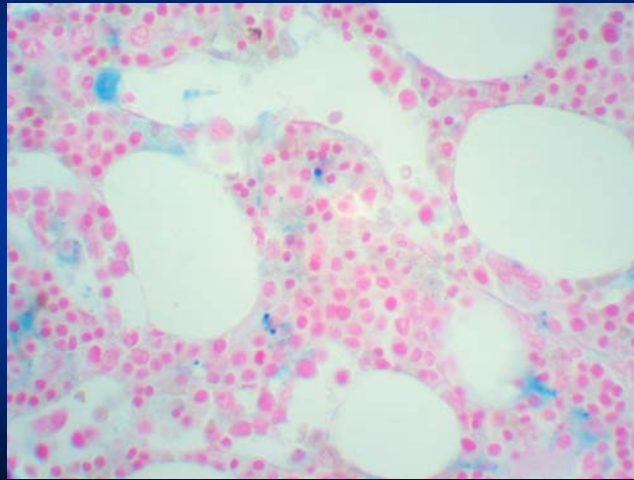
Bone Marrow Biopsy



Bone Marrow Biopsy



Marrow Iron Stain



Referral Hospital Routine Testing

- Type O Rh +
- DAT (Coomb's) negative
- Serum antibodies – negative
- All crossmatches compatible

Mycoplasma Infection

- Associated with production of a cold autoagglutinin, anti-I
- Appears by second week of diagnosis
- IgM may remain positive up to 4 yrs

Cold Agglutinin Screen

ACC. NO. R81270 TECH. 5004 Jalbert
 DATE COLLECTED 11-3-02
 DATE TESTED 11-13-02

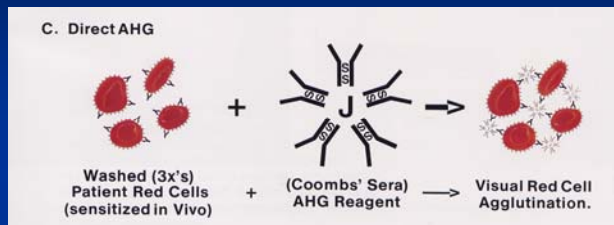
COLD ANTIBODY SCREEN Negative
 ABO DISCREPANCY _____ AN _____

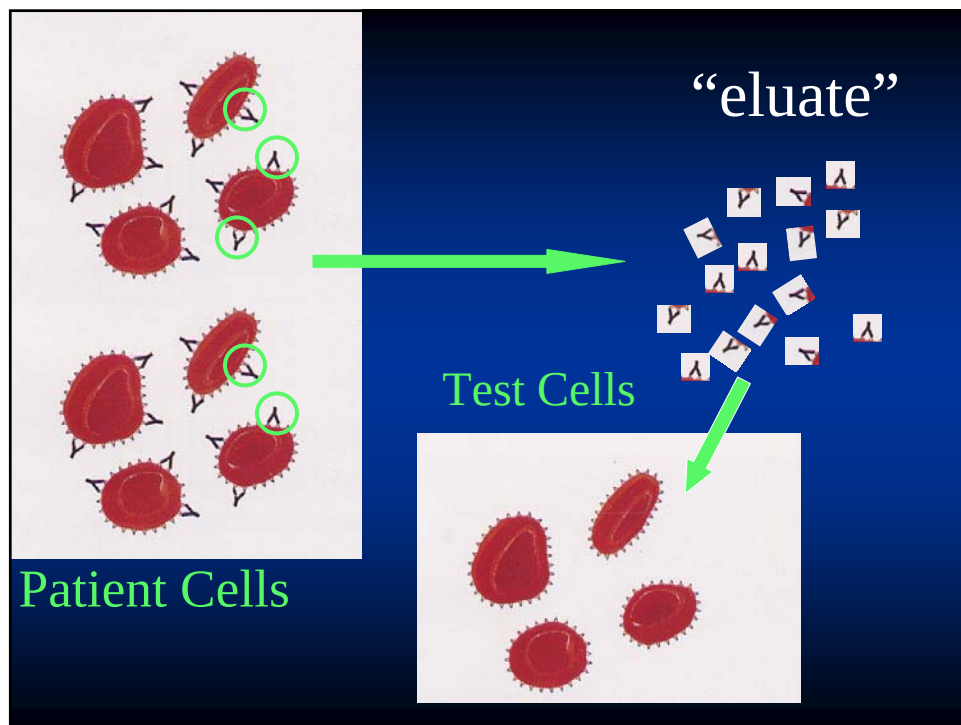
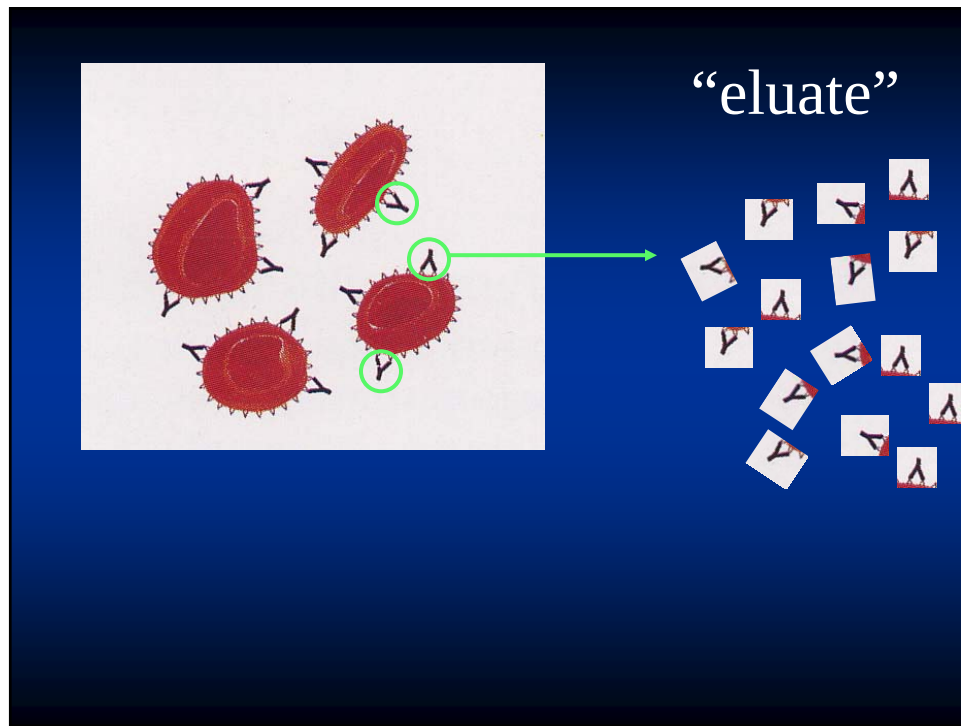
REAGENT	LOT NUMBER	OUTDATE	IMMEDIATE SPIN	ROOM TEMP (22-25 C)	16 C	4 C	IgG COOMBS SERUM	COOMBS CONTR. CELL
SCREEN CELL I (GROUP O CELL)	<u>Immucor 45914</u>	<u>11-21-02</u>	<u>0</u>	<u>0</u>	<u>ND</u>	<u>0</u>		
SCREEN CELL II (GROUP O CELL)	<u>↓</u>	<u>↓</u>	<u>0</u>	<u>0</u>	<u>ND</u>	<u>0</u>		
SCREEN CELL III (GROUP O CELL)	<u>↓</u>	<u>↓</u>	<u>0</u>	<u>0</u>	<u>ND</u>	<u>0</u>		
<u>1/6 Hetero</u> CORD CELL (GROUP O CELL)			<u>0</u>	<u>0</u>	<u>ND</u>	<u>0</u>		
<u>1/6 Hetero</u> CORD CELL (GROUP O CELL)			<u>0</u>	<u>0</u>	<u>ND</u>	<u>0</u>		
AUTOCONTROL			<u>0</u>	<u>0</u>	<u>ND</u>	<u>0</u>		
AI REAGENT CELL								

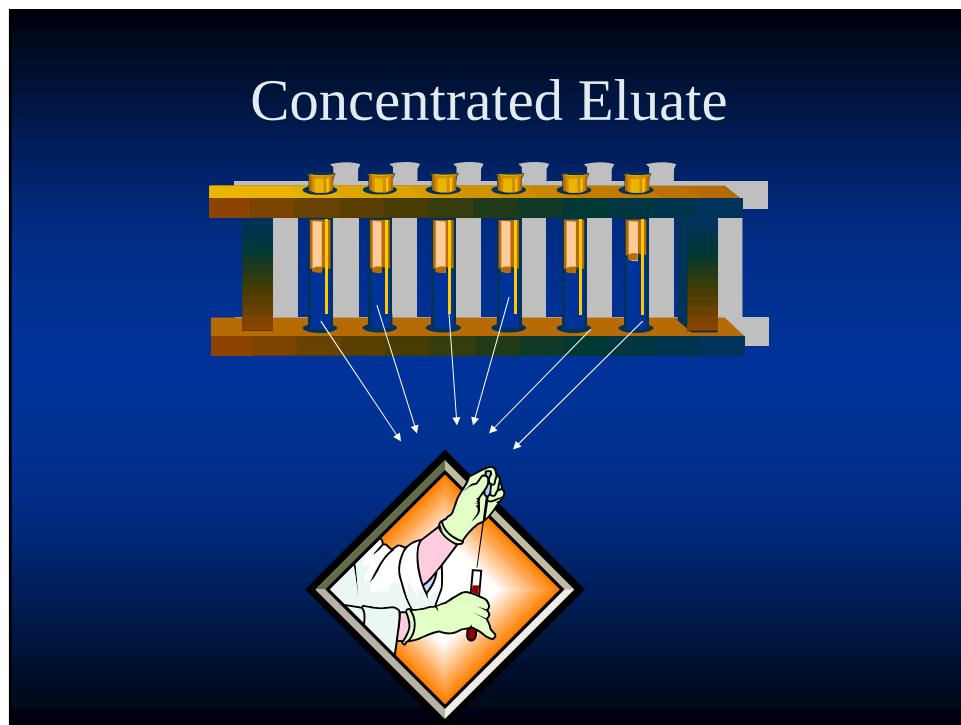
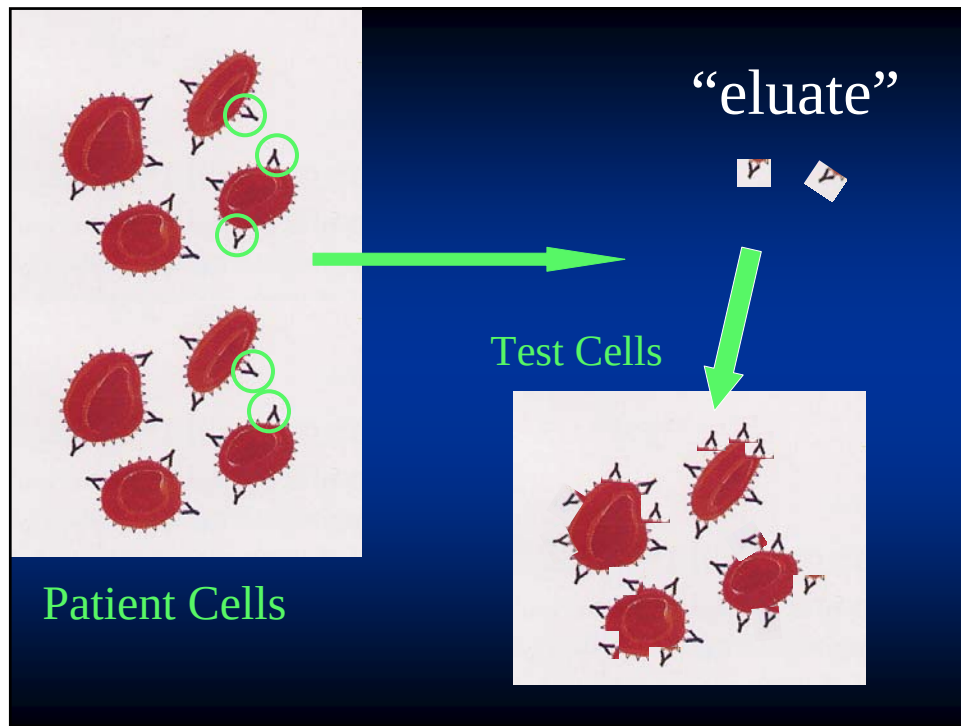
Causes of Neg DAT

- Negative DAT in 2% WAIHA cases
- Antibody coating below sensitivity of DAT
- IgA or IgM antibodies
- Altered RBC antigen expression

Direct Antiglobulin (Coomb's) Test







Concentrated Eluate

46

3 Nov 02

Donor	Rh - Hr							Kell						Duffy	Kidd	Lewis	P	MN				Lutheran	Xg	PATIENT'S SERUM T TEST METH								
	D	C	c	E	e	f	V	C ^W	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁	M	N	S	s	Lu ^a	Lu ^b	Xg ^a	Xg ^b	Am ^{CC}	CC	
R1R1 B2171	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	0	+	+	+	0	0	+	+	0	+	+	1	0	2+
R1wR1 B4798	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	0	0	+	0	+	0	+	0	+	0	+	2	0	2+
R2R2 C2702	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	+	0	+	0	0	+	0	+	0	+	0	+	+	3	2+	ND	
Ror D616	+	0	+	0	+	+	0	0	0	+	0	+	+	+	0	0	+	0	0	0	+	+	+	0	0	0	+	0	4	1+	2+	
r'r E339	0	+	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	0	+	+	5	1+	2+		
r'r F304	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	+	+	+	+	0	+	+	+	6	1+	2+		
rr G531	0	0	+	0	+	+	0	0	+	+	0	+	0	+	+	+	0	+	0	0	+	+	0	+	0	+	+	7	1+	2+		
rr H718	0	0	+	0	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	0	+	0	+	8	1+	2+		
rr N1105	0	0	+	0	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	0	+	0	+	9	1+	2+		
r'r E388	0	+	+	0	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	0	+	0	+	0	+	+	10	1+	2+		
Ror D666	+	0	+	0	+	+	+	0	0	+	0	+	0	+	0	0	+	+	0	+	+	+	+	0	+	+	+	TC				
Patient's Cells																												PC				

Concentrated Eluate

46

3 Nov 02

Donor	Rh - Hr							Kell							Duffy	Kidd	Lewis	P	MN							Luth- eran	X _b	PATIENT'S SERUM T TEST METH			
	D	C	c	E	e	f	V	C ^W	K	k	Kp ^a	Kp ^b	Js ^a	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁	M	N	S	s	Lu ^a	Lu ^b	Xg ^a	Xg ^b	Am ^{CC}	CC
R1R1 B2171	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	0	+	+	+	0	0	+	+	0	+	1	0	2+
R1wR1 B4798	+	+	0	0	+	0	0	+	0	+	0	+	0	+	+	+	+	+	0	0	+	0	+	0	+	0	+	0	2	0	2+
R2R2 C2702	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	+	0	+	0	0	+	0	+	0	+	0	+	+	3	2+	ND
Ror D616	+	0	+	0	+	+	0	0	0	+	0	+	+	+	+	0	0	+	0	0	0	+	+	0	0	0	+	0	4	1+	2+
r'r E339	0	+	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	+	+	+	0	+	+	5	1+	2+
r'r F304	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	+	+	0	+	+	6	1+	2+	
rr G531	0	0	+	0	+	+	0	0	+	+	0	+	0	+	+	+	0	+	0	0	0	+	+	0	+	0	+	+	7	1+	2+
rr H718	0	0	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+	8	1+	2+
rr N1105	0	0	+	0	+	+	0	0	0	+	0	+	0	+	+	+	0	+	+	0	+	+	0	+	0	+	0	+	9	1+	2+
r'r E388	0	+	+	0	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	0	0	+	0	+	0	+	+	10	1+	2+	
Ror D666	+	0	+	0	+	+	+	0	0	+	0	+	0	+	0	0	0	+	+	+	+	+	+	0	+	+	+	+	TC		
Patient's Cells																													PC		

Serum Antibodies in WAIHA

- Spillover of autoantibody
- Alloantibodies
 - History of prior transfusion or pregnancy

Serum Antibodies in WAIHA

- 57% have + antibody screen
- 89% positive with enzyme treated cells

Auto- or Allo- Antibody?

- Antibody coating patient's cells (mostly transfused cells):
 - Anti-c
- Antibodies in patient's serum
 - Anti-c
 - Anti-E

Auto- or Allo- Antibody?

- Antibody coating patient's cells (mostly transfused cells):
 - Anti-c
- Antibodies in patient's serum
 - Anti-c
 - Anti-E
- Patient's Rh phenotype: D+c+E+e+

SEROLOGICAL TEST RESULTS	
ABO Group:	O
Rh Type:	Positive
Probable Rh Genotype:	R ₀ r (DcE/dce)

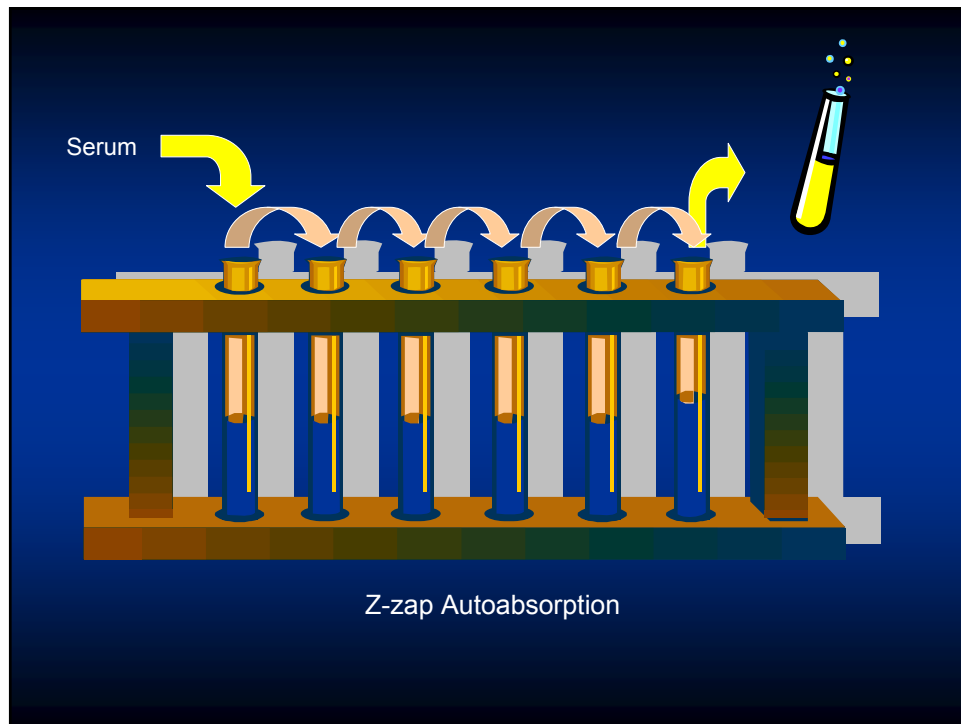
Referral Hospital Course

- 16 units pRBCs
- Decreasing transfusion requirement
- H/H: 9.4/26.6
- Steroids, IVIg
- Folate
- Splenectomy vaccines

Serologic Workup of WAIHA

- “Time-consuming complex algorithm”
 - G. Garratty
- Up to 6 hours to exclude alloantibody
- 24 to 48 hours to do a complete workup
- Requires experience, rare cells, sera.





WAIHA

- Incidence 1/80,000
- Midlife, F>M, 50% unknown cause
- 80% IgG autoantibodies

↓Retics and “missing” antibodies

- Features of WAIHA without detectable antibody
- Spleen highly sensitive to low density antibody coating
- Splenic macrophages may have upregulated Fc receptors due to infection or immunization
- Retics may have ↑ expression of offending antigen

Reticulocytopenia in WAIHA

- Portends a more serious prognosis
- Decreased retics when most anemic
- Destruction of young RBCs by antibody
- May last up to 160 days then abruptly rises.
- Recovery is the rule if red cells given to sustain life during reticulocytopenia.
- Medullary destruction

NEJM, 1982

IVIg Therapy in WAIHA

- Modulates expression and function of Fc receptors
- T-suppressor activation
- Inhibits B-cell maturation

NEJM, 2001

Splenectomy

- 50-60% show good initial response
- Cannot predict response based on age, DAT strength

Steroid Therapy

- Nonspecific – disables macrophages
- After weeks – decreases antibody production

Rituximab

- Mature plasma cells are CD20-negative
- Earlier B-cell precursors

Plasma Exchange

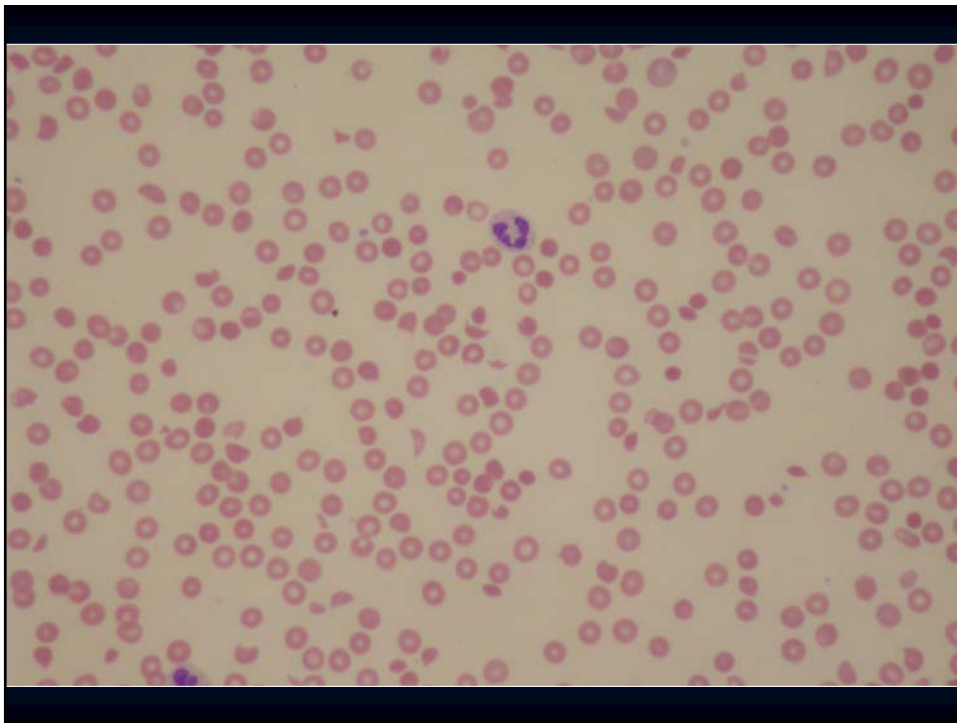
- Stabilization of critical patient prior to surgery
- Good response in some patients refractory to other therapy

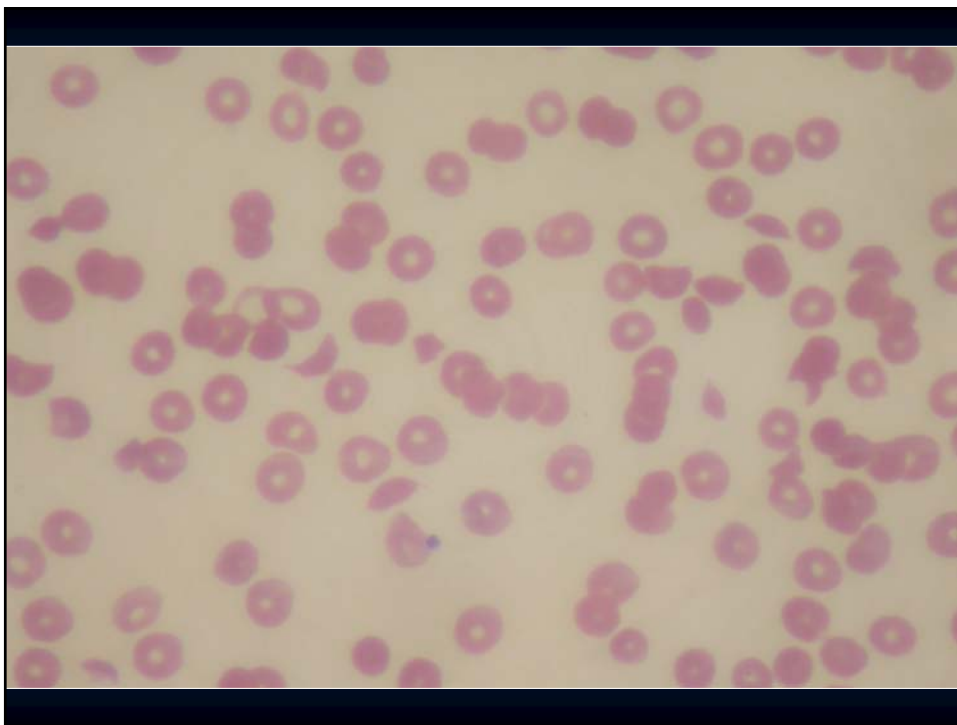
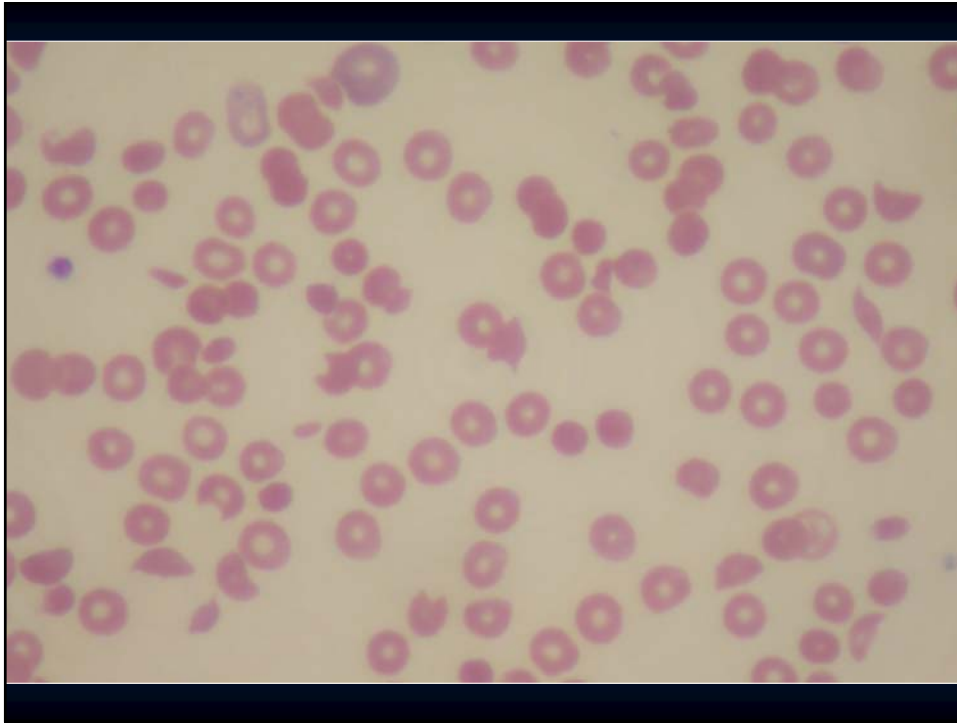
5 year old boy

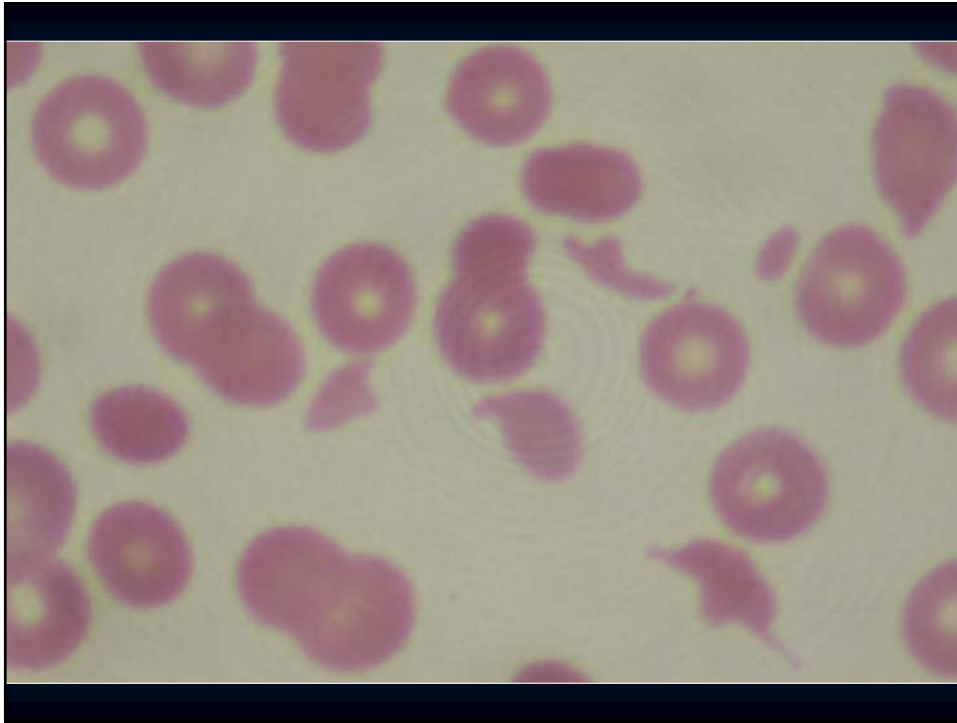
- Presented to the ED
- 3 days of severe diarrhea

CBC

- WBC 9,000
- Hb 5.5
- Hct 17
- Plt 10







Hemolytic Uremic Syndrome (HUS)

- Microangiopathic hemolytic anemia
- E.Coli 0157:H7 infection (after 2-7% infections)
- Shiga-like toxin
- Activates platelets – microthrombi
- No consumption of coag factors

ADAMTS 13

- Cleaves vW factor
- Inhibition of ADAMTS13 causes platelets to clump and adhere to capillary walls forming microthrombi
- TTP – autoantibody

Microangiopathic hemolytic anemia

- | | |
|--|---|
| <ul style="list-style-type: none">• HUS• 5-10% mortality• Children 4mo-6yr• E. Coli 0157:H7• Toxin inhibits ADAMTS 13• Plasma exchange not used | <ul style="list-style-type: none">• TTP• Adults• Mortality 95% without treatment• Autoimmune or drug related• Autoantibody inhibits ADAMTS 13• Plasma exchange with FFP or cryo-poor FFP |
|--|---|

Questions/discussion