

Hematology Volume

Thursday, August 13, 2020

Friday, August 14, 2020

Saturday, August 15, 2020

Course Organizers:

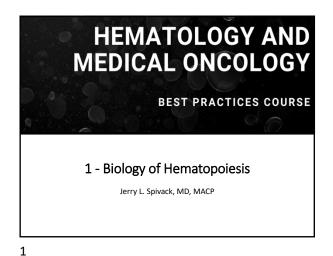
THE GEORGE WASHINGTON UNIVERSITY

WASHINGTON, DC

Biology of Hematopoiesis

Jerry L. Spivak, MD, FACP

August 13, 2020



Disclosures

Disclosures of Financial Relationships with Relevant **Commercial Interests**

None

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Hematopoiesis

☐ Hematopoiesis is the orderly continuous process by which hematopoietic stem and progenitor cells give rise to the mature circulating blood cells responsible for oxygen transport, host defense and hemostasis

Requirements of Hematopoiesis Life Span **Turnover Rate Cell Type** (cells/day) (days) 10¹² **Erythrocytes** 120 10¹¹ Granulocytes 0.5 10¹¹ **Platelets** 9

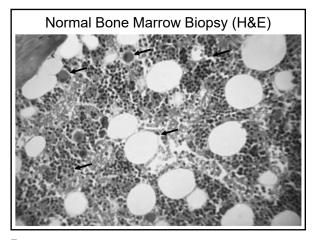


Hematopoiesis is not merely a process but a unique organ system with specific characteristics

- ☐ Hematopoiesis has a distinct ontogeny, anatomy and physiology
- ☐ Hematopoietic ontogeny repeats its phylogeny
- ☐ Hematopoiesis is hierarchical
- ☐ Hematopoiesis is clonal and normally polyclonal
- ☐ Hematopoiesis is both deterministic and random in behavior

Ontogeny of Hematopoiesis				
	% Body Weight	Site	Mature Cell	Hemoglobins
Embryonic	-	Yolk sac Intravascular	Nucleated red cells	Embryonic
Fetus	1.5	Liver, spleen Extravascular (Intravascular) Appendicular	Enucleate Red cells	Fetal
Adult	4.5	Bone marrow Extravascular (Intravascular)	Enucleate Red cells	Adult

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The Hematopoietic Microenvironment

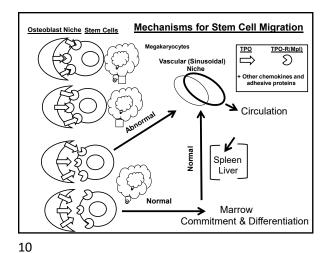
- ☐ Mammalian hematopoiesis is normally extravascular after birth
- ☐ Within the marrow, hematopoietic progenitor cells differ in their location according to their lineage
- ☐ Stromal cells essential for promoting hematopoiesis include: fibroblasts, osteoblasts, adipocytes, endothelial cells, reticular cells, and macrophages

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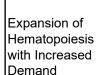


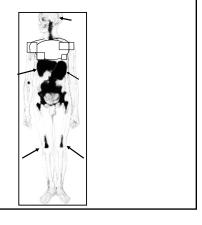
The Hematopoietic Microenvironment (Continued)

- □ Stromal elements essential for promoting hematopoiesis include: the various collagens, fibronectin, laminin and the glycosoaminoglycans
- Stromal cells synthesize soluble and membrane-bound growth factors, matrix proteins and glycosoaminoglycans that tether growth factors
- ☐ Hematopoietic progenitor cells express adhesion receptors (integrins) and homing proteins for cell-cell and cell-matrix interactions.

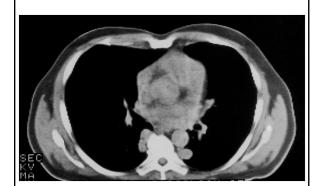


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Paravertebral Extramedullary Hematopoiesis



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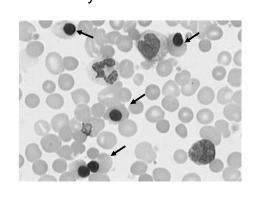
Polycythemia Vera: Extramedullary Hematopoiesis



Primary Myelofibrosis: Extramedullary Hematopoiesis

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Leukoerythroblastic Reaction



Causes of Extramedullary Hematopoiesis and Leukoerythroblastic Reactions □ Carcinoma metastatic □ Polycythemia vera to the bone marrow ☐ Chronic myelogenous ■ (prostate, breast, leukemia lung, stomach) □ Myelodysplasia □ Lymphoma involving

- the bone marrow ■ (Hairy cell leukemia,
- □ Primary myelofibrosis
- ☐ Acute hepatic injury
- □ Chronic hemolysis
- □ Recombinant hematopoietic growth factor therapy (EPO; G-CSF)

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Hematopoietic Growth Factors

- ☐ Hematopoietic growth factors (except for erythropoietin) exhibit redundancy, pleiotrophy, and synergy
 - Growth factor production is redundant since stromal cells can synthesize more than one type of growth factor
 - Some have multiple functions and stimulate more than one type of progenitor cell
 - Most have overlapping functions
 - Combinations of growth factors can be more effective than individual ones (Epo +G-CSF)



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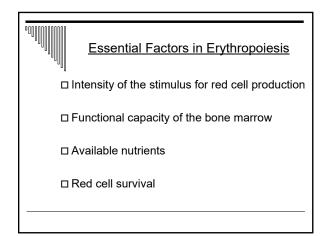
Hematopoietic Growth Factors (Continued)

- ☐ Growth factor synthesis is highly localized with growth factor tethering
- ☐ Myeloid growth factors influence both primitive progenitor cells and their mature progeny
- ☐ Growth factors act to:
 - Maintain target cell viability
 - Initiate cell cycle activity
 - Activate effector functions

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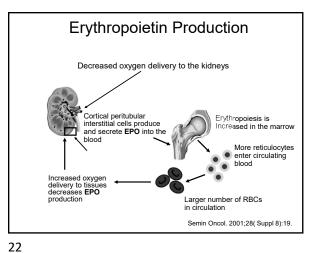
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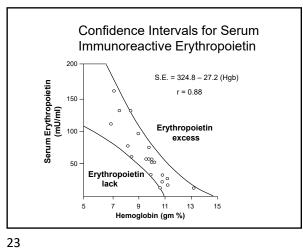
Factor	Source	Function
Erythropoietin	Kidney, Liver	Stimulates erythroid progenitor cell proliferation
Granulocyte colony Stimulating factor	Monocytes Mesenchymal cells Neutrophils	Stimulates granulocyte progenitor cell proliferation and activation
Thrombopoietin	Liver, Kidney	Stimulates megakaryocytopoiesis and thrombopoiesis and HSC quiescence and activation

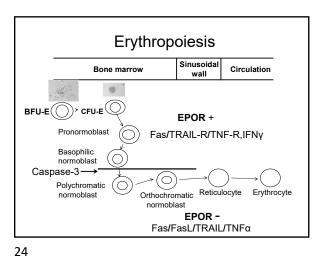


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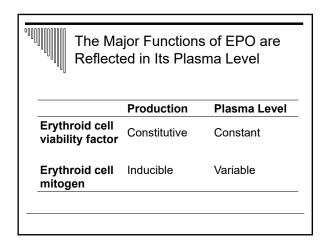
Hypoxic Regulation of Erythropoietin Production Normoxia Нурохіа HIF-1α HIF-1β HIF-la gene VEGF GLUT1 N Engl J Med 348:1282, 2003

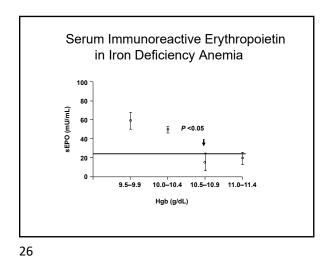


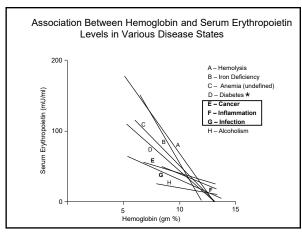




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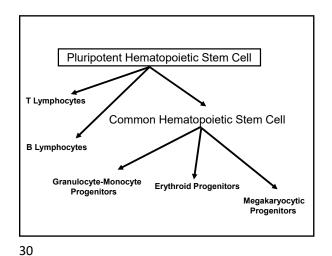


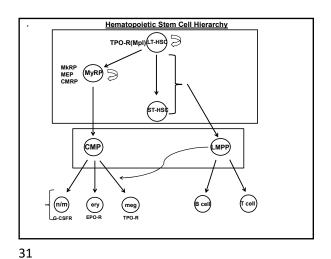


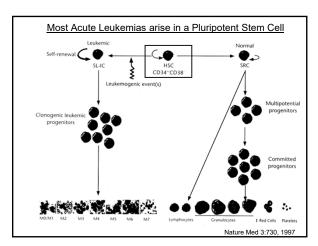


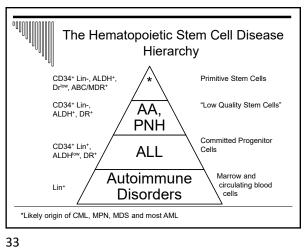
Los	ss of the Hgb-Serum EPO Correlation When the Serur Creatinine is Greater than 1.5 mg %
	70
	60 ° r = 0.32
Ŷ	50
nU/mI	40
SEPO (mU/mL)	30
-S	20
	10 8 00 00 000 000 00 00 00 00 00 00 00 0
	L 5 6 7 8 9 10 11 12 13 14 15 Hgb (g/dL)

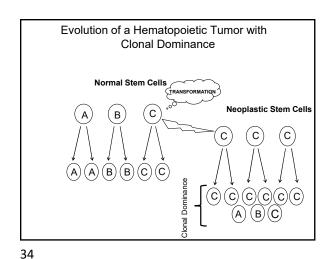
Change in Ri		e Recombinant Erythropoie er Time cı	tins
ຶ່ງ <u>2001</u>	<u>KK</u>	<u> </u>	
Hematologic Response	3.43	(3.07 - 3.84)	
Reduction in transfusions	0.64	(0.60 – 0.68)	
Risk of Thromboembolism	1.58	(0.94 - 2.66)	
Overall Survival	0.81	(0.67 – 0.99)	
<u>2007</u>			
Risk of Thromboembolism	1.67	(1.35 - 2.06)	
Overall Survival	1.08	(0.59 – 1.18)	

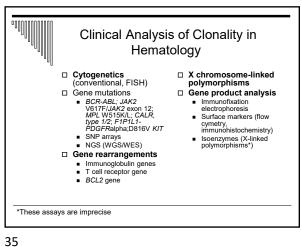


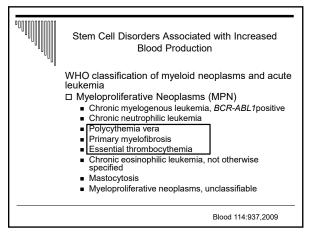


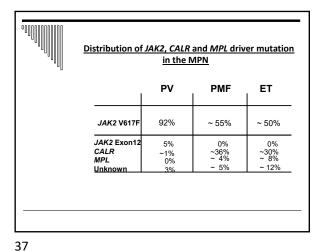












Stem Cell Disorders Associated with Increased Blood Production (Continued) ☐ Myeloid and lymphoid neoplasms associated with eosinophilia and abnormalites of PDGFRA, PDGFRB, or FGFR1 ■ Myeloid and lymphoid neoplasms

- associated with PDGFRA rearrangement
- Myeloid neoplasms associated with PDGFRB rearrangement
- Myeloid and lymphoid neoplasms associated with FGFR1 abnormalities

Blood 114:937.2009



Stem Cell Disorders Associated with Increased Blood Production (Continued)

- □ Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)
 - Chronic myelomonocytic leukemia
 - Chronic neutrophilic leukemia (CSFR2 mutations)
 - Atypical chronic myeloid leukemia, BCR-ABL1-negative (SETBP1, CSFR2 mutations)
 - Juvenile myelomonocytic leukemia (7del;NF-1)
 - Myelodysplastic/myeloproliferative neoplasm, uńclassifiable
 - Refractory anemia with ring sideroblasts and thrombocytosis (SF3B1)

Blood 114:937,2009

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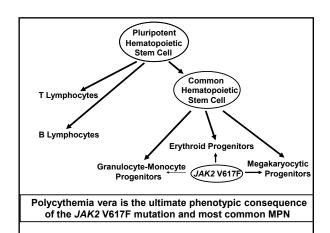


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The Chronic Myeloproliferative Neoplasms

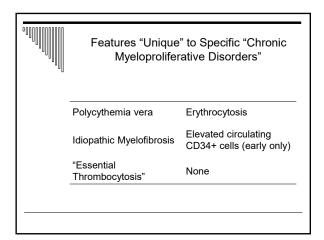
The chronic myeloproliferative neoplasms are clonal hematopoietic stem cell disorders, in which there is overproduction of one or more of the normal formed elements of the blood in the absence of a definable stimulus, extramedullary hematopoiesis and transformation to myelofibrosis or acute leukemia at variable but low rates.

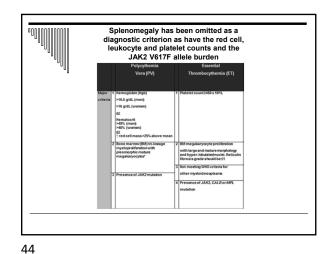
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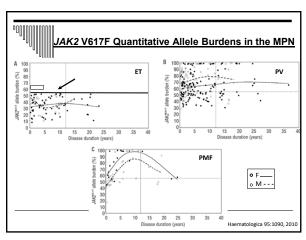
The Interrelationships Between the Chronic Myeloproliferative Disorders **Primary Myelofibrosis** ⇒ Polycythemia Vera 'Essential Thrombocytosis" 💳 "All pathways lead to polycythemia vera"

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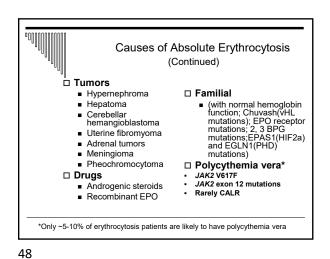


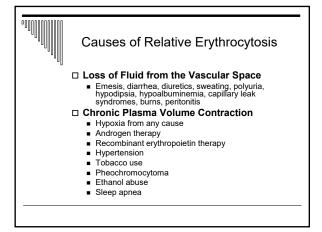
Microcytic eryth	rocytosis:a clue to p	olycythemia vera
HEMOGLOBIN (13.9-16.3)	9.3 gm %	13.2 gm %
HEMATOCRIT (41-53%)	31.9 %	42 %
RED CELL COUNT (4.5 – 5.9 x 10 ⁶ /μL)	5.53 x 10 ⁶ /μL	6.02 10 ⁶ /μL
MCV (80-100 fL)	57.7 fL	65.1 fL
RDW (11.5-14.5)	36.4	18.6
	Thalassemia Minor	Polycythemia Vera

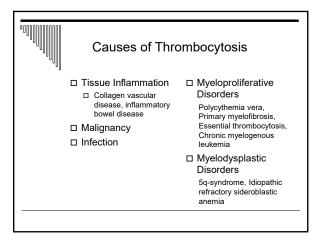


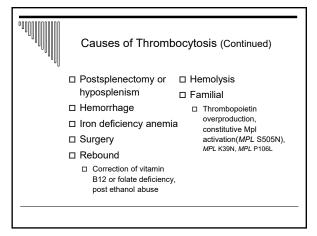
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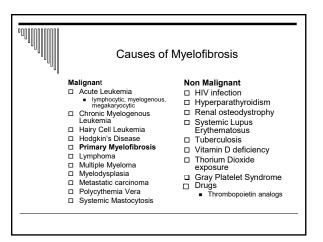
Causes of Absolute Erythrocytosis Neurologic □ Hypoxia Diseases. Carbon monoxide intoxication (tobacco□ Renal Disease abuse. ■ Renal artery environmental) stenosis High O₂ affinity hemoglobins ■ Focal sclerosing or membranous ■ High altitude glomerulonephritis ■ Pulmonary disease Renal transplantation ■ Right to left shunts ■ Sleep apnea



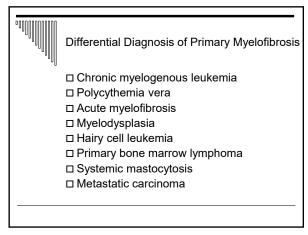


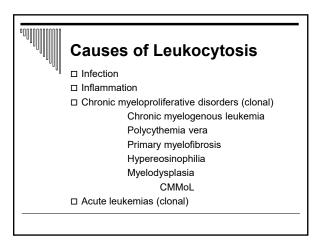


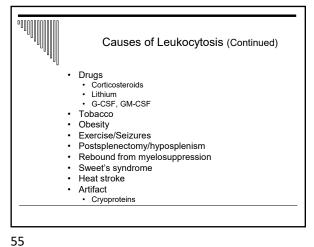


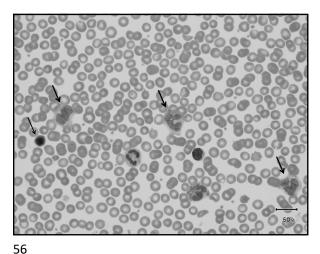


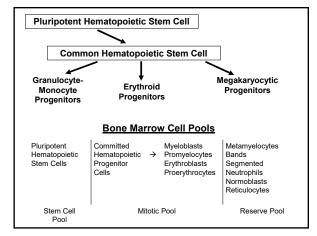
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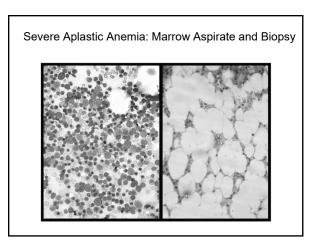


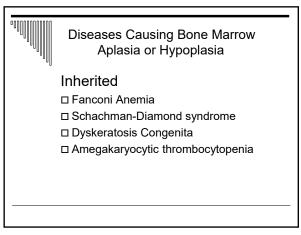


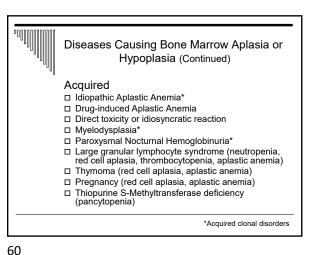




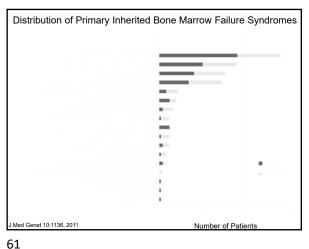


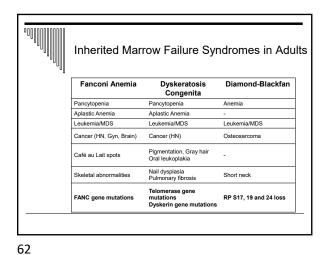


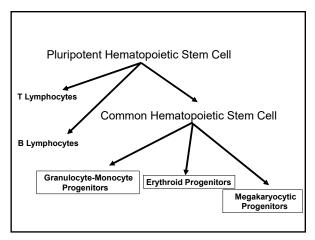


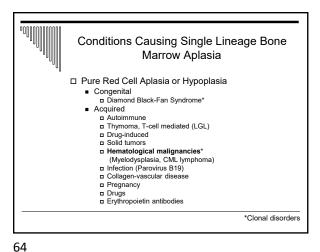


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Marrow Aplasia (Continued)

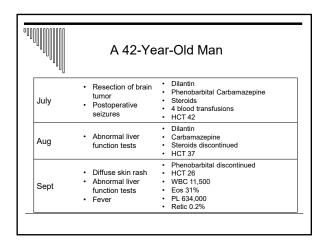
Conditions Causing Single Lineage Bone

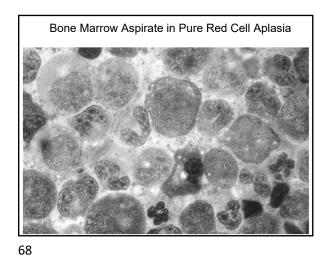
□ Pure White Cell Aplasia

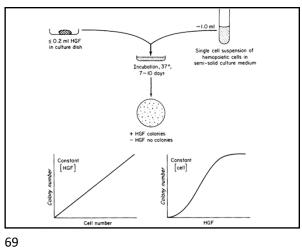
- Congenital (Kostmann's syndrome)*
- Autoimmune, T-cell mediated (LGL)
- Drugs
- □ Pure Megakaryocytic Aplasia
 - Congenital* (CAMT)
 - Thymoma, T-cell mediated (LCL)
 - Autoimmune
 - Hematological Malignancies*

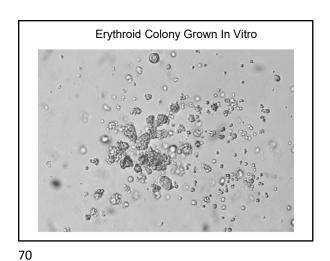
*Clonal disorders

Stem Cell Defects Causing Monocytopenias Disease Clinical Phenotype Genetic Location Diamond-Blackfan RP mutations Red Cell Hypoplasia (S17; S19; S24) ? G-CSFR mutations Kostmann's Neutropenia syndrome (Acute Leukemia) Congenital Thrombocytopenia TPO-R (MPL) amegakaryocytic (Pancytopenia) mutations thrombocytopenia Myelodysplasia Red Cell Aplasia; 5q-RP mutation (S14) , Aplastic Anemia, Thrombocytopenia

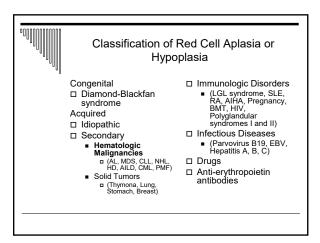


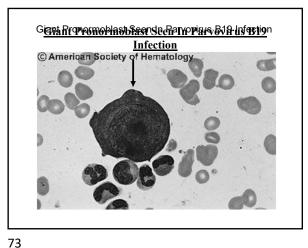




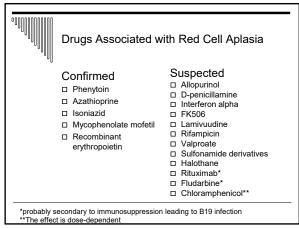


االه						
	Catamami			nse to The	se to Therapy	
	Category	Colony Growth	C.R.	P.R.	None	
	1	Normal	70%	30%	-	
	II	Reduced	25%	-	75%	
	III	Undetectable	-	-	100%	





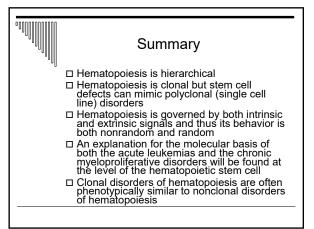


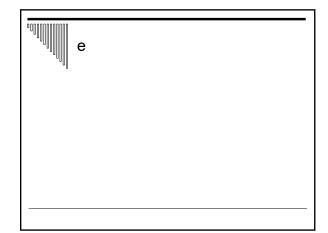


Classification of Adult Hematopoietic Disorders Clonal Noncional Aplastic anemia Aplastic anemia Red cell aplasia Megakaryocytic aplasia MDS Red cell aplasia White cell aplasia Decreased Production Megakaryocytic aplasia Anemia due to renal diseas PNH Polycythemia vera* Essential thrombocytosis 20 Erythrocytosis Primary myelofibrosis* Increased 2º Thrombocytosis Leukemoid reactions MDS (thrombocytosis; *JAK2* V617F)
CML
CMMoL; CNL Production Hemolytic anemia Immune thrombocytopenia Increased PNH Destruction *Can be JAK2 V61 Agranulocytosis

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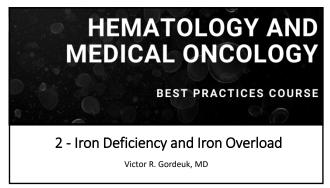




Iron Deficiency and Overload

Victor R. Gordeuk, MD

August 13, 2020



Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

- Grant support CSL Behring, Global Blood Therapeutics, Imara, Ironwood, Novartis
- Consulting- CSL Behring, Global Blood Therapeutics, Novartis, Forma

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Outline

- 1. Review of iron metabolism
- 2. Iron deficiency
- 3. Iron overload: hereditary, environmental, transfusional

Iron

- · Essential nutrient for all living organisms.
 - Reversible binding of O2: Hb, myoglobin
 - Enzyme systems:
 - heme (cytochromes, catalase, glutathione peroxidase, NO synthase)
 - non-heme (RNR, aconitase)
 - Immunity: free radicals to destroy microbes
- Highly reactive with O2; can cause toxicity

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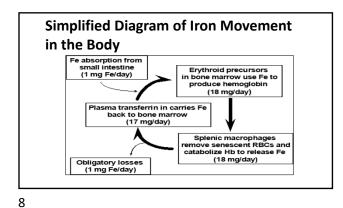
Iron Metabolism: Broad Themes

- Deficiency of iron
 - most common nutritional problem world wide
- Iron overload
 - less common
 - important health problem
- · Absorption of iron
 - highly regulated to prevent excess Fe from being absorbed
- Excretion of iron
 - There is no physiologic pathway for excreting excess

Iron Requirements

Obligatory losses	<u>Men</u> 1.0 mg/d	Women 1.0 mg/d
Menstruation	0.0 mg/d	0.5 mg/d
Total losses	1.0 mg/d	1.5 mg/d
Iron absorbed	1.0 mg/d	1.5 mg/d

	65 kg F	75 kg M
Functional compounds		
Hemoglobin	1900 mg	2500 mg
Myoglobin	310 mg	340 mg
Enzymes	170 mg	190 mg
Transferrin	2.7 mg	3.2 mg
Storage compounds		
Ferritin & hemosiderin	300 mg	800 mg
Total	~2700 mg	~3800 mg



Dietary Iron

- · Typical diet men
 - Mean of 18 mg/day
 - SD range of 4 30 mg/day
- Typical diet women

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- Mean of 13 mg/day
- SD range of 7 19 mg/day
- ~1/3 of Fe from fortification of flour

What We Eat in America, NHANES 2007-2008

Iron absorption- proximal small bowel
Hepcidin- 25 aa peptide produced by liver that suppresses

Enhanced absorption • Low hepcidin:

iron absorption

- Low hepcidin:

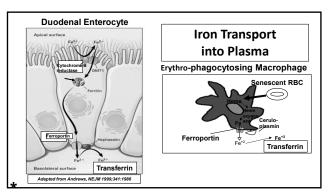
 Iron deficiency
- rythropoiesis
- Dietary factors:

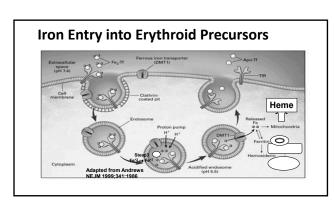
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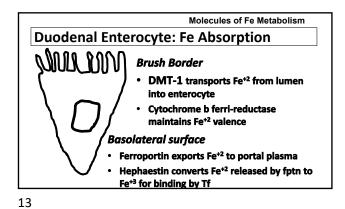
- ascorbic acid (Fe⁺² valance absorbed)
- Heme vs non-heme Fe

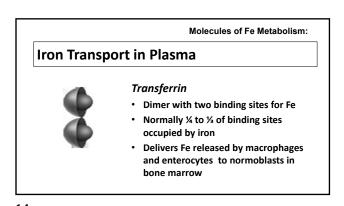
Inhibited absorption

- · High hepcidin
 - → iron stores
 - Inflammation
 Dietary factors:
 - tannins (tea)
 - phytates (bran)

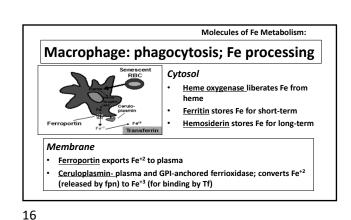




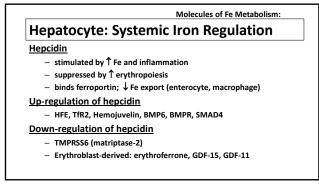


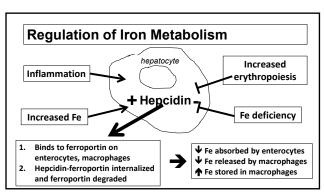


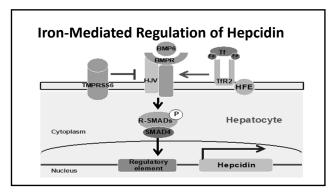
Molecules of Fe Metabolism: **Erythroid Progenitor: Hb production** Membrane Cytosol TfR1-mediated Ferritin stores excess Fe endocytosis of Tf-Fe Endosome Mitochondrion Acidification releases Fe from Tf Mitoferrin-1 imports Fe+2 Steap3 converts Ferrochelatase inserts Fe+2 Fe⁺³ to Fe⁺² into protoporphyrin to form heme DMT-1 exports Fe⁺² to cytosol

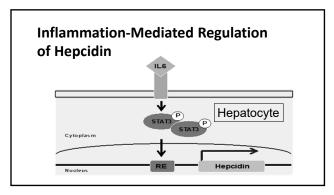


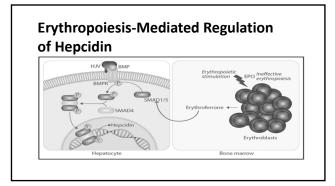
15











Indirect Indicators of Fe Status

- · Daily clinical use
 - Serum iron concentration
 - Serum transferrin conc. or TIBC
 - Transferrin saturation [(Fe/TIBC) X 100]
 - Serum ferritin concentration
- Others
 - Serum transferrin receptor conc.
 - RBC protoporphyrin concentration
 - MRI of liver

21 22

Direct Indicators of Fe Stores

- · Bone marrow aspirate
 - Prussian blue or Perls stain
- Liver biopsy
 - Prussian blue stain
 - Chemical iron concentration
- Magnetic susceptibility measurement of liver

Changes in Indirect Indicators of Fe Status

♠ Serum Fe & Tf Sat

- Iron overload
 - Hepatocellular damage
- BM suppression
- Recent ingestion of Fe preparation

↑ Serum Ferritin

- Iron overload
- Hepatocellular damage
- Inflammation
- Hyperferritinemia/cataract syndrome
 - autosomal dominant
 - 1ftn but no Fe-loading
 - mutation IRE L-ferritin mRNA

Changes in Indirect Indicators of Fe Status

◆ Serum Fe & Tf Sat

- Iron deficiency
- Inflammation

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- Diurnal variation
 - blood drawn afternoon or evening

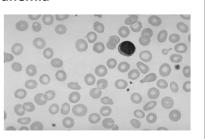
▶ Serum Ferritin

• Iron deficiency

Fe deficiency anemia

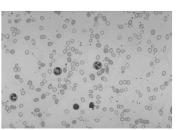
- · microcytosis
- · central pallor

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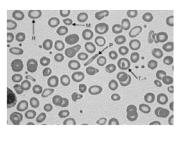
Fe deficiency anemia

- Marked microcytosis
- Marked central pallor



Fe deficiency anemia:

- H- hypochromia
- P- pencil RBC
- T- targeting;
- M- microcytosis



27 28

Fe Deficiency: Causes

1. Chronic blood loss

- GI bleeding: ulcer, tumor, cancer, diverticuli, etc.
- Heavy menses, menorrahgia
- Frequent blood donation; hemodialysis
- Hook worm infestation
- 2. Increased physiologic requirements
- Women (menstruation, pregnancy, lactation),
- Infants & adolescents (growth spurt)

Fe Deficiency: Causes

3. Decreased absorption

- Celiac disease (gluten enteropathy)
- Autoimmune (atrophic) gastritis; H. pylori
- Inflammatory bowel disease
- Gastrectomy- loss of gastric acid
- Duodenal bypass surgery

Fe Deficiency: Causes

4. Hemoglobinuria (intravascular hemolysis)

- PNH
- Runners
- · Damaged heart valves
- · Microangiopathic hemolysis

5. Sequestration

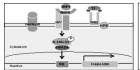
31

 pulmonary alveolar bleeding in idiopathic pulmonary hemoiderosis or Goodpasture's syndrome

Fe Deficiency: Causes

6. Genetic predisposition

TMPRSS6 mutations (Iron-refractory IDA- excessive production of hepcidin)



TMPRSS6 down-regulates hepcidin

• Inactivating mutations lead to ♠
hepcidin

Iron-Refractory Iron Deficiency Anemia

- · Severe autosomal recessive anemia
- · Lack of response to oral Fe
- · Partial response to IV Fe
- Several TMPRSS6 mutations implicated

Fe Deficiency: Clinical Manifestations

- · Impaired psychomotor development
- Fatigue, irritability, ↓ work productivity
- Pica

32

- · Koilonychiae, glossitis, angular stomatitis
- Dysphagia 2° to esophageal web (Plummer-Vinson or Patterson-Kelly Sx)
- · Increased risk of thrombosis

33 34

Fe Deficiency: Lab Findings

1. Peripheral blood cells

- − ↑ RDW, platelets
- $-\downarrow$ MCV, MCH, MCHC, RBC, Hb, Hct
- Retics not increased
- − ↑ RBC protoporphyrin

2. Serum tests

- \downarrow Fe , Tf Sat, ferritin (< 12 $\mu g/L)$
- — ↑TIBC, transferrin, transferrin receptor

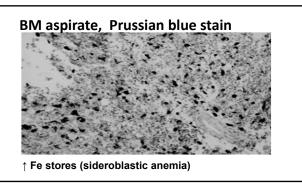
Fe Deficiency: Lab Findings

3. Bone marrow aspirate

- absent macrophage Fe; ↓ sideroblasts
- erythroid hyperplasia

4. Other tests if blood loss not found

- autoimmune gastritis: ↑ gastrin, antiparietal cell ab +
- gluten enteropathy: anti-endomyoseal ab +
- H. pylori: ab +, urease breath test +



BM aspirate, Prussian blue stain

Absent Fe stores (Fe deficiency)

37 38

Fe Deficiency: Therapy

Oral

- Ferrous sulfate: standard approach
 - 200 mg elemental Fe/d (3- 325mg tabs/d)
 - 5.0 mg elemental Fe/kg per day in children
 - risk of iron poisoning
- Once a day or alternate day dosing may be preferable

Lancet Haematol 20 4:524-33 Iron absorption from oral iron supplements given on consecutive versus alternate days and as single morning doses versus twice-daily split dosing in iron-depleted women: two open-label, randomised controlled trials Nation

Fe Deficiency: Therapy

- 2. Intravenous
 - Iron dextran (INFeD)
 - Ferric gluconate complex in sucrose (FERRLECIT)
 - Iron sucrose (VENOFER)
- Ferumoxytol- Fe oxide (FERAHEME)
- Ferric carboxymaltose (INJECTAFER)
- Risk of hypersensitivity rxns: premedicate with steroid and antihistamine in patients at risk
- Used successfully and safely during pregnancy

39 40

Other conditions with microcytosis (approximate Hb and MCV values)

	Hb	MCV
Anemia of inflammation	10	83
Thalassemia minor	12	68
Thalassemia major	2-7	48-72
Hb H disease	9	70
Hb E trait	14	73
нь сс	10	74
Hb SC	11	78
Hereditary sideroblastic anemia	6	77

Ftn cutoffs of absent BM Fe in anemia associated with inflammation

Ftn (ng/ml)	Sensitivity	Specificity	Positive pre- dictive value
<100	88-100%	46-64%	50%
<60	82-86%	84-88%	84%
<50	71-82%	84-97%	88%
<40	71%	98%	92%
<12	24%	100%	100%

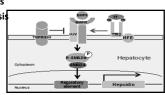
Punonnen et al, Blood 1997;89:1052 Kis and Carnes, J Gen Int Med 1998;13:455

What is Iron Overload?

- · Excessive amounts of storage iron
 - parenchymal cells liver, heart, pancreas (HH)
 - macrophages spleen, BM, liver (Txs)
 - mixed pattern
- · Quantitative considerations
 - mild: 2-4 g; moderate: > 4-10 gmarked: 10-20 g; severe: >20 g

Iron Overload: Classification

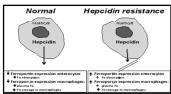
- 1. <u>Hereditary conditions causing ♥ hepcidin</u>
- HFE hemochromatosis
- TFR2 hemochromatosis
- Hemojuvelin hemochromatosis
- Hepcidin hemochromatosis



43 44

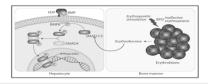
Iron Overload: Classification

- 2. <u>Hereditary conditions causing resistance of ferroportin to hepcidin</u>
 - Ferroportin disease



Iron Overload: Classification

- 3. <u>Ineffective erythropoiesis: erythroblast-derived</u> <u>erythroferrone suppresses hepcidin:</u>
 - β-thal major & intermedia; Hb E/β-thal; Hb H
 - congenital dyserythropoietic and sideroblastic anemias



45 46

Iron Overload: Classification

4. Multiple blood transfusions

- Diamond Blackfan anemia
- aplastic anemia
- thalassemia majorsickle cell anemia
- myelodysplasia



Iron Overload: Classification

- 5. Other hereditary conditions
 - Aceruloplasminemia
 - Atransferrinemia
 - · African dietary iron overload



Fe Overload: Clinical Manifestations

- 1. Fatigue, abd. pain
- 2. Liver
- ↑ ALT
 - fibrosis and cirrhosis
- Hepatoma
- 3. Heart CHF (restrictive or
 - dilated) arrhythmias
- secondary amenorrhea - Impotence

- diabetes mellitus

- 5. Hyperpigmentation 6. Certain infections
- 7. Arthritis

4. Endocrine

- esp. 1st and 2nd MP joints (HFE hemochromatosis)

Screening for hemochromatosis or increased iron stores

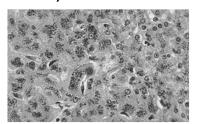
- - with family hx or compatible clinical picture
 - without inflamm., infection, trauma, surgery
- 2. Serum ferritin conc.
 - ↑ raises possibility (esp. if Tf Sat ↑ or upper nl)
 - Ferritin >1000 ug/L esp. of concern
- 3. PCR for HFE mutations, esp. Caucasians

50 49

Diagnosis of hemochromatosis or increased iron stores

- 1. Liver biopsy
 - Histology
 - Perl's stain
 - Chemical iron concentration
- 2. MRI measurement hepatic or cardiac iron
- SQUID magnetic measurement hepatic iron
- Quantitative phlebotomy

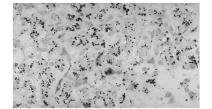
Liver Bx, HFE hemochromatosis (H&E):



Golden brown hemosiderin pigment predominantly in hepatocytes

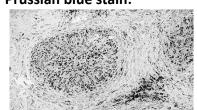
51 52

Liver Bx, HFE hemochromatosis Prussian blue stain:



Granules predominantly in hepatocytes

Liver Bx, HFE hemochromatosis Prussian blue stain:



Cirrhosis; hepatocytes in regenerating nodule heavily laden with iron

HFE Hemochromatosis

- 1. Classic triad
 - Hepatomegaly
 - Diabetes
 - Hyperpigmentation
- 2. Classically death from cirrhosis, HCC or CHF
- 3. Goal now- make dx and rx in presymptomatic stage

HFE Genetic Defect

- 1. C282Y mutation, HFE gene, 6p21.3
- Autosomal recessive
- Predominantly in N. Europeans:
 "Celtic Disease"
- high prevalence in pop.
 - homozygotes: 3-5/1000
- heterozygotes: 10-15%
- 2. HFE protein deficiency
- - ↑ Fe absorption
 - ↑ serum Fe and transferrin saturation
 - ↓ storage of Fe in macrophages
 - Fe-loading of parenchymal cells

55 56

HFE Genetic Defect

- 3. Incomplete C282Y/C282Y penetrance
 - Elevated SF
 - <50% F homozygotes
 - ≈75% M homozgotes
 - Organ damage <25%
 - Modifying factors
 - genetic
- environmental
- 4. Second mutation H63D
 - Very little iron-loading tendency
 - Fe stores may rarely develop in
 - H63D/H63D
 - C282Y/H63D
 - · C282Y heterozygote

HFE C282Y in 99,711 1º Care Pts (HEIRS)

	N	C282Y/ C282Y	No. per 100,000
Whites	44,082	0.44%	440
African Americans	27,124	0.014%	14
Asians	12,772	0.0%	0
Hispanics	12,459	0.027%	27

57 58

Management of *HFE* hemochromatosis

- 1. NI life expectancy if phlebotomy Rx started before cirrhosis or diabetes
- 2. If ferritin <1000 μ g/L and LFTs nl at dx
 - Proceed to phlebotomy therapy
- 3. If ferritin >1000 and/or LFTs abnl
 - Consider liver biopsy
 - † risk HCC in cirrhotics even with phleb. Rx
- 4. Screen first degree family members

Phlebotomy Therapy

- 1. Initial program to remove excess body Fe
 - Remove 1-2 units of blood weekly as long as Hct ≥ 35% (M) or 32% (F).
 - Continue until mild iron deficit: Pt. does not tolerate wkly phleb.; MCV declines; ftn < 50 ug/L



Phlebotomy Therapy

2. Quantitative phlebotomy

- Maintain weekly schedule and tally ml of whole blood removed until Fe deficiency develops.
- Calculate Fe stores at beginning of program (1 mg Fe/2 ml whole blood); >4g Fe- "Fe overload"

3. Maintenance to prevent the Fe reacummulation

 Remove 1 unit each 2-6 mos. to maintain serum ftn around 50 µg/L.

Other Forms of Genetic Hemochromatosis

- TFR2 hemochromatosis
 - autosomal recessive; intermediate phenotype
 - TfR2 mutation 7g22

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- · Juvenile hemochromatosis
 - autosomal recessive; severe phenotype
 - hemojuvelin mutation 1q21.1
 - HAMP (hepcidin) mutation 19q13.1

61

Other Forms of Genetic Hemochromatosis

- · Ferroportin disease
 - autosomal dominant mutations ferroportin 2q32
 - two phenotypes
 - reduced response to hepcidin: predominant parenchymal ironloading
 - increased sensitivity to hepcidin: predominant macrophage ironloading

African Iron Overload

- 1. High dietary Fe in traditional beverage
- 2. Familial pattern
 - ? "gene by environment interaction"
 - HFE mutations not present
 - Ferroportin Q24H in some iron-loaded subjects
- 3. Fe-loading macrophages and hepatocytes
- 5. Clinical associations
 - hepatic fibrosis, cirrhosis, hepatoma
 - diabetes mellitus, cardiomyopathy
 - osteoporosis, scurvyinfections
 - esophageal carcinoma
- 6. Management
 - prevention
 - phlebotomy therapy

63 64

Iron Overload in African Americans

- · Condition present; may be under-dxed
- Sometimes related to African-specific ferroportin Q248H mutation
- Screen with serum ferritin
- · Responds well to phlebotomy therapy
- Evaluate for hepatitis C- may play etiologic role by suppressing hepcidin

Transfusional Iron Overload Quantitative Considerations

- One unit blood contains about 225 mg Fe as Hb
- Hepatic Fe concentration

normal
 worrisome
 Toxic
 highly toxic
 30 μmol/g dry wt.
 180 μmol/g dry wt.
 360 μmol/g dry wt.
 100 units
 200 μmol/g dry wt.
 200 units

At 2 units/month: 50 units in two yrs

Transfusional Iron Overload Treatment

- 1. Institute iron chelation when
 - >20 units of blood transfused, or
 - hepatic iron conc. > 180 μmol/g dry wt.
- 2. Desferrioxamine, original parenteral chelator
 - 40-50 mg/kg per day
 - s.c. infusion over 8-12 hours
 - 5 days/week

Transfusional Iron Overload Treatment

- 3. Deferasirox
 - Single oral dose per day; similar potency to DFO
 - Exjade: powder; starting dose 20 mg/kg/day; advance to 30 or 40 mg/kg/day if needed
 - Jadenu: tablet; doses slightly lower
 - Monitor CBC, creatinine, LFTs, gastritis sx's q2-4wks

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Transfusional Iron Overload Treatment

- 4. Deferiprone
 - Oral chelator
 - Risk of agranulocytosis
 - Approved in US for use in thalassemia patients with transfusional iron overload who did not respond to other chelators

Monitoring Iron Chelation Rx

- 1. CBC; renal and liver function tests; GI Sxs monthly
- 2. Audiometry and ophthalmologic yearly
- 2. Serum ferritin every three months
- 3. Avoid use of phenothiazines
- Vitamin C 100 mg daily may enhance Fe excretion; do not give to heavily iron-loaded subjects
- 5. Modify dose as body Fe burden ↓'s

69 70

Other Iron Overload Conditions

- 1. Congenital atransferrinemia (3q22.1)
 - parenchymal Fe-loading; anemia
- 2. Congenital aceruloplasminemia (3q23-q25)
 - Fe-loading paremchyma, macrophages, brain
 - extrapyramidal sxs, cerebellar ataxia, DM

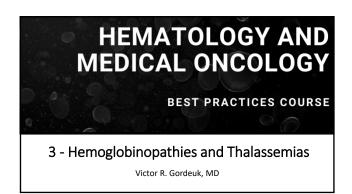
Other Iron Overload Conditions

- 4. Neuroferritinopathy
 - autosomal dominant, late-onset
 - abnl aggregates ferritin & Fe in basal ganglia
 - Mut. ftn light gene (19q13.3-13.4); ser. ftn low
- 5. Hallovorden-Spatz disease
 - autosomal recessive; onset in childhood
 - iron deposits in the basal ganglia
 - mutation in pantothenate kinase 2 gene (20p13)

Hemoglobinopathies

Victor R. Gordeuk, MD

August 13, 2020



Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

- Grant support CSL Behring, Global Blood Therapeutics, Imara, Ironwood, Novartis
- Consulting- CSL Behring, Global Blood Therapeutics, Novartis, Forma

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Outline

1

- 1. General considerations
- 2. Beta-thalassemias
- 3. Alpha-thalassemias
- 4. Hemoglobinopathies other than sickle cell disease

Ribbon structure of hemoglobin A

kobin a-gk

Tetramer

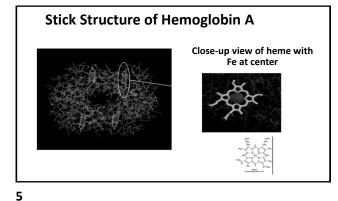
- 2 pairs of globin (polypeptide) chains
- Alpha-beta dimers aggregate to form tetramers

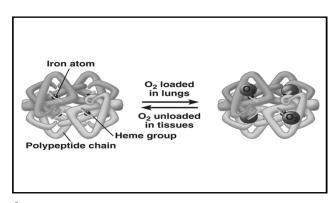
Heme

- Complex of Fe⁺² and protoporphyrin
- Covalently bound to each globin monomer
- Reversibly binds one molecule O2

3

4





Victor Gordeuk, MD

Normal Hbs found in Adults

Hb A: $\alpha_2\beta_2$

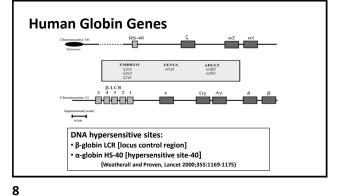
Primary structure

Hb A_2 : $\alpha_2\delta_2$

Alpha globin- 141 amino acids

Hb F: $\alpha_2 \gamma_2$ <1%

Beta globin- 146 amino acids



Chromosome 16

-----α------ α------

-----α------ α------

Chromosome 11

----^Gγ-- ^Aγ ---- δ-- β----

-----^Gγ-- ^Aγ ----- δ-- β----

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Hemoglobinopathies and Thalassemias

- Mankind's most common single gene, Mendelian diseases
- · Disorders of the synthesis or structure of Hb
- · Almost 1500 described

10

Geographical overlap in distribution

Distribution of hemoglobinopathies

Distribution of malaria

Evidence that these red cell disorders protect against malarial infection.

Thalassemias:

reduced amounts or absence of structurally nl globin chain

- α-thalassemia
- β -thalassemia

Hemoglobinopathies and Thalassemias Hemoglobinopathies:

> amino acid substitutions; structurally abnl globin

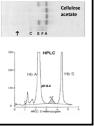
- Hb S, Hb C, Hb G-Philadelphia, Hb D, Hb O-Arabia
- Hb E
- **Unstable Hbs**
- Altered O₂ affinity
- Hb M

Hemoglobinopathies and Thalassemias

- Interactions among thalassemias and hemoglobinopathies are common
 - Hemoglobin S / beta thalassemia
 - Hb S and alpha thalassemia
 - Hemoglobin E / beta thalassemia

Hemoglobinopathies: Laboratory Dx

- · Hb electrophoresis
 - Cellulose acetate (alkaline): provisional ID of Hb A, Hb F, Hb S, Hb D, Hb C, Hb E, Hb O, Hb H
 - Citrate agar (acidic): distinguish Hb C from Hb E, and Hb C from Hb O
- HPLC
 - Retention time, peak characterisitic influenced by single aa substitutions
 - Accurately identifies 75% of Hb variants
- Molecular biology
 - PCR; gene sequencing



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Thalassemia Mutations

α-Thalassemia

- clinically expressed in fetus and at birth
- · mostly caused by gene deletion

β-Thalassemia

- expressed after several mos of age because of switching from $\gamma\text{-}$ to $\beta\text{-globin}$
- mostly caused by point mutations

(Steensma, Blood, 2005)

Beta-Thalassemias

- ↓ synthesis of β-globin chains
- Excess of α-globin chains
 - α-globin aggregates to form insoluble inclusions in erythroid precursors
 - highly toxic
 - intramedullary death of erythroid precursors: ineffective erythropoiesis

15

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Membrane Defects in β-Thalassemia

Excess cellular Fe and unstable unpaired $\alpha\text{-globin}$ chains cause

- membrane lipid oxidation
- membrane protein damage
- decreased RBC deformability
- removal from the circulation

Membrane damage leads to PS exposure and hypercoagulability

Ineffective Erythropoiesis

- High degree of erythropoietic activity
- Death of erythroid precursors in BM
- Blood tests look like hemolysis, but retics not increased for degree of anemia
 - — ↑ or high nl LDH, indirect bilirubin
 - → haptoglobin
- Thal major and intermedia
 - both ineffective erythropoiesis & hemolysis

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β-Thalassemia major

• Cooley's anemia

· homozygous or compound

heterozygous β -thalassemia

Victor Gordeuk, MD

B-Thalassemia Mutations

β⁰-thal mutations

· totally abolish expression of affected gene by critical point mutation or deletion

β+-thal mutations

- · partially abolish gene expression
- · mild, moderate, severe-depending on amount of Hb A produced

Clinical Classification of β-Thalassemia*

β-Thalassemia trait

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- uncomplicated heterozygous $\beta\text{-thalassemia}$
- B-thalassemia minor

β-Thalassemia intermedia

• no firm definition; many different genotypes

 * genotype-phenotype correlations often difficult to make: 100s of mutations, frequent interactions, role of other modifying genes and environment.

Clinical Diagnosis of **\beta-Thalassemia**

β-Thal trait

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- microcytosis
- hypochromia
- +/- mild anemia
- · elevated level of HbA₂ (>3.5%)
- β-Thal intermedia
- · microcytic anemia
- +/- Tx requirement
- high Hb F bone disease, iron loading, splenomegaly, • bone disease, iron
- many different genotypes
- β-Thalassemia major
- transfusion-dependent microcytic anemia
- very high Hb F (approaching 100%)
- pulmonary hypertension loading, splenomegaly, pulmonary hypertension
 - · many different genotypes

Beta-Thalassemias

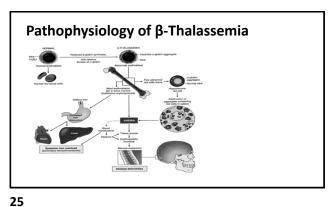
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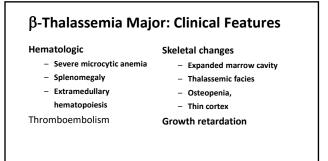
Clinical Features of β-Thal Syndromes

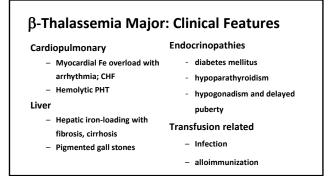
	Major	Intermedia	Minor
Severity		200	1

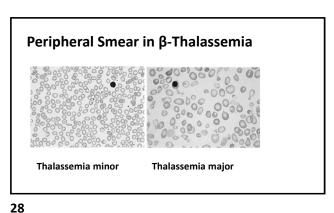
Hb Fractions in β -Thal Syndromes

	NI	Minor	Intermedia	Major
Hb A	97%	>90%	15-65%	0%
Hb A2	2.2-3.5%	3.5-8%	5.4-10%	1-5.9%
Hb F	<1%	1-2%	30-75%	>94%

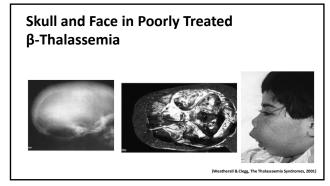


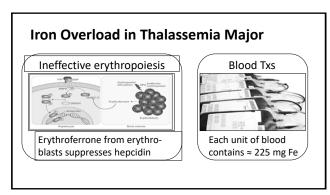


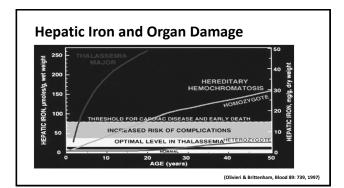




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β-Thalassemia Major: Prognosis

- No Rx:
 - death by age 5 from infections, cachexia
- Episodic blood Tx's:
 - survival into 2nd decade
- Aggressive blood Tx's:
 - death ~age 20 from iron overload (cardiac)
- Aggressive blood Tx's plus iron chelation:
 - prolonged survival

31 32

β-Thalassemia Major: Treatment

- Management in comprehensive center:
 - endocrinology
 - cardiology
 - social services
- Hypertransfusion beginning 2nd or 3rd year:
 - maintain Hb 9-10.5 g/dL
- Splenectomy for increasing Tx requirement

β-Thalassemia Major: Treatment

- · Fe chelation starting after age 3 years-
 - keep liver Fe <5 mg/g dry weight
- Also:
 - Consider stem cell transplantation
 - Increase synthesis of fetal Hb with hydroxyurea or other agents
 - Genetic counseling
 - Prenatal diagnosis

33 34

Iron Chelators

Deferoxamine

Given by prolonged infusion

Deferasirox

- Once daily oral dosing
- Can remove cardiac Fe

Deferiprone

- Orally active; limited approval in US
- Removes cardiac iron

Potential Toxicity of Iron Chelation

- Skin reactions
- Bone, bone marrow, hepatic, GI, otologic, renal, retinal damage
- · Yersinia infection
- Growth delay
- Agranulocytosis (deferiprone)

Victor Gordeuk, MD

Alpha Thalassemia

• Decreased synthesis of α-globin chains

 β_4

- Excess of beta-like globin chains
- · Potential formation of abnl Hbs:
 - Hemoglobin Barts: γ
 - Hemoglobin H:

Gene Deletion α-Thalassemia*

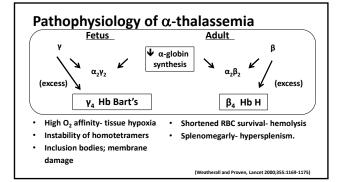
α+-thalassemia

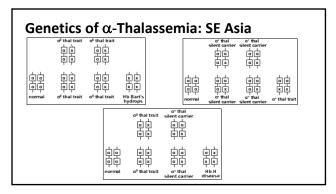
- deletion of a single gene on one chromosome 16 allele
- α⁰ thalassemia
 - deletion of both genes on one chromosome 16 alelle

*Point mutations less common cause of α -thalassemia; often associated with severe defect in α -globin synthesis

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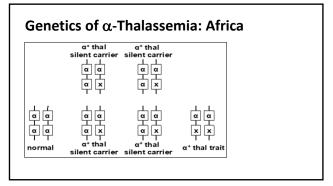
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Alpha-Thalassemias						
Geno- type	Pheno- type	Hb Barts (γ ₄)	НЬ Н (β ₄)	Heme Findings		
αα/αα	Normal			Normal		
αα/α-	Silent carrier			Normal		
αα/ or α-/α-	α-thal trait	2-10% newborn		Mild anemia		
α-/	Hb H disease	20-40% newborn	5-40% adults	Hemolysis, ineff. erythro.		
/	Hydrops fetalis	~100% cord		Anemic stillborn		

α-Thalassemia 'Silent Carrier'

- heterozygous α⁺ thalassemia
- 3 of 4 alpha genes present and functional
- +/- mild anemia
- ↓ MCV (age dependent)

Alpha-Thalassemia Trait

2 of 4 alpha genes present and functional

- Homozygous α^+ thal $(\alpha-/\alpha-)$: ~7% of Africans
- Heterozyg. α^0 thal ($\alpha\alpha/$ --): common SE Asia

Clinical features:

- +/- mild anemia
- MCV <78 fL
- Hb Barts (γ₄) 2-10% in newborns

43 44

Alpha-Thalassemia Trait

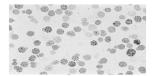
- Often Dx of exclusion
 - Compatible ethnicity and clinical picture
 - Exclude Fe def, β-thal, hereditary sideroblastic anemia
- Molecular diagnosis available thru referral labs
- Do not confuse with Fe def or treat with iron

Hemoglobin H Disease

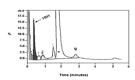
- Genotype α-/-- (SE Asia)
 - $-\alpha^+$ -thal one allele
 - $-\ \alpha^{0}\text{-thal}$ other allele
- 20-40% Hb Barts (γ_4) in newborn
- 5-40% Hb H (β₄) in adults
 - visualized by brilliant cresyl blue
 - Hb electrophoresis
 - HPLC

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Diagnosis of Hemoglobin H Disease



RBC inclusions generated by brilliant cresyl blue



Fast moving peak on HPLC

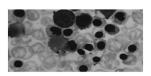
Hemoglobin H Disease

- Clinical features
 - hemolytic anemia of varying degrees
 - microcytosis
 - splenomegaly
 - ineffective erythropoiesis
 - Fe-loading

Victor Gordeuk, MD

Hemoglobin Bart's Hydrops Fetalis

- Homozygous α^0 -thalassemia (- -/- -)
- No functional α-globin genes: Hb Barts (γ₄)
- · Eclampsia in mother
- Stillbirth
- · Erythroblastosis in infant



An international registry of survivors with Hb Bart's hydrops fetalis syndrome

Dunnital Songide, "\$ "Chiefata Babbes," and Douglas R. Higgs," in collaboration with the BHPS international Consortium

"Nuclear Research Convert Melinicular Hammanitary Uni, Washington Medicar Medicare, University of Collect, C

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RBC Indices in Alpha-Thalassemia

	NI	Silent Carrier	Trait	Hb H Disease	Hydrops Fetalis
Hb	M: 14-18	M: 13-16	M: 12-15	M: 9-13	M: 3-8
(g/dL)	F: 12-16	F: 10-14	F: 10-14	F: 7-11	F: 3-8
MCV	79-99	67-95	64-79	53-69	126-146
(fL)					
мсн	27-35	22-30	21-25	16-20	22-42
(pg)					

Atypical α-Thalassemias

 α -Thalassemia-mental retardation syndromes

- ATR-16 (alpha thal. retardation associated with Chr. 16): large deletions involving α -globin genes
- X-linked- mutations in ATRX on Chr. X, which encodes a chromatin-associated protein

 α -thalassemia-MDS

• acquired α -thalassemia in myelodysplastic syndrome

51 52

Management of α-Thal Syndromes

Hb Bart's

• Screening, genetic counseling, intrauterine transfusions

Hb H disease

- Regular medical follow-up
- · Blood Tx and Rx of Fe overload as needed

Mild α-thalassemias

 Dx important for genetic counseling and avoiding misguided Rx with iron

Other conditions affecting globin chain synthesis

- Hemoglobin Lepore
 - Fusion of β and δ globin genes
 - ↓ synthesis of β-like globins
 - Homozygote: β-thal major phenotype
 - 8-30% Hb Lepore
 - 70-92% Hb F
 - Heterozygote: β-thal minor phenotype

Victor Gordeuk, MD

Other conditions affecting globin chain synthesis

- · Hb Constant Spring
 - non-deletional form of α-thalassemia
 - mutation in stop codon of α2-globin
 - poor output (1% of nl) of α -globin with 31 additional amino acids
 - homozysity leads to Hb H type clinical picture but nearly nl MCV

Other conditions affecting globin chain synthesis

- Hereditary persistence of fetal Hb
 - Up-regulation of γ chain synthesis
 - Almost 100% Hb F in homozygotes
 - Clinically silent
 - Causes:
 - deletions involving β and δ genes
 - **Ψ** expression of KLF1 transcription factor that activates BCL11A Hb F suppressor

55 56

Hemoglobin E (β26 glu→lys)

- Second most prevalent Hb variant: 30,000,000 worldwide; >80% in SE Asia
- RBC cytoplasm: precipitated α -chains, increased oxidant stress
- Carriers clinically silent; low MCV

Hb E Diso	Hb E Disorders							
<u>Condition</u>	<u>Genotype</u>	<u>Clinical</u>						
Hb E Trait	A/E	30% Hb E ± Ψ MCV						
Hb E Disease	E/E	90% Hb E, Ψ MCV						
Hb E-β-thal	E/beta ^{0,+}	Hb E 40-85%, Hb F 10-60%, Ψ MCV, Hb						
Hb SE disease	S/E	resembles Hb S-β+ thal						

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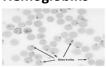
Hemoglobin E/β-Thalassemia

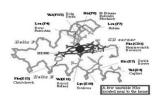
- SE Asia
- Hb E 60-85%, Hb F 15-40%
- Mild to moderate microcytic hemolytic anemia
- Ineffective erythropoiesis and iron-loading

Unstable Hemoglobin Disease

- Congenital Heinz body anemia
- Rare autosomal dominant mutations → defective binding of heme by globin
- About 200 'unstable' variants: phenotype heterogeneous
- Heinz bodies, peroxidant membrane damage, hemolysis

Unstable Hemoglobins





Heinz bodies

- RBC inclusions of denatured Hb
- · Stain: new methylene blue or bromocresol green
- Detect in RBC hemolysate: heat stability test or isopropanol stability test

Unstable Hemoglobins

Diagnosis

- · Normocellular to microcytic hemolytic anemia
- ± Distinct electrophoretic pattern
- Heinz bodies by stain, heat stability or isopropanol stability
- Mutation detection
- Hb Köln most common (β98 Val→Met)
 - anemia; retics 10-25%, splenomegaly

61 62

Unstable Hemoglobins

Hb Köln

- Most common
- β98 Val→Met destabilizes heme pocket
- · Anemia; retics 10-25%, splenomegaly

Hb Zurich

- β98 Val→Met
- · Increased affinity for CO
- · Smokers protected from hemolysis

Unstable Hemoglobins

Treatment

64

- avoid oxidant drugs
- blood Tx's
- splenectomy in severe cases

63

Hemoglobin M Disorders



Hereditary methemoglobinemia and cyanosis

Autosomal dominant

Amino acid substitution in heme pocket and allows Fe oxidation (ferrous heme 🛭 ferric heme)

Clinical: asymptomatic cyanosis, slate grey/brownish skin, no dyspnea, nl life expectancy

Hemoglobin M Disorders

Diagnosis

- abnormal pulse oximeter saturation
- distinguish from other methemoglobinemias
- Hb delectrophoresis, Hb spectra
- Methemoglobin < 30%
- Cyanosis not reversible with Vit C, Meth Blue

Treatment: major hazard is misdiagnosis and untoward treatment

Other Forms of Methemoglobinemia

Congenital deficiency of CYB5R3

Type I: most common congenital methbemia

- Autosomal recessive; defective enzymatic reduction of Fe⁺³ to Fe⁺² only in RBCs
- Methemoglobin usually < 30%
- Rx cyanosis: methylene blue or ascorbic acid

Type II: 10-15% of cases

- CYB5R3 deficiency in all cells
- Mental retardation and developmental delay
- Methylene blue improves cyanosis, not CNS

Other Forms of Methemoglobinemia

Oxidation Fe⁺² to Fe⁺³ Hb by drugs or chemicals

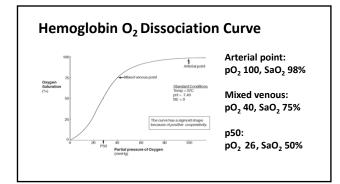
Offending agents

 Nitrites, trinitrotoluene, sulfanilamide, PAS, dapsone, primaquine, chloroquine, lidocaine, naphthoquinone, resorcinol, phenylhydrazine

Clinical

- Methemoglobin > 30% symptoms; > 50% lethal
- Emergency treatment: 1-2 mg/kg methylene blue as 1% solution IV over 10-15 minutes

67 68



Hemoglobins with Altered O₂ Affinity

High affinity Hb

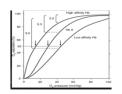
Low affinity Hb

O₂ pressure (mmHa)

69 70

Hemoglobins with High O₂ Affinity

- Familial erythrocytosis; autosomal dominant
- α or β -chain can be affected
- ± distinct electrophoretic pattern
- Left shift O₂ dissociation curve (low P50)

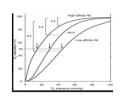


Hemoglobins with High O₂ Affinity

- Normal 2,3-DPG levels
- Diagnosis
 - Erythrocytosis in familial pattern
 - low P₅₀
 - Hb electrophoresis or HPLC
 - PCR or gene sequencing
- Treatment
 - Polycythemia mild; phlebotomy not necessary

Hemoglobins with Low O₂ Affinity

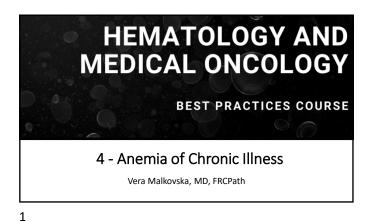
- Asymptomatic cyanosis
- Right shift in O₂ dissociation curve (high P50)
- Hb electrophoresis, HPLC
- No Rx required



Anemia of Chronic Illness

Vera Malkovska, MD

August 13, 2020



Disclosures

Disclosures of Financial Relationships with Relevant **Commercial Interests**

None

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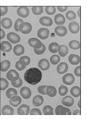
Anemia of Chronic Disease (ACD) = Anemia of Inflammation

- · Second most common anemia worldwide
- · Most frequent anemia in hospitalized and chronically ill patients

Weiss G et al. Blood 2019

Anemia of Chronic disease (ACD)

- Hypoproliferative
- Normo- or hypochromic
- · Normo- or microcytic
- Hb rarely below 8 g/dL
- · Can occur rapidly during inflammation
- · Resolves upon treatment of underlying disease



ACD is caused by diverse disorders

Infectious Tuberculosis Endocarditis HIV

Inflammatory

Rheumatoid arthritis Systemic lupus Vasculitis

Malignant

Myeloma

Miscelaneous

Metastatic cancers Lymphomas

Chronic kidney disease Congestive heart failure Advanced atherosclerosis

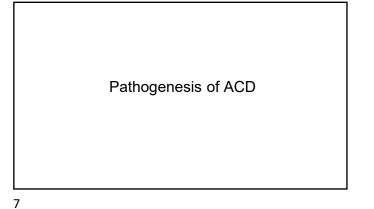
Anemia of critical illness

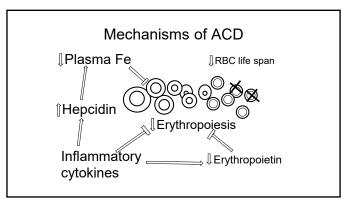
Common features of ACD

- ↑ inflammatory cytokines
- ↑ hepcidin
- iron-restricted hematopoiesis
- ↓ response to erythropoietin (Epo)
- ↓ of Epo production
- ↓ red cell survival

5

3





Hepcidin: the key iron regulator

- · 25-amino acid peptide
- · Produced by hepatocytes
- · Induced by
 - iron loading
 - inflammation (IL-6)
- · Suppressed by
 - iron deficiency
 - increased erythropoiesis
 - hypoxia

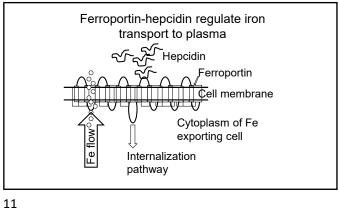
Ferroportin: the iron exporter

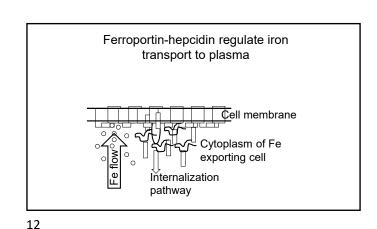
- Molecular target of hepcidin
- Transports Fe from cells
- · Large transmembrane protein
- Expressed on

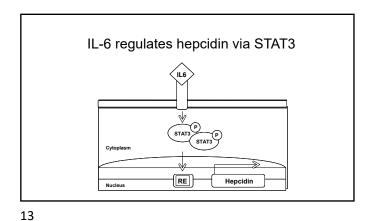
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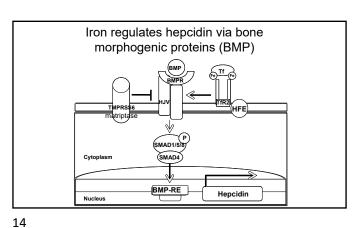
- macrophages
- enterocytes
- hepatocytes

- placental cells









Increased erythropoiesis suppresses
hepcidin

EPO

Erythroferrone & other regulators

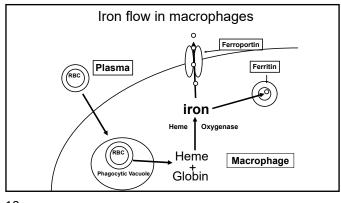
erythropoiesis

Fe availability

Hepcidin

Clinical conditions that impact hepcidin Infection ↑ Erythropoiesis Anemia/hypoxia Inflammation Liver disease ↑ iron stores Iron therapy Alcoholism Transfusion Chronic hepatitis C Renal failure Sex hormones Genetic factors Genetic factors

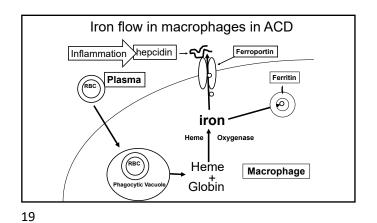
Iron regulation in ACD in macrophages

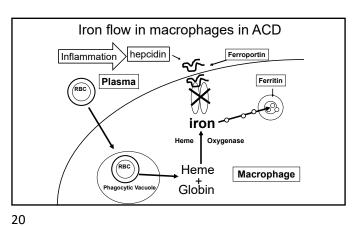


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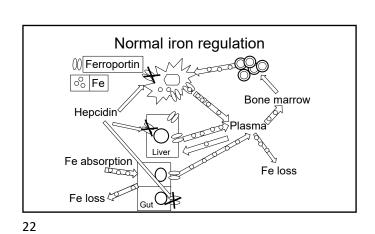
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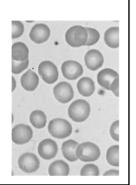
Systemic iron regulation in ACD





Blood smear

- Normo- or hypochromic, normocytic or mildly microcytic
- · Mild anisocytosis
- Rouleaux
- Low polychromasia reflects low reticulocyte count



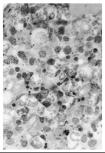
Other findings

- High ESR, CRP, fibrinogen, haptoglobin, other acute phase proteins
- Low albumin, low transferrin

25 26

Bone marrow Prussian Blue stain

Decreased siderotic granules in normoblasts



Iron studies in ACD and Fe deficiency

Anemia	Sei	rum Fe	TIBC	IBC sat.	ferritin
Fe deficier	тсу	1	†	1	1
ACD		ļ	↓or N	N or↓	† or N

27 28

Iron studies in ACD and Fe deficiency

Anemia	Ser	um Fe	TIBC	IBC sat.	ferritin
Fe deficier	псу	ļ	\bigcirc	1	①
ACD		1	or N	N or↓	(or N)

Soluble transferrin receptor ↑ in iron deficiency

Clinical findings and differential diagnosis of ACD

Clinical presentation of ACD

ACD is modified by

- -the underlying illness
- -it's treatment and complications
- -premorbid iron stores
- other disorders (e.g. thalassemia)
- -genetic make up

Anemia in patients with chronic diseases is multifactorial

Causes of cancer-associated anemia Cancer Decreased production **Blood loss** Bleeding from tumor, surgery, Marrow suppression gastrointestinal tract Inflammatory cytokines T cells (pure red cell aplasia) Fe deficiency from loss Chemo- and radiotherapy Thrombocytopenia Marrow infiltration Coag. factor def. Cell destruction Decreased erythropoietin and inhibitors DIC / microangiopathy **Nutritional deficiency** Immune hemolysis Malabsorption Hemophagocytosis (HLH)

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Anemia of infection

- · Normocytic normochromic
- Hypoferremia develops within hours in acute infection
- Granulomas in BM (mycobacteria, leprosy, brucellosis, syphillis, others)
- Shorter RBC life span (malaria, mycoplasma, bacterial toxins, other)

Anemia of chronic liver disease

- · Mildly macrocytic, acanthocytic
- · Shorter RBC life span
- · GI bleed, bleeding diathesis
- · ETOH-induced marrow suppression
- · Nutrient deficiency

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Anemia of chronic kidney disease

- \downarrow Epo is a major cause
- ↓ renal clearance of hepcidin
- Symptoms milder for degree of anemia (acidosis shifts Hb dissociation curve)
- ↓ RBC life span
- · Bleeding diathesis, GI loss
- · Aluminum excess inhibits erythropoiesis
- · Renal osteodystrophy/osteitis fibrosa

Unexplained anemia of the elderly (UAE)

- Unexplained anemia found in ~30% of elderly anemic patients
- Diagnosis by exclusion
- It is usually mild (Hb > 10 g/dL)
- Associated with ↑ mortality
- Probably a marker rather than a cause of poor outcome

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Characteristics of UAE

- · Hypoproliferative anemia
- · Hypocellular marrow
- Low Epo
- · Inflammatory markers not elevated
- · Pathogenesis is poorly understood
- · Probably distinct entity rather than "waste basket" diagnosis

Possible etiologies of UAE

- · Androgen deficiency
- · Low-grade inflammatory state
- · Altered Epo homeostasis
- · Age-related clonal hematopoiesis
- Undiagnosed MDS
- · Vitamin D deficiency
- · Other unrecognized causes incl. drugs

37 38

ACD vs. ACD combined with iron deficiency

ACD vs ACD combined with Fe deficiency

- Serum ferritin 30-100 ug/L in ACD: underlying Fe deficiency possible
- Serum ferritin >100 ug/L in ACD: Fe deficiency unlikely
- Gold standard for confirming Fe deficiency is absent Fe in BM bx
- Therapeutic trial of i.v. iron can help diagnosis

40 39

ACD vs ACD combined with Fe deficiency

- Transferrin receptor/log ferritin index >2: Likely Fe deficiency
- Transferrin receptor affected by cytokines, assays not standardized
- Hepcidin levels promising, assays not generally available and standardized

Differential diagnosis of ACD

- 1. Exclude hyperproliferative anemias
 - Hemolysis

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- ↑ reticulocytes
- ↓ haptoglobin, ↑ indirect bili and LDH
- Ineffective erythropoiesis
 - · reticulocytes not increased
 - ↓ haptoglobin, ↑ indirect bili and LDH

- 2. Exclude other hypoproliferative anemias
 - BM suppressive drugs
 - Renal failure with Epo deficiency
 - Endocrine disorders
 - Myelodysplastic syndromes
 - Aplastic anemia
 - Pure red cell aplasia

- Test acute phase proteins & other disease markers
 - CRP, ESR, fibrinogen, haptoglobin, complement
 - –↓ plasma albumin, ↓ transferrin

43 44

Useful tips for differential diagnosis

- Drug-induced marrow suppression serum Fe high
- Chronic blood loss ↓ ferritin, †transferrin receptor/log ferritin index, trial of Fe therapy
- Epo deficiency dominates in renal failure

Useful tips for differential diagnosis

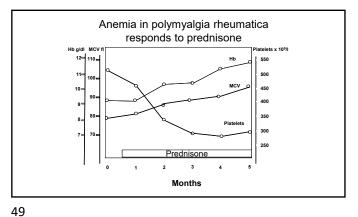
- Endocrine disorders serum Fe normal
- Marrow metastases serum Fe normal or ↑, leukoerythroblastic smear
- Dilutional anemia (pregnancy, Waldenstrom disease)

45 46

Therapy of ACD

Therapy

- 1. Therapy should be directed to underlying disease.
- 2. Most patients have self-limited anemia that needs no specific therapy.
- Correct co-existent Fe deficiency and other disorders
- 4. Transfusions should be avoided



- 5. Erythropoietin not standard therapy, but selected patients may benefit:
 - · Severe chronic inflammatory disease such as rheumatoid arthritis
 - · AIDS-associated anemia if Epo level <500 u/L

Erythropoietin warnings (FDA): "increased...risk of death when administered to target Hb of 12 g/dL in pts. with active malignant disease receiving neither chemotherapy nor radiation ...not indicated for this population.

50

- 6. I.V. iron is not standard therapy for anemia of inflammation
 - · Iron restriction is a protective response to limit availability of iron for microorganisms
 - · Iron therapy may be useful in associated iron deficiency

Consider impact of any therapy on the underlying disease!

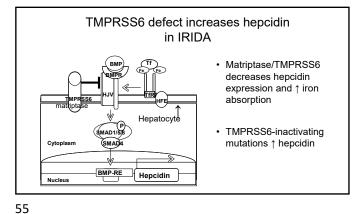
Take home points

- · ACD is a hypoproliferative anemia
- · Caused by an inflammatory process
- · Characterized by Fe-restricted erythropoiesis caused by ↑ hepcidin
- · Hepcidin is the central Fe regulator
 - is induced by cytokines & ↑ iron
 - decreases Fe absorption
 - causes Fe sequestration in macrophages

52 51

- JEpo production, Epo resistance and ↓RBC lifespan contribute to the anemia
- · Diagnostic features:
 - Normo-to-microcytic hypoproliferative anemia
 - Low serum Fe and normal to high ferritin
 - Other causes of anemia should be R/O
- · Treatment is directed to underlying condition

Iron-Refractory IDA (IRIDA): Disorder of Hepcidin Regulation



Mechanism of Iron-Refractory IDA (IRIDA)

- · TMPRSS6 loss-of-function mutations cause ↑ hepcidin production
- · High hepcidin
 - decreases Fe absorption
 - decreases Fe flow into plasma
 - causes Fe-restricted hematopoiesis

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Characteristics of IRIDA

- · Autosomal recessive
- · Diagnosed mostly in childhood
- · Improves in adults
- · No response to oral Fe
- · Partial response to i.v. Fe
- · Therapy: intermittent Fe infusions

Labs in IRIDA

- Severe microcytosis (MCV 45-65 fl)
- Moderate/severe anemia (Hb 6-9 g/dL)
- · Low serum Fe
- Very low transferrin saturation (<5%)
- · Ferritin normal or low
- · Normal or high hepcidin

58 57

Diagnosis of IRIDA

- · Fe deficiency with very low MCV and transferrin saturation
- · Exclude acquired causes of Fe deficiency
- · Confirm poor response to oral iron
- · Family history
- · Genetic testing for TMPRSS6 mutations
- · High or normal hepcidin

Promising use of hepcidin assays

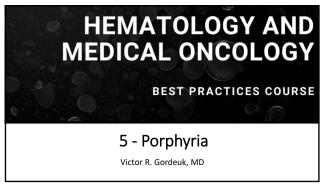
- · Diagnosis of IRIDA
- · Diagnosis of iron overload disorders
- Distinction between ACD and ACD with iron deficiency
- · Predict responsiveness to oral Fe
- · Companion diagnostic for therapies targeting hepcidin

The End

Porphyria

Victor R. Gordeuk, MD

August 13, 2020



Outline

- 1. General considerations; heme synthesis
- 2. Acute hepatic porphyrias
- 3. Cutaneous porphyrias
- 4. Summary
- 5. Case reports

Porphyrias

- Disorders of heme synthesis
- Defect of specific enzyme in heme biosynthetic pathway
 - overproduction of heme precursors formed prior to defect
 - ↑ activity of rate-controlling ALA synthase

MITOCHONDRION	CYTOSOL 2
Succinyl CuA Pos ALA synthase du	ALA coor des
04 V V V	Prophabilizagus
Spirent H-C-tota H-C-tota	
2-aminolevulinate	PRG deaminaxe
HEME	Hydroxymethyllailane
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	Uro'gen III
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6	Dely-Cody

3

E	Enzymes of Heme Biosynthesis					
	Enzyme	Chromosome	Porphyr			
1	ALA synthase 2	Xp11.2	X-linked protopo			
2	ALA dehydratase	9433	Plumbonornhyri			

	Enzyme	Chromosome	Porphyria with defect
1	ALA synthase 2	Xp11.2	X-linked protopophyria
2	ALA dehydratase	9q32	Plumboporphyria
3	PBG deaminase	11q23.3	Acute intermittent porphyria
4	Uroporphyrinogen III synthase	10q26.2	Congential erythropoietic porphyria
5	Uroporphyrinogen decarboxylase	1q34.1	Porphyria cutanea tarda
6	Coproporphyrinogen oxidase	3q12.1	Hereditary coproporphyria
7	Protoporphyrinogen oxidase	1q23.3	Variegate porphyria
8	Ferrochelatase	18q21.31	Erythropoietic protoporphyria

Porphyrins and Precursors

- Measure in urine (water soluble)
 - ALA (aminolevulinic acid)
 - PBG (porphobilinogen)
 - Uroporphyrin, Coproporphyrin
- Measure in feces (fat soluble)
- Coproporphyrin
- Protoporphyrin
- Measure in RBCs - Protoporphyrin

Broad Classification

- Acute hepatic porphyrias
 - Porphyrins proximal to defect increased
 - Earliest porphyrin precursors (ALA, PBG) increased
- Non-acute or cutaneous
 - Porphyrins proximal to defect increased
 - But, ALA and PBG not increased

Acute Porphyrias

1. Autosomal dominant

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- But rare ALA dehydratase deficiency is recessive
- 2. Heterogeneous mutations
 - Deletion, alternative splicing, Ψ mRNA stability, missence
- 3. Long quiescent periods and neurovisceral 'attacks' caused by hepatic overproduction:
 - ALA (γ-aminobutyric acid analog) and/or
 - PBG (interacts with GABA or glutamate receptors)

Acute Porphyrias

Type	Enzyme defect	Inheri- tance	Age onset	Biochemistry
Plumboporphyria	ALA dehydratase	Autosomal Recessive	Child	Urine: ↑ALA
Acute intermittent porphyria	PBG deaminase	Autosomal Dominant	Adult	Urine: ♠PBG and ALA
Hereditary coproporphyria	Copropor- phyrinogen oxidase	Autosomal Dominant	Adult	Urine: ♠ALA, PBG, coproporphyrin Stool: ♠copropor.
Variegate porphyria	Protopor- phyrinogen oxidase	Autosomal Dominant	Adult	Urine: ♠ALA, PBG, coproporphyrin Stool: ♠coproporyrin, protoporphyrin

Acute Porphyrias- Presenting Symptoms

System	Symptom or Sign	Incidence
Gastrointestinal	Abdominal pain	85-95%
	Vomiting	43-88%
	Constipation	48-84%
	Diarhea	5-12%
Cardiovascular	Tachycardia	28-85%
	Systemic hypertension	36-55%

Anderson et al. Ann Intern Med 2005;142:439-450.

9 10

Acute Porphyrias- Presenting Symptoms

System	Symptom or Sign	Incidence
Neurologic	Pain	
	(extremities, back, chest, head)	50-70%
	Paresis	42-68%
	Respiratory paralysis	9-20%
	Mental symptoms	40-58%
	Convulsions	10-20%

Anderson et al. Ann Intern Med 2005;142:439-450.

Acute Porphyrias- Precipitating Factors

- 1. Drug
 - www.porphyriafoundation.org
 - www.drugs-porphyria.org
- 2. Others
 - Females of child-bearing years
 - Fasting, dieting, stress, smoking

Drugs and Acute Porphyrias

(see www.porphyriafoundation.org and www.drugs-porphyria.org)

Unsafe	Unsafe	Unsafe	Safe	Safe
Alcohol	Ergots	Primidone	Acetominophen	Insulin
Barbiturates	Glutethimide	Progesterone	Aspirin	Penicillin
Carbamazepine	Griseofulvin	Pyrazinamide	Atropine	Penicillin derivatives
Carisoprodol	Mephenytoin	Pyrazolones	Bromides	Phenothiazines
Clonazepam	Meprobamate	Rifampin	Cimetidine	Ranitidine
Danazol	Methyprilon	Succinimides	Erythropoietin	Streptomycin
Diclofenac,	Metoclopramide	Sulfonamides	Gabopentin	
NSAIDS	Phenytoin	Valproic acid	Glucocorticoids	

Acute Hepatic Porphyrias

Early Dx of AHP in Symptomatic Patient

Urine PBG (protect from light) and ALA

- PBG Increased in AIP, hereditary coproporphyria, variegate porphyria
- PBG Levels of 20-200 mg/L (normal <2.5 mg/L)
- ALA increased in all four acute hepatic porphyrias
- Measure in an acute, symptomatic attack

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Acute Hepatic Porphyrias

Acute Porphyrias- Rx of Acute Attack

- 1. Hospitalize, withdraw unsafe meds
- 2. Provide nutrition and supportive Rx
 - IV fluids for dehydration, electrolyte imbalances
 - seizure precautions if pt hyponatremic
- 3. Use meds known to be
 - narcotics for pain
 - phenothiazines for nausea or vomiting
 - beta blockers for hypertension, tachycardia

Acute Hepatic Porphyrias

Acute Porphyrias- Rx of Acute Attack

- 4. Begin hemin urgently
 - Inhibits delta-amiolevulinic acid synthetase
 - 3-4 mg/d for 3-14 d
- 5. IV glucose

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- administer while awaiting delivery of hemin
- 10% solution, at least 300 g daily
- 6. Monitor patient closely:
 - PFTs: if vital capacity impaired, place pt in ICU
 - neurologic status, especially proximal muscle strength
 - serum electrolytes, creatinine, Mg daily
 - watch for bladder distention.

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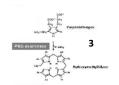
Acute Hepatic Porphyrias

Acute Hepatic Porphyrias- Chronic Rx

- 1. Manage chronic pain. Address depression or other neuropsychiatric problems
- 2. Prevent acute attacks
 - Avoid unsafe drugs
 - Maintain adequate intake of carbohydrates
- 3. Pharmacologic options to prevent acute attacks
 - Gonadotropin-releasing hormone analogues to suppress ovulation
 - Regular transfusion of hemin
 - Givosiran, approved by FDA 2019. siRNA directed at ALAS1 mRNA. Given by S.C. injection once per month.

Acute Intermittent Porphyria

- Autosomal dominant deficiency of PBG deaminase
 - >375 mutations
- Prevalence
 - ~1/10,000 European ancestry
- Also present in African Americans
- Clinical expression in <10% of those with mutation



Acute Hepatic Porphyrias

Acute Hepatic Porphyrias

Acute Intermittent Porphyria

- Symptoms and signs
 - GI (95%)
 - Neuropathy (66%)
 - Cardiovascular (70%)
 - Hyponatremia in severe attack
 - Photosensitivity not present
- . Long term- increased risk of hepatocellular CA

Acute Hepatic Porphyrias

Acute Hepatic Porphyrias

AIP- Diagnosis

- Urine
 - Clear but darkens with light exposure
 - Large excess PBG, ALA
- Blood
 - Low RBC PBG deaminase (~50%) confirms dx
 - ~10% of pts have nl RBC PBG deaminase but decreased hepatic activity
- Differential Diagnosis
 - Guillain-Barre; heavy metal poisoning; PNH

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20

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Acute Hepatic Porphyrias

Hereditary Coproporphyria



- · Autosomal dominant deficiency copro-porphyrinogen III oxidase; >60 mutations
- · AIP sx's plus photosensitivity
- Neurovisceral attacks and skin lesions usually occur together

Acute Hepatic Porphyrias

Hereditary Coproporphyria

- Diagnosis
 - Urine ALA and PBG increased
 - RBC PBG deaminase not decreased
 - — ↑ Stool coproporphyrin (> stool protoporphyrin)
 - ↑ Urine coproporphyrin

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Variegate Porphyria

· Autosomal dominant deficiency protopor-phyrinogen oxidase; >160 mutations

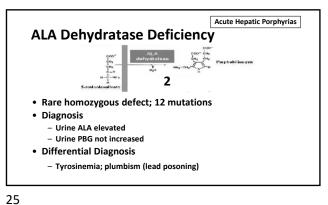
- 1/400 white South Africans
- AIP sx's plus photosensitivity,
- · Neurovisceral attacks and skin lesions may occur separately



Variegate Porphyria

- Diagnosis
 - Urine ALA and PBG elevated
 - RBC PBG deaminase not decreased
 - – ↑ Stool protoporphyrin (> stool coproporphyrin)
 - $-\uparrow$ Urine coproporphyrin (> urine uroporphyrin)

Acute Hepatic Porphyrias



Acute Porphyria Management Summary

- 1. Spot urine PBG level for rapid Dx if possible
- porphyrin synthesis
 - PO 1500-2000 kcal/24 h
- IV 10- 20% dextrose 3. IV hematin (early)
 - 3-4 mg/kg IV qd

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- 4. Avoid precipitating factors
 - Suppress ovulation with **LHRH** agonists
- 5. Consider IV hematin or SC givosiran
- 6. Mutation analysis in pts and family

Cutaneous Porphyrias

Cutaneous Porphyrias

- Variable inheritance
- Cutaneous photoxicity
 - Porphyrins deposited in upper layers of skin
 - Reactive oxygen species
 - Oxidative membrane damage to mast cells
 - Complement activation

Cutaneous Porphyrias

Cutaneous Porphyrias

Туре	Enzyme defect	Inheritance	Age onset	Biochemistry
Porphyria cutanea tarda (PCT)	Uroporphyrinogen decarboxylase	Autosomal dominant	Adult	Urine: uroporphyrin
Hepatoerythropoietic porphyria	Uroporphyrinogen decarboxylase	autosomal recessive	Infant	Urine: uroporphyrin
Erythropoietic protoporphyria	Ferrochelatase	autosomal dominant	Child to adult	RBC: protoporphyrin
Congenital erythropoietic porphyria	Uroporphyrinogen III synthase	autosomal recessive	Infant	Urine, stool: copro- porphyrin 1
X-linked protoporphyria	Aminolevulinic acid synthase 2	X-linked	Child to adult	RBC: protoporphyrin

27 28

Cutaneous Porphyrias Porphyria Cutanea Tarda urine darker than control Blistering and scarring of sun-exposed skin Fluorescence after acidification Hyper-Cloeren and Craig. NEJM 2001;245 no. 14

Porphyria Cutanea Tarda

Cutaneous Porphyrias

- 1. Sx's when hepatic uroporphyrinogen decarboxylase activity ≤20% of nl
- 2. Type 1 acquired form is most common
 - NI URO-D activity when asymptomatic
- 3. Type 2 hereditary form; <100 mutations
 - Autosomal dominant
 - ~50% URO-D activity when asymptomatic
- 4. Manifestations precipitated by co-factors

Cutaneous Porphyrias

Porphyria Cutanea Tarda

- 5. Most common porphyria
 - 1-5 cases/25,000
- 6. Precipitating factors oxidize uroporphyrinogen, which inhibits URO-D
 - frion stores; HFE, African iron overload
 - Hepatitis C (>50% of pts +), HIV
 - Alcohol, estrogens

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- Exposure to fungicide hexachlorobenzene (Turkey, 1956)

Cutaneous Porphyrias Porphyria Cutanea Tarda

7. Down-regulation of hepcidin may underlie increase in iron stores

8. Manifestations

- Bullous dermatosis (blistering skin lesions)
- Hyperpigmentation
- Hypertrichosis

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34

Cutaneous Porphyrias

Porphyria Cutanea Tarda

- 9. Evaluation
 - Elevated urinary total porphyrins (uroporphyrin >>> coproporphyrin)
 - Genetic testing
 - Screen for HFE C282Y homozygosity and other forms of iron
 - Evaluate for viral hepatitis/other liver disease; R/O

Cutaneous Porphyrias

Porphyria Cutanea Tarda

10. Treatment

- Avoid precipitating factors
 - Avoid sun and use opaque sun-block if outdoors: (responsible light is at 400 nm and not blocked by most sunscreens)
 - Phlebotomy 500 ml/wk until remission
 - Iron chelation if phlebotomy not possible

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Cutaneous Porphyrias

Hepatoerythropoietic Porphyria

- **Biallelic URO-D mutations** (homozygous PCT)
 - URO-D activity 3-10% of nl; dx
 - nuroporphyrin and heptacarboxylporphyrin in
 - − ↑ Zn-protoporphyrin in RBCs
- Management
 - Avoid sunlight including longwave UV light that passes through glass
 - Use opaque sunscreens (contain zinc oxide and titanium dioxide)
 - Avoid alcohol, estrogens, smoking, drugs that induce cytochrome P450

Erythropoietic Protoporphyria

- Deficiency of ferrochelatase
 - Autosomal dominant; >135 mutations - Sxs begin from childhood to adulthood
 - ~10% with mutant allele develop sxs;
 - related to RNA output of non-mutated
- · More common than congenital erythropoietic protoporphyria



Cutaneous Porphyrias

Cutaneous Porphyrias Erythropoietic Protoporphyria

- Symptoms & signs
 - Erythrema, urticaria, pruritus, burning sensation of sun-exposed skin
 - Hepatic dysfunction later in life from porphyrin deposition in the liver, may
- Diagnosis
 - Massive increase erythrocyte protoporphyrin



Cutaneous Porphyrias

Erythropoietic Protoporphyria

• Treatment

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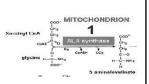
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- Photoprotection
- Beta carotene may protect from dermal toxicity
- Cholestyramine may interrupt protoporphyrin enterohepatic recycling
- Liver transplant for hepatic failure

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X-Linked Protoporphyria

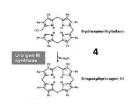
- Gain of function mutation in ALAS2
- Clincal findings identical to erythropoietic protoporphyia



Cutaneous Porphyrias

Congenital Erythropoietic Porphyria

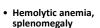
- Uroporphyrinogen III synthase deficiency
 - Autosomal recessive, rare but >35 mutations
 - Upregulation of ALAS2
- Typical onset at birth



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Congenital Erythropoietic Porphyria

- Uroporphyrin 1 in RBCs, plasma, urine, skin
 - severe cutaneous photosensitivity
 - Scarring
 - Most disfiguring porphyria
- Uroporphyrin 1 deposits in teeth
 - Discoloration of teeth





Congenital Erythropoietic Porphyria

- Treatment
 - Avoidance of sun or sunscreens
 - RBC Tx's to reduce erythropoiesis
 - Chloroquine, hematin, hydroxyurea
 - Allogeneic hemopoietic stem cell transplant
 - Iron chelation

41 42

Cutaneous Porphyrias

hyria

Porphyria: Clinical Approach Summary

- 1. General principles
 - Symptomatic porphyria always has nheme precursors; absence indicates sx's not due to porphyria.
 - During asymptomatic periods, individuals with enzymatic defect may have nl heme precursor levels.
- 2. Acute hepatic porphyria (neurovisceral sx's)
 - Measure urinary PBG- ↑ in AIP, VP, HCP
 - Measure RBC PBG deaminase; if nl, measure stool coproporphyrin and protoporphyrin

Clinical Approach Summary

- 3. Cutaneous porphyria (photosensitivity)
 - PCT- ↑ urinary uroporphyrin
 - EPP- ♠ RBC free protoporphyrin
 - CEP- ↑ uroporphyrin 1 in plasma and urine
- 4. Mutation analysis
- Mount Sinai Genetic Testing Lab-Porphyria DNA Testing 866-322-7963; Porphyria@mssm.edu
- Alnylam Pharmaceuticals, Inc. 1.800.436.3037

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Clinical Case 1:

- Hx: 24-yo female; abd. pain; progressive weakness in extremities, mild resp. distress.
- Past Hx: multiple admissions for abd. pain; moderate alcohol; estrogen-containing OCT.
- PE: diffuse abd. tenderness; generalized motor weakness; poor resp. effort.
- Lab: Na+ 129 mEq/L.
- Course: intubation, mech. ventilation in 12 h.

Clinical Case 1:

- . Findings suggestive of AIP
- Further work-up: rapid screen of urine shows ↑ PBG. RBC PBG deaminase level 50%.
- Management: 10% dextrose; panhematin; narcotics; Correct hyponatremia. Monitor resp. status and urine ALA, PBG.
- Prevention: avoid alcohol, estrogen, smoking, certain other meds; monitor for hepatoma.

45 46

Clinical Case 2:

- Hx: 52-yo female; life-long painful photosensitivity
- Past Hx: Pt. protects herself from sunlight; no alcohol; no meds.
- PE: no chronic skin changes or hepatomegaly.
- Lab: AST 96 U/L. Urine ALA, PBG, uroporph. NI; RBC protoporphyrin 18,900 ug/dL (nI <90).

Clinical Case 2:

- Findings of eyrthropoietic protoporphyria
- Management: protection from sunlight; trial of betacarotene was given.
- Clinical course: progressive hepatic dysfunction; liver bx showing cirrhosis and hepatocellular accumulation of crystalline pigment.

Clinical Case 3:

- Hx: 48-yo male; blistering lesions on sun-exposed areas 2 y; sunscreens do not help; no abd pain.
- Past Hx: Hepatitis C pos; HIV neg; regular alcohol consumption.
- PE: blistering and scarring on dorsum of hands.
- Lab: CBC and LFT's nl. Ferritin 500 ng/ml.

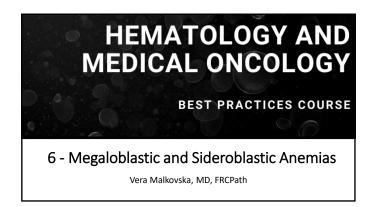
Clinical Case 3:

- Findings suggest PCT.
- Further work-up: Urinary ALA and PBG nl; urinary uroporphyrin increased; neg for HFE C282Y
- Management: use of opaque sunscreen; avoid alcohol; phlebotomy; screen for hepatoma.
- Clinical course: resolution of symptoms after four phlebotomies.

Megaloblastic and Sideroblastic Anemias

Vera Malkovska, MD

August 13, 2020



Disclosures

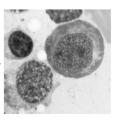
Disclosures of Financial Relationships with Relevant Commercial Interests

• None

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Megaloblastic Anemias: Definition

Disorders of DNA synthesis that lead to nuclear-cytoplasmic maturation asynchrony and characteristic morphological changes



Causes of megaloblastic anemias

- · Cobalamin (CBL) deficiency
- Folate deficiency
- Drugs: 5-flurouracil, azathioprine, hydroxyurea, zidovudine, anticonvulsants
- Hereditary disorders of DNA synthesis and other rare errors of metabolism: orotic aciduria, thiamine-responsive megaloblastic anemia, Lesch-Nyhan sy, intrinsic factor and cubilin defects, methyl- and adenosyl-CBL synthesis defects

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Morphology

1

3

Peripheral blood

- Macro-ovalocytes
- · Hypersegmented neutrophils

Bone marrow

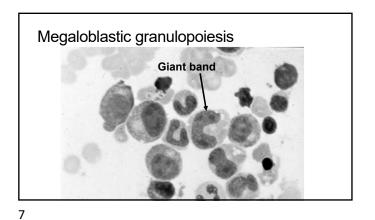
- Megaloblasts
- Giant metamyelocytes and bands
- Decreased and hypersegmented megakaryocytes

Bone marrow morphology

Macro-ovalocytes

Hypersegmented neutrophil

Megaloblasts



Labs reflect ineffective erythropoiesis

- Cytopenia in peripheral blood, ↓ retics
- ↑ MCV (masked by microcytic disorders)
- Hypercellular bone marrow
- ↑ LDH, indirect bilirubin
- Haptoglobin

8

10

• ↑ Ferritin, iron, transferrin saturation

Cobalamine (CBL) deficiency

9

Structure of methyl-CBL

Planar corrin nucleus:
4 pyrrole rings linked to central Co+

Nucleotide:
5,6-dimethyl-benzimidazolyl

Biochemistry of CBL & folate deficiency

THF: Tetrahydrofolate
DHF: Dihydrofolate
PG: Polyglutamate
DHFR: Dihydrofolate
PG: Polyglutamate
DHFR: Dihydrofolate
PG: Polyglutamate
DHFR: DHF-PG
THF-PG
THF-PG
THF-PG
THF-PG
Methylcne
THF-PG
Methylcnine
MethylTHF
Homocysteine

Adenosyl CBL-dependent reaction in mitochondria

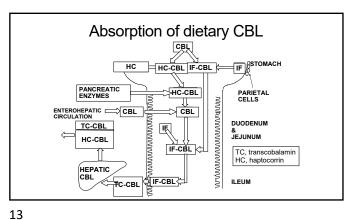
COS COA
CH2- CH - COOH
Adenosyl CBL
H

Methylmalonyl CoA

Succinyl CoA

14

16



Absorption of oral CBL

	Normal	Malabsorption
Daily loss	1 μg	2 μg
Body stores	~ 2,500 µg	variable
% absorbed fro	m single oral dose	
1 μg	0.56 μg	0.01 μg
10 μg	1.6 µg	0.1 μg
1000µg	9.7 µg	7.0 µg

Thursday, August 13, 2020

CBL retention from injection

	Normal	Malabsorption
Daily loss	1 μg	2μg
Body stores	~ 2,500 µg	variable
% absorbed froi	n single injection o	of*:
10 μg	9.7 µg	same as N
100 μg	55 µg	same as N
1000µg	150 µg	same as N

Causes of clinical CBL deficiency

- · Dietary deficiency (vegans)
- Inadequate proteolysis of food-CBL (gastric surgery)
- · Intrinsic factor (IF) deficiency pernicious anemia
- · Blind loop sy bacterial overgrowth
- Intestinal malabsorption (sprue, Crohn's, ileal resection)
- · Nitrous oxide inhalation

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Causes of clinical CBL deficiency

- Pancreatic insufficiency (rare)
- Fish tapeworm infection
- Use of metformin
- · Use of drugs blocking stomach acid
- Breastfed infants of B12 def. mothers
- · Rare genetic disorders

Pathophysiology of PA

- · Autoimmune gastritis
- Anti-parietal cell (anti-Na+/K+ ATPase)
- · Anti-IF antibodies, high gastrin
- · Decreased parietal cell function:
- Decreased IF production and CBL absorption
- Achlorhydria
- · Decreased iron absorption

PA: Clinical manifestations

Hematological

- · Symptoms of anemia
- · Pallor, lemon yellow skin

Neuropsychiatric

- Paresthesiae
- · Dementia, psychosis
- · Ataxia, weakness, spasticity

Other: Glossitis, other epithelial changes



PA: Clinical manifestations

Associated autoimmune disorders

- · Hypothyroidism, Hashimoto's
- Vitiligo, rarely hyperpigmentation
- Diabetes mellitus
- · Addison's disease

Increased risk of gastric cancer and carcinoid

19 20

Diagnosis and management of CBL deficiency

Questions to answer at diagnosis

- Is this a true clinical deficiency?
- · What is the underlying disorder?
- Is it reversible?
- · Are there other nutrient deficiencies?
- Could this be due to a rare disorder? (nitrous oxide, genetic, metabolic or transport)

21 22

Accurate diagnosis impacts therapy

- · Short vs long term therapy
- Parenteral vs oral
- Treatments of underlying and associated diseases
- Addressing long-term risks

Low B12 levels without clinical deficiency

- Lab artifact
- Folate deficiency
- Pregnancy (2nd,3rd trimester)
- Haptocorrin (HC) deficiency
- Rare: myeloma, oral contraceptives
- · Intake of megadoses of ascorbic acid

Clinical deficiency with normal B12 levels

- •TC deficiency (with normal HC level)
- Nitrous oxide exposure
- Inborn errors of CBL metabolism
- Myeloproliferative disorder with B12 malabsorption

How to diagnose "true" B12 deficiency?

- B12 should not be checked without clinical indications
- Clinical suspicion of deficiency should be investigated regardless of B12 levels
- · Reassay marginally abnormal levels
- · Confirm deficiency by 2 biomarkers
- Monitored trial of parenteral B12

25 26

Tests for the diagnosis of CBL and folate deficiency

Test	CBL deficiency	Folate deficiency
Serum CBL	Ţ	N or ↓
Serum folate*	N or 1	\downarrow
Red cell folate	↓ or N	\downarrow
MMA*		N
Plasma homocysteine	\uparrow	\uparrow
Deoxyuridine Suppression test*	Abnl.	Abnl.

*Best discriminators of CBL vs folate def.

Other tests

- Anti-IF antibody
 - Very high specificity for PA
 - · False positive within 2 days of CBL injection
- · Parietal cell antibody
- Positive in 80-90% of PA patient
- Low specificity
- Increased serum gastrin in ~ 90% of PA
- Low specificity

CBL absorption test ("Schilling test") is unavailable

Suggested therapy

Drug Hydroxycobalamin or cyanocobalamin

Route IM
Dose 1,000 μg

Regimen 8-10 x over 2 months, then monthly life-long

Route and schedule are less important than close

monitoring!

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Oral maintenance therapy

PROS

Easy and painless

Cheap

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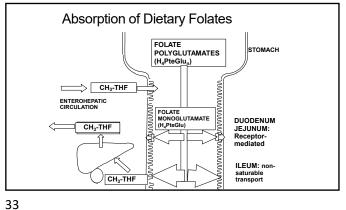
CONS

- Variable absorption with food
- Compliance problems
- Monitoring essential
- Long term experience limited

Folate deficiency

Structure of folates ĊH₂ CO-NH-CH COOH Substituted Pteridine

31



Folate in diet

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- · Destroyed by cooking
- Food folate less bioavailable than folic acid
- · Absorption 50-80% of dietary amount
- Enterohepatic recycling 100 μg/day
- All grain products supplemented in the US
- Recommended dietary intake 400 µg/day

Causes of folate deficiency

- Increased requirement (pregnancy, rapid growth, hemolytic anemia, skin disorders)
- · Dietary rare in U.S.
- Intestinal malabsorption (celiac disease)
- · Defective utilization (methotrexate, trimethoprim)
- · Anticonvulsants (decreased absorption)
- · Rare genetic disorders

Serum vs red cell (RBC) folate

Serum Folate

Unstable in vitro

 May normalize after one hospital meal

· Increased with hemolysis

RBC Folate

- Better reflect body stores
- · Low in 60% CBL deficiency
- · Increased with hemolysis

Treament of folate deficiency

Drug Folic acid Route Oral

Dose 1-5mg daily x 4 months

Maintenance if cause persists

Prophylaxis: Pregnancy, prematurity, chronic hemolysis,

dialysis

CBL deficiency must be ruled out before folic acid therapy!

Key points about folate deficiency

- Rare in the US with adequate diet and normal GI system
- Occurs with malabsorption, malnutrition, rapid cell turnover
- Cytopenia develops rapidly
- Elevated homocysteine but not MMA
- · Associated with neural tube defects
- · Neurol. manifestation very rare in adults
- Folic acid can worsen neurol. deficit in CBL deficiency

37 38

Sideroblastic anemias

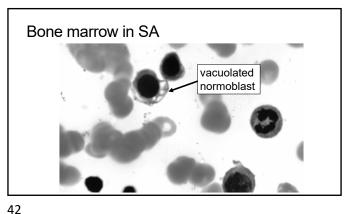
Sideroblastic anemias (SA)

- · Congenital or acquired, benign or malignant
- · Limited to erythroid defect or syndromic
- Ring sideroblasts are diagnostic, caused by iron accumulation in mitochondria
- · Ineffective erythropoiesis
- · Iron overload is common

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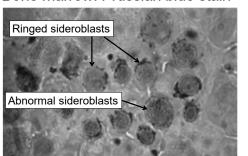
Sideroblasts: Definitions

- Normal sideroblasts
 - 1-4 siderotic granules in cytoplasm Correlate with TIBC saturation
- · Sideroblasts in SA:
 - ≥ 5 granules, coarse, form rings, reflect accumulation of mitochondrial ferritin



Vera Malkovska, MD

Bone marrow: Prussian blue stain



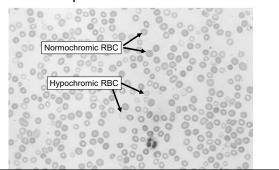
Laboratory findings

Signs of ineffective erythropoiesis

- Micro-, normo-,macrocytic anemia or dimorphic RBC
- · Low reticulocytes
- Erythroid hyperplasia in bone marrow
- •↑ LDH, indirect bilirubin
- \ Haptoglobin

43 44

Dimorphic red cells in SA



Laboratory findings

Signs of defective iron utilization

- Hypochromic RBC
- ↑ ferritin, iron, transferrin saturation
- ↑ marrow iron stores
- ↑ liver Fe content
- · Iron overload before transfusions

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Congenital SA

- Erythroid only
 - Usually microcytic
 - Iron overload
 - · Can present in adults
- Syndromic
 - Dominated by neuromuscular, metabolic and other defects
 - Macro- or normocytic
 - · Rare, diagnosed in infancy or childhood

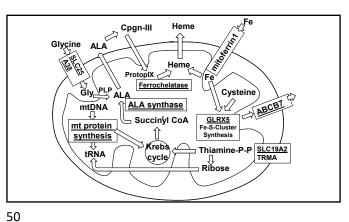
Erythroid only SA

- · Heme synthesis defects
- X-linked SA (XLSA) *
- SLC25A38 deficiency *
- · Erythropoietic protoporphyria*
- Iron-sulfur cluster generation defects
- GLRX5 deficiency
- HPSA9 deficiency
- · HSCB deficiency
- * Reported in more than 40 families

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Syndromic SA

- · Mitochondrial protein synthesis defects
- Pearson marrow pancreas syndrome (PMPS) *
- · Myopathy with lactic acidosis SA (MLASA) *
- SA with immunodeficiency, fever, developmental delay (SIFD)*
- Mitochondrial respiratory protein gene mutations
- Thiamine-responsive megaloblastic anemia (TRMA)*
- · Other rare defects
- * Reported in more than 40 families



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X-linked hereditary SA (XLSA)

- · Most common congenital SA
- Mutations in ALA-synthase 2 (ALAS2) -1st step in heme synthesis
- Mostly males < 40yrs
- About a quarter are females
- Microcytic hypochromic, Hb >7g/dL
- Iron overload

XLSA in females

- Present in mid to late adulthood
- · Normocytic or macrocytic
- Dimorphic RBC
- Associated with skewed X-inactivation
- · Can be mistaken for MDS!

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Management of XLSA

- Pyridoxine 50-100 mg/day, lower dose for maintenance
- Iron chelation
- · Small volume phlebotomy if possible
- · Transfusions rarely needed
- Splenectomy is contraindicated in hereditary SA

2. Acquired clonal/neoplastic SA*

- Myelodysplastic syndromes with ring sideroblasts (MDS-RS) with single linage dysplasia (MDS-RS-SLD)[¶]
- MDS-RS with multilinage dysplasia (MDS-RS-MLD)¶

¶Associated with SF3B1 mutation

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* The 2016 revision of the WHO classification of

55

MDS-RS-SLD (former RARS)

- Normo- or macrocytic, dimorphic RBC
- •≥15%/≥ 5%* of erythroblasts are RS, at all stages of maturation
- Favorable prognosis c/w other MDS
- Responsive to G-CSF with Epo
- Pyridoxin-refractory

*if SF3B1 mutation is present

3. Reversible acquired SA

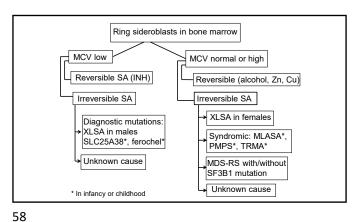
- Alcohol
- Drugs (INH, chloramphenicol, linezolid)
- · Copper deficiency:
 - · Malnourished infants
 - · Patients on parenteral nutrition
 - · Excess dietary zinc intake
- Hypothermia

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Anemia corrects upon removal of precipitating cause

Copper deficiency

- Micro- to macrocytic a. (MCV 70-116)
- ± neutropenia
- · Hypocellular marrow
- Vacuolated erythroid and myeloid precursors ± ring sideroblasts
- Ataxia, myelopathy, sensory neuropathy
- Can be mistaken for B12 deficiency!



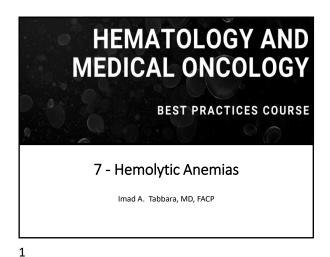
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Thank you

Hemolytic Anemias

Imad A. Tabbara, MD

August 13, 2020



Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

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HEMOLYTIC ANEMIA

- HEMOLYSIS: Premature or accelerated destruction of RBCs
- RBC survival: less than 100 days
- 2 main causes:
 - -Intrinsic RBC defects (inherited)
 - -Extra-corpuscular causes (acquired)

CLASSIFICATION

- Hereditary Hemolytic Disorders
 - * RBC enzymes defects
 - * RBC membrane defects
 - * Hemoglobinopathies
 - * Thalassemias

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CLASSIFICATION

- Acquired Hemolytic Disorders
 - * Immune hemolytic anemias
 - * Splenomegaly
 - * Microangiopathic hemolytic anemia
 - * PNH

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- * Direct toxic effect (malaria, clostridial infections)
- * Spur cell anemia

IMMUNE HEMOLYTIC ANEMIAS

- · Caused by:
 - WARM ANTIBODY
 - OR
 - COLD ANTIBODY

IMMUNE HEMOLYTIC ANEMIA

- Rare & Heterogeneous disease
- Incidence: 1 to 3 /100,000 cases per year
- Warm reactive: most common type & accounts for 70-80% of adult cases and 50 % of pediatric cases

IMMUNE HEMOLYTIC ANEMIAS

- Incidence increases with age with a dramatic increase after age 50
- There is an early childhood peak due to increased incidence of Paroxysmal Cold Hemoglobinuria (PCH)

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IMMUNE HEMOLYTIC ANEMIAS Diagnosis

- Direct Antiglobulin Test (COOMBS test) is the only test that provides definitive evidence of immune hemolysis
- Increased LDH & reduced haptoglobin: 90% specific for diagnosis
- Normal LDH & haptoglobin: 92% sensitive for lack of hemolysis

IMMUNE HEMOLYTIC ANEMIAS Diagnosis

· Direct Coombs test

- -The addition of Anti-IgG/anti-C3 leads to the agglutination of washed RBCs IF they are coated with IgG or complement
- Weakly positive test occurs in 1 in 10,000 healthy donors and in 5-10% of hospitalized patients without hemolysis and is usually caused by complement

IMMUNE HEMOLYTIC ANEMIAS Diagnosis

· Direct Coombs test

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Negative test with severe immune hemolysis can occur:

- In patients with low titers of auto Ab and/or C3. Most reagents cannot detect fewer than 100-500 Ab molecules
- In patients with auto Abs that are IgA or IgM.
 These are not detected by commonly used reagents

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IMMUNE HEMOLYTIC ANEMIAS Diagnosis

Direct Coombs test

If negative test and high suspicion of immune hemolytic process, can use enzyme-linked immunoadsorbent assay (ELISA), radiolabeled anti-immunoglobulin, or specific assays for IgA

DAT-negative Hemolysis

- 3-11% of cases
- · Mild to severe life threatening
- Can be primary or secondary similar to DATpositive disease
- Delay in diagnosis & initiation of treatment may be fatal
- Same management as DAT-positive disease

IMMUNE HEMOLYTIC ANEMIAS Diagnosis

Direct Coombs test

 Level of Coombs positivity does not predict degree of hemolysis

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IMMUNE HEMOLYTIC ANAMIAS Diagnosis

- Direct Coombs test
- A "complement-only" positive Coombs test (10%) in patients with:
 - Low titer of warm-reactive IgG
 - A warm or cold reactive IgM
 - Cold-reactive IgG : Donath-Landsteiner (D-L) (hemolysin)

IMMUNE HEMOLYTIC ANEMIAS Diagnosis

Indirect Coombs test

- -Detects Abs in the patient's serum
- Normal ABO and Rh-compatible RBCs are incubated with the patient's serum, washed and then a direct Coombs test is performed on the incubated RBCs

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A. Direct antiglobulin (Coombs') Washed (3x's) Patient crythrocytes (sensitized in vivo) B. Indirect antiglobulin (Coombs') test Human crythrocytes (not patient's) Human crythrocytes (not patient's) Sensitized crythrocytes + Human (patient serum) IgG antibody Sensitization Visual crythrocyte (in vitro) sensitization Visual crythrocyte (in vitro) sensitization Diagram of direct and indirect antiglobulin (Coombs') test

IMMUNE HEMOLYTIC ANEMIAS Diagnosis

Cold Agglutinin Assay

- Detects serum Abs which induce clumping of O \oplus RBCs in the cold
- Typically, it detects IgM cold reactive Abs

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IMMUNE HEMOLYTIC ANEMIAS Diagnosis

Cold Agglutinin Assay

- Low titer cold agglutinins are common but do not cause complement fixation

IMMUNE HEMOLYTIC ANEMIAS Diagnosis

Cold Agglutinin Assay

- Level of C3 coating does not correlate directly with hemolysis. Coombs reagents detect both biologically active C3b and inactive fragments (C3bi, C3d)
- Only C3b is associated with complementmediated lysis

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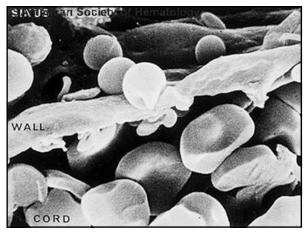
IMMUNE HEMOLYTIC ANEMIAS Warm Antibody

- Mediated by IgG Abs that react with RBCs at body temperature (37 degree C)
- These Abs do not cause lysis or agglutination of RBCs
- Ab-coated RBCs are removed from circulation by Fc receptor-expressing macrophages in the spleen

IMMUNE HEMOLYTIC ANEMIAS
Warm Antibody

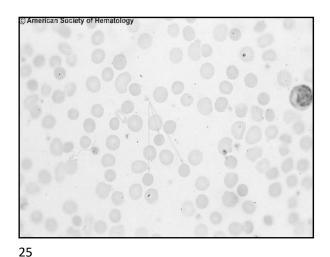
- Alteration of red cell membrane occurs when the IgG-coated RBC bind to macrophages in the spleen (partial phagocytosis), resulting in the formation of spherocytes
- Presence of C3 on RBC membrane, in addition to the Ab, behaves in a synergistic way leading to severe hemolysis

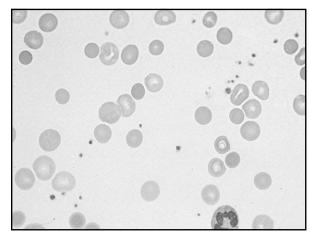
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IMMUNE HEMOLYTIC ANEMIAS Warm-Reactive

- A generalized up-regulation of the phagocytic activity of macrophages has been reported in these patients
- Lymphocytes may play a role in inducing membrane injury of the RBCs that are coated by IgG or complement





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IMMUNE HEMOLYTIC ANEMIAS Warm-Reactive

- Idiopathic or primary in 50% of cases
- Viral infections (in children)
- Neoplasia (NHL, CLL treated with purine analogs)
- Connective tissue disorders (SLE)
- Prior allogeneic blood transfusion/hematopoietic stem cell transplantation
- Drug-induced (rarely in children)

Methyldopa Quinidine

Penicillins/cephalosporins

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AIHA Purine Nucleoside Analogues

- · Fludarabine, Cladribine & Pentostatin
- AIHA reported after 1-4 courses of therapy
- Significant rate of relapse of AIHA with retreatment associated with high mortality
- Combination of Fludarabine plus cyclophosphamide and /or Rituximab protects against AIHA in CLL
- Disturbance of immunoregulatory T cells with release of a suppressed auto Ab to a native RBC Ag

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AIHA Allogeneic Blood Transfusion

- 8%-10 % incidence of auto Ab production (positive DAT)
- Mainly in patients with hemoglobinopathies receiving multiple transfusions
- · Native RBCs are hemolysed

AIHA Allogeneic HSCT

- Ab production by donor immune system against Ags on donor RBCs (autoimmune reaction of the graft against its own product)
- Incidence of 6% in pediatric population with a median onset of 4 months post transplant with high mortality
- Reported also in T-cell depleted & cord blood transplants

AIHA Orthotopic Solid Organ Transplant

- · Related to "passenger lymphocyte syndrome"
- Risk & degree of hemolysis is proportional to the mass of transplanted lymphocytes
- · Heart-lung>liver>kidney
- · Rapid onset hemolysis with positive DAT
- Hemolysis is usually transient since transplanted lymphocytes do not proliferate or engraft
- Management: Transfusion of group O RBCs, avoidance of ABO-incompatible plasma products, maintenance of adequate renal function, & RBCs exchange

31 32

- CMV Infection
 - Autoimmune hemolysis caused by warmreactive IgG

IMMUNE HEMOLYTIC ANEMIAS

Infections

- · Influenza A Infection
 - Autoimmune hemolysis caused by high-titer complement-fixing Abs to virus-produced, RBCbound polyribosome ribosylphosphate
- HSV Infection
 - Autoantibody is IgG with Rh (anti-c) specificity

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AIHA EVANS Syndrome

- Immune thrombocytopenia with acquired hemolytic anemia
- Can be a combination of any 2 or 3 autoimmune cytopenias
- Autoantibody formation due to defective B cell selection and maturation

AHIA Lymphoproliferative Disorders

- Onset may precede or follow the diagnosis of a lymphoproliferative disorder (LPD)
- Incidence of LPD is ~ 18% between 9 -76 months after onset of hemolysis
- Risk factors for LPD:
 - · IgM monoclonal gammopathy
 - · advanced age
 - · underlying autoimmune disease

AIHA Thromboembolism

- Increased risk for venous thromboembolism
- Pulmonary embolism: most common cause of death (splenectomized pts receiving corticosteroid therapy)
- · Predisposing factors:
 - HIV infection
 - Antiphospholipid antibody positivity (lupus anticoagulant)

AIHA EVANS Syndrome

- Can be a manifestation of:
 - Autoimmune lymphoproliferative syndrome
 (ALPS)
 - Classic primary immunodeficiency (PID)
 - Other novel immune dysregulation syndrome
 - *Diagnosis of Evans syndrome: initiate basic immunologic workup & screening for common variable immunodeficiency & ALPS

AIHA/ALPS

- · ALPS is caused by germline or occasionally somatic mutations in FAS, FASL or CASP10
- · Impaired FAS mediated apoptosis of activated autoreactive lymphocytes
- · In childhood, chronic ITP, AHIA
- · Lymphadenopathy, splenomegaly plus other autoimmune manifestations
- Increased risk for malignancy (lymphoma)

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DRUG-INDUCED IMMUNE **HEMOLYTIC ANEMIA**

Autoimmune Type

- Induced by α -methyldopa
- Positive Coombs test in 10% of patients receiving α-methyldopa
- AutoAb is IgG, similar to one seen in idiopathic AIHA, does not fix complement and is usually specific for Rh locus

DRUG-INDUCED IMMUNE **HEMOLYTIC ANEMIA**

Innocent Bystander Type

- Least common

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- Drugs include:
 - · Quinidine, Quinine, Sulfonamides, Isoniazid, Phenacetin and Dipyrone
- Interaction of drug with the RBC membrane produces a neoantigen
- Abs are IgG or IgM
- Drug-Ab complex adheres to RBCs membrane and can fix complement

AHIA/ALPS

Treatment

- First Line: Steroids & IVIG
 - · Splenectomy: last resort because of increased risk of fatal pneumococcal sepsis
 - · Rituximab: prolonged & severe hypogammaglobulinemia
- Second Line: Mycophenolate Mofetil (MMF) &

DRUG-INDUCED AUTOANTIBODY GENERATION



- <u>Key concept</u>:

 Drug stimulates B cell production of anti-rbc autoantibodies
- α methyl Dopa L Dopa
- Fludarabine and Chloro-deoxyadenosine
- Procainamide
- Diclofenac

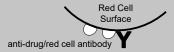
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DRUG-INDUCED IMMUNE **HEMOLYTIC ANEMIA**

Innocent Bystander Type

- Direct Coombs is positive for C3 only, since the drug-Ab complex will dissociate from RBC
- Hemolysis can be intravascular or extravascular depending on whether Ab can fix complement or not

DRUG-INDUCED FORMATION OF ABS AGAINST THE RBC -Hapten COMPLEX



Antibody forms ternary complex with the drug hapten and a specific red cell membrane component

Examples

- Quinine/Quinidine
- Stibophen
- Chlorpropamide
- Amphotericin

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DRUG-INDUCED IMMUNE **HEMOLYTIC ANEMIA**

Hapten-Induced Type

- Indirect Coombs can also be positive during rx and for many weeks after discontinuation of penicillin despite that hemolysis subsides as soon as penicillin is stopped
- Indirect Coombs test should be performed using penicillin-coated RBCs

DRUG-INDUCED ANTIBODIES -PENICILLIN-LIKE MECHANISM

DRUG-INDUCED IMMUNE

HEMOLYTIC ANEMIA

- The drug binds to the RBC membrane and

- Caused by large IV doses of penicillin or

- Direct Coombs is positive for IgG during

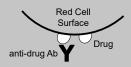
- Occurs 7-14 days after initiation of penicillin

Hapten-Induced Type

becomes the target antigen

penicillin-like antibiotics

penicillin administration



Key concept:

Drug Binding to Red cells is the critical step in targeting antibody to Red cell Membrane

Examples

- Penicillin and semisynthetic penicillins
- Cephalosporins Tetracycline, Streptomycin
- Tolbutamide

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DRUG-INDUCED IMMUNE HEMOLYTIC ANEMIA

- · Ribavirin therapy of hepatitis C has been associated with hemolysis
- · Hemolysis can be managed with erythropoietin, allowing continuation of treatment

Treatment

- Removal of drug

- Treatment of underlying condition
- Folic acid

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AIHA/ Warm-Reactive Treatment

· First line

- Prednisone: 1-2 mg/kg Q8-12 hours for 72 hours then decrease to 1-2 mg/kg/d
 - 60%-70% sustained response (20% CR)
 - Relapse occurs in 50% of responders either during steroid tapering or after discontinuation
- IVIG: 1g/kg/d for 2 days

AIHA/ Warm-Reactive Treatment

Second line

- Rituximab: 375 mg/m2 weekly for 4 weeks
- Splenectomy
 - 30%-40% of patients will be resistant to steroid rx or require excessive doses and/or prolonged administration
 - Splenectomy: 50%-60% response
 - Steroids in lower doses may be needed post splenectomy in 50% of cases

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AIHA/Warm-Reactive Treatment

Second line

– MMF: 600 mg/m2 BID– Sirolimus: 2 mg/m2 daily

 Danazol: 600-800 mg/d in 3-4 divided doses followed by maintenance 200-600 mg/d

AIHA/Warm-Reactive Treatment

Third Line

- Azathioprine: 1-2 mg/kg/d

- 6-Mercaptopurine: 50-75 mg/m2/d

 Cyclophosphamide: 1-2 mg/kg/d PO or 300/1000mg/m2 IV every 2-4 weeks

- Cyclosporine: 5mg/kg/d

51 52

AIHA/Warm-Reactive Treatment

Fourth Line

- High Dose Cyclophosphamide: 50 mg/kg/d for 4 consecutive days followed by G-CSF
- Alemtuzumab: 3 mg IV or SC on d1, 10 mg on d2, 30 mg on d3, maintenance 10-30 mg 3 X weekly for up to 12 weeks
- Autologous or allogeneic HSCT

AIHA/ Warm-Reactive Treatment

TRANSFUSION RX

- Allo-reactive Abs are present in 32% of patients with AIHA
- Allo-reactive Abs are directed against Rh, Kell, Kidd, and Duffy
- Undetected allo-reactive Abs, rather that auto- Abs, may cause increased hemolysis after transfusion

AIHA/ Warm-Reactive Treatment

TRANSFUSION RX

- Usual cross-matching is difficult because the Ab is a panagglutinin reacting with all normal donor cells
- Unlikely to find fully compatible blood

AIHA/ Warm-Reactive Treatment

TRANSFUSION RX

- Allo-reactive Abs are detected by testing the patient's serum against a panel of RBCs of known phenotypes
- The problem is that the auto Ab in the patient's serum will generally react with all RBC tested, masking the presence of an allo Ab

55 56

AIHA/ Warm-Reactive

• TRANSFUSION RX

- No patients should die because of inability to find blood for transfusion
- Most patients tolerate serologically incompatible blood

AIHA/ Warm-Reactive

• TRANSFUSION RX

- The decision to transfuse should depend on the patient's clinical status
- With appropriate precautions, survival of transfused RBC is as good as survival of the patient's own RBC
- Transfusion causes temporary benefit

57 58

Posttransfusion Hemoglobinemia & Hemoglobinuria

Result from increase in the total RBC mass available for destruction and NOT secondary to increased rate of hemolysis or alloAb- induced hemolysis

Posttransfusion Hemogloninemia & Hemoglobinuria

- Excessive & rapid transfusion of RBC should be avoided
- Transfusion of modest volumes of RBC just sufficient to maintain a tolerable Hgb/Ht

Cold Agglutinin disease (CAD)

Primary CAD

- clonal B cell lymphoproliferative disorder called primary cold agglutinin-associated lymphoproliferative disease
- Distinct from lymphoplasmacytic lymphoma, marginal zone lymphoma, and other low grade lymhoproliferative disorders

Secondary CAD

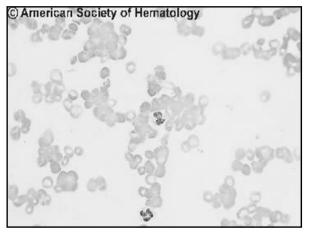
- Aggressive lymphomas
- Hodgkin's lymphoma
- Carcinomas
- Infectious conditions

61 62

Cold Agglutinin Disease

- Warming leads to quick disagglutination
- Low titers (<1:32) of this Ab can be found in normal serum with no clinical consequences
- In patients with disease, Ab titer is >1:1,000 at 4°C and 1:16 at 37°C
- · Hemolysis is intravascular

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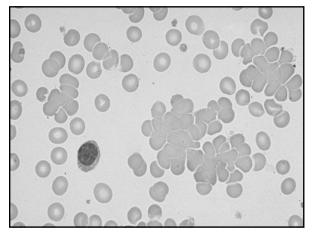


Cold Agglutinin Disease

- Caused by IgM complement-fixing Ab
- · Most common cold agglutinins are anti-l
- Ab binds to RBCs and causes agglutination at low temperatures (4°C)

Cold Agglutinin Disease

- Direct antiglobulin test detects C₃ since the bound IgM is released at 37°C
- Only red cells coated with C₃b are removed from the circulation by macrophages in liver
- Red cells coated with C₃d are not removed from the circulation and are protected from complement-mediated hemolysis because C₃d limits the sites available for C₃b activation



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Cold Agglutinin Disease

MYCOPLASMA PNEUMONIA

- Cold agglutinins are commonly detected
- Only a very small number of patients develop hemolysis
- The Ab is IgM & is directed against the I antigen
- Hemolysis usually occurs 5 to 10 days after recovery from infection and is selflimited

Cold Agglutinin Disease

MYCOPLASMA PNEUMONIA

- Cold agglutinin titers are usually > 1:256
- Direct Coombs (+) for complement only

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Cold Agglutinin Disease

• INFECTIOUS MONONUCLEOSIS

- Hemolysis is rare
- The Ab is an IgM directed against the i antigen expressed on fetal and not adult RBCs
- *i* antigen is also expressed on red cells of some patients with infectious mononucleosis
- Hemolysis results from cold agglutination of red cells or complement fixation by IgM

Cold Agglutinin Disease

CHRONIC COLD AGGLUTININ SYNDROME

• Age >60

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- Due to a "benign" monoclonal IgM
- Antibody is anti-I bearing kappa light chains
- · Indolent for many years
- In 5-10% of cases, malignant clone arises expressing the cold agglutinin

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Cold Agglutinin Disease

LYMPHOPROLIFERATIVE DISORDERS

- In pts with cold-reactive hemolysis, trisomy 3 has been associated with progression to a lymphoproliferative disorder
- Antibody is anti-I with indolent lymphomas
- Antibody is anti-i with high grade lymphomas
- Detection of anti-i Ab in the absence of a viral infection, may indicate the presence of a lymphoma

Secondary Cold Agglutinin Disease Treatment

Treat the Underlying disorder

Cold Agglutinin Disease

TREATMENT

- · Avoid cold exposure
- · Folic acid
- Corticosteroids: not effective except in IgG cold-reactive Ab, or if a concurrent warm reactive IgG is present
- · Splenectomy: not indicated

Primary Cold Agglutinin Disease

TREATMENT

- Rituximab: first line treatment with median duration of remission of 1 year in 50% of cases.
- Alpha-interferon: may play a role . Beneficial in combination with Rituximab
- Fludarabine + Rituximab : 75% response (median duration 5 years). More toxicity
- Bendamustine+ Rituximab & Bortezomib-based therapy
- · Plasmapheresis: Effective, but of temporary value
- · IV Ig: Not indicated

73 74

Cold Agglutinin Disease

Special Precautions

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- All patients needing hypothermic surgery should be tested for cold agglutinins
- Use of cooling blankets to reduce fever may worsen hemolysis and cause peripheral gangrene
- Washed RBCs transfusion should be used, since worsening hemolysis can occur if a complement-depleted patient receives plasmacontaining blood products
- Use of warm intravenous solutions

IMMUNE HEMOLYTIC ANEMIAS Cold-Reactive

Paroxysmal Cold Hemoglobinuria (PCH)

- Rare disorder

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- Used to be seen in association with tertiary syphilis
- In children, it follows a viral infection. Ab appears 7-10 days after onset of illness and persists for 6-12 weeks
- May follow other infections (Mycoplasma & Klebsiella pneumonias) and vaccination for measles

IMMUNE HEMOLYTIC ANEMIAS Cold-Reactive

Paroxysmal Cold Hemoglobinuria (PCH)

- Antibody is a polyclonal cold reactive IgG (Donath-Landsteiner) directed against the P antigen
- P antigen is also the receptor for *parvovirus* B19 suggesting a relationship
- Ab does not cause much agglutination but can fix complement
- Red cell destruction is by complement-mediated lysis upon cold exposure

IMMUNE HEMOLYTIC ANEMIAS
Cold-Reactive

- Paroxysmal Cold Hemoglobinuria (PCH)
 - Adult form is usually chronic lasting several years
 - May occur in association with other immune disorders
 - Rarely associated with lymphoproliferative disorders

IMMUNE HEMOLYTIC ANEMIAS Cold-Reactive

- Paroxysmal Cold Hemoglobinuria(PCH)
 - Treatment:
 - Usually self-limited in children
 - · Maintain warm environment
 - Prednisone, cyclophosphamide, azathioprine in chronic PCH
 - Splenectomy & IVIG of no value
 - Rituximab has been used

TRANSFUSION RX IN COLD-REACTIVE AIHA

- Compatibility testing should be performed at 37 C since autoAb does not react at this temperature but an alloAb, if present, will react
- Transfusion of warm blood is advisable despite lack of proven efficacy of this approach

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Secondary AIHA					
Underlying disease or condition	Prevalence of AIHA, %	WAIHA	CAIHA		
CLL	2.3 - 4.3	87%	7%		
NHL (except CLL)	2.6	More common	Less common		
IgM gammopathy	1.1	No	All		
Hodgkin lymphoma	0.19 - 1.7	Almost all	Rare		
Solid tumors	Very rare	2/3	1/3		
Ovarian dermoid cyst	Very rare	All	No		
SLE	6.1	Almost all	Rare		
Ulcerative colitis	1.7	All	No		
CVID	5.5	All	No		
ALPD	50	All	No		
After allogeneic SCT	4.4	Yes	Yes		
After organ transplantation	5.6 (pancreas)	Yes	No		
Drug-induced in CLL	2.9 - 10.5	Almost all	Rare		
Interferon α	Incidence: 11.5/100,000 patient- years	All	0		

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Treatment of WAIHA and CAIHA				
Disease or condition	First-line	Second-line	Third-line	
Primary AIHA	Steroids	Splenectomy rituximab	Azathioprine, MMF, cyclosporin, cyclophosphamide	
B- and T-cell NHL	Steroids	Chemotherapy ± rituximab (splenectomy in SMZL)		
Hodgkin lymphoma	Steroids	Chemotherapy (radiotherapy)		
Solid tumors	Steroids, surgery			
Ovarian dermoid cyst	Oophorectomy			
SLE	Steroids	Azathioprine	MMF	
Ulcerative colitis	Steroids	Azathioprine		
CVID	Steroids + IgG	Splenectomy		
ALPD	Steroids	MMF	Sirolimus	
Allogeneic SCT	Steroids	Rituximab	Splenectomy, T-cell infusion	
Organ transplantation (pancreas)	Discontinuation of immune suppression, steroids	Splenectomy		
Interferon a	Withdrawal	Steroids		
Primary CAD	Protection from cold exposure	Rituximab, chlorambucil		
Paroxysmal cold hemoglobinuria	Supportive treatment	Rituximab		
	NHL, non-Hodgkin lymphoma; SMZL, sp., common variable immunodeficiency; A			

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Condition	First-line	Second-line
Untreated drug-related AIHA, untreated AIHA in early stage CLL	Steroids	RCD
Untreated AIHA in active CLL	Steroids + chlorambucil	RCD; R-CVP
Steroid-refractory AIHA, non- progressive CLL	Rituximab; cyclosporin; splenectomy	RCD; R-CVP
Refractory AIHA, advanced or progressive CLL	Alemtuzumab	

c Receptor mediated Yes	No
Complement mediated Yes	Yes
Cold-reaction dependent No	Yes

MECHANISMS OF ACTION OF CORTICOSTEROIDS

- Reduce production of IgG
- May down-regulate Fc receptors (in high doses)
- Do not affect IgM production

MMUNE HEMOL THEF		N⊏IVII <i>P</i>
	IgG	IgM
Reduction in Ab production		
Corticosteroids	Yes	No
Cytotoxic agents	Yes	Yes
Reduction in available Ab		
Plasmapheresis	No	Yes
Reduction in destruction		
Splenectomy	Yes	No
IV Ig	Yes	No
Warm environment	No	Yes

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Characteristics of Anti-RBC
Antibodies

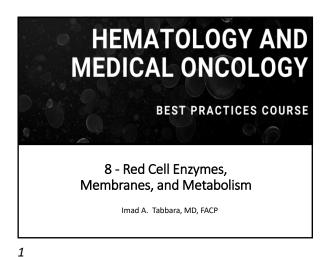
	Idiopathic AIHA	Chronic cold agglutinin disease	Mycoplasma- associated cold agglutinin disease	EBV- associated cold agglutinin disease	PCH
Class of Antibody	IgG most common	IgM	IgM	IgM	IgG
Temp. for Reactivity	Warm	Cold	Cold	Cold	Cold
Red Cell Antigen Specificity	Rh-Ag	I-Ag	I-Ag	i-Ag	P-Ag
Coombs Test for IgG	+ or rarely -	-	-	=	-
Coombs Test for C ₃	+ or -	Usually +	+	+	+

Intravascular vs. Extravascular Hemolysis				
	Intravascular Hemolysis	Extravascular Hemolysis		
Pathophysiology	Complement-mediated lysis	Fc or C ₃ b receptor mediated phagocytosis		
Clinical symptoms of acute hemolysis (fever, backache)	YES	NO		
Spherocytes	(-)	(+)		
Bilirubin	Indirect > Direct	Direct > > Indirect		
LDH	(+)	(+)/(-)		
Coombs Test	(+)C3, (+/-) IgG	(+/-) IgG, (+)C3		
Hemoglobinuria and hemosiderinuria	YES	NO		
Clincial Association	D-L Hemolysin Cold Agglutinins Drug-related hemolysis: Quinine	Warm reactive IgG-mediated AIHA, drug-mediated immune hemolysis		

RBC Enzymes, Membranes, and Metabolism

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August 13, 2020



Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

HEMOLYTIC ANEMIA

- HEMOLYSIS: Premature or accelerated destruction of RBCs
- RBC survival: less than 100 days
- O 2 main causes:
 - O Intrinsic RBC defects (inherited)
 - Extra-corpuscular causes (acquired)

CLASSIFICATION OF HEMOLYTIC ANEMIAS

- Hereditary Hemolytic Disorders
 - RBC enzymes defects
 - RBC membrane defect
 - Hemoglobinopathies
 - Thalassemias

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CLASSIFICATION OF HEMOLYTIC ANEMIAS

- Acquired Hemolytic Disorders
 - Immune hemolytic anemias
 - Splenomegaly
 - Microangiopathic hemolytic anemia
 - **O**PNH

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- Direct toxic effect (malaria, clostridial infections)
- OSpur cell anemia

ERYTHROCYTE ENZYMOPATHIES

A red cell enzyme defect is a major consideration in the differential diagnosis of inherited, non-spherocytic, Coombs' negative hemolytic anemia

ERYTHROCYTE ENZYMOPATHIES

- Enzymopathies are mostly inherited as autosomal recessive disorders
- Glucose-6-phosphate Dehydrogenase (G6PD) and phosphoglycerate Kinase (PGK) deficiencies are X-linked

ERYTHROCYTE ENZYMOPATHIES

CLINICAL FEATURES

- Family history is essential to determine the mode of inheritance
- Life-long hemolysis suggests inherited disorder
- Exposure to certain drugs or foods, surgeries or infections can lead to episodic hemolysis in some patients

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ERYTHROCYTE ENZYMOPATHIES

CLINICAL FEATURES

 Icteric sclerae and splenomegaly are present in chronic hemolysis and absent in episodic hemolysis.

ERYTHROCYTE ENZYMOPATHIES

LABORATORY EVALUATION

- Diagnosis of enzymopathy is by exclusion
- Peripheral blood smear may be helpful:
 - The RBCs are usually macrocytic and spiculated
 - ◆ Basophilic stippling is seen in pyrimidine 5'-nucleotide (P-5'-N) deficiency
 - Presence of spherocytes, elliptocytes, acanthocytes, or schistocytes suggests that the hemolytic process is not caused by an enzymopathy

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ERYTHROCYTE ENZYMOPATHIES

LABORATORY EVALUATION

- RBCs have increased osmotic fragility
- Increased unconjugated bilirubin, low or absent haptoglobin, increased LDH
- Intravascular hemolysis with hemoglobinemia, hemoglobinuria and hemosiderinuria is rare except in some patients with G-6-PD deficiency

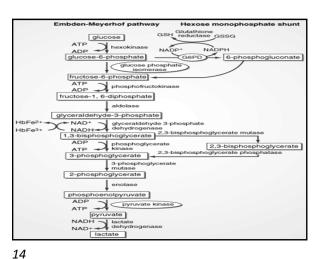
ERYTHROCYTE ENZYMOPATHIES

DIAGNOSTIC TESTS

- Oln acquired hemolysis: a direct antiglobulin (Coombs') test should be performed as initial screening
- Oin congenital hemolysis: osmotic fragility, hemoglobin electrophoresis and screening for enzyme deficiencies (G6PD and pyruvate Kinase) represent the initial evaluation

ERYTHROCYTE ENZYMOPATHIES

- During maturation, the RBC looses its ability for protein synthesis and oxidative phosphorylation
- The mature RBC generates energy through:
 - Anaerobic glycolysis: Embden-Meyerhof (EM) pathway
 - Oxidative glycolysis: Hexose monophosphate (HMP) shunt
 - ONucleotide salvage pathways



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ERYTHROCYTE ENZYMOPATHIES

- Structural & biochemical integrity of the RBC depends on:
 - The normal function of more than 20 enzymes involved in these pathways
 - The availability of five RBC substrates: Glucose, Glutathione, NAD, NAD phosphate & Adenosine diphosphate (ADP)

ERYTHROCYTE ENZYMOPATHIES

- The functions of the products of glucose catabolism include:
 - Maintenance of protein integrity, cellular deformability & RBC shape
 - Preservation of hemoglobin iron in the ferrous form
 - Modulation of the oxygen affinity of Hgb

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EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- Most of RBC adenosine triphosphate (ATP) is synthesized through this pathway.
- ●90% of the RBC glucose is converted to lactate via this pathway.
- ATP is necessary for the ATPase-linked Na+/K+ & Ca++ membrane pumps essential for cation homeostasis and RBC deformabilitiy.

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- Enzymopathy affecting this pathway leads to ATP-deficient RBCs which are rigid and removed by the spleen
- ◆ Leads to Congenital, non-spherocytic hemolytic anemia

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

O Hexokinase (HK) deficiency

- OFirst enzyme in the glycolytic pathway
- OLeast active of the glycolytic enzymes
- Activity decreases as RBC matures
- Decreased HK activity can be quantitative or qualitative
- Activity increases with reticulocytosis

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

O Hexokinase (HK) deficiency

- Activity should be measured taking into consideration the extent of reticulocytosis
- Hemolytic anemia can vary from mild to severe
- RBC morphology is normal with the presence of reticulocytosis, polychromatophilia and few spherocytes

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EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

O Hexokinase (HK) deficiency

- OAcquired HK deficiency occurs in Wilson's disease
- Elevated copper levels inhibit HK intermittently leading to brisk intravascular hemolysis

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

O Phosphoglycerate Kinase (PGK) Deficiency

- The only X-linked enzymopathy in the EM pathway
 - PGK deficient males develop normally until early childhood
 - Neuromuscular manifestations including seizures, spasticity & mental retardation
 - Cerebellar tumors within the first 4 to 5 years of age

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EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

O Phosphofructokinase (PFK) Deficiency

- PFK is a tetrameric enzyme
- Composed of 3 basic subunits; M (muscle), L (granulocyte), F (fibroblast, platelet)
- RBC PFK consists of the L4 tetramer

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

O PFK Deficiency

- O First described as a muscle glycogen storage disease
- Hemolysis is usually mild and well compensated
- Fresh blood sample for PFK assay

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

O Pyruvate Kinase (PK) Deficiency

- O Most common enzymopathy in the EM pathway
- World-wide, multi-racial distribution (more common in northern European)
- Clinical expression ranges from severe hemolytic anemia in neonates to a fully compensated anemia
- Anemia or jaundice or both are recognized in infancy or early childhood

EMBDEN- MEYERHOF (GLYCOLYTIC) PATHWAY

O Pyruvate Kinase (PK) deficiency

- RBC osmotic fragility is normal
- Small dense crenated cells (echinocytes) on blood smear
- Quantitative measurement of the enzyme can be performed by reference laboratories

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EMBDEM-MEYERHOF (GLYCOLYTIC) PATHWAY

O Pyruvate Kinase Deficiency

- Folic acid supplementation
- Splenectomy for pts with poor quality of life, chronic transfusions, need for cholecystectomy and severe anemia
- O Hgb level improves in most pts
- Postoperative thromboembolic events

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

O Glucosephosphate Isomerase (GPI) Deficiency

- Third most common enzymopathy after G6PD and PK deficiencies
- © Encoded by a single gene in all body cells However, hemolytic anemia may be the only clinical manifestation since only mature RBCs are unable to synthesize the enzyme in an accelerated fashion

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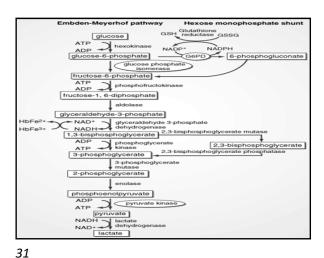
EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

O GPI Deficiency

- Peripheral smear shows poikilocytosis, anisocytosis, marked polychromatophilia and reticulocytosis.
- In rare situations myopathy, ataxia and mental retardation have been encountered in conjunction with hemolytic anemia.

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- Triosephosphate Isomerase (TPI) Deficiency
 - OTPI present in all tissues.
 - TPI deficiency: autosomal recessive inheritance.
 - Progressive multi-system syndrome:
 - O Hemolytic anemia
 - Hyperbilirubinemia
 - Neurologic abnormalities: spasticity, paraesthesia weakness, mental retardation
 - O cardiac arrhythmia
 - O Systemic infections



HEXOSE MONOPHOSPHATE SHUNT

- Major source of reduced nicotinamide-adenine dinucleotide (NADH) in RBCs
- O Reduced NADH protects Hgb and other intracellular & membrane proteins from oxidant
- 10% of RBC glucose is metabolized via this pathway

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HEXOSE MONOPHOSPHATE SHUNT

 Normal RBCs can increase the amount of glucose metabolized through this pathway upon exposure to certain oxidants. This leads to production of reduced glutathione to protect the

HEXOSE MONOPHOSPHATE SHUNT

Enzymopathy of the shunt renders the RBCs incapable of generating reduced glutathione causing precipitation of hemoglobin and Heinz bodies formation.

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HEXOSE MONOPHOSPHATE SHUNT

OGlucose-6-phosphate-Dehydrogenase (G6PD) deficiency

- Most prevalent inborn error of RBC metabolism.
- Affects more than 200 million people world wide.
- Gene located on long arm of the X-chromosome (band X q28).

G6PD DEFICIENCY

- OMany G6PD variants have been detected (>300 have been described but few are clinically significant)
- G6PD-B: is the normal enzyme (99% of whites and 70% of African-Americans)
- OG6PD-A(+): is the normal African-American variant seen in 20% of this population. Has normal activity but increased electrophoretic mobility

G6PD DEFICIENCY

- <u>G6PD-A(-)</u>: in 10% of African-Americans. Has same electrophoretic mobility as G6PD-A(+) but only 5-15% activity
- G6PD Mediterranean variant: in 5% of Arabs, Greeks, Sardinians and Sephardic Jews.

G6PD DEFICIENCY

- OClassified into 5 classes:
 - Class I: chronic hemolysis without precipitating cause
 - Class 2 + 3: Acute hemolysis associated with exposure to oxidant drugs, stress or certain foods (fava bean)
 - OClass 4 + 5: harmless

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G6PD DEFICIENCY

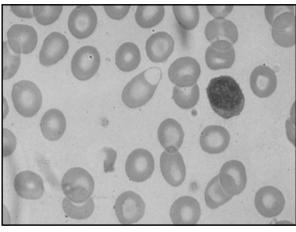
- O Mediterranean variant:
 - the most prevalent class 2 deficiency
 - almost all RBCs are oxidant sensitive and hemolysis may be fatal
- O G6PD A(-)variant:

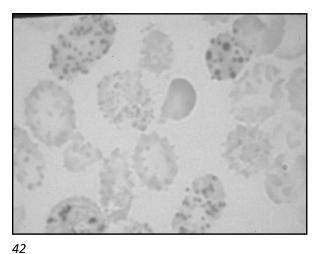
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- enzyme's half-life is 50% normal
- after a hemolytic episode, a young RBCs have near normal levels of G6PD
- African-Americans with this variant stop hemolyzing even if oxidant exposure continues

G6PD DEFICIENCY

- Neonatal jaundice and acute hemolytic anemia
- O Peak incidence: days 2 and 3
- Jaundice is more pronounced compared to the anemia
- Eccentrocytes or "bite/blister cells" are present
- Diagnostic assays should be performed during a steady state in the G6PD A- deficiency. In the Mediterranean variant the G6PD level can be checked at any time





HEXOSE MONOPHOSPHATE SHUNT

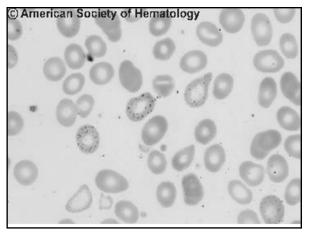
- Phosphogluconate dehydrogenase & Glutathione reductase deficiencies:
 - O Rare
 - Should be considered in cases of oxidant-induced hemolysis & normal G6PD level

DEFECTS OF NUCLEOTIDE METABOLISM

- O Pyrimidine 5'-nucleotide (P5N) deficiency
 - Basophilic stippling on Wright-stained blood smear
 - OLead poisoning inhibits this enzyme.

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DEFECTS OF NUCLEOTIDE METABOLISM

- <u>Hyperactivity of Adenosine Deaminase</u>
 (AD)
 - AD catalyzes deamination of adenosine to incsine
 - The excessive deaminase activity prevents normal salvage of adenosine and leads to ATP depletion and hemolysis
 - Autosomal dominant inheritence

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Red Cell Enzyme Deficiencies

Enzyme	Inheritance	Frequency	Anemia	Clinical Features
Hexokinase	Autosomal recessive	Rare	Mild to severe	None
Glucose phosphate isomerase	Autosomal recessive	Unusual	Moderate to severe	None
Phosphofructokinae	Autosomal recessive	Rare	Mild	Myopathy
Aldolase	Autosomal recessive	Very rare	Moderate to severe	Mental retardation
Triosephosphate isomerase	Autosomal recessive	Rare	Moderate to severe	Cardiomyopathy and neuopathies
Phosphoglycerate kinase	X-linked	Rare	Mild to severe	None
Pyruvate kinase	Autosomal recessive	Rare	Mild to severe	None

Red Cell Enzyme Deficiencies (Continued)

Enzyme	Inheritance	Frequency	Anemia	Clinical Features
Glucose-6-phosphate dehydrogenase	X-linked	Common in certain ethnic groups	Moderate to severe	None
Glutathione reductase	N/A	Very rare	Mild	None
Glutathione synthetase	Autosomal recessive	Rare	Mild	Mental retardation, 5-oxoprolinuria
Pyrimidine 5'- nucleotidase	Autosomal recessive	Rare	Mild	?Mental retardation
Adenosine deaminase (excess)	Autosomal recessive	Rare	Mild	None

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ERYTHROCYTE MEMBRANE DEFECTS

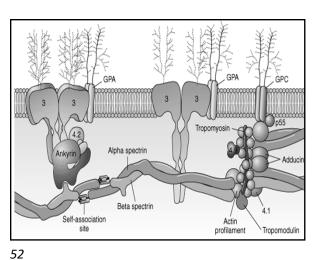
RBC MEMBRANE DEFECTS

- RBC membrane consists of a phospholipidcholesterol lipid bilayer intercalated by membrane proteins, band 3 (anion transport channel) & glycophorins
- This layer is stabilized by attachment to a membrane skeleton
- Skeleton proteins include: spectrin (75%), actin, ankyrin, protein 4.1 & adducin

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RBC MEMBRANE DEFECTS

 Defects in cytoskeletal proteins lead to abnormal shape and flexibility of RBCs causing hemolysis



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RBC MEMBRANE DEFECTS

- Spherocytes and Stomatocytes
 - Oreduced surface-to-volume ratio
 - tolerate less osmotic swelling than normal cells before they lyse
- Target cells and dehydrated RBCs:
 - Oincreased surface-to-volume ratio
 - Oosmotically resistant

HEREDITARY SPHEROCYTOSIS

- OInherited hemolytic anemia
- OMore common in Northern Europeans (1 in 2000)
- ●75% of the cases are Autosomal Dominant
- 25% are Autosomal Recessive or secondary to acquired mutations

HEREDITARY SPHEROCYTOSIS Molecular Defects

- In dominant HS, ankyrin deficiency or defect is most common
- Both ankyrin & spectrin deficiency in 30-45% of cases
- O Spectrin deficiency alone in 30%
- OBand 3 mutations alone in 20%

HEREDITARY SPHEROCYTOSIS Molecular Defects

- Mutations of the B spectrin gene are infrequent in autosomal dominant HS
- Alpha spectrin gene abnormalities identified only in autosomal recessive HS
- Mutations of protein 4.2 gene in Japanese patients with autosomal recessive HS

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Hereditary Spherocytosis Pathophysiology

- Aberrant interaction between the lipid bilayer and the skeleton
- Spectrin loss is caused by a defect in one of the membrane proteins involved in the attachment of spectrin to the membrane rather than a primary defect in the spectrin molecule

Hereditary Spherocytosis Pathophysiology

- Lipid bilayer is destabilized, with associated loss of membrane lipid and surface area leading to a spherocyte
- The reduced deformability of spherocytes leads to their retention and premature destruction in the spleen

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CLINICAL FEATURES OF HS

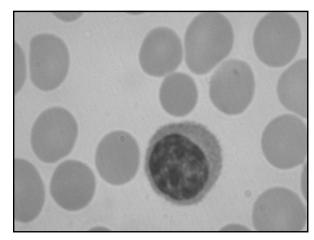
- OAnemia, Jaundice, Splenomegaly
- OMay present at any age
- O Severe in Neonates
- Partially compensated hemolysis after neonatal period

CLINICAL FEATURES OF HS

• Severe hemolysis and anemia can be precipitated by illnesses that cause splenic hypertrophy (infectious mononucleosis) and by long term intensive physical activity (increased splenic blood flow)

DIAGNOSIS OF HS

- Spherocytosis and Reticulocytosis
- Hyperbilirubinemia (increased indirect bilirubin) in 50%-60% of cases
- O Negative direct antiglobulin test
- OHS red cells have high MCHC because of cellular dehydration(MCHC ≥ 36 in 50% of HS patients)



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DIAGNOSIS of HS

O EMA (eosin-5-maleimide) binding

- © EMA is an eosin-based fluorescent dye that binds to RBC membrane proteins, especially band 3 and RH-related proteins. EMA fluorescence in HS is 2/3 of controls
- High sensitivity and specificity (93- 99 %)
- Rapid turnaround time (2 hours)
- False negative in mild HS
- Positive in hereditary pyropoikilocytosis and Southeast Asian Ovalocytosis.

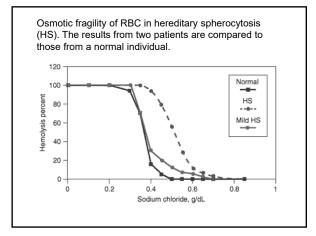
Diagnosis of HS

O Osmotic Fragility

- Increased sensitivity of spherocytes to hemolysis due to reduced surface area to volume ratio
- RBCs are incubated in hypotonic buffered salt solutions of various osmolarities, and the fraction of hemoglobin released due to hemolysis is measured
- Low sensitivity and specificity mainly in newborns
- O Positive test in 2/3 of patients with HS
- Incubation of RBCs for 24 hours prior to testing may accentuate osmotic fragility and improve diagnostic yield
- Positive in immune hemolytic anemia, hemolytic transfusion reactions, G6PD deficiency and unstable hemoglobin variants

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COMPLICATIONS OF HS

• Hyperhemolytic crises:

- Most common
- o associated with infections
- Accelerated hemolysis & splenic enlargement

O Aplastic crises:

• Rare, severe, caused by parvovirus B19. Lasting immunity after 1st infection

O Cholelithiasis:

- Increased incidence (55%-75%) after 5th decade.
- 50% are radioopaque. Ultrasonography is most reliable

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COMPLICATIONS OF HS

- Gout, indolent ankle ulcers, chronic erythematous dermatitis on the legs, extramedullary tumors in the thorax
- Distal renal tubular acidosis in HS patients with band 3 mutation (anion channel protein)
- Spinocerebellar degeneration and familial myocardiopathy have been described

TREATMENT OF HS

- Splenectomy
 - Ocorrects anemia but not the RBC defect
- Relapses can occur due to hypertrophy of accessory spleens
- Benefits of splenectomy to be weighed against the risks of post splenectomy infections

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TREATMENT OF HS

- All splenectomized patients should receive vaccination with pneumococcal, meningococcal and H-influenza B vaccines, several weeks prior to splenectomy
- Prophylactic Pen VK or equivalent post splenectomy in pediatric patients
- Folic acid therapy should be instituted at diagnosis

HEREDITARY ELLIPTOCYTOSIS

- OThree subtypes:
 - O Common HE
 - O Spherocytic HE
 - O Southeast Asia Ovalocytosis

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HEREDITARY ELLIPTOCYTOSIS

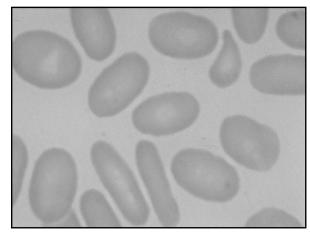
- O Heterogeneous disease
- Autosomal dominant
- Only 10%-15% have significant hemolysis
- No anemia, splenomegaly or reticulocytosis
- Osmotic fragility is normal or slightly resistant

HEREDITARY ELLIPTOCYTOSIS

- Bioconcave elliptocytes and in severe cases rod-shaped cells, poikilocytes and fragments
- Caused by defects in the interactions of red cell cytoskeletal proteins
- Spectrin abnormalities are the most common

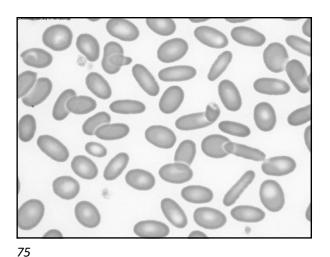
HEREDITARY ELLIPTOCYTOSIS

Splenectomy corrects the hemolysis but not the red cell abnormality. It should not be performed before the 3rd year of life



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SPHEROCYTIC ELLIPTOCYTOSIS

- ORare, autosomal dominant disorder
- Features of HS and HE
- All patients reported are from Europe
- OMild to moderate hemolytic anemia
- Round elliptocytes, and occasional spherocytes

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SPHEROCYTIC ELLIPTOCYTOSIS

- Poikilocytes and red cell fragments are uncommon
- O Positive osmotic fragility test
- O Splenectomy is curative

SOUTHEAST ASIAN OVALOCYTOSIS

- Autosomal dominant disorder
- Prevalent in Melanesia, Indonesia, Malaysia and the Philippines
- Associated with band 3 mutation
- Heterozygotes have rounded elliptocytes, some with a transverse bar that divides the central clear space (spoon-shaped)
- Elliptocytes are rigid and resist invasion by malarial parasites

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SOUTHEAST ASIAN OVALOCYTOSIS

- Rigidity appears to be caused by microaggregation of the mutant protein within the lipid bilayer
- Red cells circulate freely
- O Mild hemolysis and no anemia
- O Homozygous state is lethal

Disorders of Erythrocyte Hydration

- Heterogeneous disorders with abnormalities of RBC solute content
- O Classification:
 - Primary due to intrinsic defects of RBC hydration
 - Secondary due to other intrinsic defects of RBC that also influence RBC hydration such as dehydrated RBC in sickle cell disease
- O Primary hydration defects include:
 - Hereditary xerocytosis syndromes (dehydrated phenotype)
 - O Hereditary hydrocytosis syndromes (overhydrated phenotype)
 - O And intermediate syndromes

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Hereditary Xerocytosis

- O Most common erythrocyte hydration disorders
- Autosomal dominant
- Passive loss of intracellular K+ in excess of accumulation of intracellular Na+ leading to obligate water loss to maintain osmotic balance
- HX erythrocyte is rigid with decreased deformability leading to a shortened life span
- Missense mutations in PIEZO1 in most HX patients
- Mild to moderate, well compensated hemolytic anemia
- Splenomegaly uncommon
- Pseudohyperkalemia
- Iron overload, unrelated to chronic transfusion, requiring

Hereditary Xerocytosis

- O Increased reticulocytosis than expected for degree of anemia
- Few target cells and stomatocytes
- Elevated MCHC & MCV
- Osmotic fragility is decreased (increased osmotic resistance) in contrast to hereditary spherocytosis
- O No specific treatment is needed or available
- Splenectomy of little or no benefit associated with life-threatening thrombotic episodes

81 82

Hereditary Hydrocytosis (Stomatocytosis)

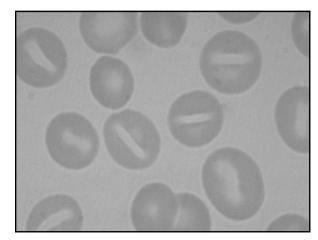
- Autosomal dominant disorder
- The red cell defect consists of a sodium leak, leading to increased intracellular sodium and water content and mild decrease in intracellular potassium
- The RBC is swollen (over hydrated), the MCHC is decreased and the MCV is increased

Hereditary Hydrocytosis (Stomatocytosis)

- Most patients have hemolytic anemia and splenomegaly
- Splenectomy decreases the hemolytic process but does not correct it completely
- Splenectomy is associated with increased incidence of thrombotic events

HEREDITARY STOMATCYTOSIS

O Stomatocytes are more commonly seen as an acquired condition without permeability defect or hemolysis in patients with liver disease or excessive alcohol abuse



86

85

Rh Deficiency Syndrome

- Absent (Rh _{null}) or markedly reduced (Rh_{mod}) expression of Rh antigen
- Associated with mild to moderate hemolytic anemia
- Autosomal recessive inheritance of either a suppressor gene unrelated to the Rh locus or a silent allele at the locus itself

Rh Deficiency Syndrome

- Stomatocytes or rarely spherocytes
- Increased osmotic fragility
- Splenectomy improves hemolysis, but is associated with thrombotic events

87

88

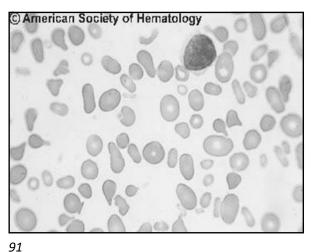
HEREDITARY PYROPOIKILOCYTOSIS

- O Rare autosomal recessive disorder
- Severe hemolysis, bizarre poikilocytosis and red cell fragmentation
- Markedly low MCV (50-60 fl)
- Marked defect in Spectrin self-association
- Heat-sensitive RBCs that fragment when heated at 45° C, secondary to mutant spectrin

HEREDITARY PYROPOIKILOCYTOSIS

- More severe variants feature Spectrin deficiency (20%-40%) and spherocytosis
- Splenectomy partially corrects the hemolysis. RBC morphology and heat sensitivity are not affected
- Splenectomy should not be performed before the 3rd year of life

89



ACANTHOCYTOSIS

- Acanthocytes or spur cells are RBCs with multiple irregular projections (vary in length, width and surface distribution
- Seen in severe liver disease (end-stage alcoholic cirrhosis-Zieve syndrome), cardiac cirrhosis, Wilson's disease, fulminant hepatitis & metastatic liver disease

92

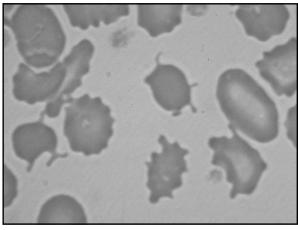
ACANTHOCYTOSIS

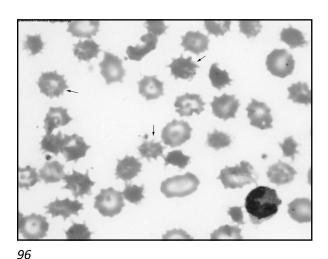
- Abetalipoproteinemia
 - Ocongenital absence of apolipoprotein-b in plasma
 - OSteatorrhea in the first month of life
 - OMarked increase in membrane sphingomyelin
 - Autosomal recessive disorder
 - ORetinitis pigmentosa, ataxia, intention tremors & death by 2nd or 3rd decade

ACANTHOCYTOSIS

- O Seen also in patients with the McLeod phenotype
- In this condition, red cells have reduced surface Kell antigen because of absence of Kx protein needed for its expression
- Kx protein encoded by the X chromosome
- Males have mild compensated hemolysis & females are identified by flow cytometric analysis of Kell antigen expression

93 94

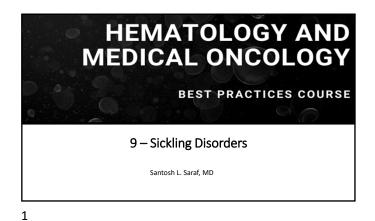




Sickling Disorders

Santosh Saraf, MD

August 13, 2020

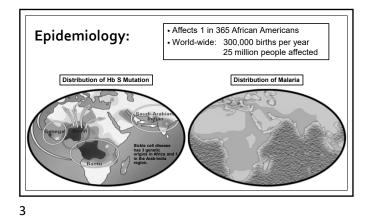


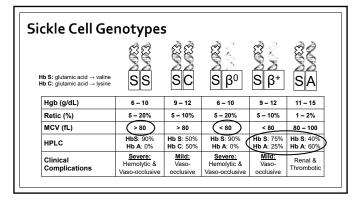
Disclosures

Disclosures of Financial Relationships with Relevant

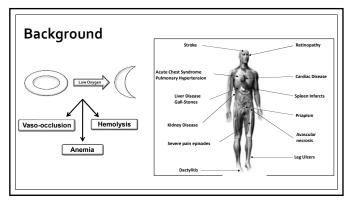
- Research Support Pfizer, Novartis, Global blood Therapeutics
- Consulting Novartis
- Advisory Board/Consulting Global blood Therapeutics

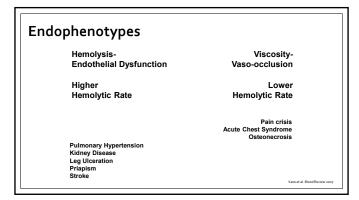
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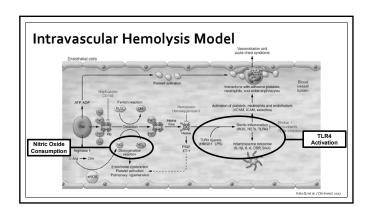


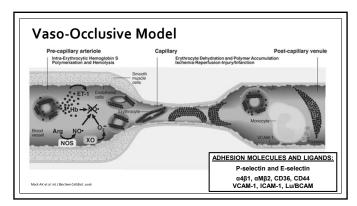


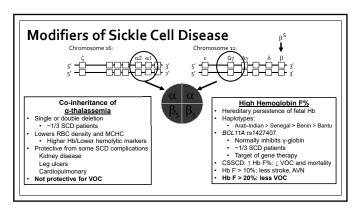
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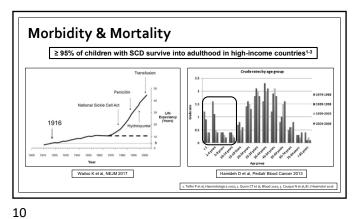










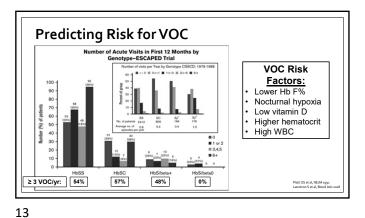


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ledian Survival	Contemporary estimates (high-income countries 38 – 67 years in Hb SS ¹⁻⁴
Cooperative Study of Sickle Cell Disease (CSSCD) 1.0 0.9 8 0.8 0.07 0.06 0.05 0.05 0.05 0.05 0.06 0.06 0.05 0.05	The second section of the section of th

	CSSCD1	MSH ²	USC ³	NIH ⁴	Brazil ⁵	UK ⁶	Meta- Analysis
Frequent VOC		Х		Х	Х	Х	
Acute Chest Syndrome	Х	Х	X		х		
Stroke			Х		Х		
High TRJV				Х			Х
Kidney Disease	Х		Х	Х		Х	

Santosh Saraf, MD



	of Anemia	
Etiology	Diagnostic Clues	Therapy
Aplastic Crisis	Very low Retic count Parvovirus IgM or PCR	IVIG Simple transfusion
Hyperhemolysis 1) Delayed hemolytic transfusion reaction 2) Nonimmune mediated	Transfusion 2 – 14 days prior, positive direct antiglobulin test, alloantibodies, low Retic count Elevated hemolysis markers (Indirect bilirubin, AST, Retic)	Steroids, IVIG Exchange transfusion
Hepatic Sequestration	Hepatomegaly Elevated ALT	Simple vs. Exchange transfusion
Splenic Sequestration	Splenomegaly Hb SC or Sβ*-thalassemia Thrombocytopenia	Simple vs. Exchange transfusion

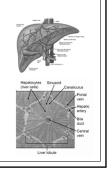
Sickle Hepatopathy

Acute hepatic crisis

- Vaso-occlusion in hepatic vasculature
- ALT ~100-300s; Normal direct bilirubin & Pt/Ptt
- Self-limited

Intrahepatic cholestasis

- Advanced hepatocyte ischemia
- ↑ Direct bilirubin & ≥ 50% of total bilirubin
- Abnormal Pt/Ptt
- · Renal dysfunction
- ~90% fatality without exchange transfusion



Acute Chest Syndrome

Definition: Fever (T ≥ 38.5°C) **New Lung Infiltrate** Hypoxia (↓O₂sat >2%)

Chest Pain

Cough, Wheezing, Tachypnea

Epidemiology:1,2

14

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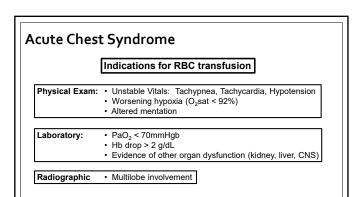
- 2nd most common cause of hospitalization
- · Leading cause of death
- $\frac{1}{2}$ of patients initially admitted for VOC
- Symptoms arise ~2.5 days after admission

Platt OS et al. NEJM 1994
 Castro O et al. Blood 199

15

Acute Chest Syndrome National Acute Chest Syndrome Study Group Etiology Proportion Infection 30% Chlamydia pneumoniae Mycoplasma pneumoniae Virus Legionella Other infection Syndrome • 7% • 6% • 1% • 9% Fat Embolism 9% Infarction 16% Unknown 46%

Prevention	Tr	reatment	
Aggressive incentive spirometery 10 breaths q2 hours	Supplemental O ₂	Minimum ≥ 92% Ideal ≥ 95%	
Avoid oversedation or excessive hydration Immunizations: • S. pneumonia • H. influenzae Influenza	Adequate pain control		
	_ Empiric antibiotics	Cephalosporin + macrolide 4th generation quinolone	
	VTE Prophylaxis	Unfractionated Heparin LMWH	
	Bronchodilators	Wheezing or Asthma history	
SCD-specific Therapies: Hydroxyurea	RBC transfusion	Simple – mild/moderate Exchange – severe	



Rapidly Progressive Acute Chest Syndrome

1) Respiratory Compromise

2) Multiorgan Failure: Liver failure (75%)

Acute kidney injury (69%)

CNS/mental status changes (44%)

- •~20% of acute chest syndrome events in adults
- Acute drop in platelets
- 10% decline or < 200k \rightarrow 5 to 7-fold greater risk^{1,2}
- Prompt exchange transfusion therapy

1. Vichinsky E et al, NEJM 2000; 2. Alhandalous C et al, AJH 201

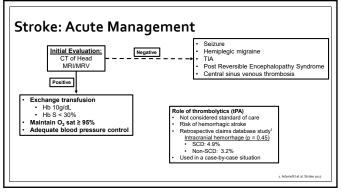
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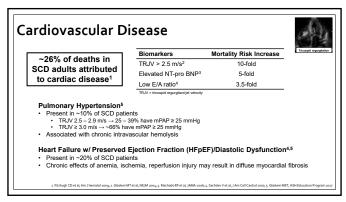
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Acute Chest Syndrome: Summary 50% develop during hospitalization for VOC • Vigilant for hypoxia, worsening hemolysis, drop in platelets Treatment: Supplemental oxygen (goal O₂sat ≥ 95%) Empiric antibiotics (cover atypical pathogens) Common transfusion triggers: Unstable vital signs O₂sat < 92% Drop in Hb > 2g/dL from baseline Multi-lobe infiltrates

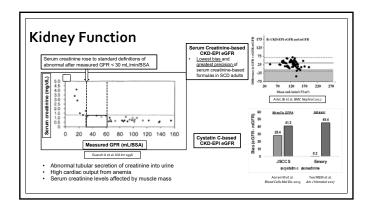
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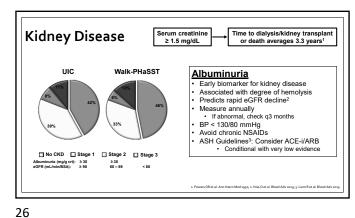


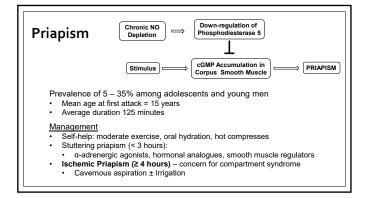


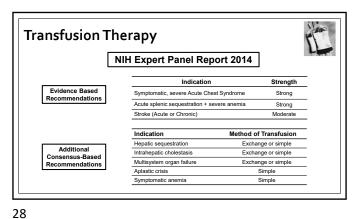
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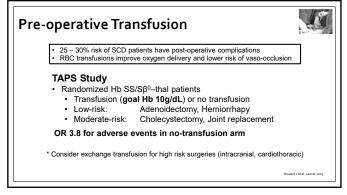


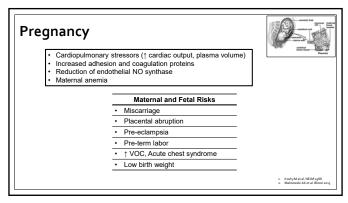






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Pregnancy



Randomized study in Hb SS1

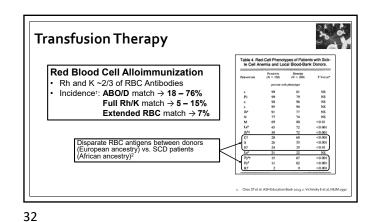
- Emergent (Hb >6 g/dL) vs. Prophylactic transfusion (Hb \sim 10g/dL)
- · No reduction in OB complications or fetal birth weight
- Significant reduction in VOC

Meta-analysis (12 studies/1291 SCD patients)² Prophylactic transfusions:

- Reduced maternal/neonatal mortality
- · Reduced VOC and acute pulmonary events

* Consider transfusions if pregnancy complicated by ↑ VOC, severe anemia, pre-eclampsia3

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Transfusion Therapy



Delayed Hemolytic Transfusion Reaction

- 2 to 14 days post-transfusion
- ~5% of transfused SCD patients experience DHTR¹
- Can lead to Hyperhemolysis + low Retic count

Milder cases:2 Corticosteroids + IVIG Severe cases: Corticosteroids + IVIG

Judicious RBC transfusions (extended-match) Limited data for Rituximab, Erythropoietin

33

Transfusion Therapy

Iron Overload

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- 1 unit pRBC = 200 250mg of iron
- Liver, pancreas, heart vulnerable to iron overload
- Ferritin > 2,500 ng/mL : 78% specificity for liver iron 7 mg/g1 Chelation: Subg/IV: Deferoxamine

Avoid acute simple transfusion > 10 g/dL Hyperviscosity

Oral: Deferasirox or Deferiprone

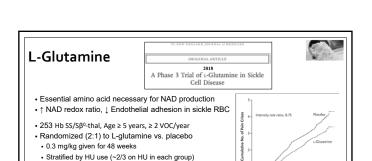
Transfusion Summary

- · Indications for transfusion
- Severe acute chest syndrome, stroke, splenic sequestration
- Pre-operative
- · Pregnancy with complications
- · ABO/Full Rh/Kell minimum typing
- · Delayed hemolytic transfusion reaction
- 2-14 days post-transfusion
- Steroids + IVIG

Hydroxyurea Multicenter Study of Hydroxyurea (MSH) in Sickle Cell Anemia • 299 Hb SS/Sβ⁰-thal adults with ≥ 3 VOC/year Start 15 mg/kg, titrate up 5 mg/kg q12 weeks if no myelosuppression Clinical Complication Placebo p-value VOC Episode 1.0/year 2.4/year < 0.001 Acute Chest Syndrome 25 patients 51 patients < 0.001 Transfusions 336 U 586 U * FDA-Approved for adults in 6/1998, children in 12/2017.

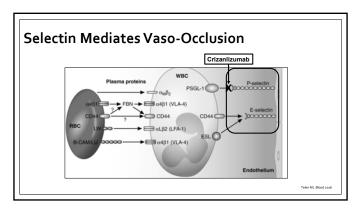
The New England

Journal of Medicine

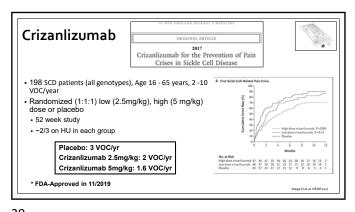


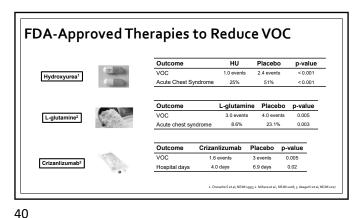
Placebo: 4 VOC/yr L-glutamine: 3 VOC/yr

* FDA-Approved in 7/2017

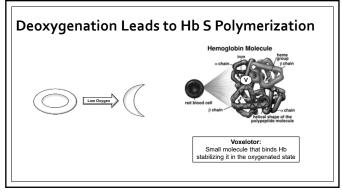


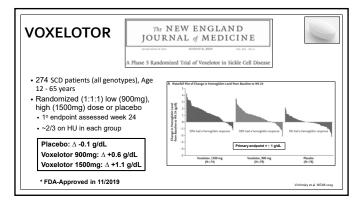
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39 4





Curative Therapies: Allogeneic HSCT*

1,000 SCD recipients from HLA-matched, sibling donors (1986 - 2013)1

• Median age 9 years (range: 1 - 54 years) & 87% myeloablative

• At 5 years: 91% cure rate

15% acute GVHD, 14% chronic GVHD

Nonmyeloablative & SCD adults:² 87 – 92% stable engraftment 0% acute or chronic GVHD

Haploidentical HSCT:2

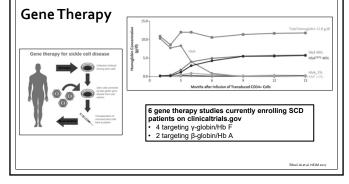
~15% of eligible patients have HLA-matched sibling
 Post-Cy: 57 - 100% stable engraftment
 0 - 25% aGVHD, 0 - 13% cGVHD

* Most common indications include stroke, recurrent VOC or acute chest syndrome despite HU therapy

wiosi confinion indications include stoke, reculterit voc or acute criess syndrome despite no trierapy

. Gudman E et al, Blood assq. a Sard St. et al. J Clin Med assq.

43



Therapy Summary

 $\textbf{Hydroxyurea, L-Glutamine, Crizanlizumab} \rightarrow \textbf{Reduce VOC}$

· L-glutamine & crizanlizumab improve VOC even in those on hydroxyrea

$\textbf{Voxelotor} \rightarrow \textbf{Improves Hgb concentration}$

Curative Approaches

- Allogeneic hematopoietic stem cell transplantation
- Nonmyeloablative approaches well tolerated in adults
- Haploidentical approaches increase donor pool
- Gene therapy
- Uses autologous cells
- Challenges –CD34 dose, transfection efficiency, myeloablative regimen, cost

THANK YOU & QUESTIONS



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46

45

Question 1

A 21 year old man with Hb SS sickle cell disease presents for a vaso-occlusive pain episode. Upon admission, he is started on IV hydration and patient controlled analgesia with adequate pain relief.

on the 2^{nd} day of hospitalization, he develops worsening chest pain, a new pulmonary infiltrate on chest x-ray, and worsening hemolysis (LDH increases 2-fold, Hb drops by 2.4 g/dL from admission values).

His vital signs are as follows: T 38.3, P 85, RR 18, BP 124/80, and O_2 saturation 91%. Which of the following are NOT indicated for his initial management:

- 1. Supplemental O₂
- 2. Continued PCA for pain relief
- 3. Dexamethasone 4mg IV q12 hours
- 4. Red blood cell transfusion
- Initiating antibiotics

Question 1: Acute chest syndrome

- Supplemental O₂: Maintain oxygen at least ≥ 92% (> 95% preferred)
- 2. Adequate pain management is essential
- Dexamethasone: Risk of rebound VOC, questionable benefit in children that are critically ill, but not considered standard management in adults
- 4. Red blood cell transfusion: Simple or exchange could be given
- Initiating antibiotics: Infection is most common identifiable cause of acute chest syndrome. Ensure that atypical bacteria are covered.

Sickling Disorders

Santosh Saraf, MD

Question 2

A 32 year old woman with Hb SS SCD presents to a follow up clinic visit doing well and without increased VOC frequency/intensity. She informs you that she is 12 weeks pregnant. She had 1 prior pregnancy that went to term and was not complicated by increased VOC or pre-eclampsia. Her blood pressure and renal function are stable and Hb = 7.3 g/dL.

Which of the following are NOT indicated for her initial management:

- . Prenatal vitamins + folic acid supplement
- 2. Referral to high-risk obstetrics service
- Genetic counseling
- 4. Red blood cell transfusion

Question 2: Pregnancy & SCD

- 1. Prenatal vitamins + folic acid supplements
- 2. Referral to high-risk obstetrics service
- 3. Genetic counseling to discuss risk of child
- 4. Red blood cell transfusion The role of prophylactic pRBC transfusions in an uncomplicated pregnancy is unclear. In a randomized study of transfusion to maintain > 6 g/dL vs. 10 g/dL, no differences in OB complications or fetal birth weight but a reduction in VOC frequency were observed in the higher Hb arm. This patient had no complications with prior pregnancy and is doing well at this time.

If the patient was having more VOC, signs of pre-eclampsia, or had prior complications during her pregnancy, would strongly consider prophylactic RBC transfusions.

The patient should be referred to high-risk OB, start prenatal vitamins + folic acid due to high demands, and monitored closely (q4weeks).

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Question 3

A 25 year old man with Hb S β^0 -thal SCD presents with L arm and leg weakness for the past day. MRI imaging demonstrates an acute stroke without hemorrhagic conversion. His Hb = 7.8 g/dL and you contact the hospital's Blood Bank to arrange an emergent transfusion.

Which of the following are the goal parameters for transfusion in the setting of an acute stroke?:

- 1. Hb 10g/dL, Hb S < 50%
- 2. Hb 10g/dL, Hb S < 30%
- 3. Hb 12 g/dL, Hb S < 50%
- 4. Hb 12 g/dL, Hb S < 30%
- 5. No exchange required, just simple transfusion to Hb 10g/dL

Question 3

50

- 1. Hb 10g/dL, Hb S < 50%
- 2. Hb 10g/dL, Hb S < 30%: This should be your initial goal. In the first two years after the initial event, the risk of another stroke is 50% and this can be substantially reduced by continuing to maintain these transfusion parameters. A hemoglobin of > 11g/dL is not recommended in the acute transfusion setting due to the risk of hyperviscosity. A simple transfusion is used pre-operatively, but will not improve blood flow rheology rapidly enough for acute situations such as stroke or severe acute chest syndrome.
- 3. Hb 12 g/dL, Hb S < 50%
- 4. Hb 12 g/dL, Hb S < 30%
- 5. No exchange required, just simple transfusion to Hb 10g/dL

51 52

Question 4

A 20 year old woman with Hb SS SCD presents for a consultation to help with her management. She had dactylitis and several VOC as a young child that had improved until the past 12 months. At that time she enrolled in college and has had 4 VOC in the past year requiring hospitalization.

Which of the following therapies could be offered to this patient to reduce the frequency of VOC?:

- Hydroxyurea
- L-glutamine
- Crizanlizumab
- 4. Voxelotor
- 5. Options 1 − 3
- 6. Any of the above

Question 4: FDA-approved therapies for SCD Outcome p-value Hydroxyurea Acute Chest Syndrome 25% 51% < 0.001 Outcome L-glutamine Placebo p-value L-glutamine Acute chest syndrome 8.6% 23.1% 0.003 Outcome Crizanlizumab Placebo p-value Crizanlizumab Hospital days Outcome Voxelotor Placebo p-value Voxelotor Increase Hb > 1g/dl

Question 5

A 25 year old man with Hb SC SCD presents to the emergency room with a painful erection that has been ongoing for 4 hours. He tried pseudophedrine and taking a cold shower without improvement.

Which of the following therapies should be immediately provided to treat this patient?

- 1. Aspiration and Irrigation of the corpora cavernosa
- 2. Exchange transfusion
- 4. IV hydration and clinical surveillance
- 5. Cavernosal artery embolization

Question 5: Ischemic Priapism

- Aspiration and Irrigation of the corpora cavernosa Priapism lasting 4 or more hours requires emergent aspiration + irrigation. An α -agonist, such as phenylephrine, is often used with the irrigation improvement is observed in > 80%
- 2. Exchange transfusion –There are case reports of ASPEN (Association of SCD,Priapism, Exchange transfusion and Neurologic events) syndrome, characterized by neurologic complications, occurring after treating priapism with exchange transfusion. Believed that vasoactive substances released after priapism detumescence leads to cerebral ischemia with symptoms ranging from headaches
- Bicalutamide Preventative measure for future priapism episodes.

 IV hydration and clinical surveillance Not recommended for ischemic priapism.
- Cavernosal artery embolization Can be used for stuttering priapism but 50% risk of erectile dysfunction.

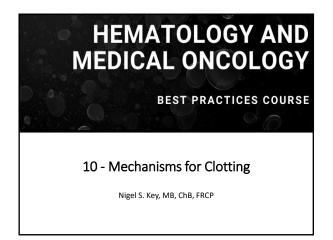
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Mechanisms for Clotting

Nigel S. Key, MD

August 14, 2020

3



Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

• None

2

Overview

- 1. Review mechanisms of primary and secondary hemostasis
- 2. Review the role of some "lesser known" coagulation proteins (thrombomodulin, TFPI, TAFI)
- 3. Review hemostasis screening assays

4

Definitions

☐ Hemostasis

Physiological blood clotting in response to injury or vascular leak

("Keeping blood where it belongs")

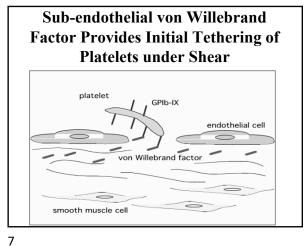
☐ Thrombosis

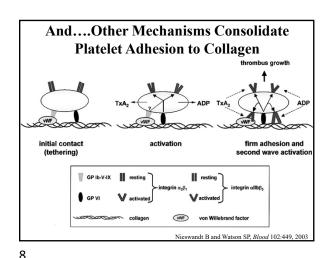
Pathological blood clotting

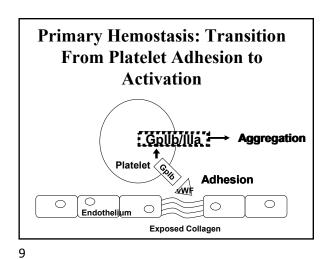
("Hemostasis in the wrong place")

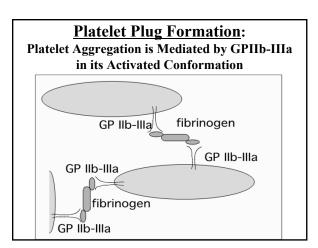
Primary Hemostasis

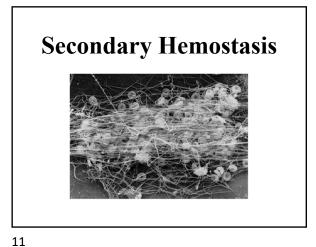
FIGURE 3-12. Scanning electron micrograph of human coronary artery planus. This photograph shows a demuded area of intima where, apparently a single endothelial cell has been lost. The raw surface is covered by a small mass of adherent and aggregated platelets which have clearly undergone shape change (× 3,570).





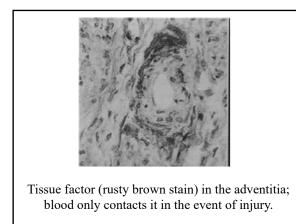


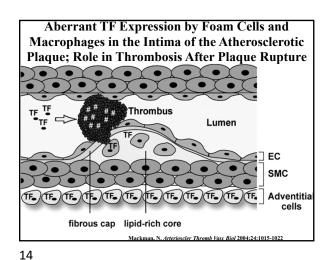


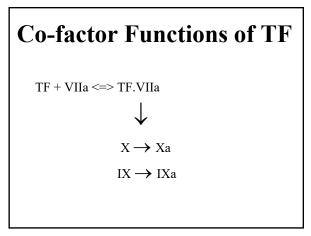


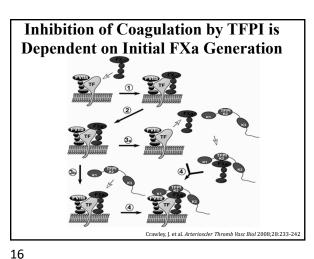
Tissue Factor

- Cell-bound glycoprotein
- Principal initiator of coagulation in vivo
- Mostly in extravascular location where it is not normally in contact with blood/clotting factors
- Exposed to blood when the endothelial barrier is breached

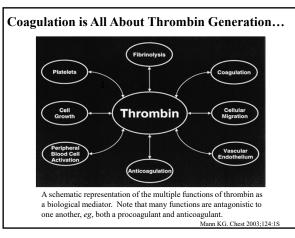


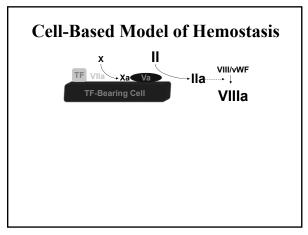


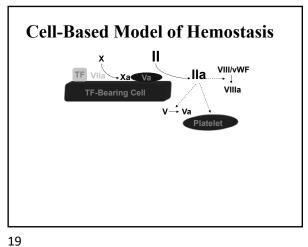


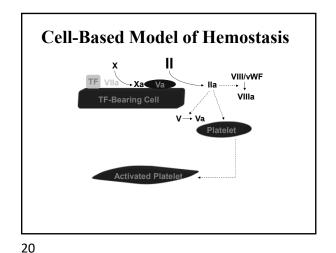


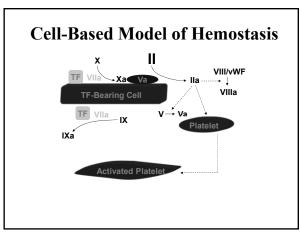
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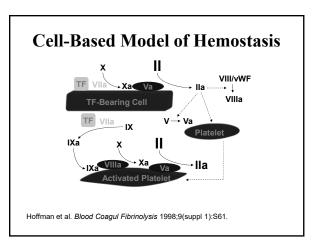


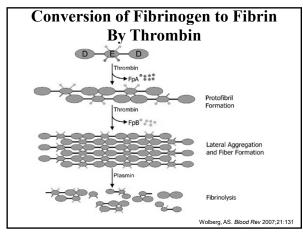


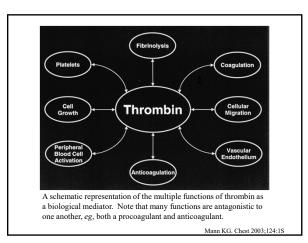


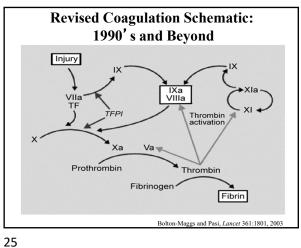


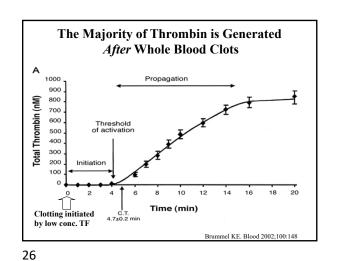


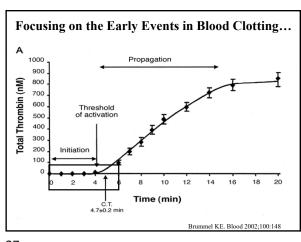


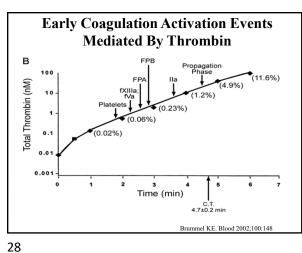


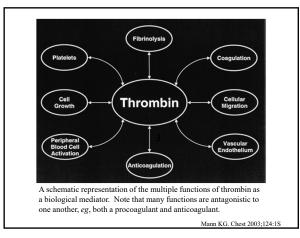


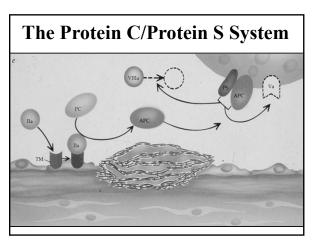








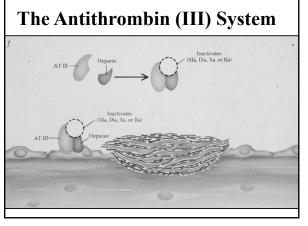




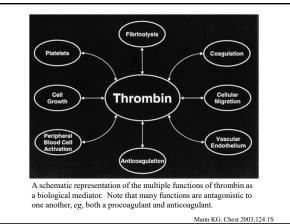
Anticoagulant Pathways

- Tissue factor pathway inhibitor
- Protein C/Protein S
- · Antithrombin (III)

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The Fibrinolytic System

- ☐ Goal: generate plasmin
- ☐ <u>Function</u>: remove/re-model fibrin clot
- ☐ Components:
 - 1. Plasminogen (in plasma)
 - 2. Plasminogen activator (tissue plasminogen activator or urokinase -- in plasma)
 - 3. Fibrin (in clot)

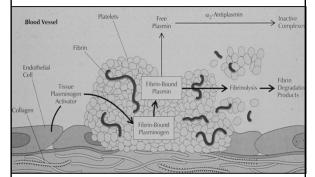
(Fibrin is therefore the co-factor for its own destruction)

Principal Inhibitors of

Fibrinolysis

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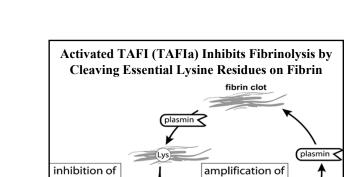
The Fibrinolytic System



1. Plasminogen activator inhibitor-1 (PAI-1)inhibits t-Pa and urokinase

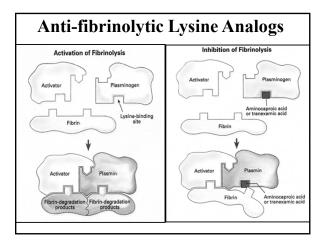
- 2. Alpha₂-antiplasmin.....inhibits plasmin
- 3. Thrombin Activatable Fibrinolysis Inhibitor (TAFI)....inhibits binding of plasminogen and tPa

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fibrinolysis

plasminoge

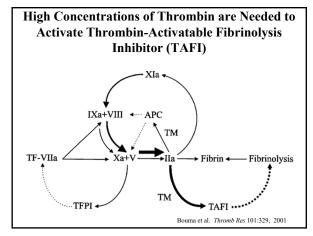


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fibrinolysis

fibrin clot

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Switching Gears

....Hemostasis Screening Assays

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Thrombin Time

- · Simplest screening test
- Bovine thrombin added to citrated plasma; no calcium or phospholipid required
- · Measures conversion of fibrinogen to fibrin
- · Prolonged by
 - Heparin
 - Hypo- or dysfibrinogenemia
 - Fibrin degradation products
 - Circulating paraprotein/light chains
- Antibody to bovine thrombin
- · Reptilase time will differentiate if due to heparin

Prothrombin Time (PT)

- "Thromboplastin" (TF + PL + Ca²⁺) added to citrated plasma
- Frequently insensitive to lupus inhibitors (excess PL)
- Usually insensitive to heparin (heparinase)
- "International sensitivity index" (ISI):
 - INR = (test PT/mean normal PT)^{ISI}

Sensitivity of PT to Single Factor Deficiencies

Factors VII, X, V, II 30-50 U/dL (60-160)

Fibrinogen 80-100 mg/dL (150-

400

Relationship of INR to Plasma
Levels of Factors
II,V, and VII:
Comparison of Liver Disease and Warfarin
Therapy

Therapy

Deticher SR.

Levels of Factors
II, V, and VII:
Comparison of Liver Disease and Warfarin
Therapy

Deticher SR.

Levels of Factors
II, V, and VII:
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Activated Partial Thromboplastin Time (APTT)

- · Add to citrated plasma
 - Activator (celite, kaolin)
 - "Partial thromboplastin" (= phospholipid)
 - Ca²

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- Variable sensitivity to lupus inhibitors, heparin
- No standardization between labs

Sensitivity of APTT to Single Factor Deficiencies

PK, HMWK, XII 30-50 U/dL (60-160)

XI, IX, VIII 30-50 U/dL (60-160)

II, V, X 10-25 U/dL (60-160)

Fibrinogen 80 mg/dL (150-400)

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Mixing Study

Principle: a 50-50 mix with normal plasma will correct if due to a factor deficiency (i.e. 50% of normal levels of any component factor should correct a prolonged screening assay) **Anti-Phospholipid Antibody Detection**

Lupus Inhibitor

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- ☐ prolongs phospholipiddependent clotting tests (APTT usually more sensitive than PT)
- ☐ failure of clotting test to correct on 1:1 mix with normal plasma
- ☐ (partial) correction with addition of phospholipid

Anti-Cardiolipin Ab

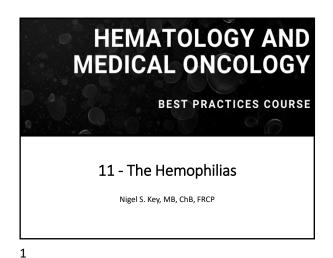
- ☐ detected by ELISA (binding of antibody to cardiolipin)
- $\hfill\square$ may be IgG or IgM

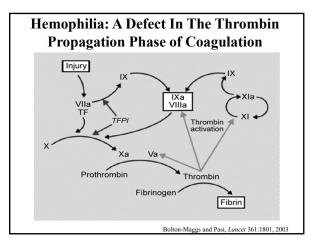
Thank you for Your Attention

The Hemophilias

Nigel S. Key, MD

August 14, 2020



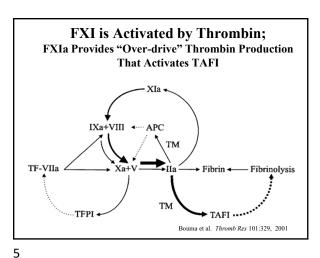


Disclosures

Disclosures of Financial Relationships with Relevant **Commercial Interests**

• None

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Prevalence of Inherited Factor Deficiency States

Fibrinogen	1:1 million	Autosomal recessive	4
Prothrombin	1:2 million	Autosomal recessive	11
Factor V	1:1 million	Autosomal recessive	1
Factor VII	1:500,000	Autosomal recessive	13
Factor VIII	1:10,000	X-linked recessive	X
Factor IX	1:60,000	X-linked recessive	X
Factor X	1:1 million	Autosomal recessive	13
Factor XI	1:1 million	Autosomal recessive	4
Factor XIII	1:1 million	Autosomal recessive	6 and 1 (2 subunits)

FXI Deficiency

- Homozygotes = 0–20% FXI; heterozygotes = 20– 70% FXI
- Ashkenazi Jews (8% heterozygous)
- Spontaneous bleeding (including hemarthrosis) rare
- Post-operative bleeding at sites of high endogenous fibrinolytic activity (mouth, urinary tract), even in heterozygotes
- Target FXI of 30-45% for surgery. Anti-fibrinolytics useful.

X-Linked Hemophilias

• Hemophilia A and B are essentially indistinguishable on clinical grounds

TT	
Hamai	nhilia
Hemo	Duna

Bleeding as a Function of Clinical Severity

Concentration of Coagulation Factor (%)	Bleeding Episodes
50 -100	None
25 - 50	Bleeding after severe trauma
5 - 25	Bleeding after surgery or moderate trauma
1 - 5	Bleeding even after slight trauma
<1	Spontaneous bleeding predominantly in joints and muscles

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Differential Diagnosis of Hemophilia A

Mild, Moderate, or Severe Forms (FVIII level: 0-30%)

Acquired hemophilia

Mild or Moderate Forms (FVIII level: >3%)

- Von Willebrand disease (types 2N, 3)
- Combined V and VIII deficiency
 - → autosomal recessive
 - → FV:c and FVIII:c in 5-30% range
- Artifact (poor sample handling)

(NOT liver disease -- FVIII levels usually elevated)

Hemophilia: Clinical Features (1)

- •Hemarthrosis
 - → beginning first year of life
 - → knees & elbows > ankles > shoulders > hips
 - → early sensation of stiffness, "bubbling", or other aura
 - → "target joints" (repeated hemarthrosis): indicative of synovitis

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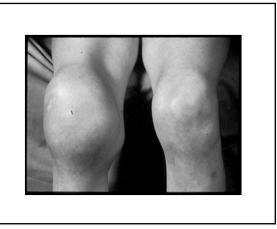
Laboratory Diagnosis of Hemophilia

Bleeding Time Normal
PT Normal
APTT Prolonged
FVIII:c (or FIX:c) <1% = severe

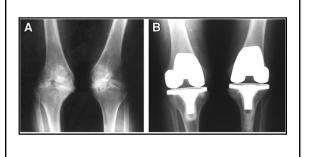
1-5% = moderate

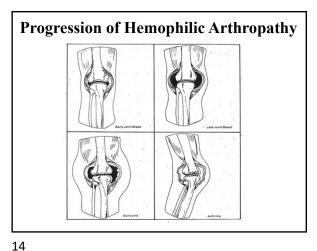
5-30% = mild

vWF:Ag Normal vWF:Rco Normal 12



End Stage Hemophilic Arthropathy





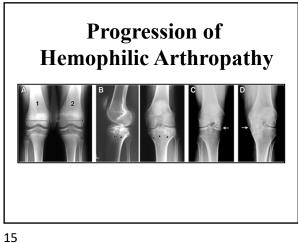
Hemophilia: Clinical Features (2)

• Muscle Bleeds

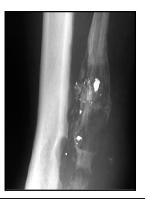
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- → flexors > extensors (ilio-psoas, quads, gastrocnemius)
- \rightarrow may require prolonged therapy to prevent re-bleed
- → pseudotumor risk if neglected



HEMOPHILIC PSEUDOTUMOR



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Hemophilia: Clinical Features (4)

- Intra-cranial bleeding
- → high index of suspicion in neonatal period, after minor head trauma, unusual headache or vomiting
- · Post-dental bleeding
- → anti-fibrinolytics useful
- Post-surgical bleeding
- → May be delayed several days

NOT excessive bleeding after minor cuts or abrasions

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Principles of Factor Dosing

- What is the desired (peak) clotting factor level?
- 1 Unit = amount of factor present in 1 ml of normal plasma
- What is the half-life of the factor?
 - FVIII = 8-12 hours
 - -FIX = 18-24 hours

Factor Replacement Therapy

- · Bolus Dosing
 - FVIII: 1 U/kg → 2% rise in FVIII activity. Usually q.12 hours
 - FIX: 1 U/kg → 1% rise in FIX activity. Usually q.24 hours
- · Continuous infusion
 - After a bolus to raise factor level to the desired target,
 - CIVI at 4U/kg/hr will usually maintain that target

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Technologies Employed to Prolong Half Life of Clotting Factor Concentrates

- Pegylation
 - varying size of PEG molecule (20kDa, 40 kDa, 60kDa)
 - varying site of PEG attachment
- · Conjugation
 - Albumin
 - Fc portion of immunoglobulin

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Experience with Long Acting Clotting Factor Concentrates

- FIX
 - significant prolongation of half-life: 2.5 to 5-fold [55-100+hours]
- FVIII
 - half-life prolongation 'capped' at 1.4 to 1.7-fold (limited by half-life of vWF) [14-19 hours]

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Adjunctive Therapies (1)

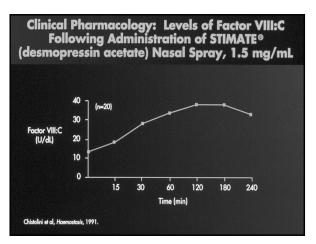
- Anti-fibrinolytics
 - Epsilon amino caproic acid (AmicarTM) or tranexamic acid (LystedaTM)
 - Inhibit fibrinolysis and increase clot stability
 - Useful especially in mucosal bleeding, after dental work
 - Contra-indicated in upper urinary tract bleeding

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Adjunctive Therapies (2)

- DDAVP (Desmopressin)
 - Releases pre-formed stores of FVIII and VWF from endothelium
 - Typically increases FVIII and VWF 3-4 fold
 - Mild hemophilia A (>5% FVIII basal level), type 1 VWD, some 2A VWD
 - Individual effect should be documented
 - Can be given iv, sc, or as a nasal spray, 150 mcg (child)-300 (adult) mcg
 - Do not confuse with much lower dose used in nephrogenic DI

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Treatment of Hemophilia

Spontaneous bleeds:

- 1-2 doses of FVIII or FIX (\approx 30 U/kg/dose) for joint or muscle bleeds, when treatment is initiated early
- Significant muscle bleeds or established hemarthroses may require more prolonged treatment (5-10 days)

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Treatment of Hemophilia

Considerations for surgery:

- Minimal plasma levels and duration depend on site of surgery
- Risk of delayed bleeding extends to 3-5 days postoperatively
- On-site factor assay capability essential
- Factor given by bolus or continuous infusion

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Treatment of Hemophilia: "Prophylaxis"

- Maintain trough FVIII/FIX >1-2% (0.01-0.02 U/ml)
- Begin at early age (1-2 years)
 - \rightarrow FVIII: 25-40 u/kg t.i.w.
 - \rightarrow FIX: 25-40 u/kg b.i.w.
- · High expense, need for reliable i.v. access
- Prevents progressive arthropathy

Gene Therapy: The "Ultimate" Prophylaxis for Hemophilia....

Hemophilia Carriers

"If she circumcised her first son and he died, and a second son and he died, she must not circumcise a third one"

"In the case of circumcision there are families in which the blood flows freely and there are families in which the blood is held tight"

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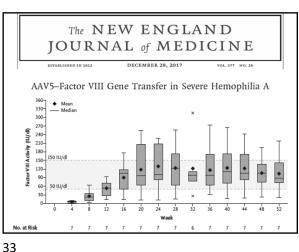
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Babylonian Talmud, 200 A.D.

31

The NEW ENGLAND JOURNAL of MEDICINE DECEMBER 7, 2017 Hemophilia B Gene Therapy with a High-Specific-Activity Factor IX Variant - Participant 5

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FVIII Levels in Women: Hemophilia Carriers vs. Normals % Factor VIII

Hemophilia Carriers May be Symptomatic Table 4. Risk of bleeding after medical interventions Carriers, event/total (%) RR (CI) Tooth extraction Prolonged bleeding more than 3 h 61/228 (27) Treatment after intervention 24/228 (11) 1/219 (0.5) 23.1 (3.1-169 Prolonged bleeding more than 3 h reatment after intervention 29/123 (24) 16/122 (13) 1.8 (1.0-3.1) Operations Prolonged bleeding; more than 3 h Treatment; ever 16/146 (11) 6/149 (4) 18/149 (12) 46/163 (28) 16/174 (9) 29/174 (17) 2.3 (0.9-5.7) 1.4 (0.8-2.4) Blood transfusion Participants who had been treated prior to the clinical intervention preparations, transxamic acid, or desmopressin were excl Plug I, et al. Blood 108:52, 2006

Hemophilia Carriers and Pregnancy

- Determine fetal sex by ultrasound; if male child..
 - → Avoid scalp sampling
 - → Avoid vacuum extraction
 - → Avoid prolonged delivery
 - → Arrange for cord blood sampling at delivery (cord blood FVIII = adult levels; cord blood FIX < adult levels)
 - → Avoid i.m. vitamin K until result of cord sampling known

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Hemophilia Carriers: Diagnosis

- 1. Family history:
 - → Obligate carrier (father has hemophilia)
 - → Presumed carrier (mother of hemophilic child)
- 2. Low FVIII:c/vWF:Ag ratio:
 - → Substantial error rate
 - → FVIII level very variable: normal in some carriers
- 3. DNA diagnosis (the gold standard):

Hemophilia: Complications

Long-term disability 2° to bleeding

- → neurologic (intra-cranial bleed)
- → arthropathy (repetitive hemarthrosis)
- → compartment syndrome or pseudotumor

Infectious 2° to blood product exposure

- → Hepatitis B, C: prior to 1983
- \rightarrow HIV: prior to 1983

Inhibitors....

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Hemophilia Carriers and Pregnancy

- Check FVIII/FIX level prior to delivery in 3rd trimester
 - → Epidural OK if FVIII/FIX >40%
 - \rightarrow Higher risk of 1° and 2° PPH:
 - maintain FVIII/FIX >50% for 5-7 days

Factor VIII Inhibitors

- Polyclonal, high affinity IgG molecules that neutralize FVIII
- Cumulative incidence ~ 25% in patients with severe hemophilia A
 - median number of exposure days = 9-15
 - median age = 0.8-3.3 years
- May occur in mild or moderate hemophilia A, usually much later in life
- · Cause significant morbidity and mortality

Indications for Inhibitor Screen: Patient with Known Hemophilia

- Poor clinical response to adequate therapy and/or sub-optimal recovery/half-life of FVIII or FIX in vivo
- Prior to all major surgeries
- Annual evaluation
- Anaphylactic reaction to FIX

High Responding

BU
100

Time

Transient

BU
100

Time

Transient

Low Responding

BU
100

Time

Time

Time

Time

Time

Hemophilic Inhibitors

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Inhibitor Screening - Principle

- Equal mix of patient and normal pooled plasma
- Incubate at 37°C for 2 hours*
- Measure residual FVIII (or FIX) activity

 (*FVIII inhibitors are time-dependent, FIX inhibitors are not)

Treatment of Bleeding in FVIII
Inhibitor Patients

High responder patients
with low inhibitor titer

High responder patients
with high inhibitor titer

Will demorrhagic episodes

FVIII concentrates
1° choice
rFVIIa or APCC

Adapted from: Haya S, et al. Haemophilia (2007), 13 (Suppl. 5), 52-60.

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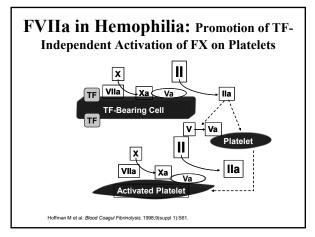
The Bethesda (Inhibitor) Assay

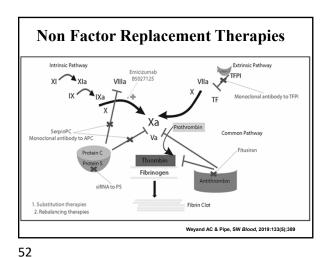
- "Gold standard" for quantifying FVIII/IX inhibitors
- 1 B.U. = inhibitor concentration that results in 50%↓ in expected residual FVIII activity

Bypassing Agents

- FEIBATM
 - 50-70% efficacy; no laboratory monitoring
 - Plasma-derived mixture of partially activated clotting factors, including II, VII(a), IX(a), X(a)
 - 50-75 units/kg IV q 8-12 hrs
 - Do not combine with Amicar (thrombosis risk)
- · Recombinant Factor VIIa
 - 70-90% efficacy; no laboratory monitoring
 - TF-independent activation of FX
 - 90-120 mcg/kg IV q2-3 hrs x 2-3 doses (half-life = 2.5 hours)

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Immune Tolerance Induction (ITI)

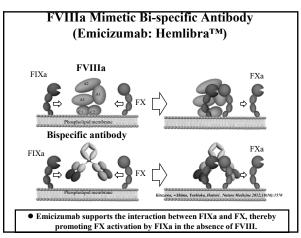
- Daily exposure to high dose factor over weeks to months to achieve immunological tolerization
- Most centers now initiate as soon as inhibitor detected in childhood – expense, compliance, i.v. access issues
- Overall, ITI is successful in \sim 70% of patients with hemophilia A
- Historical peak inhibitor titer < 200 B.U. and the presence of low inhibitor titer at the time of enrollment are best predictors of success
- Lower success rates in hemophilia B ($\approx 30\%$)

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Factor IX Inhibitors

- Rare: only 2-3% of severe hemophilia B (especially with large gene deletions)
- Associated allergic reactions to FIX products, including anaphylaxis and nephrotic syndrome
- Treatment options include high dose FIX, or by-passing therapies (FEIBA, rFVIIa); rFVIIa preferred if history of allergic reactions to FIX



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Emicizumab Prophylaxis in Hemophilia A with Inhibitors

- The annualized bleeding rate was 2.9 events in the Emicizumab prophylaxis group vs. 23.3 events in the control group, P < .001.
- The no-bleeding-event rates were 65% and 6% in the Emicizumab and control groups, respectively:
 - absolute risk reduction = 57% (95% CI, 38%-71%),
 - number needed to treat = 2 (95% CI, 2-3).

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Thank you for your attention!

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Emicizumab: Clinical Pearls

- Licensed for Hemophilia A *with or without* FVIII inhibitors (weekly subcutaneous dosing)
- Cannot be used in hemophilia B (+/- inhibitors)
- Breakthrough bleeding may need treatment:
 - if no FVIII inhibitor: use FVIII
 - if FVIII inhibitor: **avoid** FEIBA (thrombotic microangiopathy, thrombosis); FVIIa preferred
- · Limited experience in management of surgery

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Emicizumab: Laboratory Monitoring of 'Background' FVIII or FVIII Inhibitor

- aPTT is normalized at low plasma concentrations of Emicizumab....therefore....
- Emicizumab affects <u>all</u> aPTT-based assays including FVIII levels (FVIII:C) and FVIII inhibitor assays (e.g Bethesda)
 - for FVIII:C or FVIII inhibitor screen use chromogenic assays

Von Willebrand's Disease

Alice Ma, MD

August 14, 2020

Acquired Disorders of Coagulation

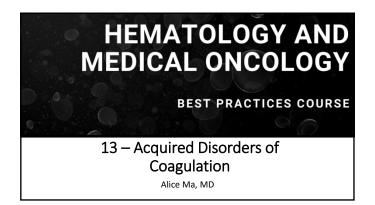
Alice Ma, MD

August 14, 2020

Alice Ma, MD

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DISCLOSURES

Off Label Usage

*Bituximab

*Profilinine

*Bebulin

*Kcentra

*Coagadex

Interests

*Advisory Boards: Novo Nordisk, Shire, BioMarin

*Honoraria: Accordant

2

Learning objectives

 Recognize the clinical presentation, diagnosis and treatment of 4 acquired bleeding disorders associated with malignancies

Case 1

- A 48 y.o. woman presents with facial swelling and oral bleeding after extraction of two molars. She had spontaneous bruising 3 weeks before tooth extractions. She had presented 3 times to local ER for care. On the third ER visit, she was intubated for airway protection and sent in for further evaluation
- PMHx significant for HIV.
- s/p cholecystectomy, NSVD x 6 with no bleeding, prior tooth extractions without bleeding.
- Meds: Atripla
- FHx: no bleeding disorders

4

6

Case 1

 PEx: intubated, sedated woman, with massive swelling of face, eyes swollen shut, massive swelling of cheeks, neck, and upper arms. Blood from mouth, lips massively swollen, nasally intubated, with blood leaking from nose. Ecchymoses over arms and legs.





The pharynx and upper trachea are near completely opacified with blood

Blood throughout the paranasal

Multiple regions of moderate to severe soft tissue swelling are seen, most notably at the right posterior skull base and overlying the left orbit.

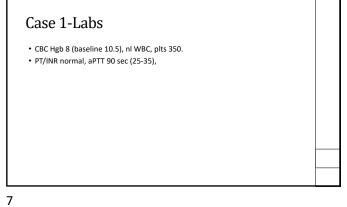
There are bilateral subdural hematomas

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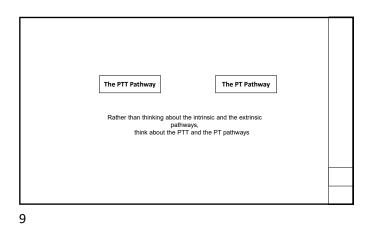
Acquired Disorders of Coagulation

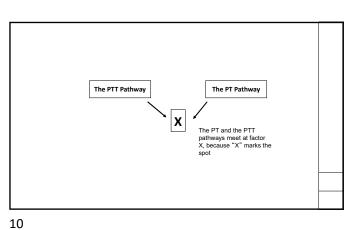
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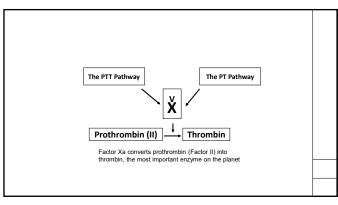


Obligatory Confusing intimidating Coagulation Cascade





The PTT Pathway The PT Pathway $\overset{\vee}{\textbf{X}}$ Factor V is a cofactor for factor X, and you can remember this because V fits into the notch of the X

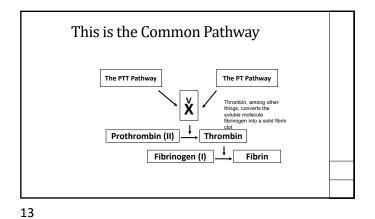


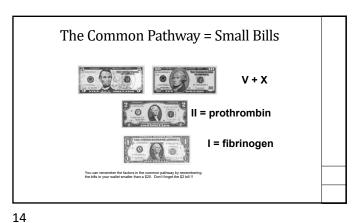
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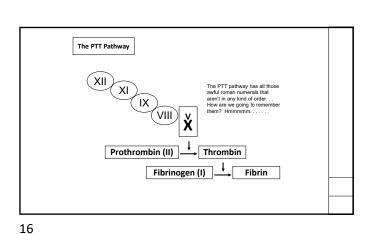
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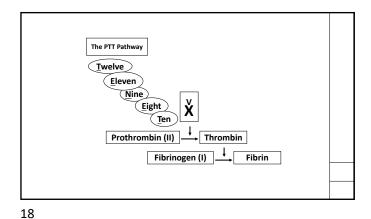


The PTT Pathway

Well, just remember that the PTT is a basic TENET of hematology, TENET stands for.....

Prothrombin (II) Thrombin

Fibrinogen (I) Fibrin



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The Mixing Study

- Used to differentiate the causes of a prolonged aPTT
- Patient's plasma mixed 1:1 with normal plasma, and aPTT repeated
 If there is a CLOTTING FACTOR DEFICIENCY, then the normal plasma supplies the deficient factor, and the aPTT corrects into the normal range
- If there is an INHIBITOR, then the patient's plasma will inhibit clotting in normal plasma, and the aPTT will NOT fully correct

Case 1

- · Labs: PT/INR normal, aPTT 90 sec (25-35), CBC Hgb 8 (baseline 10.5), nl WBC, plts 350.
- 1:1 mix of aPTT 39 sec immediately

20 19

Case 1

- Labs: PT/INR normal, aPTT 90 sec (25-35), CBC Hgb 8 (baseline 10.5), nl WBC,
- 1:1 mix of aPTT 39 sec immediately
- 1:1 mix of aPTT 52 sec after incubation at 37°C for 2 hours

Case 1

- Labs: PT/INR normal, aPTT 90 sec (25-35), CBC Hgb 8 (baseline 10.5), nl WBC, plts 350.
- 1:1 mix of aPTT 39 sec immediately
- 1:1 mix of aPTT 52 sec after incubation at 37°C for 2 hours
- FVIII activity <1%
- FIX 101%

22 21

Case 1 – Acquired Hemophilia

- · Incidence is 1 in 1 million
- Median age at presentation between 60 and 67 years
- Bleeding pattern
- · Hemarthroses are rare
- · Mucocutaneous bleeding is common
- · Gastrointestinal bleeding, epistaxis, ecchymosis, hematuria
- · Severe intramuscular bleeding, including compartment syndrome
- · Intracranial hemorrhage
- · Postsurgical or postpartum bleeding
- High mortality (8%-22%), partially due to comorbid conditions

Case 1-AH

- Associated conditions found in ~50% of cases
- · Autoimmune disorders
 - systemic lupus erythematosus, rheumatoid arthritis, Sjögren's syndrome
- Malignant conditions
 - solid tumors and lymphoproliferative malignancies
- Drugs
- Infections
- Postpartum state
- Postsurgical

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Case 1 - AH

- Screening tests—isolated prolonged aPTT, normal PT
- Mixing study—mix patient plasma 1:1 with normal plasma, then repeat aPTT
- · Fails to fully correct
- Re-prolongs (1-2 hours) at 37ºC in AH
- FVIII activity level—low (but does not have to be <1%) in AH
- · Bethesda assay

25

Our Patient • FVIII < 1% • FIX > 101%, inhibitory pattern seen • Bethesda titer 228 BU

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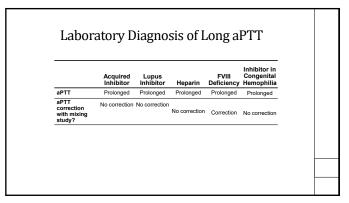
The Bethesda Assay

| Reciprocal of dilution at which 50% of normal FVIII activity is observed | Perform FVIII assay | Inhibitor Titer = 5 BU | In

Laboratory Diagnosis of Long aPTT

Acquired Inhibitor In

27 28



	Acquired Inhibitor	Lupus Inhibitor	Heparin	FVIII Deficiency	Inhibitor in Congenital Hemophilia
aPTT	Prolonged	Prolonged	Prolonged	Prolonged	Prolonged
aPTT correction with mixing study?	No correction	No correction	No correction	Correction	No correction
Mixing study prolong with incubation?	Prolongation	No prolongation	No prolongation	No prolongation	No prolongation

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Laboratory Diagnosis of Long aPTT | Acquired | Lupus | Heparin | Deficiency | Deficiency | Heparin |

	atory L	nagno	SIS Of L	ong a	PTT
	Acquired Inhibitor	Lupus Inhibitor	Heparin	FVIII Deficiency	Inhibitor in Congenital Hemophilia
aPTT	Prolonged	Prolonged	Prolonged	Prolonged	Prolonged
aPTT correction with mixing study?	No correction	No correction	No correction	Correction	No correction
Mixing study prolong with incubation?	Prolongation	No prolongation	No prolongation	No prolongation	No prolongation
FVIII activity	Low	Normal	Normal	Low	Low
aPTT correction w/ phospholipid addition	No	Yes	No	No	No

31 32

					Inhibitor in
	Acquired Inhibitor	Lupus Inhibitor	Heparin	FVIII Deficiency	Congenital Hemophilia
aPTT	Prolonged	Prolonged	Prolonged	Prolonged	Prolonged
aPTT correction with mixing study?	No correction	No correction	No correction	Correction	No correction
Mixing study prolong with incubation?	Prolongation	No prolongation	No prolongation	No prolongation	No prolongation
FVIII activity	Low	Normal	Normal	Low	Low
aPTT correction w/ phospholipid addition	No	Yes	No	No	No
Bethesda titer	Positive	0	0	0	Positive

FVIII Inhibitors: Clinical Management

- · Suspect the diagnosis
- · Make the diagnosis
- Treat the bleeding
- Increase FVIII levels—ideal goal
 Recombinant porcine factor VIII
- Use bypassing agents in an attempt to achieve hemostasis
 - rVIIa • aPCC
- Eradicate the inhibitor

33 34

Increasing FVIII Levels

- Bypassing agent
- rFVII
- aPCC
- Or Recombinant porcine FVIII
 - Only if your center can measure FVIII in real time
- Desmopressir
- In patients with very weak inhibitors (<3 BU), may increase FVIII enough to treat very minor bleeding (eg dental)

Bypassing agents

APCC | FVIIa |
Plasma derived (virally inactivated) | Recombinant |
Contains factors II, IX, X and | Contains activated factor VII |
activated VII |
Dose 50-100 U/kg | Dose 90 µg/kg (range 40-180) |
every 8-12 hours | Possible for the property of the p

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Inhibitor Eradication in AH

- Inhibitors may remit spontaneously in patients with $\ensuremath{\mathsf{AH}}$
- Most published guidelines and algorithms recommend early inhibitor eradication, unless AH associated with childbirth or drug treatment

Immunosuppression: Steroid ± Cyclophosphamide • EACH2 registry • Prednisone + oral cyclophosphamide more likely to achieve stable CR than prednisone alone **Collins P. et al. Blood. 2012;120(1)47-55.** **Collins P. et al. Blood. 2012;120(1)47-55.** **Toroid ± Cyclophosphamide or prednisone alone** **Toroid ± Cyclophosphamide or prednisone + oral cyclophosphamide or prednisone alone** **Toroid ± Cyclophosphamide or prednisone + oral cyclophosphamide or prednisone or pr

37

Case 2

- A 55 y.o. woman presented with 1 month history of progressive leg swelling and fatigue. On evaluation, she was found to be in congestive heart failure. She had 4+ edema to her thighs and had nephrotic range-proteinuria.
- Hematology was consulted because of gum bleeding and bruising.
- Her PT and aPTT were both prolonged.
- PT 25 sec, INR 2.4
- aPTT 60 sec.
- Mixing studies completely corrected
- CBC showed only mild anemia and slight rouleaux formation.
- Kidney biopsy showed amyloidosis

The Common Pathway = Small Bills

V + X

II = prothrombin

I = fibrinogen

39 40

Case 2 – Amyloidosis and acquired FX deficiency

 Patients with amyloidosis may have acquired FX deficiency due to the amyloid protein binding and removing the FX from the circulation.

Case 2

38

- This disorder is a true acquired FX deficiency, rather than an autoantibody to the clotting factor such as is seen in acquired hemophilia.
- The mixing study thus will completely correct

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Case 2

- The patients who are bleeding may require FX replacement with Prothrombin complex concentrates (PCCs).
- PCCs currently available in the United States include Bebulin VH (Baxter) and Profilnine SD (Grifols) and Kcentra (a 4 factor PCC)
- · Both products are virally-inactivated plasma-derived products
- · Coagadex is a Factor X concentrate that can also be used
- Remember that the half life of factor X in these patients may be much lower than the 40-45 hours seen in normal individuals
- PK studies may be required for optimal dosing

Case 3

- A 42 yo woman presents for evaluation of abnormal bruising over her arms and legs for the past three months. She has had nosebleeds for the past 2 weeks. She has recently had increase in menstrual flow and duration.
- No new activities, lifelong non-smoker
- Recently started on lipitor for newly diagnosed hyperlipidemia, with total cholesterol of 320.
- No prior bleeding with tooth extractions.
- Medications: lipitor, MVI
- Physical exam: young woman, NAD, normal vital signs, numerous ecchymoses over arms and legs. Coarse hair and skin.

43 44

Case 3

- VWF antigen and activity levels are found to be low at 25% and 29%.
- CBC is normal Hgb 12, MCV 88, WBC 6, normal diff, plts 190
- TSH >100!

Acquired VWD - causes

- MGUS and myeloma
- Responds to IVIg
- Hypothyroidism
- Lack of synthesis
 Myeloproliferative disorders
- Sticky platelets bind high MW VWF
- Valvular disorders (tight Aortic stenosis)
- Shearing of high MW VWF, sometimes with GI AVMs forming (Heyde's Syndrome)
- Left Ventricular Assist Devices
- Wilm's tumors
- Binds and removes VWF from circulation

45 46

Case 4

- A 35 y.o. woman presents with bleeding and joint pain. She has been having increasing ecchymoses, gum bleeding, and menorrhagia for 3 weeks. She has had worsening joint pain. ROS positive for a malar rash after sun exposure. She has had a prior history of 3 miscarriages. She is not currently pregnant.
- PT is 22 sec (13-16), aPTT is 80 sec (25-35).
- PT mix is 15 sec
- aPTT mix is 55 sec.

Case 4

- What is the most likely diagnosis?
- A. Lupus inhibitor alone
- B. Lupus inhibitor + another defect

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Case 4

- If there is another defect in addition to the lupus inhibitor, what is it likely to be?
- · A. Anti-platelet inhibitor
- B. FII deficiency
- C. FV inhibitor
- D. FVII inhibitor
- · E. FVIII inhibitor

Case 4-Acquired prothrombin deficiency and LI

- · Patients with the lupus inhibitor are at no increased risk for abnormal bleeding
- · Rare patients with the lupus inhibitor can also have an associated anti FII antibody
- This antibody leads to accelerated clearance
- The PT will thus be prolonged, and will also correct with 1:1 mix, since it is an acquired deficiency, rather than a function-blocking antibody

49

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Summary

- Acquired FVIII inhibitor
- . New onset of bleeding, no prior personal or family history of bleeding
- Isolated prolonged aPTT
- Fully diagnose with FVIII activity and Bethesda assay
- Treat bleeding with bypassing agents or recombinant procine FVIII
 - rVIIa
- Obizur (rpFVIII)
- · Eradicate the inhibitor
 - Prednisone and cyclophosphamide
- Rituximab

Summary

- Acquired VWD
- · New onset mucocutaneous bleeding
- PTT prolonged, Bleeding time or PFA-100 polonged
- VWF antigen and activity decreased
- Think of associated diagnoses Hypothyroidism
 - MGUS
 - Heart valves
 - LVADs
 - MPDs
- Wilm's tumor

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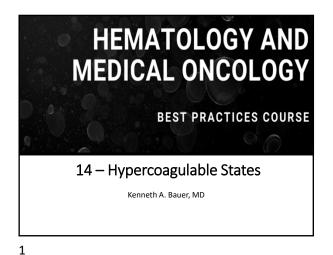
Summary

- · Amyloid causes acquired FX deficiency
- PT and aPTT are prolonged
- 1:1 mix corrects
- Patients with the Lupus inhibitor can present with acquired Prothrombin (FII Deficiency) due to a clearing antibody
- aPTT prolonged—no correction on mix
- PT is prolonged and DOES correct with mix

Hypercoagulable States

Kenneth A. Bauer, MD

August 14, 2020



DISCLOSURES

Off-Label Usage

· None

Interests

- · BMS
- · Takeda

2

Agenda

- Risk Factors and Pathophysiology
- Initial Rx and Prognosis of VTE
- Acquired and inherited thrombophilias
- Duration of anticoagulation/Risk Stratification

Risk Factors for VTE

Transient/Provoked

- Surgery
- Trauma (major trauma or lower-extremity injury)
- Acute medical illness
- Immobilization
- Estrogen-containing contraceptives or hormone replacement therapy
- Pregnancy/puerperium
- Central venous catheters
- Heparin-induced thrombocytopenia
- Prolonged air travel (I manage as unprovoked)

Persistent

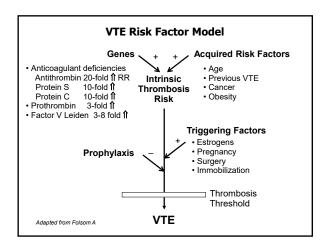
- Obesity
- Chronic Medical Illnesses
 - Cancer and its therapy
 - Inflammatory bowel disease
 - Nephrotic
 - Myeloproliferative neoplasms/PNH
- Paralysis

4

Idiopathic/

3

4



Agenda

- Risk Factors and Pathophysiology
- Initial Rx and Prognosis of VTE
- Acquired and inherited thrombophilias
- Duration of anticoagulation/Risk Stratification

6

Initial Treatment of DVT/PE

- · Parenteral AC followed by VKA or DOAC ("2 drug approach")
 - UFH (IV with PTT monitoring)/LMWH or Fondaparinux (SC without coagulation monitoring)
 - Start warfarin on day 1 overlapping with warfarin for at least 5 days until INR >2 for 1-2 days; target INR of 2-3 for at least 3-6 months
 - Stop parenteral AC after minimum of 5 days; then start dabigatran 150 mg bid or edoxaban 60 mg qd (no laboratory monitoring required)
- Oral factor Xa inhibition ("1 drug approach")
 - Start rivaroxaban on day 1 at 15 mg bid x 3 weeks followed by 20 mg daily, treat for 3-6 months
 - Start apixaban on day 1 at 10 mg bid x 1 week followed by 5 mg bid daily, treat for 3-6 months
 - · No laboratory monitoring required

Question: Now that DOACs are an oral option for initial VTE treatment, when should we consider not using them?

Pregnancy-associated VTE or post-partum if breast feeding

Massive PE (hemodynamically unstable) or DVT (phlegmasia cerulea dolens) where thrombolysis is a consideration

Very obese patients (? weight >120 kg)

Very frail patients (? weight < 50 kg)

Renal dysfunction (creatinine clearance <30 mL/min)

Patients with altered GI anatomy (gastric bypass procedures)

Highly "thrombosis-prone" patients (recurrent DVT or PE on therapeutic anticoagulation)

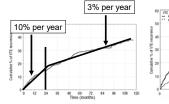
Important to ensure that patients adhere to therapy with medication (behavioral, insurance, and cost issues)

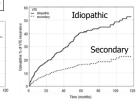
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Risk of recurrent VTE after discontinung anticoagulation in a cohort of 1626 patients

Prandoni P et al, Haematologica 2007





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Duration of anticoagulant treatment: Summary

3 months is equivalent to 6 months. Extending anticoagulation is highly effective in eliminating recurrences (>90% relative risk reduction), but only as long as treatment is continued.

Unprovoked DVT/PE is associated with a high recurrence risk after the discontinuation of anticoagulation, which is greatest during the first 2 years.

2016 ACCP Guidelines

- For VTE without cancer, we suggest DOACs instead of VKA for the first 3 months and beyond (2B)
- In patients with recurrent VTE on oral anticoagulation, we suggest switching to LMWH at least temporarily (2C).

In VTE associated with strong transient risk factors (surgery, trauma), extended treatment beyond 3-6 months not required nor is testing for thrombophilia (from 'ASH Choosing Wisely')

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Agenda

- Risk Factors and Pathophysiology
- Initial Rx and Prognosis of VTE
- Acquired and inherited thrombophilias
- Duration of anticoagulation/Risk Stratification

The Homocysteine Story

- Epidemiologic studies done 20-30 years ago found that mild hyperhomocysteinemia was both prothrombotic and proatherogenic.
- Multiple controlled randomized trials of B vitamin supplementation reduced homocysteine levels, but did not reduce the risk of thrombosis.
- Recent studies suggest that the association between mild hyperhomocysteinemia and vascular disease (particularly VTE) may have been due to unmeasured confounding factors (Ospina-Romero M, et al. Am J Epidemiol 2018).
- ⇒ No reason to measure homocysteine levels
- Never test for MTHFR polymorphisms (C677T, A1298C) Also never test for PAI-1 promoter (4G/5G) or Factor XIII polymorphisms

Antiphospholipid Antibody Syndrome (APS)

Sapporo Criteria for APS (2006):

- · Clinical criteria:
 - Vascular thrombosis
 - Pregnancy morbidity
 - Clinical manifestations include immune thrombocytopenia and livedo reticularis
- . Laboratory criteria*:
 - Lupus Anticoagulant
 - Elevated cardiolipin antibody levels (IgG or IgM) (high titer: >40 GPL/MPL or >99th percentile)
 - β₂-glycoprotein I antibodies (IgG or IgM) (>99th percentile)

*2 or more occasions, >12 wks apart.

Miyakis S, et al. J Thromb Haemost. 2006;4:295-306.

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Antiphospholipid Antibody Syndrome Lupus Anti-β2GPI ACA APS is associated with SLE, cancer, infections, drugs; often idiopathic

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Antiphospholipid Antibody Syndrome (APS)

- A clinically heterogeneous autoimmune disorder associated with thrombosis in any vascular bed; most patients present with venous thrombosis, ischemic stroke, or recurrent fetal loss
- Long-term anticoagulation with warfarin is the standard of care for thrombotic APS.
 However recurrent thrombosis remains common (10-20% on anticoagulation, 25-50% off anticoagulation).
- Two randomized trials showed that an INR of 2-3 was as effective as INR 3-4 in venous thrombosis and APS.

Rivaroxaban vs Warfarin in Thrombotic APS

 Rivaroxaban versus Warfarin in high-risk APS (TRAPS) Pengo V, et al. Blood 2018

Stopped after 120 patients were enrolled due to high event rate of 19%; recurrent thrombosis (all arterial) in 12% and major bleeds in 7% with rivaroxaban vs 3% with warfarin (all major bleeds)

 Rivaroxaban versus Vitamin K Antagonist (VKA) in APS Ordi-Ros J, et al. Ann Intern Med 2019

Recurrent thrombosis was 11.6% (17.6% for triple positive) for rivaroxaban (10 arterial, 2 venous) vs 6.3% (8.8% in triple positive) for warfarin (3 arterial, 3 venous). 9/11 recurrences on rivaroxaban were strokes vs 0 on warfarin. In non-triple positive. patients, thrombosis rates were 2.7% and 2.6% No difference in major bleeds

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How to approach DOAC use in patients with APS?

- Warfarin is the anticoagulant of choice in patients with high risk APLS (triple positive, arterial clots).
- FDA Labelling Change on 10/11/19: Warnings and Precautions

5.10 Increased Risk of Thrombosis in Patients with <u>Triple Positive</u> Antiphospholipid Syndrome

Direct-acting oral anticoagulants (DOACs) are not recommended for use in patients with triple-positive antiphospholipid syndrome (APS). For patients with APS (especially those who are triple positive [positive for lupus anticoagulant, anticardiolipin, and anti-beta 2-glycoprotein I antibodies]), treatment with DOACs has been associated with increased rates of recurrent thrombotic events compared with vitamin K antagonist therapy.

Are there APS patients in which a DOAC can be considered?

72 year old male diagnosed with unprovoked bilateral PE in 1/16 who was successfully treated with rivaroxaban for 6 months. Baseline PT/PTT wnl; LA testing not done as he was continuously on anticoagulation. Cardiolipin and B2GPI IgM antibodies persistently >40; IgG levels are normal. The patient is resistant to going on warfarin.

CONSIDER LEAVING ON DOAC AT FULL DOSE AFTER COUNSELING (DOCUMENT DISCUSSION!)

Chayoua W, et al. The (non-)sense of detecting cardiolipin and antibeta2glycoprotein I IgM antibodies in the antiphospholipid antibody syndrome. J Thromb Haemost. 2019

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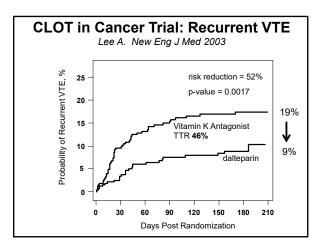
Venous Thromboembolism in Cancer

Common (~20% of all patients with VTE)

Increased risk of recurrent VTE on VKA Can occur with a therapeutic INR ("warfarin failure")

2016 ACCP Guidelines

Chronic low molecular weight heparin suggested over warfarin (Grade 2B) or DOACs (Grade 2C) In patients with recurrent VTE on long term LMWH, we suggest increasing dose of LMWH by one quarter to one third (2C).



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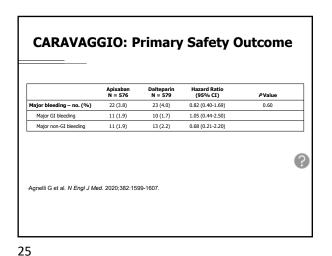
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CARAVAGGIO: Study Design o 119 sites in Europe, Israel, US; investigator-initiated, noninferiority trial with PROBE design o Largest DOAC trial in patients with cancer-associated VTE to date (1155 patients in mITT analysis) Cancer patients with objectively symptomatic or unsuspected proximal lower-imb DVT and/or PE in a segmental or more proximal pulmonary artery Randomization stratified by: • Symptomatic or incidental *VTE • Active or history* of cancer 1Each capped at 20% *Max time allowed between diagnosis and randomization Agnelli G et al. N Engl J Med. 2020;382:1599-1607.

	Apixaban (N = 576)	Dalteparir (N = 579)
iolid tumor – no. (%)		
Colorectal	121 (21.0)	113 (19.5)
Lung	105 (18.2)	95 (16.4)
Breast	79 (13.7)	76 (13.1)
Genitourinary	66 (11.5)	73 (12.6)
Gynecological	60 (10.4)	59 (10.2)
Pancreatic or hepatobiliary	44 (7.6)	43 (7.4)
Upper gastrointestinal	23 (4.0)	31 (5.4)
Head and neck	14 (2.4)	8 (1.4)
Bone/soft tissue	11 (1.9)	7 (1.2)
Skin – melanoma	4 (0.7)	7 (1.2)
Other	16 (2.8)	15 (2.6)
łematological malignancy – no ((%) 33 (5.7)	52 (9.0)

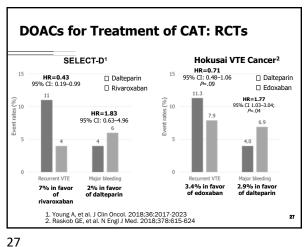
	Apixaban N = 576	Dalteparin N = 579	Hazard Ratio (95% CI)	P Value
Recurrent VTE, n (%)	32 (5.6)	46 (7.9)	0.63 (0.37-1.07)	<0.001 for non-inferiority 0.09 for superiority
Recurrent DVT, n (%)	13 (2.3)	15 (2.6)	0.87 (0.34-2.21)	
Recurrent PE, n (%)	19 (3.3)	32 (5.5)	0.54 (0.29-1.03)	
Fatal PE, n (%)	4 (0.7)	3 (0.5)	1.93 (0.40-9.41)	
nelli G et al. <i>N Engl J M</i> ed	. 2020;382:1599-	1607.		



CARAVAGGIO: Secondary Outcomes Apixaban N = 576 0.70 (0.45-1.07) Recurrent venous thromboembolism or major bleeding 51 (8.9) 1.42 (0.88-2.30) 52 (9.0) 70 (12.2) 1.16 (0.77-1.75) Death from any cause 135 (23.4) 1.36 (1.05-1.76) Event-free survival 422 (73.3) 397 (68.6) Agnelli G et al. N Engl J Med. 2020;382:1599-1607.

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Hokusai VTE: Bleeding GI vs non-GI Cancers Patients with Patients with nongastrointestinal cancer Major (%) ₋ 60 90 330 360 35 31 28 27 25 23 22 19 17 17 14 12 7 5 4 1 5 4 0 0 9 1 4 3 88 Kraaiipoel N. et al. Thromb Haemost, 2018:118:1439-1449

Cancer-Associated Thrombosis: Duration of Treatment?

Data after 1st 3-6 months of treatment are limited for type of anticoagulant as well as duration of therapy.

General consensus is to continue anticoagulation in the setting of active/persistent cancer and treatment, which can be years for some patients.

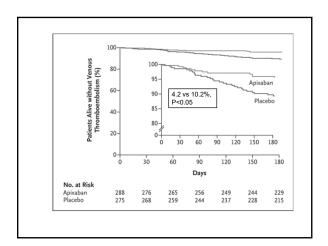
Must individualize based on severity of initial thrombotic event, initial/ongoing risk factors, quality of life considerations, and patient preference

Primary Prevention of CAT in Ambulatory Patients on Chemotherapy: Risk Score Development and Validation Characteristic

Site of Cancer Very high risk (stomach, pancreas)	2	8% ☐ □ Deve 7% - ■ Valid 6% -
High risk (lung, lymphoma, gynecologic, GU excluding prostate)	1	5% - 1 > 4% - 2 3% -
Platelet count ≥ 350,000/mm³	1	2% - 1% - 0.8%
Hb < 10g/dL or use of ESA	1	0% n=734
Leukocyte count > 11,000/mm ³	1	Lou
BMI ≥ 35 kg/m ²	1	

Nkrudqd#DD#hw#dd#Earrg#533;

	CASSINI	AVERT
Drug	Rivaroxaban 10 mg qd	Apixaban 2.5 mg BID
Duration	6 months	6 months
Inclusion criteria	Khorana score ≥ 2	Khorana score ≥ 2
Types of cancers	Solid tumors + lymphomas	Solid tumors + lymphomas + myelomas + primary brain tumors
Baseline LENIs	Yes	No
Primary events definition	Symptomatic + screen- detected + incidental	Symptomatic + incidental
Reduction in primary endpoint	NNT=27; on-treatment, 17 including secondary efficacy endpoints	NNT=17
Bleeding risk	NNH=101	NNH=59; on-treatment,100



31 32

Evaluation for occult malignancy in the patient presenting with unprovoked VTE

Available data do not support an extensive search for occult malignancy (i.e., CT scans); it is however important to pursue symptoms or signs which suggest an underlying malignancy and to ensure that age-appropriate screening tests have been performed.

Carrier M. New Eng J Med 2015

What do guidelines say about the thrombophilia testing and the management of venous thromboembolism?

ACCP - The presence of hereditary thrombophilia should not guide duration of anticoagulation because its presence is not a major determinant of recurrence risk.

Australia/NZ (Tran HA, et al. Med J Aust 2019)

Routine thrombophilia testing not indicated

Most patients with proximal DVT/PE should be treated with a factor Xa inhibitor and assessed for extended anticoagulation (if unprovoked).

33 34

C:t	-6	There	b	:	_

ABNORMALITY	ARTERIAL	VENOUS			
Factor V Leiden	-	+			
Prothrombin 20210A	-	+			
AT Deficiency	-	+			
Protein C Deficiency	-	+			
Protein S Deficiency	-	+			
Lupus Anticoagulant	+	+			

Prevalence of Defects in Caucasian Patients with Venous Thrombosis

Factor V Leiden (FVL)	12-40%
Prothrombin Gene Mutation (PGM)	6-18%
Deficiencies of AT, Protein C, Protein S	5-15%
Antiphospholipid Antibody Syndrome	~5%
Unknown	20-70%

Several variants have been found by candidate and genomewide screens – all common and weak (OR < 1.5) (Smith NL, JAMA 2007; Bezemer ID, JAMA 2008; Li Y, JTH 2009

Hereditary Thrombophilia and Obstetric Complications

- Significantly increased risk for second and third trimester fetal loss (~3-fold ↑)
- No association with preeclampsia, IUGR
- Role of LMWH to prevent recurrent fetal
 - One positive trial in thrombophilic women
 - o Multiple negative trials in women with recurrent losses before 20 weeks including thrombophilic women (TIPPS, Lancet 2014)

The "Hypercoagulable Workup"

- Genetic test for Factor V Leiden mutation
- Genetic test for Prothrombin G20210A mutation
- Functional assay of Antithrombin
- Functional assay of Protein C
- Free Protein S Antigen (best assav)
 - o Total Protein S Antigen
 - o Protein S Activity
- Tests for Antiphospholipid Antibody Syndrome
 - Lupus anticoagulant
 - Cardiolipin/β2-glycoprotein I IgG/IgM (not IgA)
- Factor VIII Coagulant Activity (I don't order this)

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Assay Measurements in Deficiencies of Antithrombin, Protein C, or Protein S

TYPE	ANTIGEN	ACTIVITY
I	Low	Low
II	Normal	Low

Protein S levels and the risk of venous thrombosis: results from the Mega Study - a population-based case-control study (Blood 2013)

N=5,317, protein S deficient if protein S level < 2.5th percentile of controls

Total protein S < 68 U/dL (Odds ratio 0.90, 95th CI 0.62-1.31) Free protein S < 53 U/dL (Odds ratio 0.82, 95th CI 0.56-1.21)

Using a lower cut-off for free protein S ($<0.10^{th}$ percentile): Free protein S < 33 U/dL (OR 5.4, 95^{th} CI 0.61-48.8)

Conclusion: Low protein S levels were not associated with VTE in this study. In the absence of a family history, hereditary protein S deficiency is a rare risk factor for VTE.

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Acquired Deficiencies in Antithrombin, Protein C, or Protein S

ANTITHROMBIN	PROTEIN C	PROTEIN S
Pregnancy		Pregnancy
Liver Disease	Liver Disease	Liver Disease
DIC	DIC	DIC
Nephrotic syndrome		
Major surgery		Inflammation
Acute thrombosis	Acute thrombosis	Acute thrombosis
Treatment with:		
Heparin	Warfarin	Warfarin
F		Estusana

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Don't draw these tests when patients present acutely with VTE or are receiving

anticoagulants. DOACs can result in erroneous results in some functional assays (e.g., oral factor Xa inhibitors in testing for antithrombin deficiency). Abnormal results drawn at presentation with VTE must be confirmed. Draw protein C and S levels after discontinuing warfarin for a minimum of 1 week.

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Risk of Recurrent Venous Thrombosis in Patients with Inherited Thrombophilia

- Heterozygosity for Factor V Leiden (FVL) or Prothrombin G20210A do not substantially increase recurrence risk.
- Retrospective data indicate that risk is not even higher in homozygotes with FVL and heterozygotes with both FVL and PT G20210A (Lijfering WM, Circulation 2010).
- Antithrombin, Protein C, Protein S Deficiency
 - · High in selected kindreds with strong clinical penetrance (retrospective studies)
 - · Less data in unselected patients

How do DOACs compare to standard therapy (LMWH/warfarin) in patients with hereditary thrombophilia?

- · Retrospective data show similar low rates of recurrence and bleeding (JTH 2019; 17:645-656)
- · No mechanistic reason to believe that DOACs will not be as effective and as safe as LWMH/warfarin in patients with common thrombophilias (1 copy of Factor V Leiden or Prothrombin G20210A mutations); might be preferable to heparin/LMWH in AT deficiency or warfarin in protein C deficiency
- However I generally don't switch thrombophilic patients with recurrent VTE from warfarin to DOACs if they have done well on warfarin for years (with good INR control)

Testing for Hereditary Defects in Patients with Thrombosis and No Family History

Improve understanding of pathogenesis of VTE Identify and counsel affected family members

Infrequently identify patients in whom the identification of an abnormality should alter their management

No evidence of "direct particular benefit to family members" (because of low absolute risk of an initial VTE)

Potential for overaggressive management of propositus and asymptomatic affected relatives (if screening undertaken)

Cost of testing/consultations

Create undue anxiety

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Agenda

Risk Factors and Pathophysiology

Initial Rx and Prognosis of VTE Acquired and inherited thrombophilias

Stratification

Duration of anticoagulation/Risk

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ACCP Evidenced-Based Clinical Practice Guidelines (10th Edition)

Indication Proximal DVT/PE secondary to a transient risk factor 1st isolated.

Duration of Anticoagulation

3 months

unprovoked distal DVT

Idiopathic proximal DVT or PE

0

3-6 months

2nd Unprovoked DVT or PE

VTE in setting of active cancer: LMWH for at least 3 months

Long-term

45

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Extension of warfarin treatment beyond 3 to 6 months in 1st unprovoked/idiopathic VTE Douketis JD et al. Ann Intern Med 2007;147:766-774 Linkins LA et al. Ann Intern Med 2003; 139:893-900

In year 1 following cessation of anticoagulation, for 1000 patient-years

Death by PE recurrence

80 VTE recurrences Case-fatality rate 4-12% 3 to 10 deaths

Death by major bleed 20 to 60 bleeds

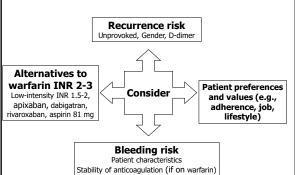
Case-fatality rate 10% 2 to 6 deaths

NO MORTALITY BENEFIT FROM LONG-TERM ANTICOAGULATION WITH VITAMIN K-ANTAGONISTS (WARFARIN)

⇒ In the warfarin era, a recurrent VTE rate of <5% per year was considered "acceptable" (risk of bleeding on anticoagulation > benefit)

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Extended (or Chronic) Treatment - an Individualized Management Decision



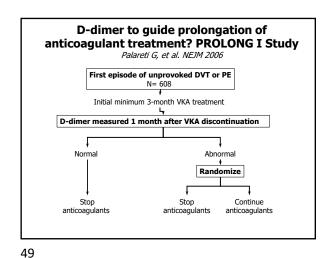
Duration of anticoagulant treatment Additional determinants of the risk of recurrence

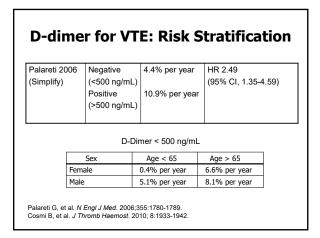
Other elements that increase the risk of recurrence after stopping anticoagulant treatment include:

- Persistently elevated D-dimer levels off anticoagulants
- Male gender
- Residual thrombus on ultrasound (conflicting data difficult to standardize)

Clinical Prediction Models

- Vienna Prediction Model (gender, type of VTE, D-dimer on VKA)
- HERDOO (Hyperpigmentation, edema/leg redness, D-dimer, BMI, patient age)
- DASH (D-dimer post-VKA, age, gender, hormonal therapy)





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D-Dimer for VTE Risk Stratification

Kearon C, et al. Ann Intern Med 2015

D-dimer to select patients with a first unprovoked VTE who have anticoagulants stopped at 3-7 months: a multicentre management study (D-Dimer Optimal Duration Study, DODS)

410 patients enrolled, mean age 51. 319 patients with negative D-dimer on VKA and 4 weeks later off VKA

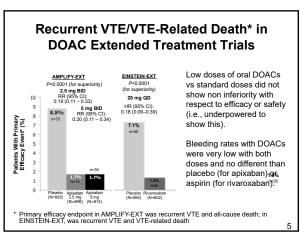
	Recurent VTE	95% CI
Entire Cohort (n=318)	6.6% per year	4.8-9.0
Men (n=180)	9.7% per year	6.7-13.7
Women (no estrogens=81)	5.4% per year	2.5-10.2
Women (estrogens=58)	0% per year	0.0-3.0

DODS Results: Extended Follow Up

Kearon C, et al. JTH 2019

	VTE rate per 100 patient- years	Risk of recurrence at 5 years
Total: no ac	5.1 (3.9, 6.5)	21.5%
Men: no ac	7.5 (5.5, 10.0)	29.7% (22.1-37.3)
Women: no ac		
no estrogen	3.8 (2.0, 6.6)	17.0% (8.1-25.9)
estrogen	0.4 (0.0, 2.3)	2.3% (0.0-6.8)

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Should the superior safety profile of direct oral anticoagulants (DOACs) lead to the treatment of more has been patients with a 1st unprovoked VTE with extended therapy?

"Greater net clinical benefit with DOACs than VKAs"

Major bleeds
~0.5% per year

DVT/PE recurrence
17-30% at 5 years without anticoagulation

Slide 54

KAB9 KENNETH A BAUER, 10/1/2014 **KAB10** KENNETH A BAUER, 10/1/2014

Kenneth A. Bauer, MD

Other Considerations in Deciding on **Long-Term Oral Anticoagulation**

- Site of thrombosis
 - $_{\odot}\,$ PE more likely to recur as PE, DVT as DVT
- Severity of Thrombosis
 - Massive PE
 - o Ilio-femoral DVT
 - o Severe post-phlebitic syndrome
- Age of patient
 - Prefer not to commit young patients to lifelong anticoagulation after a 1st event unless clearly very high risk for recurrence
- IVC filters (>75% of retrievable filters never retrieved)

 - o ↑ risk of recurrent DVT especially if VTE unprovoked

 Randomized clinical trial of retrievable IVC filters in severe acute PE with DVT showed no benefit in preventing recurrent PE (Mismetti P, et al. JAMA 2015)

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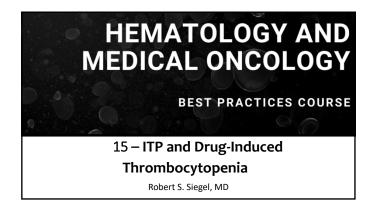
August 14, 2020

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DISCLOSURES

Off-Label Usage:

*None

Interests:

*Consultant: CVS/Caremark (Formulary Review)

*Research Funding: Janssen Pharmaceuticals, Medivation, Pfizer, Suncoast Community Clinical Oncology

2

Primary Immune Thrombocytopenia

Thrombocytopenia with otherwise normal CBC

Normal peripheral smear

No congenital disorders

No drugs, MDS or carcinomatosis

No viral infection (including HIV)

No SLE or other autoimmune disease

No lymphoproliferative disease

Age and Sex
Distribution of ITP

Average annual ITP
incidence by age group
and gender (n=1145)
diagnosed between 19902005 as reported by the
General Practice Research
Database, United
Kingdom.

Marieke Schoonen W, Kuzera G, Coolson J, et al. Epidemiology of immune
thrombocytopenic purpuru in the general practice research darabase. Br J Haematol.

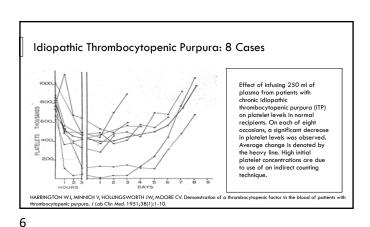
ITP Background: Important Findings

1951: Harrington found anti-platelet factor in the plasma of ITP patients

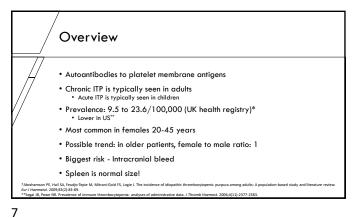
1965: Shulman identified the anti-platelet factor in the 7S gamma region

1968: Karpatkin identified platelet associated lg

1975: Rosse quantitated PAIg, His findings were reproduced in other labs



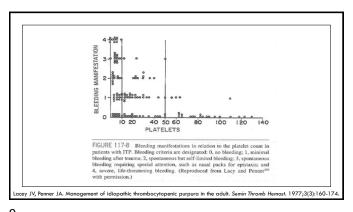
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Immune Thrombocytopenia Findings • Thrombocytopenia • Increased megakaryocytes • Increased PAIgG • Increased platelet destruction • Decreased platelet production

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ğ PATHOPHYSIOLOGY Immune MAKING THE DIAGNOSIS Thrombocytopenia: Major Sections TREATMENT & TREATMENT GUIDELINES

10 9

/ ITP Pathophysiology (Cont.): Platelet Associated Antibodies
 Increased in 90% of ITP patients Non-specific finding in other disorders Auto-antibodies to membrane glycoproteins: GP IIb/IIIa GP Ib/IX

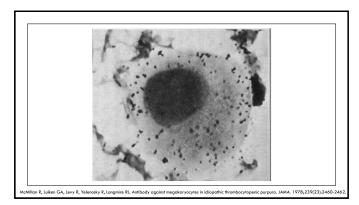
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ITP Pathophysiology (Cont.): Impaired Immune Regulation • Antibodies to platelet antigens • More rapid platelet destruction • Requires an intact reticuloendothelial system • Antibodies to megakaryocyte antigens • Suppression of thrombopoiesis • Altered T cell function

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Heterogeneity of Platelet Turnover: Indium Studies in the 1980s

SIEGEL, BLOOD 1982;50:191a GROSSI, SCAN J HAEM 1983;31:206 SCHMIDT, SCAN J HAEM 1985;34:47 STOLL, BLOOD 1985;65:584 HEYNS, BLOOD 1986;67:86 BALLEM, JCI 1987;80:33



13 14

ITP Pathophysiology (Cont.)

- Complement and immune complexes usually are not involved
- The sensitizing event is obscure in adults
- Platelet count is a dynamic equilibrium between production & destruction rates

Causes of Secondary ITP

- Antiphospholipid syndrome
- Autoimmune thrombocytopenia (eg Evans syndrome)
- Common variable immune deficiency
- Drug administration side effect
- Infection with cytomegaloviraus, Helicobacter pylori, hepatitis C, human immunodeficiency virus, varicella zoster
- Lymphoproliferative disorders

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• Bone marrow transplantation side effect

Causes of Secondary ITP (Cont.)

- Vaccination side effect
- Systemic lupus erythematosus
- Myelodysplasia
- AFB disease

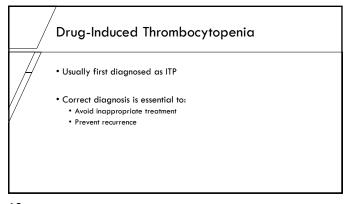
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- Other Granulomatous disease
- Drug-induced
 - Decreased production
 - More rapid destruction

Drug-Induced Thrombocytopenia

- Frequency is uncertain
- Frequency of medication use increases with age
- Frequency of alternative medicine use is increasing at all ages

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Drug-Induced Thrombocytopenia: Initial Diagnosis as ITP • 343 patients registered as ITP, 1993-1999 • 28 (8%) excluded because of subsequent diagnosis of drug-induced thrombocytopenia • Quinine most common cause (13 of 28, 46%) Neylon AJ, Saunders PW, Howard MR, Proctor SJ, Taylor PR; Northern Region Haematology Group. Clinically significant newly presenting autoimmune thrombocytopenic purpura in adults: a prospective study of a population-based cohort of 245 patients. Br J Homentol. 2003;12(2)6)96-674.

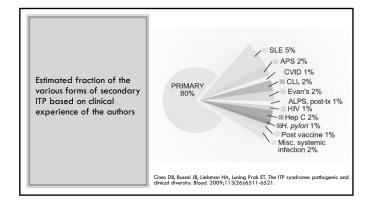
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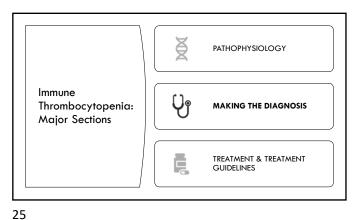


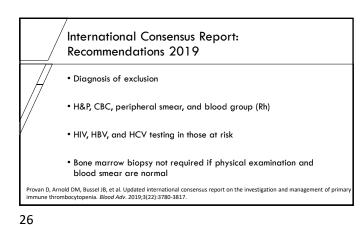
Acute and Chronic ITP Acute Chronic 20-40 Years Peak Age 2-6 Years **Sex Predilection** Women 3:1 None **Prior Infection** Nο Yes Onset Abrupt Insidious <20,000 20,000-80,000 Platelet Count 6-8 Weeks Chronic Duration Spontaneous Remission Usual Unusual

	Physiology of Thrombopoietin
	• A constant amount is produced by the liver
/ /	Binds to TPO receptors of:
Y	Megakaryocyte precursors Megakaryocytes
	• Platelets
23	

Physiology of Thrombopoietin (Cont.) Effect on megakaryocyte precursors • Increases megakaryocyte production Effect on megakaryocytes • Causes more platelet production • TPO binds to platelets and is internalized • Platelet numbers determine plasma TPO level

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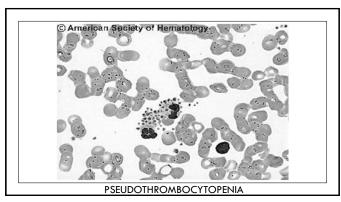


International Consensus Report Recommendations 2019 (Cont.) Tests of potential utility: • Glycoprotein-specific antibody (poor sensitivity) • Anti-phospholipid antibodies • Anti-thyroid antibodies and thyroid function • Pregnancy test • Viral PCR for EBV, CMV, and parvovirus • Bone marrow examination for specific indications* • Helicobacter pylori testing (dependent on geography) Provan D, Arnold DM, Bussel JB, et al. Updated international consensus report on the investigation and management of prima immune thrombocytopenia. Blood Adv. 2019;3(22):3780-3817.

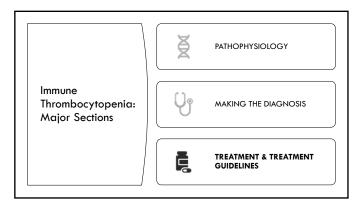
International Consensus Report Recommendations 2019 (Cont.) Tests of unproven or uncertain benefit: • Reticulated platelets/immature platelet fraction • Bleeding time Serum complement Provan D, Arnold DM, Bussel JB, et al. Updated international consensus report on the investigation and management of prima immune thrombocytopenia. *Blood Adv.* 2019;3(22):3780-3817.

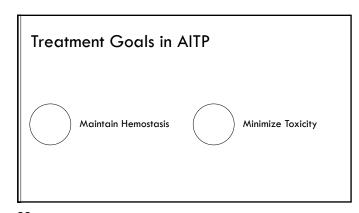
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American Society of Hematology Guideline Update Neunert C, Terrell DR, Arnold DM, et al. American Society of Hematology 2019 guidelines for immune thrombocytopenia [published correction appears in Blood Adv. 2020 Jan 28;4(2):252]. Blood Adv. 2019;3(23):3829-3866.



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31 32

Recommendations for Surgery		
	SURGERY	RECOMMENDED PLATELET COUNT
///	Dental Prophylaxis (Cleaning/Scaling)	>20,000
//	Simple Dental Extraction	>30,000
ľ	Complex Dental Extraction	>50,000
	Minor Surgery	>50,000
	Major Surgery	>80,000
	Major Neurosurgery	>100,000
Cuke	er A, Cines DB. Immune thrombocytopenia. Hematology A	um Soc Hematol Educ Program. 2010;2010:377-384.

Immune Thrombocytopenia: Emergency Therapy

•Platelet Transfusion

•IVIG

•Corticosteroids

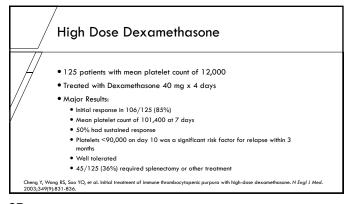
33 34

	ASH Practice Guidelines - Adults
	• Tx given when platelet count less than 30,000 (1A)
	 Recommend against a prolonged course (>6 weeks including treatment and taper) and in favor of a short course (<!--= 6 weeks) (3)</li-->
//	• Use IVIg with steroids when rapid response is needed (2B)
ľ	• Either IVIg or anti-D used first line if steroids are contraindicated (2C)
	 If IVIg is used, initial dose should be 1gm/kg as a one- time dose, it may be repeated if necessary (2B)
	• Hospitalization for initial diagnosis with platelets count less than 20,000
	errell DR, Arnold DM, et al. American Society of Hematology 2019 guidelines for immune thrombocytopenia orrection appears in Blood Adv. 2020 Jan 28;4(2):252]. Blood Adv. 2019;3(23):3829-3866.

Prednisone

•1-2 mg/kg qD at diagnosis
•Improvement usually within 3 days
•Maximal improvement within 2 weeks
•Allows increased platelet production
•Reduces rate of platelet destruction

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High Dose Dexamethasone vs. Prednisone

Observations:

Dexamethasone has a higher anti-inflammatory effect and more effectively modulates T-cell abnormalities*

Results of a meta-analysis including 9 randomized studies showed initial response is higher with dexamethasone but there is no significant difference between durable response rates**

**Uu Z, Wang M, Zhou S, et al. Pulsed high-dose dexamethasone modulates Th1-7Th2-chemokine imbalance in immune thrombocytopenia. J Transl Med. 2016;4(1):301. Published 2016 Oct 24.

**Mithoewah, Graggery-Meller, Groy Let al. High-dose desamethasone compared with prednisone for previously untreated primary immune thrombocytopenia.

37 38

High Dose Dexamethasone vs. Prednisone (Cont.) Prospective multicenter trial: • DXM 40 mg/d for 4 days or prednisone 1.0 mg/kg daily for 4 weeks with taper afterwards • 1-2 courses of HD-DXM resulted in higher incidence of overall initial response (82.1% vs. 67.4%, p=0.044) and complete response (50.5% vs. 26.8%, p=0.001) compared with prednisone • Incidence of sustained response and sustained complete response showed no significant difference • Either recommended as first line therapy, however dexamethasone recommended for rapid response (ASH Guidelines 2019) Wei Y, JXB, Wang YW, et al. High-dose dexamethasone vs prednisone for treatment of adult immune thrombocytopenia: a prospective multicenter randomized trial. Blood. 2016;127(3):296-370.

IVIg in ITP

Mechanism

• Reticuloendothelial blockade
• Anti-idiotype antibodies

Advantages

• Low toxicity

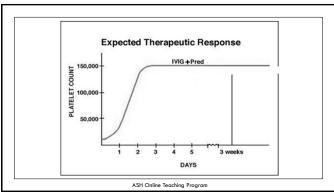
• Effective (pre-op)

• Can be used when steroids contraindicated
• Indicated when rapid elevation in platelet count needed (i.e. active bleeding)

Disadvantages

• Temporary Effect
• Expensive

39 40



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Intravenous Anti-D Therapy

• Minimal toxicity
• Anti-D is effective only in Rh + patients with intact spleens
• Ig binds to erythrocyte D Ag
• Immune clearance of sensitized RBCs occupies the Fc receptors in the RE system
• Minimizes removal of Ab coated platelets
• Potential for hemolytic anemia after multiple treatments
• Do not use in pts with Pos Coombs or Hgb < 10.
• Steroids vs Anti-D

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Second-Line Therapy AHS Guidelines 2019* Treatment of ITP lasting >3 months in patients who are corticosteroid dependent or have no response • Thrombopoietin receptor agonist (Romiplostim, Eltrombopag, Avatrombopag) • Rituximab • Splenectomy International Consensus Report 2019** • Thrombopoietin receptor agonist • Rituximab • Fostamatinib • Fostamatinib • Wait at least 1-2 years from diagnosis before considering splenectomy **Assumer C, Terrell SP, Amdid DM, et al. American Society of Hematology 2019 guidelines for Immuse thrombocytopenia [published cirrection appears in Blood Adv. 2019:3(2):3537-3866.

Rituximab in ITP Patients • Long-term durable responses occur in 20% to 25% of adult patients • Used after glucocorticoid failure • Used before or after splenectomy • Usually well tolerated, but small risks of progressive multifocal encephalopathy and hypogammaglobulinemia • Must be tested for HBSag, Hepatitis B core antibody • Prior treatment with IVIG may confound anti-HBc results • Vaccination against gram positive encapsulated bacteria should be given

• NOT FDA APPROVED for ITP, but has a compendial listing

43 44

Safety and Efficacy of Rituximab in Adult ITP Patients • Prospective registry of 248 patients • 173 received 375 mg/m² q4 weeks, 72 received two fixed 1gm infusions q2 weeks • Infusions stopped in 3 patients • 7 showed infection • 3 died of infection 12-14 months later, but role of Rituximab unclear • 61% showed overall initial response (platelets >30,000 and >2x baseline platelet count) • At 24 months, 39% showed a durable response • Good response was associated with: • ITP of <1 year • Previous complete response to corticosteroids Khellaf M, Charles-Nelson A, Fain O, et al. Safety and efficacy of rituximab in adult immune thrombocytopenia: results from a prospective registry including 248 patients. Blood. 2014;124(22):3228-3236.

Rituximab + rhTPO vs. Rituximab alone

• Multicenter trial including 115 patients with steroid resistant or relapsed ITP

• Randomized 2:1 for combination

• OR/CR in combination group 79.2%/45.4%

• OR/CR in Rituximab alone group 71.1%/23.7%

• Shorter time to response in combination group

• No difference in long term response

Zhou H, Xu M, Qin P, et al. A multicenter rendomized open-label study of rituximab plus rhTPO vs rituximab in corticosteroid-resistant or relapsed ITP. Blood. 2015;125(10):1541-1547.

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Second Line Therapies in Recent Years • Splenectomy rates have fallen with Rituximab and TRAs* • Rituximab and TRAs are used earlier and more frequently in adults and children** • Long-term treatment with TRAs is safe*** * Chaturedis, Arnold DM, McCrae KR. Splenectomy for immune thrombocytopenia: down but not out. Blood. 2018;131(11):1172-1182. * * Chapin J, Lee CS, Zhang H, Zehnder JL, Bussel JB. Gender and duration of disease differentiate responses to rituximab-dexamethasone therapy in adults with immune thrombocytopenia. Am J Hematol. 2016;91(9):907-911. * * ** Kuter DI, Bussel JB, Newland A, et al. Long-term treatment with romiplostim in patients with chronic immune thrombocytopenia: safety and efficacy. Br J Haematol. 2013;161(3):411-423.

Sustained Responses to TRAs

• Among 28 patients treated with a TRA, 20 (28.5%) who achieved a complete response had a sustained response after stopping drug.*

• Among 46 patients who received a TRA, 11 (23.9%) had a sustained response after stopping.**

• Among 31 patients who received Romiplostim, 9 (29%) had a sustained remission, but 4 of 9 relapsed.***

• Of 75 patients treated with romiplostim, long term remission occurred in 32%.***

• Of 49 patients treated with eltrombopag, 26 (53%) had a sustained response after stopping drug with 9-month F/U.******

• Of 49 patients treated with eltrombopag, 26 (53%) had a sustained response after stopping drug with 9-month F/U.*******

• Of 49 patients treated with eltrombopag, 26 (53%) had a sustained response after stopping drug with 9-month F/U.*******

• Of 50 patients received with eltrombopag, 26 (53%) had a sustained response after stopping drug with 9-month F/U.*******

• Of 50 patients received with eltrombopages are special supplementation of transference with the patients of the special supplementation of transference with the patients of the special supplementation of transference with the patients of the special supplementation of transference with the patients of the special supplementation of transference with the patients of the special supplementation of transference with the patients of the special supplementation of transference patients and the special supplementation of transference patients and the special supplementation of transference patients and transference that the special supplementation of the special supplem

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Sustained Responses to TRAs Conclusion TRA's have a high rate of response No major toxicity from long term use 25-33% maintained remission after TRA was stopped Predictive features for maintenance of platelet count of TRA are not known

Available TRAs/TPO-RAs • Romiplostim (Nplate) Starting dose 1 mcg/kg/wk SQ • Eltrombopag (Promacta) Starting dose 50 mg PO qd • Avatrombopag (Doptelet) Starting dose 20 mg PO qd

49 50

Plang J, Liang Y, Ai Y, et al. Eltrombopag versus romiplostim in treatment of adult patients with immune thrombocytopenia: A systematic review incorporating an indired-comparison meta-analysis. PLoS One. 2018;13(6):e0198504. Published 2018 Jun 1.

Avatrombopag (Doptelet)

• FDA approval in May 2018: thrombocytopenia associated with chronic liver disease prior to invasive procedure

• Oral thrombopoietin receptor agonist (TPO-RA)

• Indication: ITP that hasn't responded responded to previous Tx

• Reduced need for platelet transfusions

• FDA approved June 2019: chronic ITP which has not responded to prior therapy

• Most common adverse events: HA, fatigue, confusion, epistaxis

51

Phase 3 Trial: Majority of patients achieved a platelet count of at least 50,000 per mcg after 8 days of therapy.

Efficacy was also superior to placebo in the maintenance of platelet counts during the 6-month treatment period

Dosage: Initiate at 20 mg once daily. Adjust dose/frequency to maintain platelet count >50,000

Do not exceed 40 mg per day

Fosamatinib

Fosamatinib (Tavalisse)

• FDA approved in April 2018

• Oral SYK (spleen tyrosine kinase) inhibitor

• Indication: Adult ITP patients who have had an insufficient response to a previous treatment

• Start at 100 mg PO BID

• Must monitor BP, LFTs, diarrhea, neutropenia

• Avoid use of Fosamatinib with CYP3A4 inhibitors

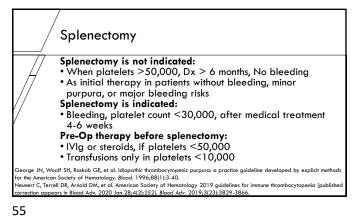
Bussel J, Arnold DM, Grossbard E, et al. Fostamatinib for the treatment of adult persistent and chronic immune thrombocytopenia:

Results of two phase 3, randomized, placebo-controlled trials. Am J Hemotol. 2018;93(7):921-930.

54

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Splenectomy: Definitive Therapy for ITP • Remember immunizations for pneumococcus, H. flu, and N. Utilized when more than low dose prednisone is needed for maintenance • Removes main site of platelet destruction AND anti-platelet antibody production • Decreased PAIg after splenectomy Carpatkin S, Strick N, Karpatkin MB, Siskind GW. Cumulative experience in the detection of antiplatelet antibody in 234 patients with idiopathis irombocytopenic purpure, systemic lupus erythematosus and other clinical disorders. Am J Med. 1972;52(6):776-785.

Ackillan R, Longmie RJ, Velenosky R, Smith RS, Craddock CG. Immunoglobulin synthesis in vitro by splenic tissue in idiopathic thrombocytopenic urpura. N Engl J Med. 1972;286(13):681-684.

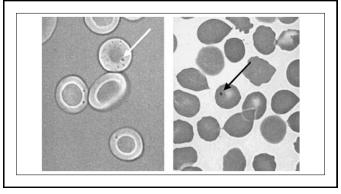
56

Splenectomy: Definitive Therapy for ITP (Cont.) Postpone in children until after 6 y.o. Remission is most likely in patients with: Short platelet survival High platelet turnover - rigii piarelet turnover • Post-op platelet count > 500,000 plts/ul • Good response to corticosteroids • Good response to IVIg • Patients < 60 y.o.

Use of IVIg Followed by Splenectomy Retrospective study of 30 patients who received IVIg, then had splenectomy 9 had poor response • 19/21 had good/excellent response to both • Response to high-dose IVIg is an important predictive factor for success of splenectomy .aw C, Marcaccio M, Tam P, Heddle N, Kelton JG. High-dose intravenous immune globulin an thrombocytopenic purpura. N Engl J Med. 1997;334(21):1494-1498.

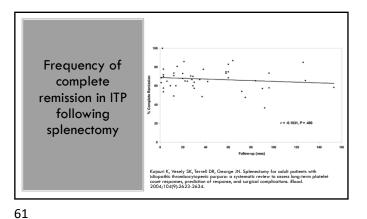
TAO CV, Kim SS, Soe JH, et al. Response to high-dost intravenous immune globulin as a val thronic idiopathic thrombocytopenic purpura patients. Am J Hematol. 2001;66(3):197-202.

57 58



Schwartz et al. 0.7 0.3 0.2 Fig. 1. Kaplan–Meier curve of remission rate (CR+PR) in 56 adults with ITP following splenectomy.

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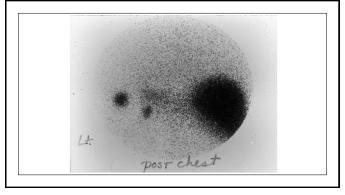
Laparoscopic Splenectomy • Longer procedure time • Earlier tolerance of fluids • Diminished need for narcotics • Shortened hospital stay • Open procedures if necessary

62

Splenectomy Guidelines Laparoscopic vs. Open Splenectomy • Recommendation: For medically suitable patients, both laparoscopic and open splenectomy offer similar efficacy (1C) Treatment of ITP after Splenectomy • No further treatment in asymptomatic patients with platelet counts greater than 30,000 (1C) Neunert C, Lim W, Crowther M, et al. The American Society of Hematology 2011 evidence-based practice guideline for immun

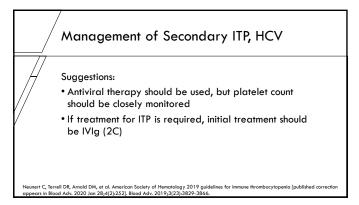
Accessory Spleens • Seen in 3/6 patients with good response to splenectomy • In refractory patients approximately 50% will respond to accessory splenectomy

63 64



Splenic Radiation • 11 Older ITP patients with AITP studied • 8 pts with secondary ITP studied • All pts given XRT 1-6 Weeks (Dose 75-1370 cGy) Of 11 older pts with ITP, 8 Responded
3 for >52 weeks
4 pts had platelet count increase for 8-25 weeks • 2 of 8 pts with secondary ITP responded Conclusion:
• Splenic XRT can be a safe means for raising the platelet count in patients with steroid resistant AITP

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Recommendations:

• Eradication therapy be administered in patients who are found to have H pylori infection (based on urea breath tests, stool antigen tests, or endoscopic biopsies) (1B)

Suggestions:

• Screening for H pylori be considered in patients with ITP in whom eradication therapy would be used if testing is positive (2C)

• H. Pylori is uncommon in N. America

67 68

Management of Secondary ITP, HIV • Steroids • IVIg • Anti-D (winRho) • Splenectomy • Effective • No difference in CD4 decline

Patients who do not respond to splenectomy

• Patients who recur after remission from splenectomy

69 70

High Dose Dexamethasone
 10 refractory ITP patients All had received >2 prior treatments Given dexamethasone 40 mg qdaily x 4 days every month 6 cycles Mean pre-tx platelet count: 12,000 Mean post-tx platelet count: 248,000 Minimal toxicity
Response of resistant idiopathic thrombocytopenic purpura to pulsed high-dose dexamethasone therapy (published correction Engl J Med 1994 Jul 28,331(4):283]. N Engl J Med. 1994;330(22):1560-1564.

Danazol

Dose: 50 mg qD to 800 mg qD

Decreases number of monocyte receptors

Alters clearance of Ig coated platelets

Alters clearance of Ig coated platelets

Alters (1983)308(23)1396-1399.

Alters (1983)308(23)1396-1399.

Effect of danazol in immune thrombocytopenic purpura. N Engl J Med. 1987;316(9):503-508.

Alters (1987)107(2):177-181.

Control RD, Kland C, Charle DB. Effect of danazol in immune thrombocytopenic purpura. N Engl J Med. 1987;316(9):503-508.

Alters (1987)107(2):177-181.

Control RD, Kland C, Charle DB. Effect of danazol in immune thrombocytopenic purpura. N Engl J Med. 1987;107(2):177-181.

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Danazol - Dose Related Problems Expensive Voice change Increased weight Increased LFTs • Other androgen consequences: hirsutism

Vinca Alkaloids After Splenectomy

- Vincristine 0.02 mg/kg or Vinblastine 0.1 mg/kg
- Platelet count rises 5-10 days later
- Series of 3 infusions
- Mechanism unknown, Possible macrophage inhibitor
- · Infusion better than bolus

Ahn YS, Harrington WJ, Mylvaganam R, Allen LM, Pall LM. Slow infusion of vinca alkaloids in the tree purpura. Ann Intern Med. 1984;100(2):192-196.

Cytotoxic Agents Cyclophosphamide (Cytoxan) Azathioprine (Imuran) Advantages: Salvage therapy · Usually no acute toxicity Disadvantages: • Up to 2-month lag period • Possible leukemia

Pulse Cyclophosphamide

- 20 refractory AITP patients
- Received 2-8 (mean 4.8) prior treatments
- Cytoxan 1.0-1.5 mg/m² (1-4 doses, mean 2.0)
- 13/20 (65%) had complete response
 - · 8 in remission, median 2.5 years later
 - Of 5 that relapsed, 2 were successfully retreated
- 4/20 (20%) had partial response
- 2 remain in PR 10 months & 4 years later
- 3/20 (15%) had no response

siner A, Gernsheimer T, Slichter SJ. Pulse cyclophosphamide therapy for refractory autoimmune thrombocytopenic purpura. Blooc 995,85(2):351-358.

76

74

Drugs Under Investigation: Rozanolixizumab

- Anti-neonatal Fc receptor (FcRn) recycling agent that decreases circulating pathologic IgG by blocking FcRn (the receptor primarily responsible for recycling IgG and prolonging its half-life)
- Studies in murine autoimmune disease demonstrated favorable results. Based on preliminary data in humans showing the effect of this agent on reducing circulating IgG
- Phase II multiple-dose study of rozanolixizumab in adult ITP (subQ administration) completed in 2019

 - 66 partients enrolled and given 4, 7, 10, 15, or 20 mg/kg SQ Infusion
 30% of partients previously received IPO-Ras
 Dose-dependent increases in platelet count were observed with peak median counts
 >100x10°/L in the 15 and 20 mg/kg groups
 - Dose-dependent decreases in mean serum IgG concentrations were observed by Day 8
- Headache most common adverse effect

nus Robok, Model Kafmierczek, Isidro Jarque, Vosile Musteata, Jozek Trelinák, Nichola Cooper, Peter Kiesling, Ute Massov, Franz Wolfering, Rose Snipes, Joan K nt Langdon, Brigh Holer, James B. Bussel, Stephen Joles, Rozanolkizamab, an Anti-Fañ Antibody: Pinal Results from a Phase II, Multiple-Dose Study in Patients with ny Immunic Thromotorypepenia. Blood 2016 j. 134

Drugs Under Investigation: Efgartigimod

- FcRn antagonist
- Multicenter Phase II Randomized Trial:
 - Randomized to receive placebo (n=12) vs. efgartigimod at 5 mg/kg (n=13) and 10 mg/kg (n=13)
 - Median number of previous treatments = 2
 - Patients permitted to concurrently receive corticosteroids, TPO-RA, or immunosuppressants
 - 46% on efgartigimod vs 25% on placebo achieved a platelet count \geq 50,000 on at least two occasions
 - 38% on efgartigimod vs 0% on placebo achieved a platelet count ≥ 50,000 for at least 10 consecutive days

ewland AC, Sánchez-González B, Rejtő L, et al. Phase 2 study of efgartigimod, a novel FcRn antagonist, in adult patients with imary immune thrombocytopenia. Am J Hematol. 2020;95(2):178-187.

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Drugs Under Investigation: Histone Deacetylase Inhibitors - Chidamide Novel therapeutic avenue for patients with refractory disease who need alternative therapies Pathophysiology: CD4*CD25*Foxp3* regulatory T (Treg) cells are reduced in ITP

- Use of low-dose HDACi restores Treg cell populations in patients w/ GVHD and other autoimmune conditions
- Led to hypothesis that these agents may be useful as therapy for ITP

hao HY, Ma YH, Li DQ, et al. Low-dose chidamide restores immune tolerance in ITP in mice and humans. Blood. 2019;133(7):730-42. doi:10.1182/blood-2018-05-847624

79

Drugs Under Investigation: Histone Deacetylase Inhibitors (Cont.) • A translational experiment showed that PMNs from patients with ITP treated w/ low-dose HDACi responded by increasing the number of Treg cells in culture • Chidamide tx of macrophages decreased macrophage phagocytosis of Ab-coated platelets • Supports role of HDACi's in modulating macrophage activity and providing a potential additional mechanism by which these drugs might be effective • Similar to therapy aimed at modulating monocyte and macrophage activity against platelets through the splenic tyrosine kinase (SYK) inhibitor fostamatinib

Zhao HY, Ma YH, Li DQ, et al. Low-dose chidamide restores immune tolerance in ITP in mice and humans. Blood. 2019;133(7):730-742. doi:10.1182/blood-2018-05-847624

80

Drugs Under Investigation: Histone Deacetylase Inhibitors (Cont.)

- Although they do not alter the production of antiplatelet antibodies, strategies intended to reduce phagocytosis hold promise for patients with refractory disease, many of whom have been treated with therapies that modulate platelet count without success
- Zhao et al study is the first to propose the mechanism of CTLA4 impairment in ITP as it relates to histone acetylation
- This study also provides some intriguing preliminary data for exploring mechanisms of loss of tolerance

Zhao HY, Ma YH, Li DQ, et al. Low-dose chidamide restores immune tolerance in ITP in mice and humans. Blood. 2019;133(7):730

-21 doi:1011102/Blood 2010 00 04/02

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Drugs Under Investigation: Bruton Tyrosine Kinase Inhibitor - PRN1008

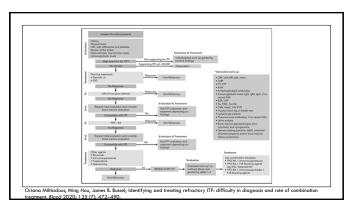
- Preclinical data: inhibits B-cell receptor-mediated activation of human B cells, Fc-receptor activation of immune cells, and dose-dependent reduction in platelet loss in mouse ITP model
- Does not interfere with platelet agonists unlike ibrutinib
- Ongoing Phase I/II of PRN1008 in patients with relapsed/refractory primary or secondary ITP
- Active in 33% of patients with refractory ITP across all doses tested

David J. Kuter, Ralph V. Boccia, Eun-Ju Lee, Merlin Efraim, Nikolay Tzvetkov, Jiri Mayer, Marek Tměný, Milan Kostal, Roman Holek, Vickie McDonald, Olga Bandman, Regan Burns, Ann Neale, Dolca Thomas, Nichola Cooper, Phase Jili, Open-Label, Adaptive Study of Oral Brutan Tyrosibe Kinson. Milhilbiror PRNI Ols in Protents vitils Relapsed Referaciory Primary or Secondary Immune Thombocytopenia. Blood 2019, 134 (Supplement, 1): 87.

82

Time to Platelet Count Response for ITP Treatments Initial response (days) Peak response (days) 1 to 3 2 to 7 Anti-D 1 to 3 3 to 7 Splenectomy 1 to 56 7 to 56 Prednisone 4 to 14 7 to 28 5 to 14 14 to 60 Romiplostim 14 to 180 7 to 28 14 to 90 7 to 42 Vincristine 7 to 14 7 to 42 14 to 90 28 to 180 30 to 90 30 to 180 F. F. (03/2009). Blood: St

purpura of adults and children: Report from an international working group. American Society of Hematology. doi:10.1182/blood-2008-07-16250



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Conclusions

- Do not allow treatment of ITP to be worse than the disease
- Identify whether there is secondary cause for ITP in refractory cases
- Rituximab and TPO-RAs recommended prior to splenectomy
- Several immunosuppressive and immune modulating agents are possible alternatives

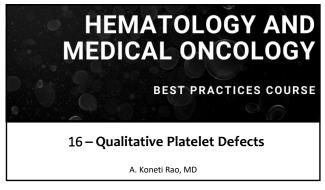


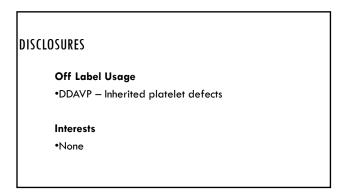
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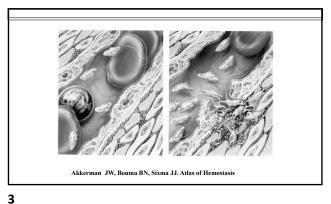
Qualitative Platelet Defects

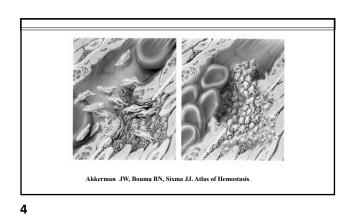
A. Koneti Rao, MD

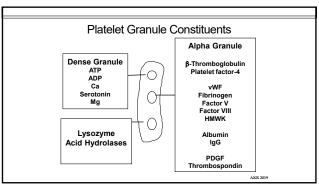
August 14, 2020

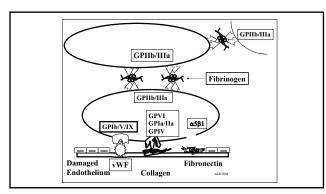


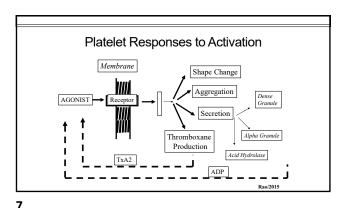


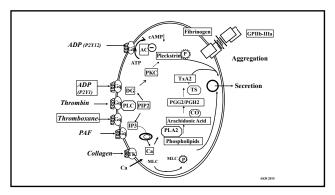


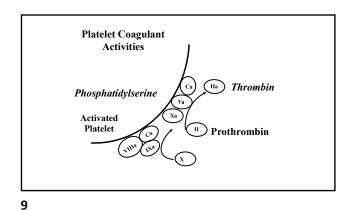








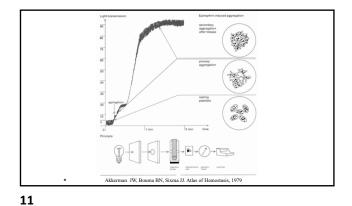


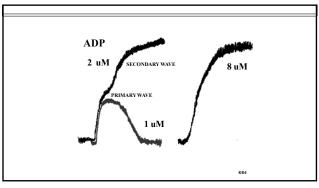


Platelet -Vessel Wall Disorders
Laboratory Tests

Platelet Counts
Bleeding time
Platelet Aggregation Studies
Platelet Secretion Studies
Platelet Function Analyzer (PFA-100)
New Generation Sequencing

10





Congenital Disorders of Platelet Function General Characteristics

- Mucocutaneous bleeding manifestations
- · Markedly variable in bleeding manifestations
- · Normal platelet counts in most, not all
- · Prolonged bleeding times
- Abnormal platelet aggregation/secretion responses



Congenital Disorders of Platelet Function

- Studies using new generation sequencing and whole genome sequencing show association with mutations in numerous genes in patients with impaired platelet number and function.
- These studies provide important new information into the pathogenesis of inherited platelet defects and platelet/megakaryocyte biology
- The diagnostic evaluation encompasses NGS in the work-up Lentaigne, C, et al, Blood 2016, 23:2814-23 Simeoni, I, et al, Blood 2016, 23: 2791-803

KR 8/2020

16

15

Inherited platelet disorders: toward DNA-based diagnosis

Late Megakaryopolesis
& Pro-platelet formation

HSC Early MK

Late MK Pro-platelet formation

Late MK Pro-platelet formation

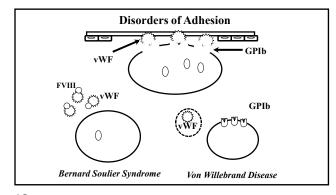
Platelet formation

Transcription
Regulation
Re

Congenital Disorders of Platelet Function

- 1. Defects in Platelet-Vessel Wall Interaction
 - von Willebrand Disease
 - Bernard-Soulier Syndrome
- 2. Defects in Platelet-Platelet Interaction
 - Congenital AfibrinogenemiaGlanzmann Thrombasthenia
- 3. Defects in Platelet Secretion and Signal Transduction
 - · Abnormalities of Granules
 - · Signal Transduction Defects
 - Abnormalities in Arachidonate Pathways and Thromboxane synthesis
- 4. Disorders of Platelet Coagulant-Protein Interactions
- 5. Miscellaneous Disorders

AKR 8/2014



Bernard Soulier Syndrome

- · Autosomal recessive inheritance
- · Deficiency of GPIb-IX-V complex
- · Thrombocytopenia; increased platelet size
- Absent or markedly reduced aggregation response to ristocetin
- Normal response to ADP, epinephrine, collagen
- Impaired response at low thrombin concentration

20

22

AKR 2012

von Willebrand Disease/ Bernard Soulier Syndrome

ADP P Epinephrine N Ristocetin N P N

19

Bernard-Soulier Syndrome and vWD

	ADP NL	Epi NL	Collagen NL	AA NL	Ristocetin
Aggregation		econd	rimary ai lary Wav resent		Decreased

Congenital Disorders of Platelet Function

- 1. Defects in Platelet-Vessel Wall Interaction
 - · von Willebrand Disease
 - Bernard-Soulier Syndrome
- 2. Defects in Platelet-Platelet Interaction
 - · Congenital Afibrinogenemia
- 3. Defects in Platelet Secretion and Signal Transduction
 - Abnormalities of Granules
 - Signal Transduction Defects
 - Abnormalities in Arachidonate Pathways and Thromboxane synthesis
- 4. Disorders of Platelet Coagulant-Protein Interactions
- 5. Miscellaneous Disorders

AKR 8/2014

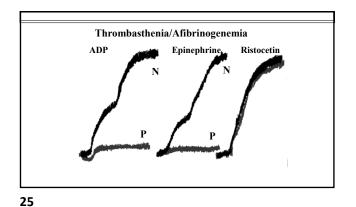
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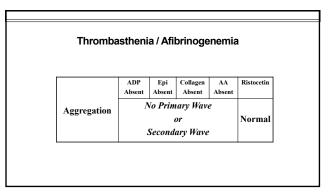
Disorders of Aggregation FIBRINOGEN Afibrinogenemia AGONIST R C Thrombasthenia AKR2010

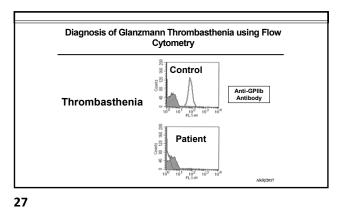
Glanzmann Thrombasthenia

- · Autosomal recessive inheritance
- · No bleeding in heterozygotes
- Absent or markedly reduced aggregation responses: no primary wave
- · Normal aggregation with ristocetin
- · Impaired clot retraction in most

AKR 2006

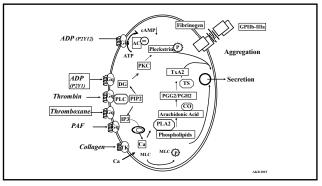






Congenital Disorders of Platelet Function 3. Disorders of Platelet Secretion/Signal Transduction a. Abnormalities of Granules Storage Pool Deficiency $(\delta, \alpha, \alpha\delta)$ Gray Platelet Syndrome (α) Quebec Platelet Disorder b. Signal Transduction Defects (Activation Defects) c. Abnormalities in Arachidonic Acid Pathways and in Thromboxane Synthesis AKR/2014

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Storage Pool Disease/ Secretion Defects Epinephrine

Congenital Disorders Of Platelet Function

- 3. Disorders of Platelet Secretion/ Signal Transduction
 - Abnormalities of Granules
 Storage Pool Deficiency (δ, α, αδ)
 Quebec Platelet Disorder
 - b. Signal Transduction Defects (Activation Defects)
 - c. Abnormalities in Arachidonic Acid Pathways and in Thromboxane Synthesis

AKR/201

32

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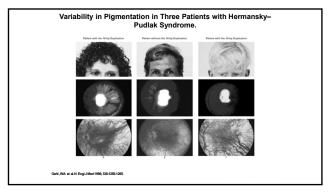
Disorders of Platelet Granules Storage Pool Deficiency

- *Platelets may be deficient in dense granules (DG), alpha granules or both types
- Gray Platelet syndrome: deficiency of only alpha granules
- •DG deficiency syndromic or non-syndromic

Hermansky-Pudlak syndrome (HPS)

- DG-SPD with Oculocutaneous albinism, nystagmus
- Autosomal recessive; Pocket in Puerto Rico
- Colitis and pulmonary fibrosis

AKR/2017

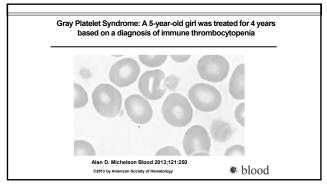


Disorders of Platelet Granules Gray Platelet Syndrome

- Deficiency of only α -granules
- Heterogeneous disorder
- Thrombocytopenia
- Gray appearance of platelets in the blood smear
- Genetic basis: mutations in NBEAL2 and other genes (RUNX1, GATA1)
- NBEAL2 protein is involved in vesicle trafficking
- Predisposed to myelofibrosis.

AKR/2019

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SPD/Secretion Defects/Activation Defects

Aggregation	ADP	Epi	Collagen	AA	Ristocetin
Aggregation	Only Pri Wave Pre		Decrease	ed	Normal

Secretion of Dense Granule Contents Decreased

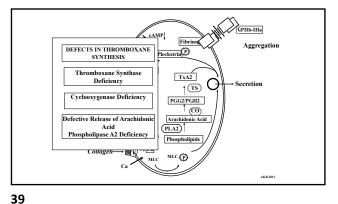
Disorders of Platelet Granules Quebec Platelet Disorder

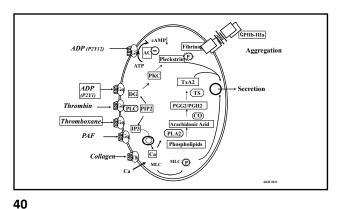
- · Autosomal dominant disorder of a granules
- · Normal to reduced platelet counts
- Decreased platelet Factor V, multimerin, vWF, fibrinogen, fibronectin, thrombospondin
- $\boldsymbol{\cdot}$ Abnormal proteolysis of α granule proteins
- · Increased urokinase plasminogen activator in platelets: increased proteolytic activity
- Defective aggregation to epinephrine; normal to impaired with ADP and collagen
- · Responsive to fibrinolytic inhibitors

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Receptor Defects ADP (P2Y12) ADP P2Y12 Thromboxane A2 GPVI, GP1a/IIa PKC Epinephrine ADP (P2Y1) G-Proteins Gaq, Gai2, Ga Effector Enzyme Defects Phospholipase C-β2 PKC-θ Thromboxane-Other Signaling Proteins CalDAG-GEFI Kindlin3

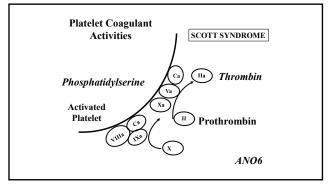
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Congenital Disorders of Platelet Function

- 1. Defects in Platelet-Vessel Wall Interaction
 - von Willebrand Disease
 - Bernard-Soulier Syndrome
- 2. Defects in Platelet-Platelet Interaction
 - · Congenital Afibrinogenemia
 - Glanzmann Thrombasthenia
- 3. Defects in Platelet Secretion and Signal Transduction
 - Abnormalities of Granules
 - Signal Transduction Defects
 - Abnormalities in Arachidonate Pathways and Thromboxane synthesis
- 4. Disorders of Platelet Coagulant-Protein Interactions
- 5. Miscellaneous Disorders



Congenital Disorders of Platelet Function

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Congenital Disorders Of Platelet Function Miscellaneous Disorders

- 1. Abnormalities of Cytoskeletal/ Structural proteins
 - Wiskott-Aldrich Syndrome
 - · 61-Tubulin Deficiency
- 2. Defects in Transcription factors: Thrombocytopenia and Platelet Function Defect

Songdej, N and Rao, AK, Blood, 2017;129:2873-2881

AKR 8/2019

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Congenital Disorders of Platelet Function Defects in Transcription factors

- RUNX1: Familial platelet defect –AML
 - Thrombocytopenia
 - Platelet function defects (Aggregation, secretion, SPD)
 - Predisposition to Acute Leukemia
- FLI1: Dysmorphic platelets- giant α-granules
- GFI1B

Songdej, N and Rao, AK, Blood, 2017;129:2873-2881

Congenital Disorders of Platelet Function: Management Principles

- · Individualized based on clinical features
- Platelet transfusions indicated in the management of bleeding episodes or surgical procedures
- Risk of developing specific anti-platelet antibodies
- · DDAVP (Desmopressin)
- · Recombinant Factor VIIa

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Congenital Disorders of Platelet Function **Therapeutic Options**

- DDAVP (Desmopressin)
 - Shortens the bleeding time in some patients with platelet function defects
 - · Induces a rise in plasma VWF. FVIII, tPA
- · Recombinant Factor VIIa

Acquired Disorders of Platelet Function

- Drugs
 - Aspirin
 - Nonsteroidal Anti-Inflammatory Agents

 - Antibiotics Other Drugs
- · Chronic Renal Failure
- · Bone Marrow Disorders Myeloproliferative Neoplasms
 - Dysproteinemias Leukemias; MDS
- · Cardiopulmonary Bypass
- · Liver Disease
- · Antiplatelet Antibodies
- · Acquired Storage Pool Deficiency

NSAIDS and Bleeding

- Inhibit cyclooxygenases reversible, short lived compared to aspirin
- Effect of indomethacin not detectable after 6 hrs.
- · Relative risk of bleeding varies between various NSAIDS
- Combined with other platelet inhibitors may make bleeding worse e.g SSRI's
- Interfere with the antithrombotic effect of aspirin

Pharmacologic Agents Effecting Platelet Function

NSAIDS

NSAIDS
Antibiotics
Cardiovascular Drugs
β-Adrenergic Blockers (propranolol)
Vasodilators (Nitroprusside, nitroglycerin)
Calcium Channel Blockers (Verapamil)

Quinidine

Psychotropic Drugs

Selective serotonin reuptake inhibitors (SSRIs) Tricyclic Antidepressants (Imipramine) Phenothiazines (Chlorpromazine)

Local (e.g., Dibucaine); General (e.g., Halothane)

Chemotherapeutic Agents Mithramycin, BCNU, Daunorubicin

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Radiographic Contrast Media

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Antibiotics and Platelet Function <u>β-Lactam Antibiotics</u> (Penicillins and Cephalosporins)

- _□ Share β-lactam ring structure
- Dose and duration related effect on bleeding time
- Generally with large doses; ill-patients
- Mechanisms: Associate with the membrane and inhibits platelet receptor-agonist interaction; Altered stimulus-response coupling
- □ Some (Moxalactam) Inhibit synthesis of vitamin K dependent coagulation factors

Pharmacologic Agents Effecting Platelet Function

Cardiovascular Drugs

Ardiovascular Drugs β-Adrenergic Blockers (propranolol) Vasodilators (Nitroprusside, nitroglycer Calcium Channel Blockers (Verapamil)

Psychotropic Drugs Selective serotonin reuptake inhibitors (SSRIs)

Tricyclic Antidepressants (Imipramine) Phenothiazines (Chlorpromazine)

Anesthetics
Local (e.g., Dibucaine)
General (e.g., Halothane)

Chemotherapeutic Agents Mithramycin, BCNU, Daunorubicin

Radiographic Contrast Media

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Selective Serotonin Re-uptake Inhibitors (SSRI's)

and Bleeding

• Platelets incorporate serotonin into dense granules - by uptake from plasma

 SSRIs inhibit platelet function – prolong BT, inhibit aggregation - secretion, prolong PFA CT.

· Associated with increased risk of GI bleeds, transfusions following orthopedic surgery, and hospital admissions for bleeding

Chronic Renal Failure

- Serious spontaneous hemorrhage is less common than previously encountered
- GI hemorrhage is a common complication
- Primary hemostatic defect: platelet dysfunction due to accumulation of substances (guanidinosuccinic
- Increased nitric oxide production leading to platelet inhibition
- Medications (e.g. aspirin) contribute to bleeding
- Bleeding time appears to correlate with clinical bleeding in some studies

Chronic Renal Failure: Therapeutic Approaches

- Dialysis (hemodialysis and peritoneal) effective in correcting the BT and platelet aggregation defect
- Delatelet transfusions
- ^a DDAVP shortens BT in 50-75% patients
- ¹ Cryoprecipitate shortens the BT in some studies
- Increase in Hct by RBC transfusions and erythropoietin is associated with correction of BT and diminished
- Conjugated estrogens (IV or oral) reported to shorten

Acquired Disorders of Platelet Function

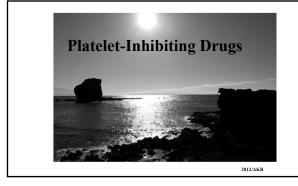
- Drugs

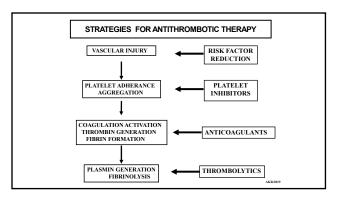
 - Nonsteroidal Anti-inflammatory Agents Antibiotics
- Other Drugs
- Chronic Renal Failure Bone Marrow Disorders
- Myeloproliferative Neoplasms
 - Dysproteinemias
- Leukemias; Preleukemia
- Cardiopulmonary Bypass
- Liver Disease

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- **Antiplatelet Antibodies**
- **Acquired Storage Pool Deficiency**

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Platelet Inhibiting Drugs

- Dipyridamole
- P2Y12 Antagonists
 - Thienopyridines
 Ticlopidine, Clopidogrel, Prasugrel (Effient)
 - Non-Thienopyridine
- Ticagrelor (Brilinta), Cangrelor (Kengreal)
- GPIIb/IIIa Inhibitors
 - Abciximab (ReoPro);
 - Eptifibatide (Integrilin);
- Tirofiban (Aggrastat) PAR1 Thrombin Receptor Antagonist: Vorapaxar
- Cilostazol (Pletal)

Cyclooxygenase **Arachidonic Acid** Ser ОН 529 соон PGG2, PGH2 OCOCH, Aspirin Cyclooxygenase Thromboxane A2 Ser OCOCH₃

Aspirin Pharmacokinetics

- · Rapidly absorbed in stomach and small intestine
- Peak levels at 30-40 min after ingestion
- · Plasma concentration decays: half-life of 15-20 min
- Inhibition of platelet function evident at 1 hour
- · Irreversibly inactivates COX in platelets
- · Acetylates the enzyme in megakaryocytes as well

AKR/2010

Aspirin as Antithrombotic Agent

- · Effective in a wide range of arterial disorders
- Reduces vascular deaths by 15% and vascular events by 30% in high risk patients
- · Effective when used long-term in doses 50-100 mg/day
- No convincing evidence of superiority of higher doses over lower doses
- Lower doses (300 mg) produce fewer GI side effects than higher doses (1200 mg)
- Bleeding risks increased with increasing aspirin dose (75-325 mg/d) with or without clopidogrel (CURE Trial)

AKD/2010

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Dipyridamole/Aspirin Combination (Aggrenox)

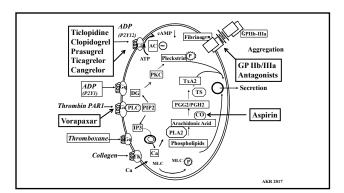
- Dipyrimidole pyrimido-pyrimidine derivative
- Increases platelet cAMP
- Inhibits cyclic nucleotide phosphodiesterase and blockade of adenosine uptake
- Formulation: Aspirin (25 mg); Extended release Dipyridamole (200 mg); twice daily
 European Stroke Prevention Study 2:
- 6602 patients with stroke or TIA
- · Randomized placebo, aspirin, Aggrenox

2 yr stroke rates: Placebo 15.2%

Aspirin 12.5% (Reduction 19%; p = 0.0009) Aggrenox 9.5% (Reduction 37%; p < 0.001)

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P2Y12 Antagonists: Thienopyridines Ticlopidine, Clopidogrel, Prasugrel

- Prodrugs
- Converted to active metabolites by liver cytochrome P450 isoenzymes
- · Irreversible P2Y12 Inhibitors

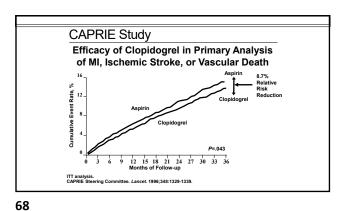
AKR 2019

P2Y12 Antagonists: Thienopyridines Clopidogrel

- The effects on platelet aggregation may be seen within 24 hours but are maximal after 4-6 days.
- The effects may last 4-10 days after discontinuation.
- The antiplatelet effects of thienopyridine and aspirin are additive.

AKR 2019

<u>C</u> lopidogrel versus <u>A</u> spirin in <u>P</u> atients at <u>R</u> isk of <u>I</u> schaemic <u>E</u> vents				
CAPRIE Study: Design				
Prospective, randomized, blinded				
19,185 patients with atherosclerotic vascular disease				
Recent ischemic stroke (≤6 mo) Recent MI (≤35 d) Established peripheral arterial disease				
Clopidogrel: 75 mg qd Aspirin: 325 mg qd				
Up to 3 y (mean, 1.6 y)				
304 in 16 countries, including the US				



Clopidogrel: Indications

- For reduction of atherosclerotic events (MI, stroke and vascular death) in patients with recent stroke, recent MI, or established peripheral arterial disease
- Acute coronary syndromes and coronary stenting (dual therapy with aspirin)
- Dose: 75 mg once daily; ACS loading dose 300 mg

AKR/2017

Prasugrel (Effient)

- Thienopyridine
- · Converted to active metabolite
- · More effective inhibitor of platelet ADP receptor
- TIMI-38 TRITON trial in ACS patients showed it More effective than clopidogrel (CV events); associated with greater bleeding.
- · Indications: ACS; stents (DAPT)

Wiviott et al. N Engl J Med 2007;357:2001-5

AKR/2019

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Ticagrelor and Cangrelor: Reversible P2Y12 Inhibitors

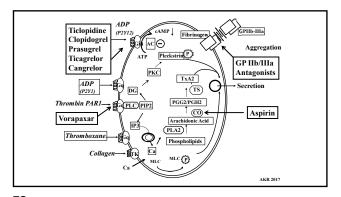
Ticagrelor

- Not a thienopyridine
- · Direct acting; More rapid onset of action;
- · Oral
- ACS Patients, as a part of dual therapy with aspirin
- · ATP analog; Not a thienopyridine
- · Direct acting; rapid onset of action
- · Given IV
- · PCI

AKR/2019

Clopidogrel: Adverse Effects

- · Bleeding events
- ${\bf \cdot} {\bf Thrombotic\ thrombocytopenia\ purpura}$



GPIIb/Illa Inhibitors

Abciximab (ReoPro)

- A chimeric human-mouse Fab specific for GPIIIa
- · Given IV, bolus and infusion
- Inhibits platelet aggregation for 18-24 hours

Eptifibatide (Integrilin)

- Synthetic peptide inhibitor; binds RGD site
- · Given IV bolus and infusion

Tirofiban (Aggrastat)

Small molecule inhibitor, nonpeptide

CD_2019

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GPIIb-IIIa Antagonists

- Indications: Acute coronary syndromes and in conjunction with percutaneous coronary interventions
- · Major side effects:
 - Hemorrhage
 - Thrombocytopenia

GPIIb-IIIa Antagonists and Thrombocytopenia

- · Occurs with all GPIIb-Illa Antagonists
- · May occur in patients not previously exposed
- · May be acute (within hours) or delayed
- Acute profound thrombocytopenia < 20,000/ul

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Thrombocytopenia with Tirofiban and Eptifibatide: Mechanisms

- · Antibody mediated and drug-dependent
- Drug binds to GPIIb-IIIa and induces neoepitope recognized by Ab
- "Naturally occurring" antibodies: present in persons not previously exposed

Rao 8/2007

Thrombocytopenia with Abciximab: Mechanisms

- Abciximab is a chimeric human-mouse Fab specific for GPIIIa
- Antibodies causing thrombocytopenia recognize murine component of chimeric Abciximab
- "Naturally occurring" antibodies
- In some patients thrombocytopenia onset delayed over several days

Rao 8/2007

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PAR-1 Antagonist: Vorapaxar (Zontivity)

- Thrombin receptor PAR-1antagonist
- · Inhibits platelet activation by thrombin
- Does not inhibit thrombin cleavage of fibrinogen
- · Orally active
- Indications: Preventing thrombosis in patients with atherothrombotic disease, PCI.

Cilostazol (Pletal)

- · Phosphodiesterase III inhibitor
- · Increases cAMP levels in platelets
- Inhibits platelet aggregation induced by a variety of stimuli, including thrombin, ADP, collagen, arachidonic acid, epinephrine, and shear stress
- Indicated for the reduction of symptoms of intermittent claudication (PAD)

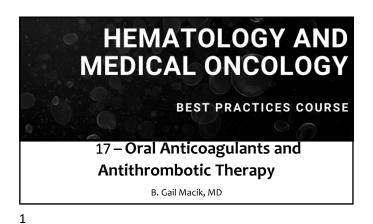
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Oral Anticoagulants and Antithrombotic Therapy

B. Gail Macik, MD

August 14, 2020



DISCLOSURES

Off-Label Usage

•Novel dosing for anticoagulants for which labelled indications are not available

Interests

- •Pfizer Speaker bureau for apixaban
- •Bristol-Myers Squibb Speaker bureau for apixaban

2

ANTICOAGULATION -BALANCE THE RISK Evidence-based guidelines address population effect, take years to develop and tweak

Doctors use guidelines to determine management of individual

3

- Guidelines lag behind current needs and do not address individual patient profile

Pay CLOT

THE "IDEAL" ANTICOAGULANT

- Oral, once daily fixed dose
- · Rapid onset and offset of action
- Predictable pharmacokinetics (PK), and pharmacodynamics (PD)
- Low propensity for food and drug interactions
- No side effects or organ toxicities
- · Wide therapeutic window
- · No need for monitoring
- · Rapidly reversible if bleeding

DRUG	PARENTERAL	ORAL
Vitamin K antagonist (Inhibits II, VII, IX, X, & PC,PS)	Warfarin – All indications	Warfarin – All indications (grandfathered)
Indirect inhibitors of Ila and / or Xa (require ATIII)	Heparin – All indications anti-lla = anti-Xa (grandfathered) LMWHs – varies by drug	NONE
	anti-Xa > anti-lla	
	Fondaparinux- anti-Xa only 1. Prevent DVT/PE TKA/THA	
	2. Treatment DVT/PE	

ANTICOAGULANTS- Approved US DRUG PARENTERAL Direct inhibitors Xa NONE RIVAROXABAN -1. Prophylaxis of DVT /PE - TKA/THA Reduce risk of stroke/systemic embolism in nonvalve afib 3. Treatment of DVT/PE 4. Reduce risk recurrent DVT/PE Prophylaxis of VTE in acutely ill medical 6. Reduce risk major CV event in CAD/PAD APIXABAN -1 Prevent stroke/embolism in nonvalve afib Reduce risk DVT/PE - TKA/THA 3. Treatment of DVT/PE 4. Reduce risk recurrent DVT/PE

B. Gail Macik, MD

DRUG	PARENTERAL	ORAL
Direct		EDOXABAN -
inhibitors Xa (cont)		Treatment of DVT/PE after 5-10 days of parenteral anticoagulant
		Prevent stroke/embolism in nonvalvular afib
		Betrixaban – (discontinued)
		Prevent VTE in adult patients hospitalized for acute medical illness at risk for VTE due to moderate or severe restricted mobility and other risk factors for VTE.

DRUG	PARENTERAL	ORAL
Direct inhibitors ila	Argatroban Bivalirudin	DABIGATRAN- 1. Prevent stroke/emboli in nonvalve after 5-7 days of parenteral anticoagulant 3. Reduce Risk of recurrent DVT/PE 4. Prevent DVT hip surgery

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WARFARIN

- * Began use in the US in 1951 (grandfathered by FDA)
- * Half-life of 36-42 hours

9

- Circulates bound to plasma proteins
- Only FREE warfarin is active (major drug interaction)
- Inhibits vitamin K pathway DOES NOT INHIBIT but LOWERS factors II, VII, IX, X, PC, PS
- Intra- & Inter-patient variable dose responses
 - * pharmacokinetics absorption or metabolism
 - * pharmacodynamics different dose response
 - pharmacogenetics genetic variation response

WARFARIN

- Mixture of two active isomers, (R) and (S)
- ❖ (S) is 3-5 fold more potent than R

8

10

- Both isomers are metabolized by multiple cytochrome P450 (CYP450) enzymes in the liver
- Reduced clearance of (S) leads to warfarin sensitivity, less drug but more activity
- CYP2C9*2 requires a 14-20% reduction and CYP2C9*3 variant a 21-49% reduction of drug
- CYP2C9 variants had an approximately 3-fold higher risk of bleeding

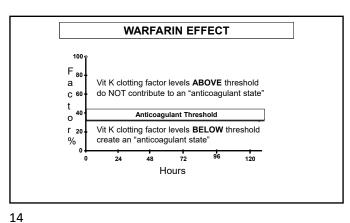
WARFARIN

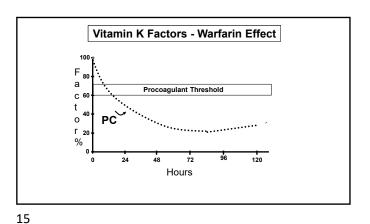
- Warfarin inhibits vitamin K epoxide reductase (VKOR), an enzyme that recycles reduced vitamin K, through interaction with subunit protein 1 (VKORC1)
- VKORC1 genetic variants effect warfarin dosing, decreasing OR increasing dose requirement
- Warfarin dosing algorithms based on age, height or weight, CYP2C9*2, CYP2C9*3, and VKORC1 (1639GA) genotypes estimate the warfarin dosage for patients
- Use of pharmacogenetic dosing has been advocated by the FDA (but is not practical due to expense/time)

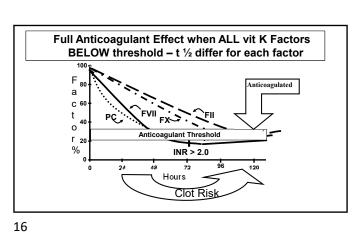
Factors That Influence Warfarin Dose Effect

- * Altered vitamin K balance due to
 - ✓ Diet
 - √ Biliary obstruction (no bile to absorb fat soluble vitamin)
 - √ Change in gut flora (prevents conversion of dietary vit K)
- Impaired liver function decreased clotting factor production
- Hypermetabolic states fever or hyperthyroid





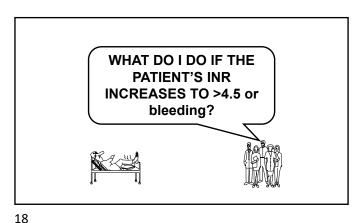


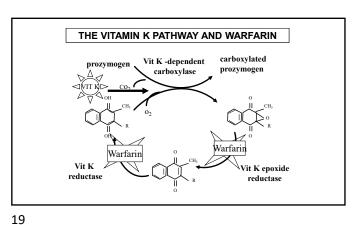


INITIATION OF WARFARIN RULE 1:

While initiating warfarin give another immediately acting anticoagulant for AT LEAST 5 days UNTIL the INR > 2.0 on 2 consecutive days (the time it take Factor X & II to reach anticoagulant levels which is about 2 days after FVII nadir which IS the INR)

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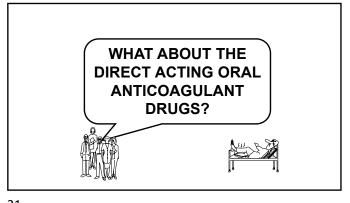


TREATMENT OPTIONS TO REVERSE WARFARIN

- Vitamin K (assuming good liver function)
 - IV effective within 6-12 hours (diluent may cause reaction)
 - ♣ PO effective 12-24 hours

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- Low dose (1-2.5 mg) reverses effect and less likely to interfere with re-initiation of warfarin
- Avoid SQ/IM studies suggest erratic absorption
- ❖ FFP Not very effective due to low concentration of vit K factors/mL of plasma, reserve for bleeding patient or patient with high bleeding risk WHO CAN TOLERATE VOLUME
- ❖ Prothrombin Complex Concentrates II/IX/X +/- VII (3 vs 4 factor) concentrate, AVOID as routine therapy due to thrombotic risk. Use "4-factor" product not "3 factor" which does not have factor VII and will not change the PT INR
- * rFVIIa Rarely use. Associated thrombotic risks.

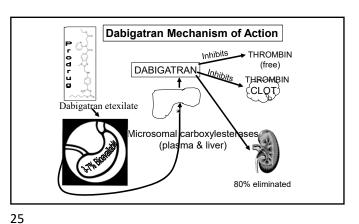


	Apixaban	Rivaroxaban
	Anti-Xa	Anti-Xa
Effective life Half-life	10-14 hours 8-15 hours	20-36 hours 6-9 hours
Elimination	~27%	~66 % unchanged & inactive metabolite
Reversible	Yes	Yes
Monitor	Not Yet	Not Yet
Peak effect	3-4 hours	2-4 hours
Absorbed	Small Bowel/Colon	Stomach(w /food)

22 21

	Edoxaban	Dabigatran
	(anti-Xa)	(anti-IIa)
Effective life	24 hours	12-17 hours
Half-life	6-11 hours	12-17 hours
Elimination	50%	~80%
Reversible	Yes?	YES
Monitor	Not yet	ECT/dTT?
Peak effect	1-3 hours	<2 hours
Absorbed	Proximal Gut	Proximal Gut

DOAC Characteristics			
Betrixaban ?? (discontinued 2020)			
	(anti-Xa)		
Effective life	19 to 27 hours		
	8		
Elimination	Mostly gut,		
Reversible	Yes		
Monitor	Not Yet		
Peak effect	3-4 hours		
Absorption	Effected by fatty food		



Dabigatran (Pradaxa)-Oral **Antithrombin**

Key Points

- >Peak level occurs 1-2 hrs post dose with/without food
- ➤The oral bioavailability of dabigatran INCREASES by 75% when the pellets are taken without the capsule shell.
- >PRADAXA capsules SHOULD NOT BE BROKEN, CHEWED, OR OPENED BEFORE ADMINISTRATION.
- >Must have acid environment for absorption- packaged

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Dabigatran (Pradaxa)-Oral Antithrombin

Gastrointestinal Adverse Reactions

- > Patients on PRADAXA 150 mg had an increased incidence of GI adverse reactions (35% vs 24% warfarin)
- > These were commonly dyspepsia (including abdominal pain, upper abdominal pain, abdominal discomfort, and epigastric discomfort) and gastritis-like symptoms (including <u>GERD</u>, esophagitis, erosive gastritis, gastric hemorrhage, hemorrhagic gastritis, hemorrhagic erosive gastritis, and GI ulcer)
- > Likely due to tartartic acid in capsule to aid absorption not endogenous stomach acid

DABIGATRAN DOSING

- > Prevention of stroke and systemic embolism nonvalvular a-fib
 - CrCl >30 mL/min: 150 mg PO BID
 - CrCl 15-30 mL/min: 75 mg PO BID
 - CrCl <15 mL/min or dialysis: not recommended
- > Dosing modifications (atrial fibrillation)

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- Renal impairment and co-administration with P-gp
- · CrCl 30-50 mL/min and coadministration with dronedarone or ketoconazole: Consider reducing dose to 75 mg BID (dose adjustment is not necessary when coadministered with other P-gp inhibitors)
- CrCl <30 mL/min with concomitant use of any P-gp inhibitor: Avoid coadministration

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DABIGATRAN DOSING

- > Indicated for treatment of deep vein thrombosis (DVT) and pulmonary embolus (PE) AFTER treatment with a parenteral anticoagulant for 5-10 days. Also to reduce the risk of recurrence of DVT and PE in patients who have been previously treated
 - CrCl >30 mL/min: 150 mg PO BID
 - CrCl ≤30 mL/min or on dialysis: AVOID (no data-highly
 - CrCl <50 mL/min with concomitant use of P-gp inhibitors: Avoid coadministration
- > Tablets available 75mg, 110 mg (for prevention), 150 (for

Dabigatran (Pradaxa)-Oral Antithrombin

Gastrointestinal Adverse Reactions

TAKE HOME PPI/antacid may interfere doesn't help

Oral Anticoagulants and Antithrombotic Therapy B. Gail Macik, MD

Overview of Rivaroxaban (Xarelto)

- Rapid onset of action 1 hr, peak 2-4 hrs
- > Half life 5-9 hrs at steady state, longer in elderly
- Elimination: Renal(66%)
- Dose 20 mg/d CrCl > 50, 15mg/d CrCl 30-50, NOT recommended CrCl < 30 and contraindicated CrCl < 15
- > FOOD IMPROVES absorption (66vs76%) for 20mg
- > Absorbed in stomach, should not be given by G/J-tube
- ▶ Bioavailability high 80-100% for 10mg dose, 70-80 for 20
- No dosage adjustment with mild liver impairment but not recommend for moderate / severe liver impairment

RIVAROXABAN DOSING

- 1. Reduction risk of Stroke/embolism non-valve a-fib
- CrCl > 50: 20 mg daily with evening meal
 CrCl < 50: 15 mg daily with evening meal
 Treatment of DVT and/or PE
- CrCl> 15: 15mg TWICE daily X 21 days then 20 mg DAILY + food CrCl< 15: Avoid use
- Reduction in Risk of Recurrent DVT/PE with continued risk for DVT/PE
- CrCl> 15: After 6 months of full dose then 10 mg daily +/- food
- Hip Replacement Surgery
 CrCl > 15: 10 mg daily X 35 days +/- food, start 6-10 hrs post surg
- CrCl < Avoid Use
- Knee Replacement Surgery
 CrCl > 15: 10 mg daily for 12 days +/- food, start 6-10 hrs post surg
 CrCl < 15: Avoid Use
- 6. Reduction Risk of Major CV Events in chronic CAD/PAD
- 2.5 mg TWICE DAILY PLUS QD ASPIRIN (75-100) any CrCl, +/- food
 7. Prophy of VTE in acute ill med patient at risk for VTE and low risk bleed
 - CrCl > 15: 10 mg daily in hospital and post D/C 31-39 days

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Rivaroxaban (Xarelto) - Warnings and Precautions (SAME FOR ALL DOACS)

- 1. Premature discontinuation of the drug increases risk of thrombotic
- 2. Spinal / epidural hematomas may occur with neuraxial anesthesia or spinal puncture/injury
- 3. Bleeding is the most common adverse reaction and may be serious or
- 4. Use with caution in pregnant women, little data with potential for obstetric hemorrhage
- 5. Not recommended for prosthetic heart valves
- 6. Increase Risk of Thrombosis in Patients with Triple Positive Antiphospholipid Syndrome use not recommented

KEY POINTS APIXABAN (Eliquis)

- » Bioavailability approximately 50% not effected by food
- > Maximum concentrations 3-4 hours after oral intake
- > Prolonged absorption and short clearance half-life result in effective half-life ~12 hr = twice a day dose
- > Dual modes of elimination: feces and renal(27%), FDA approval for use in renal insufficient for VTE indication
- > Absorbed distal small bowel/colon feeding tube OK
- > Avoid in moderate-severe liver dysfunction

33 34

APIXABAN DOSING

- Reduction risk of Stroke/embolism non-valve a-fib
 5 mg twice a day

 - 2.5 mg twice a day for patients with 2 of the following Age > 80
 Body weight < 60 kg
 Creatinine > 1.5
 Age > 1.5
- Creatmine > 1.5
 Treatment of DVT and/or PE
 Initial therapy 10 mg twice a day for 7 days (NO PARENTERAL NEEDED)
 Maintenance therapy 5 mg twice a day
 No dose adjustment for age or creatinine
 Reduction in Risk of Recurrent DVT/PE with continued risk for DVT/PE
- After at least 6 months of full therapy, 2.5 mg twice a day Hip / Knee Replacement Surgery
- Start 12-24 hrs after surgery
- 2.5 mg twice a day
 Treat hip for 35 days and knee for 12 days

APIXABAN ADVERSE EVENTS

- > Bleeding most common but less than comparator
- >Other reported side effects

36

- ✓ GI symptoms: nausea (3%), increase LFTs
- ✓ Reported at < 1%: syncope, rash, anaphylaxis</p>

KEY POINTS EDOXABAN (Savaysa)

- > Bioavailability ~50% not effected by food
- > Maximum concentrations 3-4 hours after oral dose
- > Dual modes of elimination: renal 50%
- > Absorbed proximal gut feeding tube unknown.
- Unusual finding, more strokes in afib patients with Creatine Clearance > 95 (subset analysis afib only)
- Only p-glycoprotein not CYP3 / CYP4 like others, less drug interactions

EDOXABAN ADVERSE EVENTS

- ➤Bleeding most common, similar to warfarin
- ➤Other reported side effects
 - ✓ Rash 3.6%
 - ✓ Increase LFTs 7.8%
 - ✓ Anemia 1.7%

37 38

EDOXABAN DOSING

- ➤Treatment of NVAF: Assess CrCL before initiating therapy - 60 mg once daily with CrCL >50 to ≤ 95 mL/min
 - Do NOT use in patients with CrCL > 95
 - 30 mg once daily CrCl 15 to 50
- Treatment of DVT and PE: AFTER 5-10 days of another drug
 - 60 mg once daily
 - 30 mg once daily for CrCL 15 to 50 mL/min or weight < 60 kg or with certain P-gp inhibitors

Betrixaban Key Points

NOTE: MAY NO LONGER BE MARKETED

- ➤Only medical prophylaxis as indication, no treatment
- Mostly cleared GI 82-89%, biliary; therefore interruption of biliary system may effect clearance
- ➤ Longest half life 37 hours / 20 hours effective
- ➤ Least hepatic metabolism
- ➤ Fatty food decreases absorption
- ➤ Bioavailability 34%

39 40

MONITORING OF DOACS

➤ Dabigatran my be monitored by ECT or dilute Thrombin Time but difficult to acquire

41

➤ Apixaban/rivaroxaban/edoxaban may be monitored with anti-Xa level BUT therapeutic range not established DO NOT USE LMWH RANGES!!

TAKE HOME MESSAGE
PT and PTT prolong but highly variable
and should not be used to assess
anticoagulant effect

IS BRIDGING NECESSARY FOR PROCEDURES? - NO

- ✓ The clinically effective life of DOAC are about the same as enoxaparin. Why bridge to similar drug?
 - Renal insufficiency clinically prolongs effect of Lovenox/Pradaxa/Xarelto but not Eliquis
 - Lovenox has advantage of reliable lab monitor
 - Reversing drugs now approved for all DOACs but may be difficult to obtain
- ✓ For Pradaxa hold 1-2 days CrCl > 50; 3-5 CrCl > 50
- ✓ For Xarelto hold at least 24 hours

- ✓ For Eliquis High risk bleeding 48hr; low risk 24 hours
- Key: factor in surgical risk of bleeding, patient risk of clotting, renal function – recent review suggests same 24-48 hr hold before for all DOAC

Oral Anticoagulants and Antithrombotic Therapy B. Gail Macik, MD

Bleeding Risk Associated with DOAC

✓ ALL ANTICOAGULANTS HAVE A RISK OF BLEEDING

- ✓ All DOAC trials same or less bleeding than warfarin
- ✓ DOACs are associated with ~50% less ICH than warfaring
- ✓ Meta-analysis of 19 trials, DOAC associated with a significantly lower risk of major bleeding than warfarin (odds ratio = 0.77; 95%CI: P+0.003)
- ✓ Pooled analysis of 42 trials found no significant difference in major bleeding between DOACS and all comparators include warfarin, LMWH, and aspirin
- ✓ For acute DVT lower risk of bleeding DOACs vs warfarin
- ✓ For afib no significant difference in major bleeding DOACs vs warfarin/aspirin

TREATMENT OF BLEEDING DABIGATRAN

- Idarucizumab (Praxbind) has 350 X greater affinity for dabigatran than dabigatran for thrombin
- ✓ Reversal effects immediately after administration
- ✓ 100% reversal in first 4 hours (9/10 patient achieved complete reversal)
- May have abnormal coag tests 12-24 hours after administration
- ✓ Dose 5 grams (100 mL), second dose rarely indicated
- ✓ Hereditary Fructose Intolerance warning 4 g sorbital
- Most frequent reported side effect HA, hypokalemia 9/123), delirum (9/123) constipation 8/123) fever (7/123 and pneumonia (7/123)

43 44

ANDEXANET ALFA - REVERSING AGENT FOR ORAL Anti-Xa INHIBITORS

- · Andexanet Alfa (Andexxa) is a recombinant modified human factor Xa
- · Indicated for use with apixaban or rivaroxaban only
- Accelerated approval based on change from baseline in anti-FXa activity in healthy volunteers not evidence of clinical efficacy

WARNING

- May be associated with serious and life-threatening adverse event
 - Arterial and venous thromboembolic events
 - · Ischemic events including MI and ischemic stroke
 - Cardiac arrest
- Sudden death

 Regulation or incomplete
- Re-elevation or incomplete reversal can occur
- Monitor for thromboembolic events and start anticoagulation when medically appropriate

ANDEXXA DOSING Bolus Followed by Infusion

DOSE	Initial IV Bolus	IV Infusion post Bolus	# of 200mg Vials
Low	400 mg @ 30	4 mg/min up to 120 min	5 vials
Dose	mg/min	(480 mg)	(2 bolus + 3 infusion)
High	800 mg @ 30	8 mg/min up to 120 min	9 vials
Dose	mg/min	(960 mg)	(4 bolus + 5 infusion)

- Anti-Xa activity decreases rapidly after start of bolus / infusion
- Anti-Xa activity increases about 2 hours after drug bolus or infusion completed back to placebo levels
- Anti-Xa activity 2 hours after completion of drug corresponds to clearance of anticoagulant drug

45 46

ANDEXANET ALFA – DOSING

(revised 3/2020)

· Dose determined by

47

- Drug patient takingDose patient taking
- Timing of last dose

FXa Inhibitor Drug	Drug Dose	Last Dose < 8 Hours or Unknown	Last Dose > 8 hours
Rivaroxaban	< 10 mg	Low Dose	
Rivaroxaban	> 10 mg or unknown	High Dose	Low Dose
Anivahan	< 5 mg	Low Dose	Low Dose
Apixaban	> 5 mg or unknown	High Dose	

TREATMENT OF BLEEDING ON DOACS WHEN REVERSING AGENT NOT AVAILABLE

- FFP/PCC do NOT reverse but replace clotting factors consumed with bleeding or "dilute" drug
- PCC/rFVIIa do NOT reverse but cause a "hypercoagulable state" that may clot bleeding site at risk of unwanted thrombotic event
- ✓ Antifibrinolytics do NOT reverse but may slow clot break down and slow bleeding
- Dabigatran may be dialysed but inefficient, time consuming and requires line no one will place

Overview of Clinical Scenarios

- ✓ Small studies in morbid obese patients suggest DOACs may be used same dose but still no large prospective trials and ISTH guidelines recommend avoidance in patients > 120kg OR if used that trough level checked after 5th dose
- Recent addition to package inserts for DOACs recommend against use in antiphospholipid syndrome particularly "triple positive" APS (NOTE: these patients may also fail warfarin but warfarin is the recommended drug)

Extended Treatment to Reduce VTE risk

- > Apixaban for Reduction of Risk of Recurrence
 - 2.5 mg and 5 mg both significantly decreased risk
 - 2.5 mg was similar to placebo for bleeding risk
 - 2.5 mg twice a day for long term secondary prevention approved by FDA
- ➤ Rivaroxaban for Reduction of Risk of Recurrent VTE*
 - ✓ 20 mg & 10 mg vs aspirin 100mg after 6-12 mon
 - ✓ Both doses significantly decreased thrombotic events compared to aspirin
 - ✓ No significant difference in bleeding.
 - √ 10 mg daily approved by FDA for long term prevention after > 6 months treatment dose

*Weitz, J, et al. Rivaroxaban or Aspirin for Extended Treatment of Venous Thromboembolism, N Engl J Med 2017; 376:1211-1222

50

52

49

Reduction of Risk of Major Cardiovascular Events in Patients with Chronic CAD or PAD – COMPASS TRIAL

- Compared
 - Rivaroxaban 5 mg twice daily
 - Rivaroxaban 2.5 mg twice daily with aspirin 100 mg daily
 - Aspirin 100 mg once daily
- Primary outcome composite of cardiovascular death, stroke, or myocardial infarction.
- Study stopped for superiority of the rivaroxaban-plus-aspirin group after a mean follow-up of 23 months
- > Most frequent adverse reactions Bleeding events
 - 2.7% for rivaroxaban 2.5 twice daily + 100mg aspirin daily
 - 1.2 % for aspirin 100 mg once daily





Tune in again for more
- Cascade of Caveats during the next COAG
HOUR

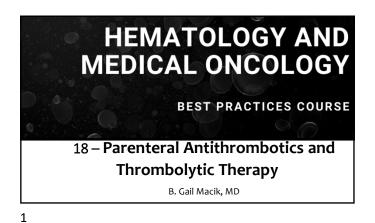
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Parenteral Antithrombotics and Thrombolytic Therapy

B. Gail Macik, MD

August 14, 2020



DISCLOSURES

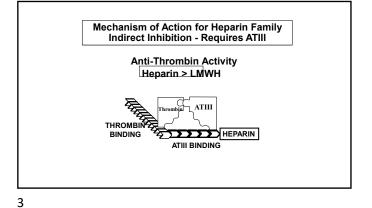
Off-Label Usage

•Novel dosing for anticoagulants for which labelled indications are not available

Interests

- •Pfizer Speaker bureau for apixaban
- •Bristol-Myers Squibb bureau for apixaban

2



Mechanism of Action for Heparin Family
Indirect Inhibition - Requires ATIII

Anti- Xa Activity
Fondaparinux > LMWH → Heparin

ATIII Factor
Xa

ATIII BINDING

4

Mechanism of Action for Heparin Family Indirect Inhibition - Requires ATIII

CHAIN LENGTH DETERMINES PROFILE

❖ Less than 18-saccharides = anti-FXa only

❖ 18 or more saccharides = anti-Ila + anti-Fxa

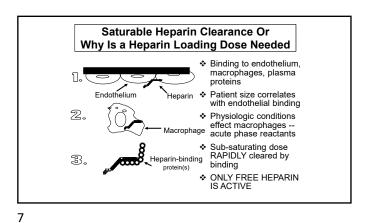
Source Of Heparin

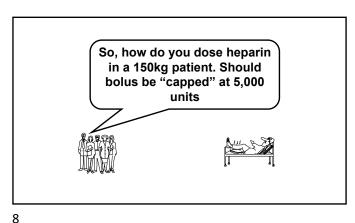




- Biologic Product "infectious??"
- Variability Lot to Lot only 1/3 of heparin in a vial is active
- "Refined" to LMWH all brands differ in characteristics/profile

5





Tidbits Regarding Heparin Therapy

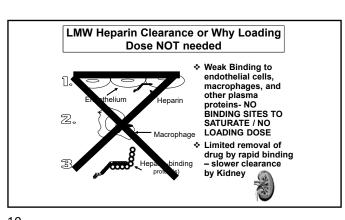
* Bolus/infuse by nomogram (blood vessels in fat can bind/clear heparin!)

* use best dry weight (water doesn't have blood vessels!)

* 150kg = bolus (80/kg) 12,000 units & drip (18/kg) 2700 units (dry weight)

* Patient may be "sensitive/resistant" to heparin, monitor PTT / anti-Xa every 6-8 hours to increase/decrease dose until therapeutic then once a day

* Dose may decrease as "acute phase reaction" of patient clears



9 10

Myths Regarding LMWH 1. Never need monitoring – WRONG! Patients with changing kidney function Very large, very small, and pregnant patients 2. Bleeding is not a problem with LMWH - WRONG! Treatment dose - bleeding equivalent to heparin LMWH only partially reversible with protamine LMWH have longer t 1/2 – longer bleeding risk 3. HITT not a problem with LMWH - WRONG! Less likely if ONLY LMWH (no UFH) given but still associated with HITT

Fondaparinux (ARIXTRA)
Indirect Inhibition Factor Xa - Requires ATIII

Pure Anti- Xa Activity

ATIII

Factor Xa

Synthetic Pentasaccharide
Antithrombin Binding Site Only

11 12

. Cross reacts with antibodies caused by UFH

FONDAPARINUX CLEARANCE

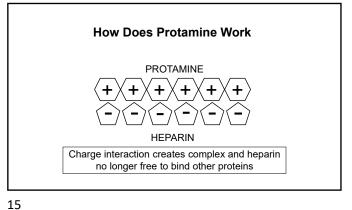


- Highly cleared by Kidney
- * Must have normal Kidney function to use the drug

Chain Length Determines Properties

- * The longer the heparin chain then the
 - > Greater the antithrombin activity
 - More charge (= better target for protamine)
 - > Greater binding to endothelium/ macrophages with rapid clearance
 - > More effect on PTT
- The shorter the heparin chain
 - > OPPOSITE E OF ALL POINTS ABOVE

13 14



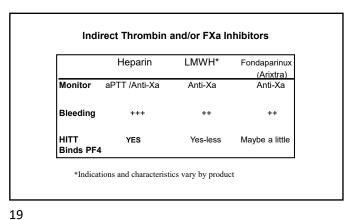
HEPARIN (Like) FAMILY PROTAMINE ANTI IIa:ANTIXa REVERSAL UNFRACTIONATED HEPARIN (-)(-) (-) (-) (-) (-) (-) (-) (-) (-) Excellent Some 1:2 **ENOXAPARIN** (-) (-) (-) 1:4 ALL Anti-Xa FORGET IT

16

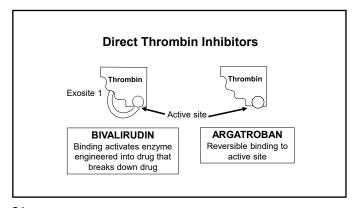
How to Reverse Heparin (family) Anticoagulation?

- > The Heparin/pentasaccharide family PROTAMINE
 - ✓ Heparin is the only rapidly reversible drug
 - ✓ LMWH may be 20-30%, Fragmin>Lovenox>>>Arixtra
 - ✓ CAUTION Do you know your protamine?
 - Has weak anticoagulant effect AND prolongs PT/PTT at high dose
 - Giving high dose protamine to "MAYBE" reverse Lovenox / Arixtra may worsen coagulopathy/PTT
 - Protamine has a shorter half-life and interaction with Heparin/LMWH is reversible; therefore there is a rebound anticoagulant effect as protamine cleared faster than high dose heparin or LMWH

	Heparin	LMWH*	Fondaparinux (Arixtra)
Clinical Effect	1-6 hrs	12-24*hrs	17-24 hrs
Excretion	Endothelium		
Δ T ½	Higher dose longer t ½	Kidney function	Kidney function
Reversible?	Protamine HIGHLY	Protamine* MINIMAL	Little if any





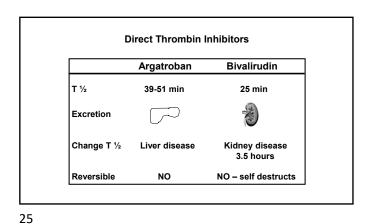


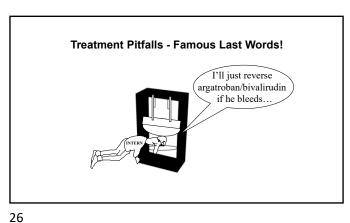
Direct Thrombin Inhibitors Do NOT need cofactor (ATIII) for activity ❖ Do NOT bind to endothelium or other proteins . Do NOT have known drug interactions Do NOT cause thrombocytopenia ARE NOT REVERSIBLE DO inhibit clot bound thrombin DO effect platelet function

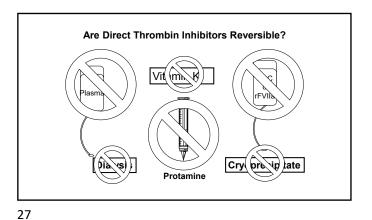
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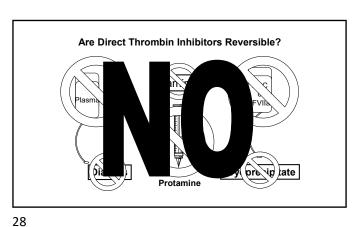
	Argatroban	Bivalirudin
Product	Synthetic arginine analogue	Synthetic peptide
FDA	Approved for HITT	Approved for PC
MW	506	2180
Clinical Effect	Direct anti-lla Irreversible	Direct anti-lla Self-destructs

	Argatroban	Bivalirudin
Monitor	aPTT 1.5-3.0	ACT 250-350
Effects PT	YES	Yes
Bleeding	++	++

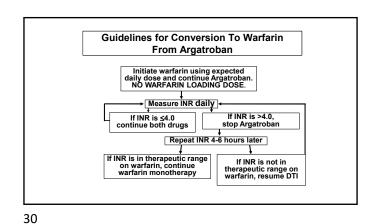






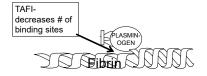


Treatment Pitfalls - Famous Last Words! DTIs EFFECT ALL CLOT Patient is on DTI not BASED ASSAYS heparin I'll do hyp Beware False Results w/u before Warfarin **FIBRINOGEN** is started PT/PTT RVVT Silica Clot time StaClot **Protein C activity** Protein S activity Reptilase Thrombin clot time 29



FIBRINOLYTIC CASCADE

- · Plasminogen binds to fibrin as it polymerizes.
- Number of plasminogen binding sites controlled by Thrombin Activator Fibrinolysis Inhibitor (TAFI)
- The more binding sites, the more plasminogen & the faster clot lysis



FIBRINOLYTIC PROCESS

- Fibrin settles on injury site, forms clot that supports vessel repair
- When fibrin settles on surrounding intact endothelium then it initiates the fibrinolytic process to prevent propagation of clot past site of injury

31 32

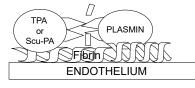
FIBRINOLYTIC CASCADE

- Fibrin provokes release of tissue Plasminogen Activator (tPA) and Single Chain Urokinase (Scu-PA) from intact endothelium
- Fibrin catalysis its own destruction.

TPA OGEN OGEN OGEN ENDOTHELIUM

FIBRINOLYTIC CASCADE

 tPA and/or Scu-PA "clips" plasminogen and creates the active enzyme Plasmin.



33 34

FIBRINOLYTIC CASCADE

- · Plasmin clips off pieces of fibrin
- The smallest subunit that cannot by broken is the d-dimer.



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FIBRINOLYTIC CASCADE

• As tPA released from Fibrin it is bound and neutralized by Plasminogen Activator Inhibitor (PAI)

• As Plasmin released from Fibrin pieces it is bound and neutralized by alpha 2 plasmin inhibitor (aka alpha 2-antiplasmin)

PAI

PAI

PAI

PLAMIN plasmin inhibitor

THROMBOLYTIC AGENTS

- Streptokinase and Urokinase no longer marketed in the US
- Tissue Plasminogen Activator derivative drugs available US
 - _o Alteplase
 - 。Reteplase
 - _o Tenecteplase

THROMBOLYTIC AGENTS

- ✓ Alteplase (Activase®; rtPA)
 - ∘ recombinant form of human tPA rare immune response
 - o short half-life (~5 min)
 - administered as an intravenous bolus followed by an infusion.
 - Indications: Acute myocardial infarction (risk of stroke may be higher than benefit), Acute ischemic stroke, Acute massive Pulmonary emboli
 - Also available as Cathflo Activase indicated for the restoration of function to central venous access devices as assessed by the ability to withdraw blood

37 38

THROMBOLYTIC AGENTS (continued)

- ✓ Retaplase (Retavase®)
 - o Less fibrin specific than alteplase
 - Genetically engineered smaller derivative of recombinant tPA
 - Increased potency and is faster acting than rtPA
 - o Indicated for acute STEMI only
 - o Administered as IV bolus injections

THROMBOLYTIC AGENTS (continued)

- ✓ Tenecteplase (TNK-tPA)
 - ₀ Mutant form of rtPA
 - Indication: acute myocardial infarction only

 - Greater binding affinity for fibrin and greater resistance to PAI -1 than rtPA
 - o Administered by IV bolus

39 40

INDICATIONS FOR THROMBOLYTIC THERAPY

- Accepted Indications
 - Acute myocardial infarctions
 - Thrombotic stroke within 3 hours
 - Acute peripheral arterial occlusive disease
 - Massive pulmonary embolism with hemodynamic instability

INDICATIONS FOR THROMBOLYTIC THERAPY

QUESTION

Does therapy do more than provide accelerated clot lysis and short-term physiologic improvement?

THROMBOLYTIC THERAPY CONTRAINDICATIONS

ABSOLUTE?

- > History of hemorrhagic stroke
- > Major internal bleeding in previous 6 months
- > Intracranial or intraspinal neoplasm
- > Recent (<2mon) intracranial surgery / trauma

THROMBOLYTIC THERAPY CONTRAINDICATIONS

RELATIVE

- > Surgery or biopsy in the preceding 10 days
- > Hypertension (>200 systolic, >110 diastolic)
- > Thrombocytopenia (< 100,000)
- > Nonhemorrhagic stroke within 2 months
- > Presence of a bleeding disorder

43 44

THROMBOLYTIC THERAPY - DVT

- Increasing use of Catheter directed pharmocomechanical thrombolysis, systemic therapy RARE for this indication
- Patients with massive iliofemoral or proximal femoral DVT with risk of limb compromise best candidate
- "ATTRACT TRIAL" designed to answer question if catheter thrombolysis improved development of PTS

THROMBOLYTIC THERAPY - DVT

ATTRACT TRIAL -NEJM 2017; 377(23):2240-2252

- Designed to evaluate whether pharmacomechanical cathether-directed thrombolysis (PCDT) decreased the occurrence of Post Thrombotic Syndrome (PTS) vs anticoagulation alone for acute proximal DVT
- PCDT did not lower the risk of PTS, and resulted in higher risk of major bleeding.
- » PCDT decreased severity of PTS, 2nd endpoint
- > TO DO: limit and define group for intervention

45 46

THROMBOLYTIC THERAPY - DVT

Inadequate fibrinolytic response

- May occur in patients with baseline or acquired low plasminogen levels (ie, consumed by therapy)
- > Patients with old clot (> 14-21 days) less
- Premature termination of therapy monitor progress
 - √Stop therapy at 24 hrs if no lysis has occurred
 - ✓ Continue therapy after 24 hours if partial lysis

PE - Indications For Thrombolytic Therapy

- > Massive acute pulmonary emboli
- > Patient hypotensive
- > Patient hypoxic despite oxygen
- Echocardiographic evidence of right ventricular failure?
- > Non-invasive treatment results in less bleeding
- ie avoid angio/catheter directed therapy

Laboratory Evaluation In Thrombolysis

- > Common laboratory findings
 - √low plasma fibrinogen
 - ✓ elevated fibrinogen degradation products inclusive of d-dimer
 - ✓ reduction in platelet count
 - ✓ Prolongation of PT and PTT
- > No laboratory finding predicts bleeding risk

Risk Factors For Bleeding

- > CNS bleeding more common in elderly (>70years)
- > Trend towards HTN as a risk factor for CNS bleed
- > Dose effect CNS bleed lower with 100mg vs 150mg
- > CVA/TIA patients have greater risk of CNS bleed
- Co-morbidity especially liver or kidney disease
- > ASA, NSAID, B-blockers, nitrates may increase risk
- > Heparin increases bleeding but improves mortality

49 50

Treatment Of Bleeding After Thrombolytic Therapy

- > Apply local control measures, i.e., pressure to site
- > Reverse heparin with protamine if applicable
- Obtain coag tests to guide replacement therapy
 PT / aPTT to determine if plasma needed to replace clotting factors destroyed by plasmin (FV, FVIII)
 - Fibrinogen level to determine if cryoprecipitate needed to replace fibrinogen destroyed by plasmin
 - √ Platelet count if count < 100,000 then transfuse. Platelets are activated by plasmin
- > Avoid additional invasive procedures

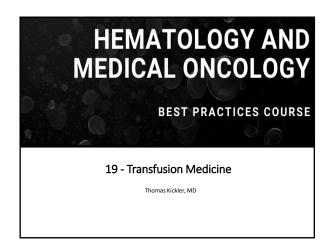




Transfusion Medicine

Thomas S. Kickler, MD

August 15, 2020



Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

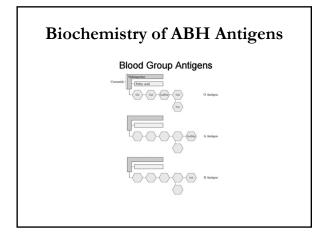
None

Topics

- Blood Group Alloantigen Systems Relevant to Common Clinical Problems
- Blood Components
- Transfusion Practices in Common Hematologic Disorders
- Adverse Effects of Transfusion

ABH Blood Group Antigens

- Antigens of Blood Group A and Blood Group B are trisaccharides with a terminal immunodominant group
- · Group A 's sugar is N-acetylgalactosamine
- · Group B 's sugar is galactose
- Group O lacks the addition of these sugars to the glycolipid
- ABO genes on Chromosome 9 and code for transferases that covalently links the saccharide to subterminal galactose

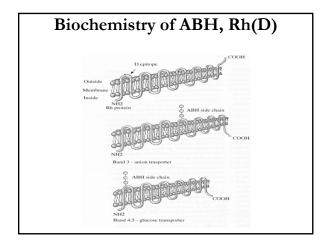


ABO Immunology

Cell Type	Isoagglutin Abs in serum	Class of Ab	All ABO Abs
A	Anti-B	IgM & IgG	Fix complement
В	Anti - A	IgM & IgG	Agglutinate RBCs
0	Anti-A Anti-B	IgM & IgG	Cause I.V. Hemolysis
AB	None	IgM & IgG	"Naturally Occurring"

Implications of ABO Immunology

- · Group O packed cells universal donor
- Group O plasma cannot be given to Group A, B or AB recipients
- Platelets from Group O donor given to Groups A, B, AB may cause hemolysis (250 ml of Group O plasma in Plt bag)
- Group O marrow donor to Groups A,B or AB may cause hemolysis due to production of anti A, B acting on patients own remaining RBCs (see below)
- Passenger lymphocytes in stem cell transplant or solid organ transplants may make issoagglutins and cause hemolysis



Rhesus Blood Group

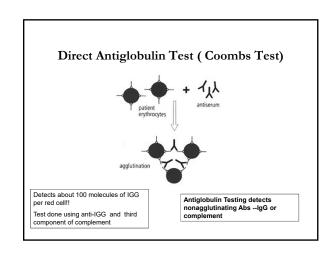
- Antigen System Comprised of D, C, c, E, e..... NO (d)
- Rh D is the most immunogenic and all recipients are typed for D (15% are Neg)
- · The Rh proteins are integral to RBC membrane
- Absence of Rh proteins lead to Rh Null state, associated with stomatocytes and hemolysis
- If make Abs to these Ags give antigen (-) blood (HbS patients) match before make an antibody to C,c,E, e)
- In Warm Auto Immune Hemolytic Anemia- Auto Ab directed toward these proteins

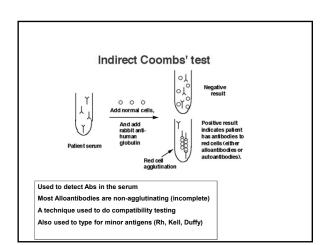
Blood Groups & Disease Associations

- Rh Null cells- stomatocytes, chronic hemolytic anemia
- Duffy Antigen Receptor for Plasmodium vivax; Duffy A negative, B Negative less likely for malaria, BUT 40% more likely to contract HIV.
- Colton- associated with water transport protein

Key Points on Blood Groups

- The ABO system is the most important for blood compatability
- Rh (D) compatability is necessary because of high immunogenecity and potential role in hemolytic disease of the newborn and delayed reactions
- Other relevant Blood groups include kell, kidd, duffy and mns





Causes of Positive Direct Antiglobulin Test

- · Autoantibodies to intrinsic red cell antigen
- Alloantibodies in a recipient's circulation, reacting with antigens on recently transfused red cells * Hallmark of Hemolytic Transfusion Reaction
- Antibodies directed against drugs that bind to red cell membranes
- Nonspecifically adsorbed proteins including immunoglobulins associated with hypergammaglobulinemia
- · Passive administration of alloantibodies in IGIV
- Antibodies produced by passenger lymphocytes in transplanted organs or hematopoietic components
- Red cell bound complement. This may be due to complement activation by alloantibodies, drugs or bacterial infection

Blood Components

Blood Components What are their contents?

- RBCs
- Platelets
- Pheresis Plts
- FFP
- Cryoprecipitate
- Hct < or = 80%,
- $> 5.5 \times 10^{10}$
- 3 X 10 ¹¹
- 250 ml
- 150 mg fibrinogen

High WBC contamination of RBCs and Platelets, remove to prevent febrile reactions, HLA abs, CMV

Red Cell Components: Characteristics & Indications Component Packed Cells, stored up Red Cell to 42 days reactions :HLA alloimmunization Reduces CMV Prevent severe allergic reactions Washed Red Cells Plasma depleted Frozen Red Cells 150 ml of RBC;plasm and WBCs depleted Rare donor or Whole Blood Massive transfusion, exchange transfusion

Leucodepletion through Blood ___Filtration__



Leucocyte poor advantageous in reducing febrile reactions, alloimmunization, second line approach in prevention of CMV, may reduce immunosuppressive effects of transfusion

RBC Transfusions in Selected Clinical Situations

- Transfusion of Autoimmune Hemolytic Anemia Do not be afraid to transfuse if needed!
- Transfusion in HPSCT Remember ABO, minor and major mismatches determine course of action

Supplying Red Cells to Transfusion Dependent Patients

APPROACHES--- <u>Rationale</u>-with repetitive transfusion patients make red cell antibodies leading to difficult crossmatching

- Provide antigen negative blood after the patient has made an alloantibody (the traditional approach)
- Provide phenotype matched blood after the patient makes the first Ab
- Provide blood matched for D,C, E, K antigens prior to alloAb forming
- Provide blood with extended phenotype matching to include D,C, E, c, e, K, Fy, Kidd

CONSIDERATIONS—

The approach a joint decision of consulting hematologist, transfusion service director, and blood donor centers.

It may not be possible for the community to meet the request

Autoimmune Hemolytic Anemia

- · All crossmatches will be incompatible
- Lab ABO and Rh types patient, if unable give Group O , Rh Neg
- If patient previously transfused, should exclude the possibility of an alloantibody
- This is done by autoabsorbing the patient's serum with autologous RBCs
- · May need to give extended phenotype blood
- For Cold Autoimmune Hemolytic anemia- use blood warmer

Transfusion Support in Hematopoietic Stem Cell Transplant

- Prevent CMV, serologically negative blood products, or leukocyte depleted products
- · Gamma irradiated to prevent GVHD
- Leucocyte depleted blood products to prevent HLA alloimmunization
- What ABO type of Red cells, plasma should be given? Major & Minor Incompatible Transplants

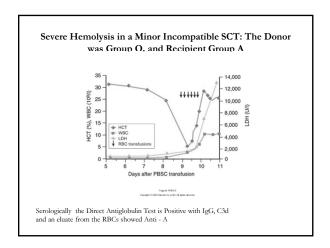
Major ABO Incompatibility

- Occurs when the recipient has ABO antibodies against the Donor Red Cells
- The HPC can be processed to remove RBCs thereby reducing risk of immediate hemolytic reaction
- The group O recipient who gets Group A graft may make anti A, & B for 3-4 months
- · Will delay RBC engraftment
- Group A RBCs will appear in circulation when isoagglutins disappear
- Transfuse with RBCs that are compatible with donor and recipient, to avoid confusion we use Group O

Delayed RBC engraftment in the recipient of a Major ABO incompatible SCT Major ABO incompatible SCT

Minor ABO Incompatibility

- Minor ABO incompatibility occurs when the graft makes antibodies against the recipient RBCs
- For example a Group O donor to a Group A recipient
- Clinical hemolysis abrupt onset at 7-10 days and may last 2 weeks
- Transfuse with Group O cells and use plasma compatible with Donor and Recipient
- · We start to transfuse Group O cells prior to transplant



Adverse Effects of Transfusion

- Infectious
- Immunological
- Metabolic
- Volume

Warning Transfusions are Hazardous Package Insert

• Disease transmission

• AIDS

Hemolysis

Malaria Immune suppression

• Allergies

Sepsis

Allergies Metabolic abnormalities

• GVHD

Volume overload

Febrile reactions

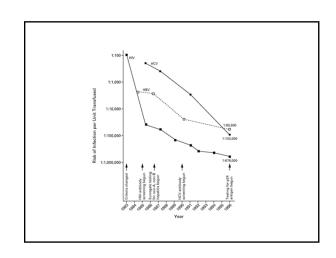
• Dementia (?)

Death

• Hepatitis

• CMV

Disease Transmission by Transfusion



Blood Tested For:

HIV-1, HIV-2 - Test for antibodies to HIV-1 and HIV-2 (Human Immunodeficiency Virus)

HBc - Test for antibody produced during and after infection with HBV (Hepatitis B)

HCV - Test for antibody to HCV (Hepatitis C)

HTT.V-I and HTT.V-II - Test for antibodies to HTT.V-I and HTT.V-II (Human T-cell Lymphotropic Virus)

HBsAg (Hepatitis B Surface Antigen — Screens for HBV)

PKTP (Syphilis) - Test for syphilis

Nucleic Acid Testing – Detects Viremia Before Antibody Testing

NAT (Nucleic Acid Testing) - NAT is a technology that can detect the genetic material of Hepatitis C virus, HIV and West Nile Virus faster and more accurately than other tests, which react to antibodies of those viruses.

All of the blood collected by the American Red Cross for transfusion is now subjected to NAT for Hepatitis C, HIV and West Nile Virus.

Infectious Risk of Transfusions Due to Viruses

Viral Agent	Estimated Risk/Unit	Estimated % infected units that transmit
HIV-1 & 2	1:2,300,000	90
HTLV 1 & 2	1:2,930,000	30
HAV	1:1,000,000	90
HBV	1:220,000	70
HCV	1:1,800,000	90
B19 Parvoviurs	1:40,000	low

West Nile virus- estimated risk- depends upon

Infectious Risk Due to Bacteria

Blood Product	Estimated Risk/Unit	Estimated % if infected units that transmit
RBCs	1:1,000	1:10,000,000 fatal
Platelets	1:2,000	1:2,500 result in sepsis
Platelets Pheresis	1:2,000	1:13,400 result in

Estimated Risk of Collecting Blood During the Infectious Window Period (repeat donors)

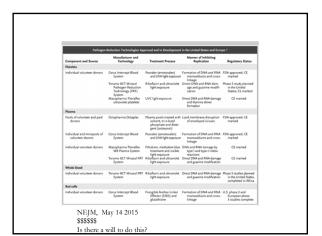
- HCV , Ab test only
- 70 days
- HCV, + NAT
- 10 days
- HIV AB + p24
- 16 days
- HIV AB + NAT
- 11 days
- HTLV
- 51 days
- HBV
- 59 days

Infectious Risks of Transfusion Due to Parasites

Parasite	Estimated Risk/Unit	Estimated % infected units that transmit
Malaria and Babesis	<1:4,000,000/ Babesia 1,800 in endemic areas	unknown
Trypanoso ma Cruzi	1:42,000	<10



patient with fevers and babesia



Immunologic Transfusion Reactions

Immunologic Reactions

• Immune suppression

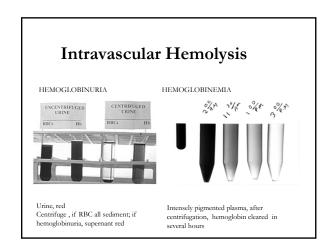
- Hemolytic
- Allergic
- WBC & Cytokine mediated
- GVHD
- Transfusion Related Acute Lung Injury
- Anaphylactic

Hemolytic Transfusion Reactions

- Acute Hemolytic: ABO mismatched blood, and some IgG antibodies to other Alloantigens, such as E, C, e, Fy
- Delayed Hemolytic Reactions: IgG antibodies form anamestically in previously immunized people
- Passive administration of isoagglutins in FFP and platelets

ABO Hemolytic Reactions

- Acute pain, arm, back
- Fever, chills
- DIC, in O.R. unexplained bleeding
- hemoglobinuria, and hemoglobinemia
- DAT positive for Complement



Conditions Mimicing a Hemolytic Reaction

- · Over heating of blood in blood warmer
- · Adding hypotonic solutions to blood
- Mechanical Trauma of membrane oxygenator or ventricular assist device
- Addition of medications to bag of blood that lysis red cells
- Unit of blood is from a G6PD deficient donor

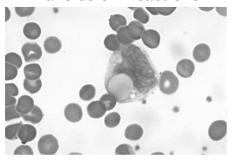
Lab Findings of A Hemolytic Transfusion Reaction

- Positive direct antiglobulin test, due to isoagglutins on red cells and or complement on the red cells
- Repeat incompatible crossmatch
- New blood specimen typically shows an ABO discrepancy
- Hemoglobinemia, hemoglobinuria
- DIC

Delayed Hemolytic Transfusion Reactions (DHTR)

- Seen in previously transfused or pregnant patients
- The Ab is below detection in pretransfusion testing
- 7-10 days post-TRX the Ab titer increases resulting in extravascular hemolysis
- · At this time the DAT and Indirect DAT are Positive

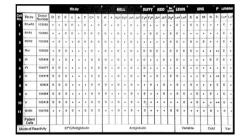
Spherocytes in Delayed Transfusion Reactions



Laboratory Findings in Delayed Hemolytic Reaction

- Antibody screening positive this signals a alloantibody is present
- Next step is to identify the antibody is it Anti E, Kell, Duffy a etc.
- This identification is done by performing a specificity panel by testing the patient serum against a panel of 10 well phenotyped red cells.
- Those cells that do not react, yield the identification of the antibody

What a RBC Panel Looks Like



From a panel of red cells one can identify the specificity of the antibody. This takes specialized medical personnel to do. Multiple antibodies may do, and is not something a hematologist is credentialed to do.

Clinical Issues of DHTR

- · Fever, jaundice, spleen may increase
- · Renal failure unlikely
- · Falling hematocrit, increased Reticulocyte count
- Need to give RBCs lacking the Ags promoting the immune response
- For example if have anti E, and Kell give blood negative for these antigens
- Give the patient a card stating the patient has these ABs

Febrile Reactions

- Due to patients HLA or antigranulocyte antibodies
- · Cytokines from WBCS in blood
- · Prevent by giving leucocyte depleted blood
- Antipyretics: no clinical trials showing efficacy
- If have febrile reaction: STOP TRANSFUSON AND WORKUP

Transfusion Reactions – Most Common Immunologic					
Usu: Urgei		Reaction Type	Approx Incidence	Clinical significance/ Burn points	
++++	Acı	ite Hemolytic	1:12,000- 35,000	~50-75% of Txn fatalities (1:100,000 – 600,000)	
+	Dela	yed Hemolytic	1: 1000- 12,000	• Severe symptomatic hemolysis: 1: 250,000; death: rare; •↑ risk sickle cell	
+/- to ++	Feb Noi	rile n-Hemolytic	0.5-1.4% 15% recur	 Frequent: 43-75% of Txn Rxns Frightening to patient Specters of AHTR + bacterial sensis 	

Allergic Reactions

- Hive:
- Caused by allergy to proteins, or some substance in the donor cells
- Prevent by giving antihistamines
- Washed RBCs for persistent, severe allergic reactions

Anaphylactic Reactions

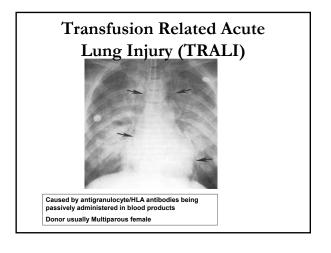
- Antibodies to IgA in totally IGA deficient patient
- May occur in previously untransfused patient
- Give IgA deficient plasma products, extensively washed RBCS
- May occur due to antibodies to haptoglobin

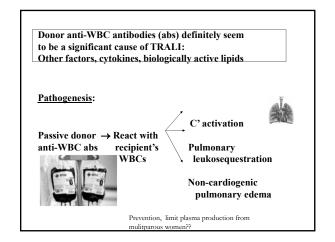
Transfusion Related Acute Lung Injury(TRALI)

- severe respiratory compromise (clinically ARDS:
- severe hypoxemia + non-cardiogenic pulmonary edema)
- Occurs within 4-6 hrs of txn; no other cause evident

Other usual clinical concomitants:

- Rapid onset: 15-20 mins
- · Fever almost invariable
- ↑ bp initially; most severe → ↓ bp
- ↑ pulmonary artery pressures
- Resolution usually rapid (hrs) ≠ ARDS





Transfusion Associated GVHD

- Seen in severe immune suppressed patients
- Patients with congenital cellular immunodeficencies
- · Premature babies
- · Give irradiated blood to these patients
- Patient groups, <u>BMT</u>, Lymphoma, leukemics undergoing induction therapy

Usual Urgency	Reaction Type	Approx Incidence	Clinical significance/ Burn points
+-+++	Volume overload	? 1:100	Rarely fatal
+++	Hypotension from bedside filters	?	Bradykinin implicated; ↑ risk ACE inhibitors
++	Non-Immune Hemolysis	Rare	Hypotonic solutions; intra op salvage; defective warmers; rarely serious
++ -	Metabolic imbalar	ices	
++++	 ↑ K Acidosis ↓ Ca⁺⁺ (Mg⁺⁺) 	Rare S	Rapid infusion of old RBCs; renal insufficiency • Massive transfusion Special attn: young children

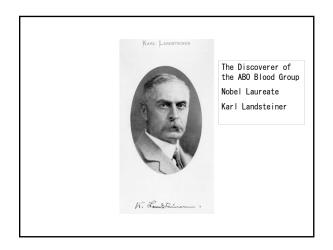
Usual Urgeno		Approx Incidence	Clinical significance
+++	Hypothermia	-	Massive Txn; traumacoagulopathy
	Fe++ overload	-	Chronic Txn -250 mg/uni

Hemoglobin Carriers

- Solutions of Hb from outdated Human RBCs or Cows (Biopure: what vets use)
- The Hb is chemically modified to make the molecule larger to prevent renal clearance
- Some interfere with NO leading to hypertension
- None approved clinically, yet but available on IND

Summary

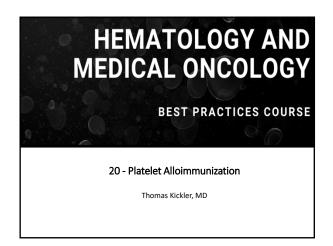
- Blood Group Alloantigen Systems Relevant to Common Clinical Problems
- Blood Components
- Transfusion Practices in Common Hematologic Disorders
- Adverse Effects of Transfusion
- Reducing the Risks of Transfusion



Platelet Alloimmunization

Thomas S. Kickler, MD

August 15, 2020



Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

None

Topics

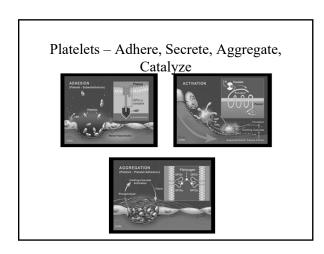
- Alloimmune Thrombocytopenias
- Important Alloantigen Systems
- Clinical and Laboratory Issues
- Platelet Transfusion Therapy in Alloimmune Disorders
- Platelet Transfusion Therapy
- Alternatives to Transfusion

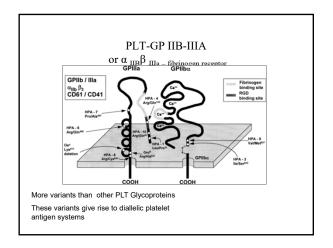
Immune Cytopenias

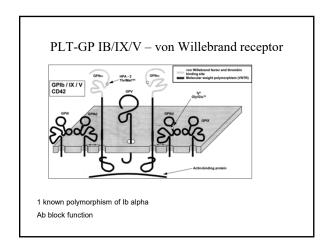
RED CELLS	PLATELETS
AUTOIMMUNE HEMOLOYTIC ANEMIA	AUTOIMMUNE THORMBOCYTOPENIA
HEMOLYTIC DISEASE OF THE NEWBORN	FETAL ALLOIMMUNE THROMBOCYTOPENIA
TRANSFUSION ALLOIMMUNIZATION	TRANSFUSION REFRACTORINESS
DRUG INDUCED AIHA	DRUG PURPURA
DELAYED HEMOLYTIC TRANSFUSION REACTION	POST TRANSFUSION PURPURA

PLATELET ALLOANIGENS

- HLA CLASS I ANTIGENS Transfusion Refractoriness
- HUMAN PLATELET ALLOANTIGENScause Post Tranfusion Purpura, Neonatal Alloimmune Thrombocytopenia, Link to platelet hyper-reactivity??
- · ABO BLOOD GROUPS







Human Platelet Alloantigens (HPA)

- Human Platelet Antigens, formerly called platelet specific antigens represent variations of platelet glycoprotein integrins
- These integrins are present on platelets, endothelial cells
- Conformational immunogens
- Give rise to alloimmune mediated thrombocytopenic disorders of:

ANTIBODIES TO HPA LEAD TO:

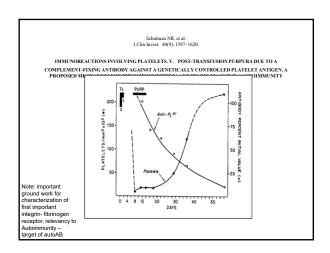
- POST TRANSFUSION PURPURA
- FETAL/NEONATAL ALLOIMMUNE THROMBOCYTOPENIA
- PLATELET TRANSFUSION REFRACTORINESS

Human Platelet Alloantigens (HPA)

HPA System	Original	Plt Glyco-	Amino Acid	DNA
	names	protein	Substitution	substitution
la	Pl(A1)	IIIa	Leu33	T196
lb	Pl(A2)		Pro33	C196
2a	Ko(b)	Ib	Thr145	C524
2b	Ko(a)		Met145	T524
3a	Bak(a)	IIb	Ile843	T2622
3b	Bak(b)		Ser843	G2622
4a	Pen(a)	IIIa	Arg143	G526
4b	Pen(b)		Gln143	G526
5a	Br(b)	Ia	Glu505	G1648
5b	Br(a)		Lys505	A1648

+ 10 other less common

		Immunogenetic Studies on 5 Human Platelet Antigens Most Frequently Associated with Alloimmune Disorders						
	Antigen System	*Caucasian	African American	**Asian				
	HPA-1a	0.89*	0.92	0.95				
	HPA-1B	0.17	0.08	0.005				
	HPA-2a	0.92	0.82	0.87				
	HPA-2b	0.09	0.18	0.13				
	HPA-3a	0.67	0.63	0.67				
	HPA-3b	0.33	0.37	0.33				
	HPA-4a	0.99	0.99	0.99				
	HPA-4b	0.01	0.01	0.01**				
	HPA-5a	0.89*	0.79	0.97				
	HPA-5b	0.11	0.21	0.03				
Kickle	r, et al. Blood 199	<u>13</u>						



Post Transfusion Purpura (PTP)

- Characterized by sudden onset of severe thrombocytopenia 7-10 days after transfusion after blood or plasma
- The Enigma of PTP, at the time of thrombocytopenia an alloantibody to HPA develops and persists despite the resolution of the thrombocytopenia
- Seen in females previously transfused or pregnant 0nly 1 male reported!

Clinical Presentation in PTP

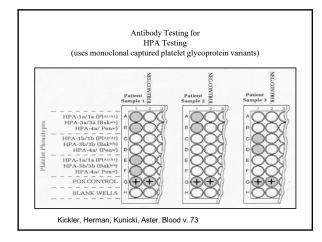
- · Petechiae
- · Wet Purpura
- Bleeding from multiple operative sites



JHH 45 cases from 1981-1995

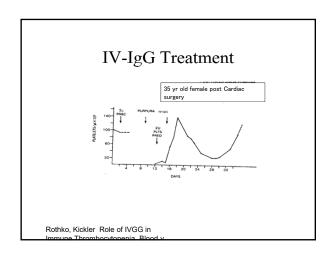
- Platelet counts < 5,000/ul
- Development of Thrombocytopenia is abrupt 24 hours!!
- Persists for mean duration of 7 days with range of up to 35 days; suggests possibility of different mechanisms
- Anti- HPA-1a> 90% of cases- never anti HPA-1b
- In our series no African Americans, Asians
- · Associated with severe, acute febrile reactions transfusions
- · Unmatched Platelet Transfusions Ineffective
- Of 8 patients transfused with platelets developed thrombosis, both arterial and venous; no thrombosis in non transfused patients

Vogelsang G, Kickler TS. Am J Hem 1996



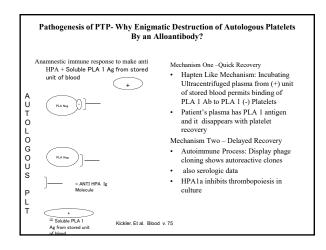
PTP Treatment

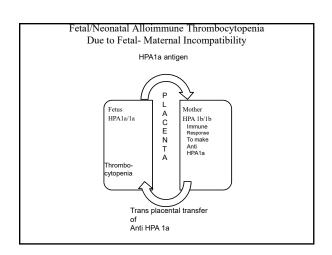
- Steroids no effect on platelet count but wet purpura, petechiae improve
- Plasma exchange in use at JHH until 1987
- IV-IgG** Treatment of Choice
- Platelets transfusions if necessary HPA matched, and HLA compatible?

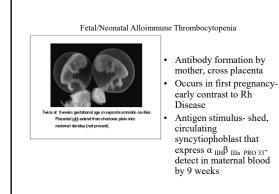


Prevention- future elective operations

- · Use autologous blood
- Antigen negative if available Red Cross has several HPA-1b donors, the result of readily available HPA genotyping of donors
- Any woman who has child with neonatal alloimmune thrombocytopenia should be cautioned of risk of post transf purpura in future with transfusion





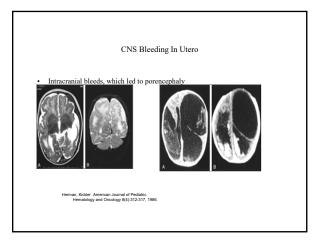


Fetal/Neonatal Alloimmune Thrombocytopenia

- · Caused by alloantbodies to HPA
- Most cases are diagnosed at birth (NAIT)
- In contrast to Rhesus Hemolytic Disease Newborn frequently seen in first pregnancy
- HPA1a (Caucasians) alloimmunization most frequent, followed by HPA-3a or b or HPA-5a or b in African Americans, Asians
- Occurs in 1 of 1000 2000 pregnancies

Clinical Characteristics

- Serious fetal consequences are common Intra Cranial Hemorrhage occurs in ~20% (10% fatal)
- Risk of ICH in subsequent fetal-antigen + pregnancies is virtually 100%
- · A cause of recurrent pregnancy loss
- Fetus small, with small placenta gives rise to Intrauterine growth retardation

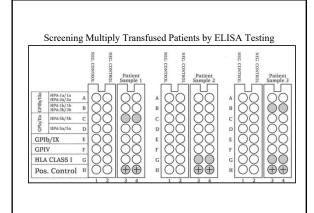


FMAIT, contd

- There is a strong association with HLADRw 52a (HLADR3*0101)
- HLADRw 52a (HLADR3*0101) is present in 1 in 3 Caucasians
- The negative predictive value of making anti HPA1a in the absence of HLADRw 52a (HLADR3*0101) is >99%
- Positive predictive value of HLADRw 52a (HLADR3*0101) is < 30%
- Kickler TS, Tissue Antigen: 45

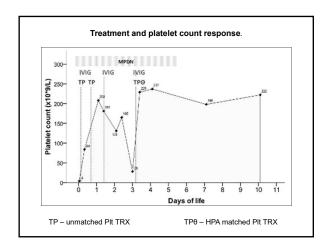
FMAIT Testing- How to Workup suspected case of NAIT

- First choice to genotype both parents for HPA-1
- Screen maternal serum against a panel of phenotyped glycoproteins, much like a RBC panel
- Where father is heterozygous genotype the fetus for prenatal management
- Do not utilize newborn sample for antibody testing, antibody frequently undetectable
- Ante Natal Screening no proven cost effectiveness, unlike Rh



Provision of Platelet Support

- · Historically, washed maternal platelets
- Platelets need to be available soon after delivery or at time of delivery since severe bleeding occurs in first 48 hours
- Centers developing typed donor pools of HPA-1a and HPA-5b donors; if none matched random platelets may be used
- Absence of HPA, HLA antibodies, incompatible isoagglutinins, CMV negative + other donor requirements and testing



Guidelines For Platelet Transfusions

- · Bone marrow failure
- Threshold of 10,000/ul if no other risk factors) sepsis, coagulopathy)
- Threshold of 20,000/ul of other risk factors present

continued

Guidelines for PLT Transfusion

- Invasive Procedures
- A count of 50,000/ul for LP, indwelling lines, epidural anesthesia, gastroscopy and Biopsy, transbronchial biopsy, laporotomy or liver biopsy
- A count of 100,000 for brain and eye surgery

continued

Guideline for PLT Transfusion

- DIC
- Aim for count > 50,000/ul
- · Massive Transfusion
- Keep count >50,000
- · Cardiac Surgery
- Reserve PLTs for those with severe bleed and surgical cause ruled out

continued

Guideline for PLT Transfusion

- Qualitative PLT Disorder
- DDAVP
- Correct HCT to >30%
- Consider PLT Transfusion when other measures failed
- IF Glanzmann's consider rVIIa

Dose of Platelets

- · Depends on therapeutic goal
- If prophylaxis in myelosuppressed, keep above trough of 10,000/ul
- Factors affecting dose, size, bleeding, splenomegaly, DIC, antibodies, microvascular damage
- Single donor platelts have at least 3 x log 11 plateletsincrease count by 20-30,000/ul
- If lower dose may still decrease bleeding, but more transfusion episodes needed!

Platelet Refractoriness

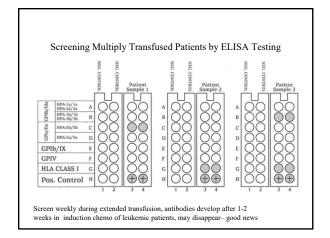
- Immune Refractory- Alloimmunization to HLA major cause
- Non Immune refractory
 DIC, Sepsis, fever, splenomegaly
 No identifiable cause
 poorly preserved platelet transfusion

Alloimmune Platelet Transfusion Refractoriness

- HLA Abs account for the majority of immune mediated PLT transfusion refractoriness
- 15-25% of Patients become HLA alloimmunized, maybe more if in patient group non alloimmunized
- 8-35% of HLA matched platelet transfusions have poor increments not related to clinical factors
- 3-8 % caused by antibodies to HPA
- · Rarely caused by ABO antibodies

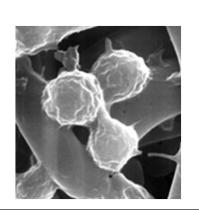
OTHER CAUSES OF IMMUNE MEDIATED REFRACTORINESS

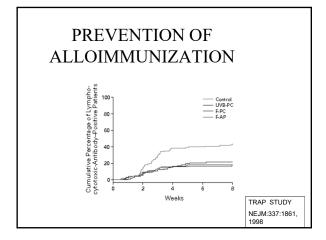
- AUTOANTIBODIES
- DRUG INDUCED ANTIBODIES
- ANTIBODIES TO PLATELET GLYCOPROTEINS, IN GLANZMAN'S OR BERNARD SOULIER DISEASE ***



Prevention of Alloimmunization

- Limit transfusion exposure- no dose response
- Only use pheresis platelets not those prepared from units of whole blood- not effective
- Leukocyte depletion- removing WBCs, the immunizing agent in blood or platelets, works!





HLA Matching of Platelets

- Ideal situation would be to give HLA identical platelets to everyone
- Genetic polymorphisms of this complex antigen system makes this impossible
- Strategy is to prevent alloimmunization if cannot do this
- Circumvent the antibodies by HLA matching or selective mismatches

HLA Transfusion Strategy

- Platelets have HLA-A , and HLA-B antigens on them
- It is not necessary to match for HLA-C antigens since these are weakly expressed
- Try to give HLA A and B antigens that are identical or cross-reactive with the recipient, once the recipient has HLA antibody
- This will allow successful transfusions > 60-70% of the time

When HLA Matched Platelets are not Available

- Laboratory can do platelet crossmatching of the donor pool available
- This approach is not only practical but highly successful in > 78-80 % of difficult to match transfusion recipients
- Alternatively HLA serologic identification of the antibodies may permit finding donors who lack the Antigens that the patient has antibodies to I.e. give "antigen negative platelets"

Alternative Management Strategies

- Reticuloendothelial Blockage—High Dose IgG- only transiently helpful
- Immunosuppressive Therapy not helpful
- Growth Factors if no megakaryocytes, no benefit
- Repeated Platelet Transfusions benefit?
- Amicar- no control trials showing benefit

Recombinant VIIa in Congenital Platelet Disorders

- 28 patients with Glanzmann's thrombasthenia treated, 2 with Bernard Soulier and 2 with pseudo vWD
- rVIIa used in 3 major and 10 minor surgical procedures, and for 57 bleeding episodes
- Red cells needed in 24 and Amicar used in 54 cases

rVIIa, continued

- For 13 invasive procedures, results good in 11, not evaluable in 2
- 9 GI bleeds, 1 had recurrence after 36 hours
- Of 42 others, 11 were failures
- For 34 non GI bleeds total doses received 1-14

rVIIa Continued

- Dose recommended 85 mcg/kg given every 2 hours
- Effective in covering invasive procedures and stopping bleeding episodes
- Poon et al. Blood, Abstract 1102, 2000

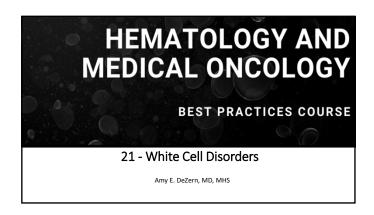
SUMMARY

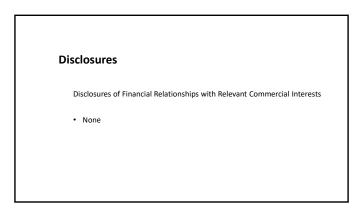
- •Alloimmune Thrombocytopenias
- •Important Alloantigen Systems
- •Clinical and Laboratory Issues
- Platelet Transfusion Therapy in Alloimmune Disorders
- •Alternatives to Transfusion

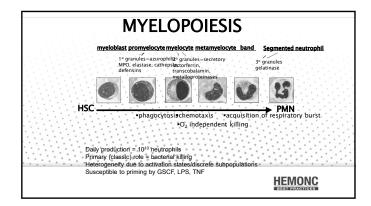
White Cell Disorders

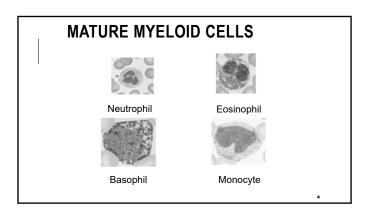
Amy DeZern, MD

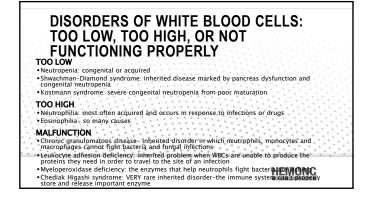
August 15, 2020

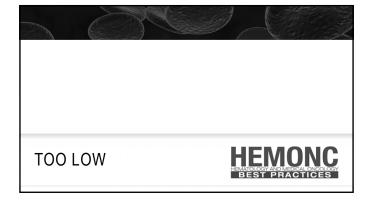












MANAGEMENT OF NEUTROPENIA Different in children and adults BM if not obviously secondary to other medical disease, infection, etc. Genomics for germline mutations in suspect and younger patients In adults: LGL, agranulocytosis are most frequent and treatable Broad spectrum parenteral antibiotics for suspected infection Specific therapy based on diagnosis G-CSF; appropriate utilization desirable--but a trial almost always performed! avoid overuse (as in benign neutropenia) **HEMONC**

Decreased Production	Increased Destruction	Shift to Marginating Pool
Bone marrow	Peripheral circulation	Move from the circulating pool to attach along the vessel wall
Medication induced	> Autoimmune diseases (RA, SLE)	Severe infection Endotoxin release Hemodialysis, Cardiopulmonary bypass

BENIGN ETHNIC NEUTROPENIA ALSO CALLED CONSTITUTIONAL NEUTROPENIA

- Classically seen in those of African ancestry, some Jewish and Arabic populations
- Prevalence varies according to racial/ethnic group: Blacks (4.5%), Whites (0.8 %), and Mexican-Americans (0.4 %)
- Defective release from bone marrow

Hsieh MM et al. Ann Intern Med 2007; 146:486.

- Associated with a single-nucleotide polymorphism of the ACKR1 gene, which encodes the Duffy antigen receptor for chemokines and is a component of the Duffy RBC blood group system
- No increased risk of infections throughout life

HEMONC

NEUTROPENIA CAUSED BY TOXINS/ DRUGS

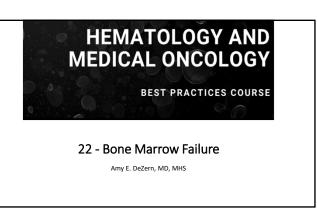
- Antibiotics
- Macrolides
- Chloramphenicol Anti-malarials Anti-virals
- Dapsone
- Psychotropics
- Clozapine
- •TCAs
- Anti-convulsants
- Phenytoin
- Carbamazepine

- ■H2 blockers
- Diuretics
- •Cardiovascular drugs •Flecainide
- Deferiprone
- Rituximab

Bone Marrow Failure

Amy DeZern, MD

August 15, 2020



Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

• None

DAMESHEK'S RIDDLE

What do aplastic anemia, paroxysmal nocturnal hemoglobinuria, and "hypoplastic" leukemia have in common?



Dameshek. Blood. 1967 Aug;30(2):251-4.

ISSUES IN STUDYING, DIAGNOSING AND TREATING BONE MARROW FAILURE

Present with pancytopenia

Overlapping diseases with distinct pathophysiology

Difficult to distinguish in early stages Serial evaluations often needed

Appropriate therapy depends on accurate and early diagnosis

No reliable disease model for AA, PNH, MDS, LGL

Relative paucity of curative

Clonal evolution and transfusional iron overload cause severe morbidity and

APPROACH TO DIFFERENTIAL DIAGNOSIS VARIES

Acute versus Chronic

Inherited versus Acquired (Younger vs Older)

Neoplastic versus not

Immune mediated versus non immune

Cellular versus hypocellular marrow

Primary stem cell disorder versus stem cell toxic

HEMONC

BONE MARROW FAILUR Lyelodysplastic syndrome (MDS) Hypoplastic MDS Aplastic anemia (AA) Large granular lymphocytosis (LGL) Pure Red Cell Aplasia (PRCA) Paroxysmal nocturnal hemoglobinuria (PNH) Inherited syndromes **Nutritional deficiencies**

Amy DeZern, MD

DIFFERENTIAL DIAGNOSIS OF PANCYTOPENIA

With hypocellular BM

- · acquired versus constitutional (FA, DKC)
- Mvelodvsplasia (<15% cases)
- · Myelofibrosis ("dry tap")
- · Rarely: ALL (children), AML (elderly) as aleukemic leukemia), lymphoma

With normo- or hypercellular BMV

- Primary marrow disease
- · MDS
- PNH
- Myelofibrosis
- · Leukemias and lymphomas
- · Hairy cell leukemia
- · Large granular lymphocytosis
- Hypersplenism
- · Systemic lupus erythematosus
- · Vitamin deficiency (B12, folate) · Alcohol
- Sarcoidosis
- Tuberculosis and other mycobacteria; brucellosis;
 Sarcoidosis

PHYSICAL MANIFESTATIONS OF PANCYTOPENIA

MASQUERADERS OF MDS...

Myeloid Disorders

- Aplastic Anemia
- · Paroxysmal Nocturnal Hemoglobinuria (PNH)

Lymphoid Disorders

- · Large granular leukemia/lymphocytosis (LGL)
- · Autoimmune Disorders (SLE)

Genetic disorders

· Dyskeratosis congenita

Nonclonal disorders

- · Vitamin deficiencies
- Toxins

HEMONC

NUTRITIONAL DEFICIENCIES

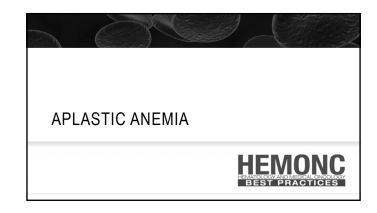
Post bariatric surgery deficiencies can manifest with hematologic and neuro deficits

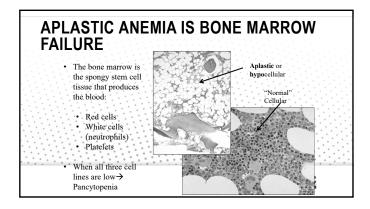
- B12→ advanced stages can look just like MDS
- B12 assays can vary, MCV can be WNL
- IM supplementation
- Iron Deficiency
- Copper Deficiency

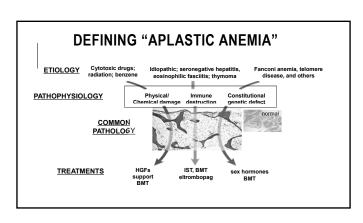
All require monitoring 2–3 times per year postesurgery

COPPER DEFICIENCY

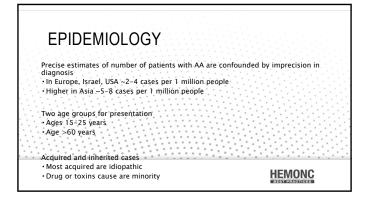
- · Absorbed in the stomach
- · Required cofactor in many redox reactions
- 90% bound to ceruloplasmin
- · Heme manifestations rapidly reversible with supplementation but neuro may not be
- · Worsened by Zinc supplementation over 150-450 mg of zinc per day (dental creams, cold remedies)
- Carriers of the 5,10-methylenetetrahydrofolate reductase A1298C gene polymorphism also affect copper metabolism

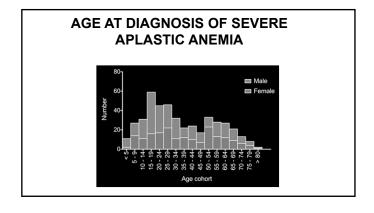


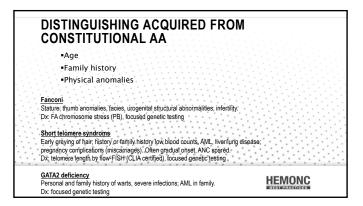


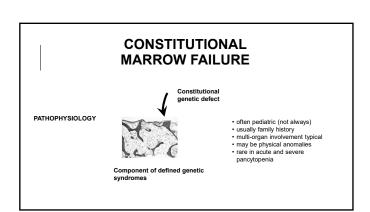


DRUG/ TOXIN CAUSES Carbamazepine Indomethacin Phenytoin Methimazole Hydantoins Propylthiouracil Sulfonamidse Gold Chloramphenicol Arsenicals Phenylbutazeon Benzene







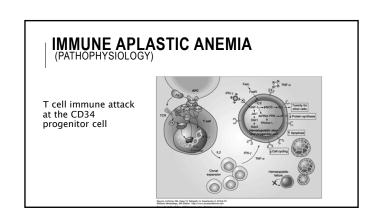


	<u> </u>	Inheritance.	Predominant	RROW F	7 (I - O) (L		
Syndrome	Gene(s)	mechanism Mutation types	age of reported MDS/AML onset (range)		Extra- hematopoletic features	Other cancers	Implications fo management
Fanconi anemia	21 genes to date	AR AD (FANCR) X-linked (FANCB), loss-of- function Missense, FS/NS, splicing, CNV	(range 1-57)	AA, MDS, AML ALL with FANCD1	Short stature, developmental delay, skeletal and renal abnormalities	SCC of head, neck and anogenital region	Require attenuated therapy, radiosensitive
Short telomere syndromes	13 genes to date	AD, AR, X- linked; variable mechanisms resulting in telomere shortening Missense, FS/NS, splice.	Adult > Ped (range 2–77)	AA, MDS, AML	oral leukoplakia,	SCC of head, neck and anogenita	Attenuated regimen, radiosensitive

	IN HU	MAN BO	Predominant	RROW F			
Syndrome	Gene(s)	mechanism Mutation types	age of reported MDS/AML onset (range)	Heme features	Extra- hematopoletic features	Other cancers	Implications for management
Familial MDS/AML with GATA2 mutation	GATA2	AD, haploinsufficien t Missense, NS/FS, duplication, splicing, regulatory, CNV	(range 3-78)	AA, MDS, AML, CMML Increased prevalence of monosomy 7	Lymphedema Pulmonary alveolar proteinosis Hearing loss		
Familial platelet disorder with propensity to AML	RUNX1	AD, haploinsufficien t or dominant negative, depending on mutation Missense, FS/NS, duplication,	(range 5-72)	MDS, AML, T- ALL, hairy cell leukemia, CMML Mild-to- moderate thrombocytopen iaNormal plt size Abnormal function	Case report of co- occurring eczema		

Syndrome	Gene(s)	inheritance, mechanism	Predominant age of reported MDS/AML	Heme features	Extra- hematopoietic features	Other cancers	implications for management
		Mutation types	onset (range)				
Germline SAMD9/SAMD9L	SAMD9 SAMD9L	AD, gain-of- function	adult	AA, MDS, AML, CMML ²	(SAMD9)		
		Missense	(range 1-56)	Increased	Ataxia-		
		IWII33EII3E		prevalence of	Pancytopenia		
				monosomy 7	(SAMD9L)		
Diamond-	22 genes to	AD, X-linked			Growth	Osteosarcoma,	
Blackfan anemia	date	(GATA1, TSR2)	(range 2-57)	red cell aplasia,	retardation,	colon, possibly	
		haploinsufficie		MDS, AML, ALL	congenital	others	
		nt, loss-of-			malformations in		
		function			<50% (head,		
		Missense.			limbs, GU, heart)		
		FS/NS, splicing,					
		CNV. 3' UTR					
Shwachman-	SBDS	AR, loss-of-	AYA	Neutropenia.	Exocrine		
Diamond	DNAJC21	function	(range 2-53)		pancreatic		
	EFL1			- '	insufficiency,		
		Missense,	l	1	neurodevelopment		
		EC/MC enlice			al & chalatal		

SCREENING FOR GERMLINE MUTATIONS IN HUMAN BONE MARROW FAILURE								
Syndrome Gene(s		Inheritance, mechanism Mutation types	Predominant age of reported MDS/AML onset (range)		Extra- hematopoletic features	Other cancers	Implications for management	
ANKRD26-related ANKRD thrombocytopenia		AD, suspected to be gain-of-function SNV or small deletion in 5' UTR, N-terminal truncating variants, missense ^a	Adult (range 26-70)	Thrombocytope nia Normal plt size Abnormal function			Mild bleeding tendency	
ETV6-related thrombocytopeni a	ETV6	AD, incompletely understand, possibly dominant negative	Ped + Adult (range 8-82)		Not shared across pedigrees (developmental delay, dysmorphisms, autoimmunities)	Colon, breast, meningioma	Mild-to- moderate bleeding tendency	

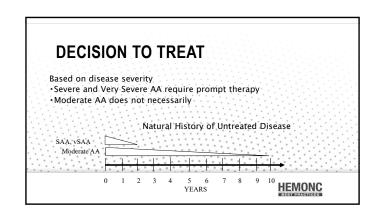


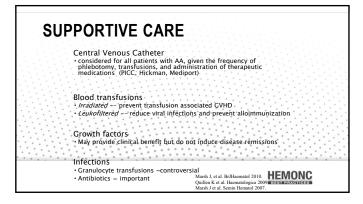
CLASS	IFICATIO	N OF AA:	CAMITTA
Peripheral	Non-severe	Severe	Very-severe
Blood	(Moderate)	aplastic anemia	aplastic anemia
Cytopenias	aplastic anemia (not meeting criteria for severe disease)	(any 2 of 3)	(meets criteria for severe disease and absolute neutrophil < 200)
Bone marrow cellularity	< 25%	< 25%	< 25%
Absolute neutrophil count		< 500 / μΙ	< 200 / µl
Platelet count		< 20,000 / µl	
Reticulocyte		< 1.0% corrected or	

< 60,000 / µl

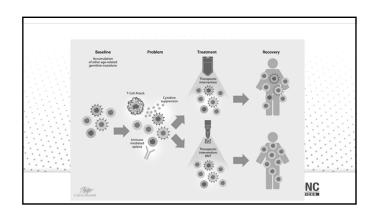
CLASS CRITER	IFICATIO RIA	N OF AA:	CAMITTA	
Peripheral	Non-severe	Severe	Very-severe	
Blood	(Moderate)	aplastic anemia	aplastic anemia	
Cytopenias	aplastic anemia	(any 2 of 3)	(meets criteria for	
	(not meeting		severe disease and	
	criteria for severe		absolute neutrophils	
	disease)		< 200)	
Bone marrow	< 25%	< 25%	< 25%	
cellularity				
Absolute		< 500 / μl	< 200 / µl	
neutrophil				
count				
Platelet count		< 20,000 / μl		
Reticulocyte		< 1.0% corrected or		
count		< 60,000 / μl		
		Camitta BM et al Blo	od. 1976:48:63-70	

	<u>Aplastic</u>	<u>MDS</u>
	<u>Anemia</u>	
Cellularity	Decreased	Increased or normal* (* 15% hypoplastic MDS)
CD34 count	Decreased (< 0.1%)	Normal or increased
Dyserythropoiesis	Common	Common
Ringed sideroblasts	Never	Common
Myeloid dysplasia or blasts	Never	Common
Dysplastic megakarocytes	Never	Common
PNH population	Common	Rare
Abnormal karyotype	Rare	Common

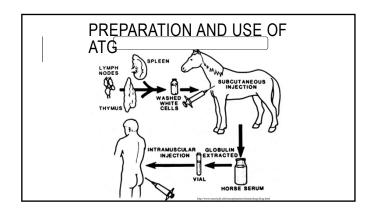


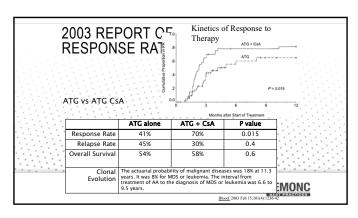


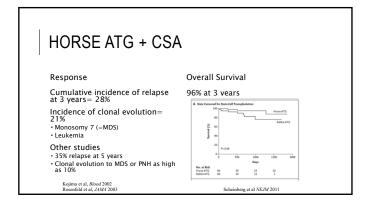
IDEAL THERAPY FOR SAA Available to all patients •Not limited by age and donor status Low toxicity •Rapid hematopojetic reconstitution •Low risk for graft failure/GVHD/infections Reduces or eliminates risk of secondary clonal disease •MDS/Leukemia •PNH

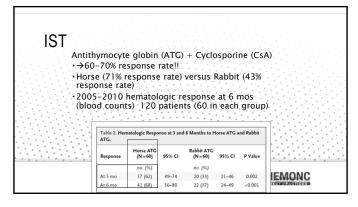


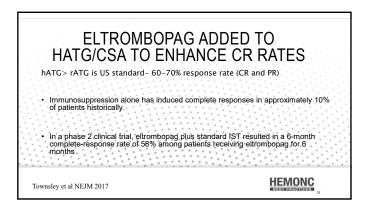


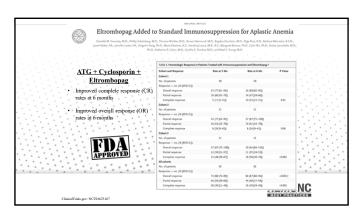


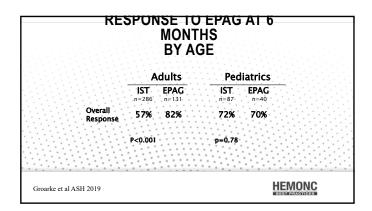


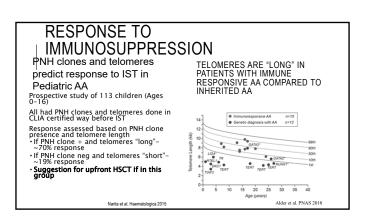


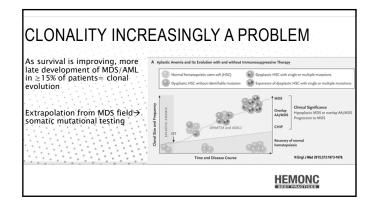




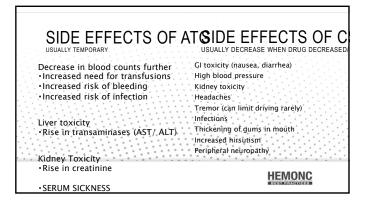


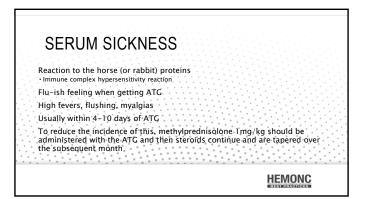






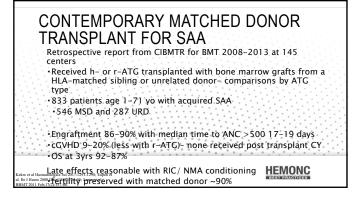
Paper	N	Dx Age of patients (yrs)	N genes/ Method		found found	•	Most Detected	VAF	MDS/AML evolution
Lane 2013	39 (20 SAA, 9 vSAA) 4 pts with PNH clone	34.8 (4-65.7)	219 Targeted NGS: exon capture followed by deep sequencing4 of 219 Genes	Ī	9 (24.2 %)		ASXL1, DNMT3A, BCOR	<10 % in 7	n/a
Heuser 2014	38 (12 SAA, 15 vSAA) 17pts with PNH clone	30 (9-79)	42 Targeted NGS: Coding exons in 33, NGS in 9	Ī	2 (5.3)		SLITTI, SETBP1 + ASXL1	25-50%	n/a
Kulasekararaj 2014	150 (51 SAA, 23 vSAA) 6 pts with PNH clone	44 (17-84)	835 Targeted NGS: (in 1st 57 samples; targeted sequencing detected) Genotyping was performed on DNA from BM mononuclear cells by using the Affymetrix 250K or SNPG platform		29 (19.3 %)		ASKL1, DNMT3A, BCOR	20 % (<10 % in 41 % patients)	17 (11.3%)
Huang 2015	138 (10vSAA, 71 SAA, 57 NSAA) 20 pts with PNH clone	30.7 (5-76)	6/ Sanger sequencing (focus on TET2 and ASLXL1)	I	24(17.4%)	I	ASXL1, TET2	n/a	9 (9%)
Yoshizato 2015	439 (187 SAA, 160 vSAA) 257 pts with PNH clone	29 (2-82) 56 (7-88)	106 Targeted NCS: whole-exome and targeted sequencing and single-nucleotide polymorphism (SNP)-array karyotyping		24 % (35.5 % including PIGA)		ASXL1, DNMT3A, BCOR/BCORL1, PIGA	9.5%	47 (11.2%)
Babushok 2015	22 (16 SAA, 5 vSAA) 10 pts with PNH clone	14.5 (1.5-61)	WES	ı	16 (72 %); 2 (9 %) with an MDS- associated SM		PIGA, LOH6p, STATSB, CAMK2b	>20%	n/a
Negoro 2017	258 AA and 59 PNH PRE and POST IST	Need supplemen ta	WES and targeted deep sequencing # genes	1	69/133 A4 (32/71 presentation n vs. 42/74 ofter IST		ASXL1, DNMT3A, PIGA	Р	N/A

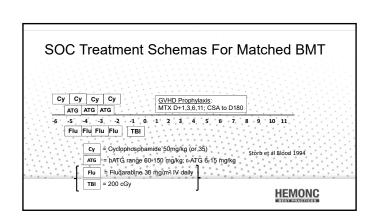




Addition of eltrombopag to IST was associated with higher rates of hematologic response among patients with SAA compared to historical cohorts Overall survival rate at 2 years was 97% Essentially newer SOC in adult patients Less clear for pediatric patients—trial data did not show this

	NOI L	١١٧٤ ر	: ON MA	ATCHE	SIBL	ING TF	RIALS	\
Institutio n	Years	N	Median Age (Range)	Engraftme nt (%)	Survival (%)	Median Follow- Up (Years)	Acute GVHD (%)	Chronic GVHD (%
IBMTR	1988- 1992	471	20 (1-51)	84	66	3	19	32
EBMT	1991- 1998	71	19 (4-46)	97	86	5	30	35
Seattle	1988- 2004	81	25 (2-63)	96	88	9	24	26
Seoul	1995- 2001	113	28 (16-50)	85	89	6	11	12
Taipei	1985- 2001	79	22 (4-43)	92	74	5	7	35
Sao Paulo	1993- 2001	81	24 (3-53)	82	56	6	37	39
EBMT	1998-	239	42 (30-67)	86	61	4	20	25

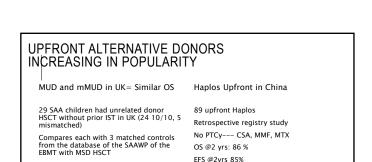




No PTCy CSA alone or CSA + MTX

1 failure to engraft @ 1 yr CI of cGVHD 19%

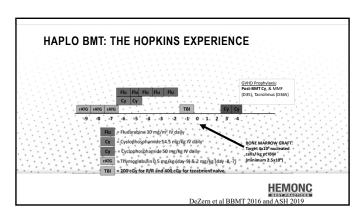
compared to 91% in MSD and 94% in IST

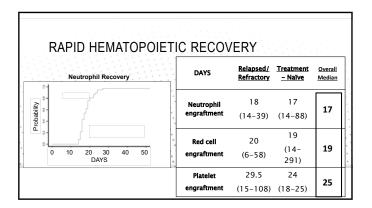


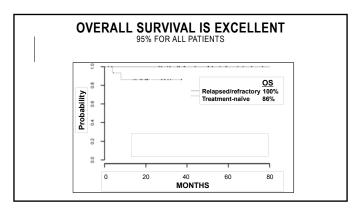
Increased CI grades II-IV aGVHD 30%

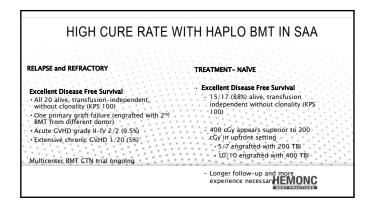
Xu et al Hematol Oncol 2017

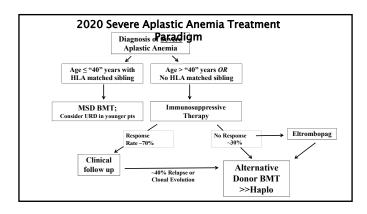
extensive cGVHD 3.4%

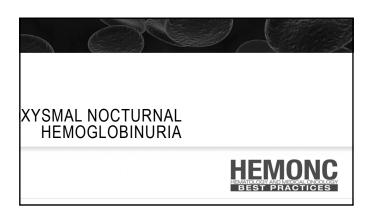


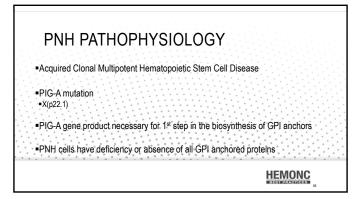


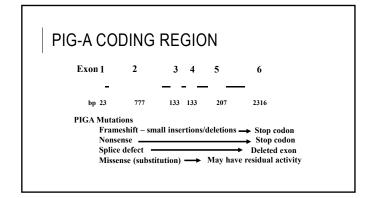


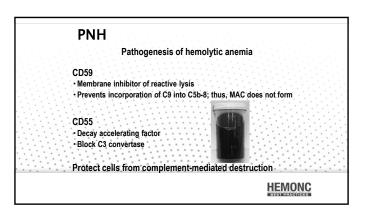


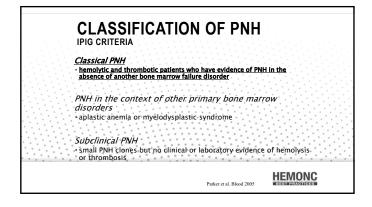


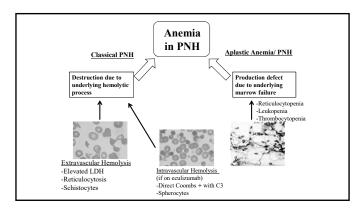




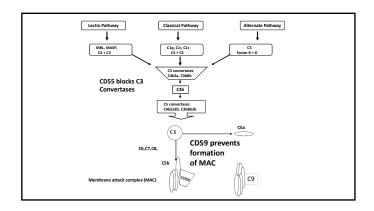


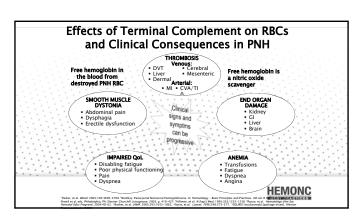


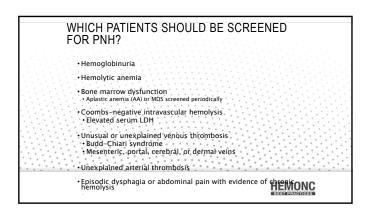


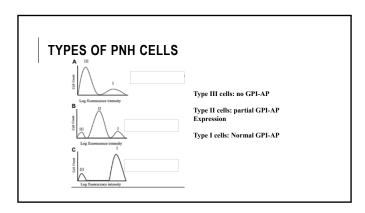


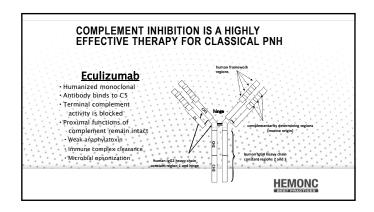
Amy DeZern, MD

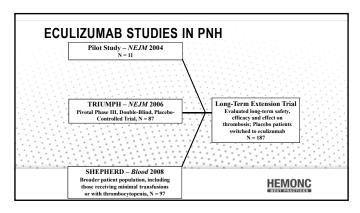


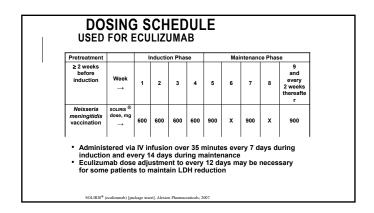


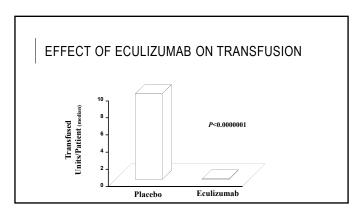


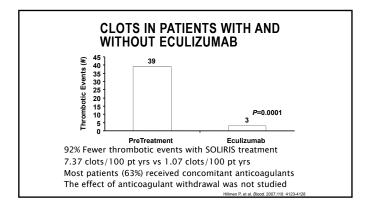


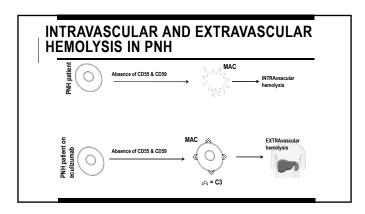


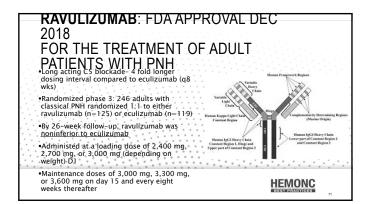






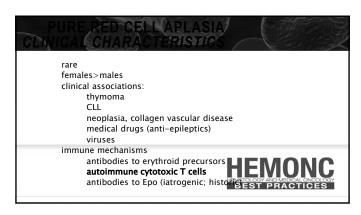


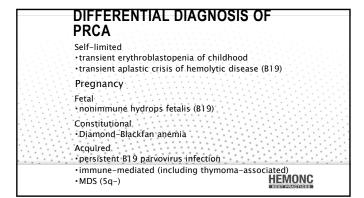


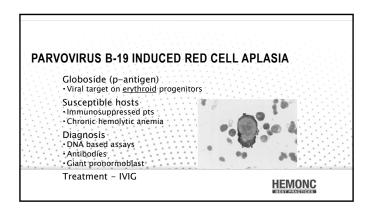


WHAT C5 BLOCKAGE DOES NOT DO Does not improve genetic defect Does not treat extravascular hemolysis Does not improve impaired hematopoiesis (bone marrow dysfunction) • Low white count or low platelet count persist









Patient	RBC lifespan	Immune Status	Time to mount immune response	Outcome
Normal	120 days	Normal	14-21 days	Normal Hgb usually maintained
Chronic hemolysis	5-10 days	Normal	14-21 days	Acute PRCA (transient)
lmmuno- deficient	120 days	Abnormal	Unable to clear virus	Chronic PRCA

PRCA: CLINICAL APPROACH Look for LGL in peripheral blood • Smear • flow cytometry if indicated Marrow culture • BFU-E maturation in vitro predicts response to immunosuppression Thymoma rule out with imaging Consider other associated diseases (SLE, MDS, thymoma, Rheumatoid Arthritis) Treat underlying cause! (usually with immunosuppression) HEMONC DEZem et al. Expl Rem 2013.

TREATMENT OF ACQUIRED IMMUNE PRCA

- Corticosteroids
- •Azathioprine/cyclophosphamide
- •Cyclosporine
- Antithymocyte globulin
- Plasmapheresis
- •anti-CD20; anti-IL2R
- Thymectomy (for tumor excision)

HEMONC

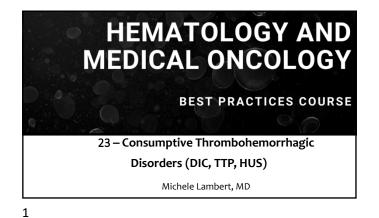
Consumptive Thrombohemorrhagic Disorders (DIC, TTP, HUS)

Michele Lambert, MD

August 15, 2020

Consumptive Thrombohemorrhagic Disorders (DIC, TTP, HUS)

Michele Lambert, MD



DISCLOSURES

Consultant: Novartis, Dynamed(EBESCO),
 Octapharma, Sysmex, CSL Behring, Dova,
 Shionogi, Bayer, Rigel, Principia, Argenx,
 DOI:

Off Label Usage

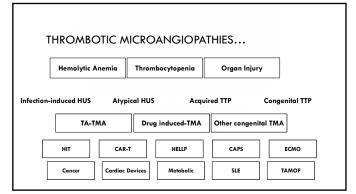
 \circ Eculizumab for TMA (not aHUS)

 Research Funding: OctaPharma, Novartis, Astra Zeneca, Quansys, Sysmex;

 Medical Advisor: PDSA, 22q11.2 Society, CdLS Foundation;

• Medical-legal firms: Consulting, Testimony

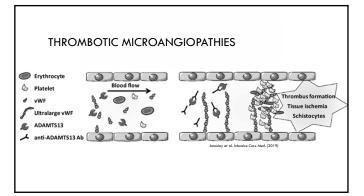
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THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)

- Dr. Moschowitz (1924)
 - Fever, thrombocytopenia, hemolytic anemia, neurologic dysfunction, renal involvement
- 1982 identified ultra-large vWF multimers as responsible for disease process
- 1998 ADAMTS-13 deficiency

3



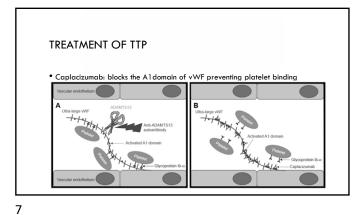
TREATMENT OF TTP

- Replenish ADAMTS13 activity (FFP, plasma exchange)
- Remove inhibitors (if acquired) (steroids and rituximab, plasma exchange)
- Remove ULVWF (plasma exchange)
- If complement activation is playing a role, strategies to block complement may be necessary as adjunctive therapies

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Consumptive Thrombohemorrhagic Disorders (DIC, TTP, HUS)

Michele Lambert, MD



TREATMENT OF TTP • ISTH recommendations: $^{\bullet}\,$ Plasma exchange for newly diagnosed TTP (strong recommendation) and addition of steroids and rituximab are recommended as well (conditional) Conditional recommendation for treatment with caplacizumab, • bleeding is a risk and relapses are delayed • For congenital TTP, plasma infusion during pregnancy and other high risk times is

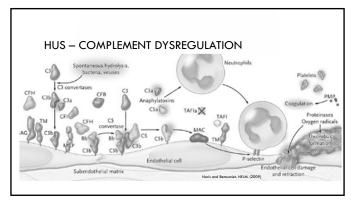
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CLASSICAL DIFFERENTIAL DIAGNOSIS HUS Renal dysfunction Neurologic symptoms (Can have renal failure) ave neurological dysfunction) Hemolytic anemia Hemolytic anemia Thrombocytopenia ADAMTS13 deficiency (congenital or Thrombocytopenia (E coli, Strep) ment-mediated (atypical) acquired)

HEMOLYTIC UREMIC SYNDROME • infection associated • Shiga-toxin producing E.Coli - STEC s. pneumonia • Others (Influenza, H1N1, HIV) atypical HUS • 50-60% alternative complement pathway • 5% thrombomodulin mutations • Some mediated by anti-complement factor H antibodies

9 10

HEMOLYTIC UREMIC SYNDROME • Metabolic Disease related Cobalamine C defects DGKE mutations • Underlying conditions Transplant associated Malignancy Autoimmune disorders (SLE, CAPS) • Medication induced (calcineurin inhibitors) Malignant HTN



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DIAGNOSIS OF HUS

- ADAMTS13 levels are >10%
- Low C3 and normal C4 found in 40% of HUS patients along with elevated C5a and sC5-b9 but these are not specific for complement driven HUS
- Identification of etiology is important and differentiation of infection driven vs not by culture/PCR (30% atypical HUS have GI symptoms and 5% STEC-HUS have no GI symptoms)

MANAGEMENT OF HUS

- If secondary, treat or remove underlying cause
- If complement mediated, anti-C5 complement blockade (eculizumab) is standard of care
 - Meningococcal vaccine is recommended for all patients
 - Risk counseling
 - Antibiotic prophylaxis for first 2 weeks after starting therapy
- Often start with plasma exchange while awaiting results of testing

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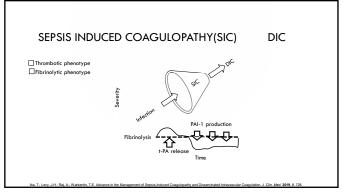
SO WHAT ABOUT DIC...

How does that fit in with the TMAs and how do we tell the difference?

DISSEMINATED INTRAVASCULAR COAGULATION (DIC)

- Acquired syndrome of intravascular activation of coagulation
- Loss of localization
- Microvascular damage end organ damage
- Combination of inflammation and coagulation leading to endothelial dysfunction

15 16



PATHOGENESIS OF DIC

- Multiple triggers but same final pathway:
 - Depletion of hemostatic factors (both pro-coagulant and natural anti-coagulants)
 - Depletion of the inhibitors (antiplasmin, antithrombin, protein c/s) that help stop the process
- $^{\bullet}$ Based on the trigger, DIC may look different
 - $\bullet\,$ Sepsis mediated DIC more organ dysfunction and thrombosis, higher fibrinogen
 - Fibrinolytic (non-sepsis) DIC have more bleeding (malignancy, placental abruption)

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CLINICALLY RELEVANT DIC

- Causes bleeding or thrombosis
- Many patients may have lab abnormalities that do not require intervention
- But morbidity and mortality are high for patients with DIC and bleeding/thrombosis



SEPSIS INDUCED COAGULOPATHY SCORE

- Platelet coun
- INI

20

 SOFA score (sequential organ failure assessment) (used to confirm presence of sepsis and is limited to 2 points);

IF SIC SCORE POSITIVE, TREAT AND DO DIC SCORE

Laboratory Value	Score
Platelet Count:	
>100 x 10 ⁹ /L	0
50-99 x 10 ⁹ /L	1
<50 x 10°/L	2
Elevated Fibrin split product (d-dimer, FDP):	
No increase	0
Increased (2000-9999)	2
Markedly increased (>10,000)	3
Elevated INR:	
≤1.2	0
1.3-1.4	1
≥1.5	2
Fibrinogen*: *the sensitivity of a low fibrinogen level for the	diagnosis of DIC
≥100 mg/dL was only 28%	0
<100 mg/dL	1

TREATMENT

- Consider heparins in coagulopathic patients with sepsis to prevent progression to DIC and LMWH is preferred over UFH (if able)
- Would consider antithrombin replacement if antithrombin is sufficiently low in DIC patients

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A FEW NOTES

- DIC, TTP and HUS and have overlap
- Management is different for the different disorders because of different underlying pathophysiology
- $^{\bullet}$ Important to make the right diagnosis

CONCLUSIONS

- ADAMTS13 activity can differentiate TTP from HUS
 - Clinically can be hard to distinguish
- Plasma exchange initially until labs come back
- If complement activation, eculizumab; if not, ? Caplacizumab
- If suspect DIC, use a score

Review on DIC, TTP, HUS and HIT and Limb Gangrene

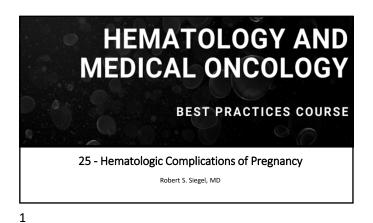
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August 15, 2020

Hematologic Complications of Pregnancy

Robert S. Siegel, MD

August 15, 2020



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Disclosures of Financial Relationships with Relevant Commercial Interests

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- · Research Funding: Janssen Pharmaceuticals, Medivation, Pfizer, Suncoast Community Clinical Oncology

1. Anemia 2. Thrombocytopenia and **Topics** Other Bleeding Disorders 3. Venous Thromboembolism 3

Anemia

2

Anemia in Pregnancy

- Common complication
- Often dilutional:
 - Plasma volume increases by 40%-50%
 - \bullet Red cell mass increases by $20\%\mbox{-}30\%$
- Prevalence of iron deficiency anemia in pregnant women in the United States estimated to be 19%

Mei Z, Cogswell ME, Looker AC, et al. Assessment of iron status in US pregnant women from the National Health and Nutrition Examination Survey (NHANES), 1999-2006. Am J Clin Nutr. 2011;93(6):1312-1320.

Anemia in Pregnancy

Definition of anemia in pregnancy by ACOG and WHO:

- First trimester Hemoglobin <11 g/dL
- \bullet Second trimester Hemoglobin <10.5 g/dL
- \bullet Third trimester Hemoglobin <10.5 to 11 g/dL
- Postpartum Hemoglobin < 10 g/dL

Anemia in Pregnancy

- Association between severe anemia and poor pregnancy outcomes in multiple observational studies
 - $^{\bullet}$ Severe antenatal or postnatal maternal anemia is associated with increased risk of maternal death (OR = 2.36) 1
- Strong recommendation for iron supplementation for pregnant women with anemia
- Benefit of iron supplementation in pregnant women who are not anemic unclear, however recommended for all pregnant women by CDC, WHO, and ${\sf ACOG}^2$
- Daru J, Zamora J, Fernández-Félik BM, et al. Risk of maternal mortality in women with severe anaemia during pregnancy and post multilevel analysis. Lancer Glob Health. 2018;6(5):e548-e554.

 Pena-Rosos JP, Viter IF. Effects of routine aral tins supplementation with or without folic acid for women during pregnancy. Cockra of systematic reviews. 2006;3(3):C0004736-.

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Folate Requirements

- Macrocytosis due to vitamin B12/folate deficiency can be masked by simultaneous iron deficiency
- B12 deficiency is rare
- Pregnant women require 600 mg of folate daily
 - Daily supplementation with 400 mg recommended by CDC and ACOG
- Avoids maternal megaloblastosis
- Avoids fetal neural tube defects

1. Achebe MM, Gafter-Gvili A. How I treat anemia in pregnancy: iron, cobalamin, and folate. Blood. 2017;129(8):940-949

Folate Requirements

Iron Requirements

nd Quality (US); 2015 Mar. (Evidence Syntheses, No. 123.)

recommended if patient is not anemic1

be treated with 60-120 mg/day of iron²

• Pregnant women have increased iron requirements, 27 mg per day • In the United States, daily supplementation with 30 mg is

 \bullet Women with IDA and hemoglobin <9 g/dL or hematocrit <27% should

I. Institute of Medicine (US) Committee on the Prevention, Detection, and Management of Iron Deficiency Anemia Among U.S. Children and Women of Childboarring Age, Earl R. Woteki C.F., editors, Iron Deficiency Anemia. Recommended Guidelines for the Prevention, Detection, and Management Among U.S. Children and Women of Childboarring Age, and Varbington (DC): National Academies Press (US); 1993. Recommended Guidelines For Preventing And Treating Iron Deficiency Anemia in Pregnant Women.

2. McDonagh M, Cantar A, Bougatsos C, et al. Routine Ion Supplementation and Screening for Iron Deficiency Anemia in Pregnant Women.

2. McDonagh M, Cantar A, Bougatsos C, et al. Routine Ion Supplementation and Screening for Iron Deficiency Anemia in Pregnant Women. A youtenant Review to Update the U.S. Preventive Service Task Force Recommendation [Internet]. Rockville (MD) Agency for Healthcare Research

Higher folate doses needed:

- Hemoglobinopathy
- Patients on anti-convulsants
- Multiple gestation
- Neural tube defect in prior pregnancy

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Sickle Cell Disease

Pregnant women have increased risk of:

- Vaso-occlusive crises
- Anemia
- Hypertension
- Stillbirth
- Spontaneous abortions
- Infection, especially UTIs and pulmonary infection (Mycoplasma, Haemophilus, Salmonella)

Sickle Cell Disease

Infants of SCD mothers have increased risk of:

- Prematurity
- Small for gestational age

Sickle Cell Disease

Transfusions/exchange transfusions:

- Not routinely used throughout pregnancy
- May be useful in final weeks
- Individualized therapy

Early & aggressive therapy for:

- Infection
- Pain crisis

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Sickle Cell Disease

Prophylactic transfusion for pregnant women with sickle cell disease: a systematic review and meta-analysis

- · Prophylactic vs "on demand" transfusions
- Twelve studies involving 1291 participants included
- Netra-analysis: prophylactic transfusion associated with a reduction in:

 Naternal mortality (7 studies, 955 participants; odds ratio [OR] = 0.23)

 Vasa-occlusive pain episodes (11 studies, 1219 participants; OR = 0.26)

 Pulmonary complications (9 studies, 1019 participants; OR = 0.25)

 Pulmonary embolism (3 studies, 237 participants; OR = 0.07)

 Neonatal death (5 studies, 374 participants; OR = 0.06)

 Preterm birth (9 studies, 1123 participants; OR = 0.59)

wski AK, Shehata N, D'Souza R, et al. Prophylactic transfusion for pregnant women with sickle cell disease: a systematic and meta-analysis. Blood. 2015;126(21):2424-2437.

Thrombocytopenia

Thrombocytopenia of Pregnancy

- Gestational Thrombocytopenia
- Immune Thrombocytopenic Purpura
- Human Immunodeficiency Virus
- Preeclampsia/HELLP Syndrome
- SLE/ Various Vasculitides
- TTP/HUS

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Gestational Thrombocytopenia

• Dilutional issue vs mild ITP

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- Platelet count is 100-150,000/dl¹
- Mothers can be delivered normally
- No association with fetal thrombocytopenia
- Occurs in 5-7% of all pregnancies
- Patients should receive routine OB care

Immune Thrombocytopenia

- Occurs in 1 to 10 in 10000 women1
- Incidence of severe ITP (platelet count <50,000 or thrombocytopenia requiring treatment) occurs in 0.83 per 10000
- A diagnosis of exclusion
- Platelet count lower than in gestational thrombocytopenia
- Diagnosis is suggested when a mother with pre-existing ITP develops profound thrombocytopenia
- Provan D, Stasi R, Newland AC, Blanchette VS, Bolton-Maggs P, Bussel JB, et al. International consensus report on the investigation and management of primary immune thrombocytopenia. Blood 2010;115:168–86.
 Care A, Pavord S, Knight M, Alfirevic Z. Severe primary autoimmune thrombocytopenia in pregnancy: a national cohort study. BJOG. 2018;125(5):604-612.

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Immune Thrombocytopenia

Retrospective study of 92 women with ITP:

- Most pregnancies were uneventful
- · Bleeding was uncommon and not correlated with platelet count
- No association between maternal & infant's platelet count
- 32% required ITP therapy
- Platelet count in 2nd child was predicted by platelet count in first
- Intracranial bleed/fetal loss in ITP mothers = 1-2%

bert KE, Mittal R, Sigouin C, Heddle NM, Kelton JG. A retrospective 11-year analysis of obstetric patients with idiopathic thrombocytop pura. Blood. 2003; 102(13):4306-4311.

Immune Thrombocytopenia

- Concern for fetal thrombocytopenia
- Fortunately, a rare problem
 Among ITP mothers, 10% have platelets <50,000
- 0.04% have platelets <20,000
- * Higher rates of preterm delivery (6.7% vs. 2.2%) and perinatal mortality (4.8% vs. 1.3%) 2
- ullet Scalp vein & percutaneous umbilical cord sampling should not be done 3
 - Hemorrhagic risk to fetus
 - · Underpredicts platelet count
- Burrows RF, Kelton JG. Fetal thrombocytopenia and its relation to maternal thrombocytopenia. N Engl J Med 1993;329:1463–6.
 Belkin A, Levy A, Sheiner E. Perinatal outcomes and complications of pregnancy in women with immune thrombocytopenia purpura. J Mater Neconatal Med. 2009;22(11):1081-1085.
 Burrows RF, Kelton JG. Fregnancy in patients with idiopathic thrombocytopenia assessing the risks for the infant at delivery. Obstet
 - col Surv. 1993;48(12):781-788.

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Immune Thrombocytopenia

- Prednisone/IVIg used for bleeding or platelets <20,0001
- Splenectomy can be considered second-line for thrombocytopenia refractory to steroids and IVIg²
 - Other therapies (immunosuppressants, cytotoxic agents) teratogenic
 - Done during second trimester
- ITP is NOT an indication for C-section, mode of delivery should be based on obstetric indications¹
- Platelets rise after delivery

 Mohey R, Kaur SD, Chumber S, Kriplani A, Bhatla N. Splenectomy during pregnancy: treatment of refractory immune thrombo purpura. BMJ Case Rep. 2013;2013:ber2013201778. Published 2013 Dec 20. doi:10.1136/bcr-2013-201778
 Neunert C, Lim W, Crowther M, et al. The American Society of Hematology 2011 evidence-based practice guideline for immune. mbocytopenia, Blood, 2011:117(16):4190-4207.

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Preeclampsia

- BP > 140/90, proteinuria > 0.3 gms/24 hrs after 20 weeks gestation
- · Primigravidas at increased risk
- Platelets low in 15-50%
- Platelet count < 50,000 seen in < 5%

Preeclampsia

Consider starting low dose ASA in the second trimester for patients at high risk for preeclampsia:

- · Antiphospholipid antibody syndrome
- Prior history of preeclampsia
- Age <20 or >30 y.o.
- BMI >35
- Hypertension
- History of insulin resistance
- Twin pregnancy

Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prab Antithrombotic Therapy and Prevention of Thrombosis, Guidelines. Chest. 2012;141(2 Suppl):e691S-e736S. tra DL, Prabulos AM, Vandvik PO. VTE, thrombophilia, antithrombotic therapy, and pregnan Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice **HELLP Syndrome**

- Hemolysis (microangiopathic)
- Elevated (Bili>1.2, SGOT>70)
- Liver enzymes (LDH >600)
- Low Platelets (<100,000/dl)

HELLP Syndrome

- 12% of preeclamptic pts have HELLP
- Typical platelet count < 100,000
- Pts have nausea, malaise, abdominal pain
- HELLP pts should be delivered ASAP
- HELLP pts may maintain low platelet count for days after delivery
- Plasmapheresis for refractory patients

Thrombotic Thrombocytopenic Purpura

• Seen with pregnancy or post partum

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 Thrombocytopenia, microangiopathic hemolytic anemia, impaired renal function, fever, neurologic abnormalities

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Thrombotic Thrombocytopenic Purpura

- •Widespread endothelial damage seen
- •Therapy:
 - Daily plasmapheresis
 - Delivery ASAP
 - Splenectomy for resistant cases

Neonatal Alloimmune Thrombocytopenia (NAIT)

- Fetal inheritance of paternal platelet isoantigens that are not present in the mother
- Fetal thrombocytopenia when maternal isoantibodies cross the placenta
- •Most common Ag: HPA-1a & HPA-5b

27 28

Neonatal Alloimmune Thrombocytopenia (NAIT)

Women at highest risk:

- Related to other women with NAIT
- Prior pregnancy complicated by NAIT
- Lacking HPA-1a and HPA-5b Ag

Diagnosis: well infant with a very low platelet count

• Later pregnancies more affected than the first

Neonatal Alloimmune Thrombocytopenia (NAIT)

- Most severe complication is <u>intracranial bleeding</u>
- 10-50% occurs in utero
- •Tx: washed maternal platelets or from blood bank
- Antenatal Tx: early C-section, IVIg, prednisone, intrauterine platelets

Other Bleeding Disorders

Von Willebrand Disease

- Types 1 & 2A patients have safe levels of von Willebrand factor by delivery
- Type 2B patients may have worsening thrombocytopenia during pregnancy because of platelet aggregation
- Type 3 disease is the least common, but most severe
 - VWF levels do not rise throughout pregnancy, bleeding risk is unchanged

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Von Willebrand Disease

Patients with Types 2B, 2M, 2N, & 3

- Do NOT use DDAVP
- Use Factor replacement until VWF and Ristocetin cofactor levels reach 50 IU/mL

Hemophilia A and B Carriers

- In Factor VIII Deficiency, factor levels <30 IU/dL
 - Use DDAVP or recombinant Factor VIII for 3-4 days
 - Rarely required by delivery
- In Factor IX deficiency, factor levels <50 IU/dL
 - Use recombinant factor IX for 3-4 days
 - Factor IX levels typically do not rise during pregnancy

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Factor XI Deficiency

- Deficiency levels
 - Severe: 20%
 - Partial: 20%-60%
- Mostly seen in Ashkenazi Jewish population
- Heterozygous frequency is 1 in 12
- May bleed heavily during C-section

Factor XI Deficiency

- Bleeding risk doesn't always correlate with factor XI level
- Past bleeding history is important
- Treatment is FFP, antifibrinolytic agents
- possibly DDAVP
- rVIIa (15-20ug/kg)

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Factor XI Deficiency

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- Post partum bleeding noted in 18% of factor XI deficient patients (versus 1-5% in average risk population)
- No consistent change in Factor XI levels during pregnancy
- Fewer bleeding episodes in women who received prophylactic agents
- Tx: FFP, DDAVP, antifibrinolytic agents, factor XI concentrate in Europe

Wiewel-Verschueren S, Arendz IJ, M Knol H, Meijer K. Gynaecological and obstetrical bleeding in women with factor XI deficiency - a systematic review. Haemophilia. 2016;22(2):188-195.

Venous Thromboembolism

Essential Thrombocytosis, JAK2, and Pregnancy

Retrospective single-institution analysis of 63 pregnancies in 36 women with ET

- 61% live births, 39% fetal loss
- 10/20 patients studies had JAK2 mutation

Predictors of outcome:

- No correlation with maternal age, JAK2, WBC, HGB, platelet count
- Only factors that affected results: use of ASA
- ullet 75% of patients who took ASA had successful delivery

1. Gangat N, Wolanskyj AP, Schwager S, Tefferi A. Predictors of pregnancy outcome in essential thrombocythemia: a single institution study of 63 pregnancies. Eur J Haematol. 2009;82(5):350-353.

pregnancies. Eur 7 naemaroi. 2009;62(3):330-333

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Platelets
Clotting
Anti Clotting Factors Clotting

Based on packets of proteins in blood in:
Platelets
Vessel walls
Tissues (brain, heart)

Thrombosis & Pregnancy

- Pregnancy & 6 weeks postpartum
 - 5-6x increased risk of DVT
 - •1/1000 pregnancies
- Most DVT (90%) involve left iliac vein
 - From L iliac vein compression by R iliac artery and ovarian artery
 - Iliac VTE more likely to embolize

Hypercoagulability

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Virchow's Triad: Hypercoagulability, Stasis, Endothelial Damage

Hypercoagulability

- •Increased coagulation factors
- Acquired resistance to APC, Protein S
- •Impaired fibrinolysis
- Increased plasminogen activator inhibitors 1&2
- High Factor VIII levels

Hypercoagulability

Virchow's Triad: Hypercoagulability, Stasis, Endothelial Damage

Stasis

43

- Diminished venous flow to lower extremities
 - 50% reduction by end of 2nd trimester
 - Nadir at 36 weeks
 - Full recovery 6 weeks post delivery

Endothelial Damage

• Damage to pelvic vessels during vaginal delivery or C-section

Normal Pregnancy & Postpartum Period as an Acquired Hypercoagulable State

- ↑ concentration of coagulation factors II, V, VII, VIII,IX, X, XII, fibrinogen and vWF
- ullet levels of free protein S, and \uparrow acquired resistance to APC
- ↑ markers of coagulation: D-dimer and Prothrombin fragment F1+2
- ↓ venous flow in the extremities

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Thrombophilia and VTE in Pregnancy

- Inherited thrombophilia is present in 15% of Western populations
- Thrombophilia seen in 50% of pregnant VTE pts
- Level of risk dependent on:
 - Underlying thrombophilia
 - History of thrombotic events
 - Additional risk factors (obesity, smoking, DM)

Pregnancy-Associated Thrombosis

- PE account for 15% of maternal deaths in developed countries
- \bullet In women of reproductive age, > 50% of VTE related to pregnancy
- Incidence rate of VTE = 3.24/1000 women-years (Glasgow retrospective study of > 72,000 deliveries)¹
- 84% of deep vein thrombosis = left leg
- Highest VTE Risk in 3rd trimester & in first 6 wks post-partum

McColl MD, Ramsay JE, Tait RC, et al. Risk factors for pregnancy associated venous thromboembolism. Thromb Hoemost. 1997;78(4):1183-1188.

45

Pregnancy-Associated Thrombosis

MEGA Study

- 285 pregnant women vs. 857 controls
- 4 to 5 x increased risk of VTE during pregnancy
- 8.8x increased risk in 3rd trimester
- 52 x increased if patient V Leiden carrier
- 31 x increased if patient II G20210A carrier
- 84 x increased in the first 6 weeks post partum

Pomp ER, Lenselink AM, Rosendaal FR, Doggen CJ. Pregnancy, the postpartum period and prothrombotic defects: risk of venous thrombosis in the MEGA study. J Thromb Haemost. 2008;6(4):632-637.

Pregnancy-Associated Thrombosis

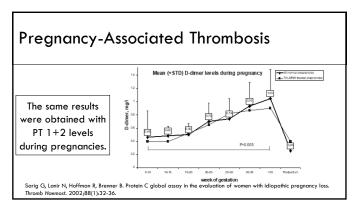
Mean (±STD) of free protein S levels during 28 pregnancies

During pregnancy, levels of the anticoagulants antithrombin (AT) and protein C (PC) are unchanged, but free protein S levels decrease markedly.

Sarig G, Lanir N, Hoffman R, Brenner B. Protein C global assay in the evaluation of women with idiopathic pregnancy loss.

Thromb Hosemost. 2002;88(1):32-36.

48



Thrombosis & Pregnancy

Inherited

- Protein C and S
- Deficiency
- APC Resistance
- Antithrombin Deficiency Factor V Leiden
- Prothrombin G20210A

- Antiphospholipid Antibodies
- Ovarian Hyperstimulation
- Obesity
- Immobilization
- Age > 35

49

51

50

Prevalence of Congenital Thrombophilia (European Populations)

Prevalence (%)
0.25 - 0.55
0.20 - 0.33
2 - 7
2
10

VTE Risk in Thrombophilic Patients

Thrombophilia ¹	Odds ratio for VTE ²	
AT III Deficiency (quant)	282	
AT III Deficiency (qual)	28	
Factor V Leiden (heterozygous)	4.5	
Prothrombin gene mutation	4.4	
MTHFR C677T (homozygous)	No change (pregnancy only)	

- NcColl MD, Ellison J, Reid F, Tait RC, Walker ID, Greer IA. Prothrombin 20210 G-->A, MTHFR C677T m. Promboembolism associated with pregnancy. BI.OG. 2000;107(4):565-569. Seharat A, Scharl RE, Beckmann MW, et al. Prothrombin and factor V mutations in women with a histor and the puerperlum. N Engl J Med. 2000;342(6):374-380.

52

Assisted Reproductive Treatment & DVT Risk

Ovarian Hyperstimulation Syndrome (OHSS)

Increased risk of DVT, especially after treatment cycle that results in pregnancy

Systematic Review of 54 patients with ovarian stimulation

- 75% had DVT (60% of which were in internal jugular vein/neck vein or upper
- · 25% had arterial clots (mostly intracerebral)
- 6 had a history of prior thrombosis
- · 2 had a strong family history of thrombosis
- Prophylaxis indicated for high risk women

art JA, Hamilton PJ, Murdoch AP. Thromboembolic disease associated with ciques. Hum Reprod. 1997;12(10):2167-2173.

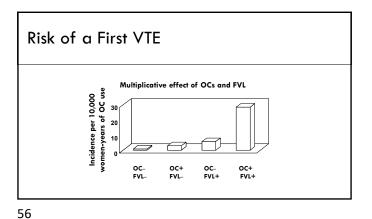
Assisted Reproductive Treatment & DVT Risk

- Estradiol levels > 10x normal
- Polycystic ovaries increase risk
- Thrombi occur at 7 to 10 weeks
- · Mechanism(s) for thrombosis is unclear
- Underlying thrombophilia generally has not been detected in these
- Prophylactic anticoagulation is not recommended for average risk women

Bottes SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvík PO. VTE, thrombophilla, antithrombotic therapy, and pregnanc Antithrombotic Therapy and Prevention of Thrombosis, 9th eds American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141(2) Supplies69715-2736.

Managing Patient at Risk for VTE

- Risk of VTE must be established
- No evidence to support universal screening for inherited thrombophilia in pregnant patients
- Use of thromboprophylaxis is often a judgment decision



Vitamin K Antagonists

55

59

- Women on Vit K antagonists contemplating pregnancy should have:
 - Frequent checks for pregnancy
 - Change to LMWH when pregnancy is achieved
 - Limit use of fondaparinux
 - Avoid oral direct thrombin inhibitors and anti-Xa inhibitors

Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvík PO. VTE, thrombophilla, antithrombotic therapy, and pregnancy: Antithrombotic therapy and Prevention of Thrombosis, 7th eds. American College of Chest Physicians Evidence-Based Clinical Practice Quidelance. Chest. 2012;14 |12 Supplie-901 S-a7365.

Use of Anticoagulants in Lactation

- The following can continue during breastfeeding:
 - Warfarin
 - UFH
 - LMWHDanaparoid
 - R-Hirudin
 - Low dose aspirin

Avoid fondaparinux, oral antithrombin, and anti-Xa inhibitors

Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO. VTE, thrombophilla, antithrombotic therapy, and pregnancy: Antithrombotic Therapy and Prevention of Thrombosis, 9th edi. American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141(2 Suppl):e6915-e7365.

57 58

VTE Prophylaxis after C-section

- Pre-op risk DVT risk assessment to determine need for prophylaxis
 - In absence of VTE risk factors, use only early mobilization
- For women at increased risk because of 1 major risk factor or 2 minor risk factors:
 - Prophylactic LMWH or UFH or compression stockings
- For women with > 2 risk factors & at high risk for VTE:
 - Prophylactic LMWH + compression stockings and/or INT pneumatic compression
 - High risk pts should continue prophylaxis for 6 wks post discharge

Botes SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO. VTE, thrombophilla, antithrombotic therapy, and pregnancy-Antithrombotic Therapy and Prevention of Thrombosis, 9th ed. American College of Chest Physicians Evidence-Based Clinical Practice Guidelines Chest. 2012;141(2) supplies6915-27456.

Safety of Withholding Heparin

Previous VTE is a risk factor for recurrence:

- 125 pregnant women with prior VTE were prospectively followed
- Antepartum heparin was held
- Labs for thrombophilia sent on 95 women
- Warfarin or other anticoagulant given for 6 weeks post partum

Brill-Edwards P, Ginsberg JS, Gent M, et al. Safety of withholding heparin in pregnant women with a history of venous thromboembolism. Recurrence of Clot in This Pregnancy Study Group. N Engl J Med. 2000;343(20):1439-1444.

Safety of Withholding Heparin

Results:

61

- 3/125 women had recurrent VTE
- No VTE in 44 women with no evidence of thrombophilia and prior VTE associated with temporary risk factor
- No VTE In 51 patients with positive lab evaluation for thrombophilia and/or idiopathic prior VTE
 - 5.9 % had VTE

Brill-Edwards P, Ginsberg JS, Gent M, et al. Safety of withholding heparin in pregnant women with a history of venous thromboembolism Recurrence of Clot in This Pregnancy Study Group. N Engl J Med. 2000;343(20):1439-1444.

VTE Prophylaxis: Single Past VTE

VTE with temporary risk

- Antenatal: clinical surveillance, no therapy
- Postpartum: anticoagulant treatment for 6 weeks
 - Enoxaparin 40 mg qd
 - Dalteparin 5000 IU qd
 - Warfarin (INR 2-3)

Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO. VTE, thrombophillia, antithrombotic therapy, and pregnancy; Antithrombotic Therapy and Prevention of Thrombooks, 9th ed. American College of Chest Physicians Evidence-Basec Clinical Practice Societaleines. Chest. 2012; 141(2) Suppl)e691 Se-27365.

VTE Prophylaxis: Single Past VTE

Idiopathic VTE associated with higher risk thrombophilia (antithrombin deficiency, antiphospholipid antibody, prothrombin gene mutation, factor V Leiden, homozygosity for these conditions)

- Antenatal
- Prophylactic or INT dose LMWH
- Postpartum
- Therapeutic anticoagulation for 6 weeks

Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvík PO. VTE, thrombophilla, antithrombotic therapy, and pregnancy: Antithrombotic therapy and Prevention of Thrombosis, 7th eds. American College of Chest Physicians Evidence-Based Clinical Practice Quidelance. Chest. 2012;14 |12 Supplie-901 S-a7365.

VTE Prophylaxis: ≥ 2 VTE

Not associated with thrombophilia

- Antenatal: LMWH
- Prophylactic or INT or ADJ dose LMWH
- Postpartum

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- Therapeutic anticoagulation for 6 weeks
- Prophylactic, INT, or ADJ dose LMWH OR
- Warfarin with INR 2-3

Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO. VTE, thrombophillo, antithrombotic therapy, and pregnancy. Antithrombotic therapy and Prevention of Thrombosis, 9th ed. American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;14 1(2 Supplied) 15–27365.

63 64

VTE Prophylaxis: Previous VTEs on Long Term Anticoagulation

Stop oral anticoagulants, make change to

- ADJ dose LMWH
- 75% of therapeutic dose of LMWH

Resume long term anticoagulation post partum

Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO. VTE, thrombophilla, antifhrombotic therapy, and pregnancy: Antifhrombotic therapy and Prevention of Thrombosis, 7th eds: American College of Chest Physicians Evidence-Based Clinical Practice Quidelines: Chest. 2012;14 1(2 supplied) 15-e7365.

Acute VTE Diagnosed in Pregnancy

Antenatal

- ADJ dose LMWH
- S.Q. therapy should continue through pregnancy
- Hold for 12-24 hours before delivery

Postpartum

- Continue for at least 6 weeks after delivery for a total duration of at least 3 months
- months
- Warfarin (INR 2-3)

Bates SM, Greer IA, Middeldorp S, Veenstra DL, Probulos AM, Vondvik PO. VTE, thrombophilla, antithrombotic therapy, and prepancery Antithrombotic Therapy and Prevention of Thrombosis, 9th eds American College of Chest Physicians Evidence-Based Clinical Protate Guidelines. Chest. 2012;141(2 Supplied) 518-2736.

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DVT: Making the Diagnosis

- Compression ultrasonography:
 - Difficult in calf and iliac vein DVT
- MRI is useful for iliac vein DVT
 - CT causes radiation exposure to fetus
- Negative D-Dimers are helpful
- Limited venography is acceptable
- For suspected PE, CT or Ventilation/perfusion scan
- Making the correct diagnosis is critical

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Thrombophilia and Pregnancy Complications/Loss

- A woman should be screened for antiphospholipid antibodies when she has:
 - Recurrent pregnancy loss (≥3) before 10 weeks

Botes SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvík PO. VTE, thrombophillo, entithrombotic therapy, and pregnancy: Antithrombotic Therapy and Prevention of Thrombotis, 9th ed. American College of Chest Physicians Evidence-Based Chincal Practice Guidelines. Chest. 20.12/j.141(2 Suppl)e6915-87365.

Anti-phospholipid Antibodies (APLA)

Occurs in association with

- Systemic lupus
- Drugs
- Without explanation in an otherwise healthy woman

APLA is associated with increased risk of thrombosis and pregnancy loss

Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO. VTE, thrombophilla, antifhrombotic therapy, and pregnancy: Antifhrombotic therapy and Prevention of Thrombosis, 7th eds: American College of Chest Physicians Evidence-Based Clinical Practice Quidelines. Chest. 2012;141(12 Supplied)15-e7365.

Prevention of Pregnancy Complications in Women with Thrombophilia

Patients with APLAs & recurrent pregnancy loss (>3)

Recommendations:

- Prophylactic or INT dose UFH or LMWH +
- Aspirin 75 to 100 mg qd

Bates SM, Greer IA, Middeldorp S, Veenstra DL, Probulos AM, Vandvík PO. VTE, thrombophilla, antithrombotic therapy, and pregnancy: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012; 141(2 Supplied) 518-2735.

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Prevention of Pregnancy Complications in Women with Thrombophilia

Aspirin + Heparin vs. Aspirin Alone in Women with Recurrent Miscarriage (ALIFE)

- 364 patients with history of unexplained miscarriage (>2) and <6 weeks pregnant
- Patients were enrolled into 3 arms
 - ASA 80 mg qd + open label LMWH
- ASA 80 mg alone
 Placebo
- Primary measure: live births

Kaandorp SP, Goddijn M, van der Post JA, et al. Aspirin plus heparin or aspirin alone in women with recurrent miscarriage. N Engl J Med. 2010;362(17):1586-1596.

Prevention of Pregnancy Complications in Women with Thrombophilia

Aspirin + Heparin vs. Aspirin Alone in Women with Recurrent Miscarriage (ALIFE)

- Proportions that gave birth by group
 - ASA: 50.8%
 - ASA + LMWH: 54.5%
- Placebo: 57%
- Even patients with thrombophilia did not benefit
 I imitation, subgroups was a small
- Limitation: subgroups were small
- Conclusion: Neither ASA nor ASA + Heparin improved live birth rate

Kaandorp SP, Goddlip M, van der Post JA, et al. Aspirin plus heparin or aspirin alone in women with recurrent miscarriage. N Engl J Med. 2010;362(17):1586-1596.

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Prevention of Pregnancy Complications in Women with Thrombophilia

The Scottish Pregnancy Intervention Study

- 294 participants, presenting at <7 weeks gestation, with a history of 2 consecutive previous pregnancy losses at or before 24 weeks gestation
- Patients excluded if they had APLA, LAC, ACA, hx of DVT, or hx of arterial thrombosis
- Randomized to:
 - Enoxaparin 40 mg sq (start at 6 weeks) + 75 mg ASA qdaily
 - Surveillance only

Clark P, Walker ID, Langhorne P, et al. SPIN (Scottish Pregnancy Intervention) study: a multicenter, randomized controlled trial of low-molecular-weight heparin and low-dose aspirin in women with recurrent miscarriage. Blood. 2010;115(21):4162-4167.

Prevention of Pregnancy Complications in Women with Thrombophilia

The Scottish Pregnancy Intervention Study

- 147 participants randomized to pharmacological intervention
 - 32 (22%) pregnancy losses occurred
- 147 participants with surveillance only:
 - 29 losses (20%)

74

76

 Conclusion: No evidence to support pharmacologic intervention based on miscarriages alone

Clark P, Walker ID, Langhorne P, et al. SPIN (Scottish Pregnancy Intervention) study: a multicenter, randomized controlled trial of low-molecula weight heparin and low-dose aspirin in women with recurrent miscarriage. Blood. 2010;115(21):4162-4167.

73

Prevention of Pregnancy Complications in Women with Thrombophilia

Randomized trials and meta-analysis with similar findings:

- Pasquier E, de Saint Martin L, Bohec C, et al. Enoxaparin for prevention of unexplained recurrent miscarriage: a multicenter randomized double-blind placebo-controlled trial. Blood. 2015;125(14):2200-2205.
- Schleussner E, Kamin G, Seliger G, et al. Low-molecular-weight heparin for women with unexplained recurrent pregnancy loss: a multicenter trial with a minimization randomization scheme. Ann Intern Med. 2015;162(9):601-609.
- Rodger MA, Gris JC, de Vries JIP, et al. Low-molecular-weight heparin and recurrent placenta-mediated pregnancy complications: a meta-analysis of individual patient data from randomised controlled trials. Lancet. 2016;388(10060):2629-2641.



ABO Incompatibility and Other Transfusion-Related Issues in Hematopoietic Transplantation

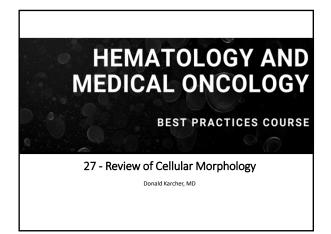
Shelley Kalsi, MD

August 15, 2020

Review of Cellular Morphology

Donald Karcher, MD

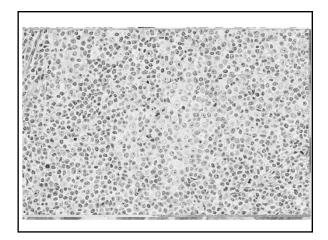
August 15, 2020



Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

None

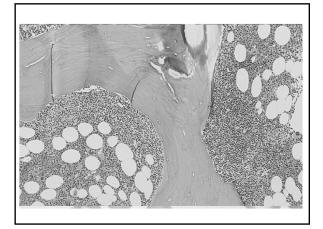


Answer:

Chronic lymphocytic leukemia

Special features -

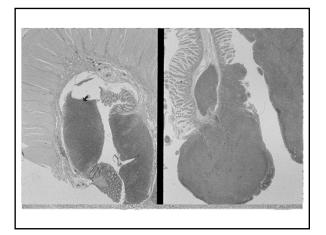
- Small to medium size lymphocytes with densely clumped chromatin
- "Smudge" cells on peripheral smear
- May progress with an increase in prolymphocytes
- "Proliferation centers" in lymph nodes



Answer:

Follicular lymphoma, low-grade (grade 1-2 of 3)

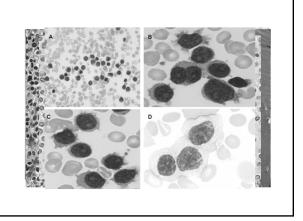
- "Buttock" cells in peripheral blood
- Multiple uniform follicles in lymph node
- Predominance of centrocytes; less than 15 centroblasts per HPF
- Paratrabecular bone marrow infiltrate



Mantle cell lymphoma

Special features -

- · Circulating lymphoma cells
- · Vaguely nodular growth pattern in lymph node
- "Naked" germinal centers in some cases
- · Small lymphocytes with moderate mitotic activity
- · Admixed histiocytes in some cases
- Blastoid variant → Fine chromatin, nucleoli, ↑ mitoses
- · Cyclin D1 positivity
- In gut, "lymphomatous polyposis"

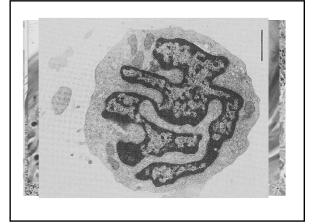


Answer:

Hairy cell leukemia

Special features -

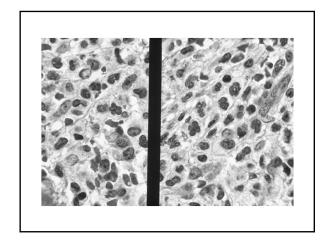
- Indistinct cytoplasmic margins (rarely see "hairs")
- Round, bean-shaped, to irregular nuclei
- Cytoplasmic "ruffles" and ribosome lamellar complexes on EM
- Tartrate-resistant acid phosphatase (TRAP) positive
- "Fried egg" appearance on tissue sections
- Variant hairy cell leukemia \Rightarrow Higher WBC count, larger nucleoli

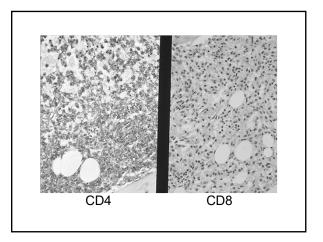


Answer:

Cutaneous T-cell lymphoma; mycosis fungoides

- Circulating Sezary cells with "cerebriform" nuclei
- High dermal lymphocytic infiltrate with intraepidermal Pautrier microabscesses
- Complex nuclear shape on EM

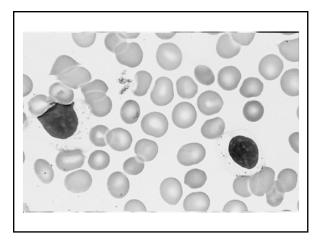




HTLV-I-associated adult T-cell leukemia/lymphoma

Special features -

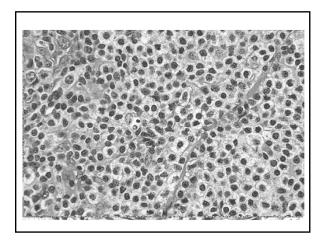
- Lymphocytes with "cloverleaf" or "flower" nuclei in peripheral blood, bone marrow, and other sites
- •CD4+, CD25+ neoplastic cells



Answer:

T-cell large granular lymphocytic leukemia (formerly "large granular lymphocytosis")

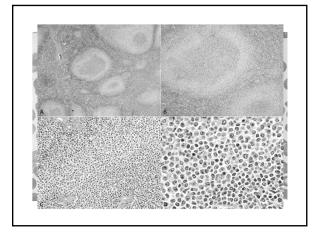
- Typical large granular lymphocytes
- •Immunophenotype: CD3+, CD8+, CD16+, CD57+



Marginal zone B-cell lymphoma

Special features -

- <u>Extranodal marginal zone B-cell lymphoma (MALT lymphoma)</u> Gastric and pulmonary examples shown
 - Small lymphocytes ± plasma cells
 - "Lymphoepithelial" lesions
 - In stomach, H. pylori often seen in crypts
- Nodal marginal zone B-cell lymphoma
 - Expanded marginal zones around follicles
 - "Monocytoid" B cells

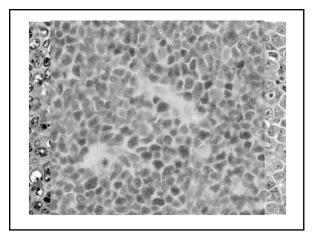


Answer:

Splenic marginal zone lymphoma

Special features -

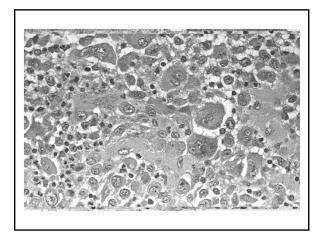
- Circulating small lymphocytes with "villi", sometimes polar
- In spleen:
 - Increased and enlarged white pulp nodules
- Biphasic nodules: Dark centers, light marginal zones
- Marginal zone cells → Monocytoid B cells
- Red pulp often diffusely infiltrated

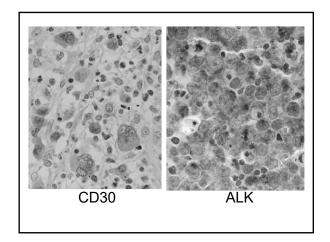


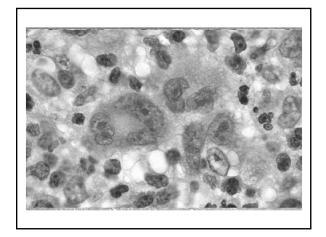
Answer:

Diffuse large B-cell lymphoma

- · Diffuse growth pattern
- · Large cells with:
 - "Vesicular" to fine chromatin
 - Small to large nucleoli; near nuclear membrane to central → Centroblastic vs. immunoblastic morphology
 - Variable mitotic activity
- Some cases have very high Ki-67 and/or MYC expression



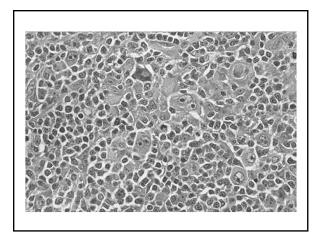


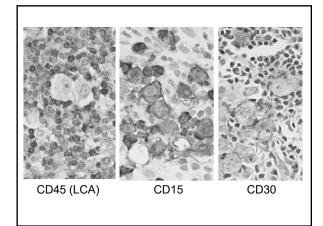


Anaplastic large cell lymphoma

Special features -

- Large pleomorphic lymphocytes, some large to very large cells with horseshoe-shaped multilobulated nucleus ("hallmark" cells)
- CD30+ by definition
- ALK+ in most systemic cases; ALK- in most cutaneous cases

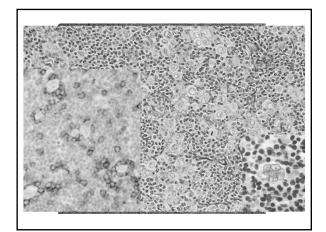




Answer:

Classical Hodgkin's lymphoma, nodular sclerosis type

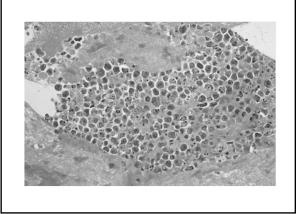
- •Nodules surrounded by fibrotic bands
- Lacunar ± classical Reed-Sternberg cells, often loosely clustered
- Reed-Sternberg cells: CD45-, CD20-/+, CD15+, CD30+, PAX5 dim+, Oct2-, BOB.1-
- Background: Mostly CD4+ T cells



Hodgkin's lymphoma, nodular lymphocyte predominant type

Special features -

- · Vaguely nodular growth pattern
- "LP" or "popcorn" cells: CD45+, CD20+, CD15-, CD30-, PAX5 bright+, Oct2+, BOB.1+
- Background: B cells ± T cells
- Rosettes of CD57+, PD1+ T cells around LP cells

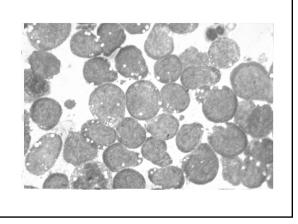


Answer:

Primary effusion lymphoma

Special features -

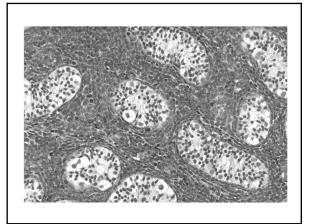
- Frequently confined to body cavities
- · Large immunoblastoid cells
- Negative for most B-cell antigens and surface immunoglobulin
- Strong association with HHV-8



Answer:

Burkitt's lymphoma

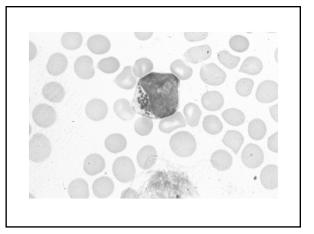
- Frequently extranodal
- Uniform, medium size, blast-like cells with basophilic cytoplasm and clear vacuoles
- "Starry sky" cells (tingle-body macrophages)
- · Very high mitotic activity
- Lymphoma cells often in peripheral blood and/or bone marrow ("Burkitt cell ALL", "ALL, L3")



Acute lymphoblastic leukemia

Special features -

- Variable blast morphology, may include mature-appearing cells
- PAS positivity in blasts
- "Hand-mirror" cells in some cases
- Testis as "sanctuary site"

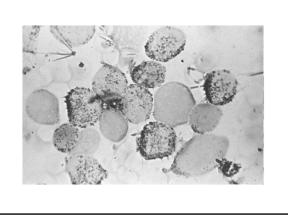


Answer:

"Granular" precursor B-cell ALL

Special features -

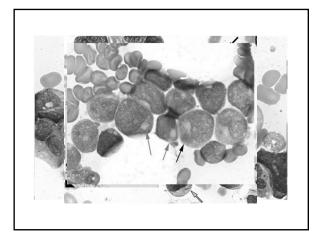
- •5-15% of childhood ALL; <5% of adult ALL
- Large cytoplasmic granules (3 or more)
 - Myeloperoxidase (MPO) negative
 - Sudan black positive (light gray)
- Slightly worse prognosis than conventional precursor B-cell ALL



Answer:

Acute myeloid leukemia

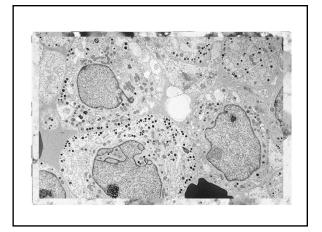
- "Classical" myeloblast morphology
 - Fine, "reticular" chromatin
 - Prominent "cookie-cutter" nucleoli
 - Auer rods in some cases
 - Myeloperoxidase (MPO) positive [cytochemical technique shown]



Acute myeloid leukemia with t(8;21)(q22;q22.1) (RUNX1-RUNX1T1)

Special features -

- Large myeloblasts with fine cytoplasmic granules
- · Auer rods, sometimes thick with tapered ends
- · Some blasts have perinuclear hofs
- · Large salmon-colored granules in some cases

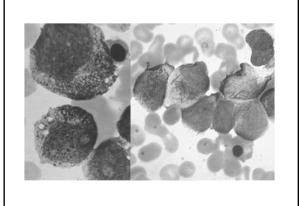


Answer:

Acute promyelocytic leukemia with *PML-RARA*, microgranular subtype

Special features -

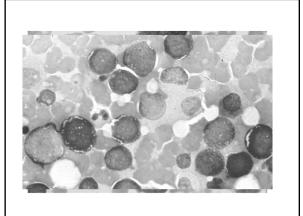
- Neoplastic cells with deeply lobulated nuclei and very find cytoplasmic granules
- Intense myeloperoxidase (MPO) positivity [cytochemical technique shown]
- Small lysosomal granules by EM



Answer:

Acute promyelocytic leukemia with *PML-RARA*, typical "hypergranular" subtype

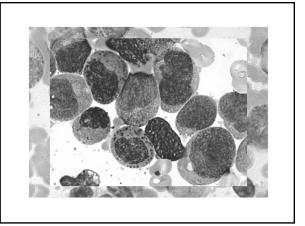
- Promyelocytes with very large primary granules
- Cells with multiple Auer rods ("faggot" cells)



Acute myelomonocytic leukemia

Special features -

- Myeloblasts with round to lobulated nuclei, often with abundant cytoplasm ± vacuoles
- Intense alpha naphthyl butyrate esterase ("NSE") positivity [cytochemical technique only]

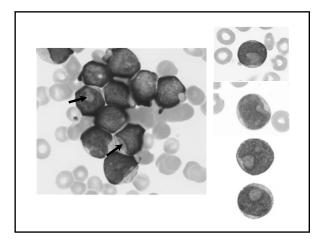


Answer:

Acute myeloid leukemia with inv(16) or other 16q22 breakpoint abnormality (CBFB-MYH11)

Special features -

- Myeloblasts with lobulated nuclei, abundant cytoplasm ± vacuoles
- Numerous eosinophilic precursors with pink to purple/violet cytoplasmic granules

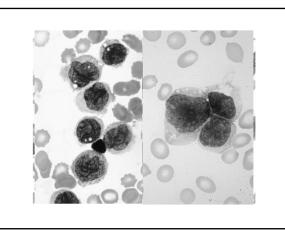


Answer:

Acute myeloid leukemia with NPM1 and/or FLT3 mutations

Special features -

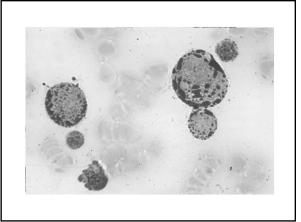
• Myeloblasts with nuclear invaginations, socalled "cup-like" or "fish mouth" nuclei



Acute monoblastic/monocytic leukemia

Special features -

- Large blasts with ± lobulated nuclei, abundant cytoplasm, ± vacuoles
- Monoblasts less mature-appearing; promonocytes more "monocytoid"
- Intense alpha naphthyl butyrate esterase ("NSE") cytochemical positivity [not shown]

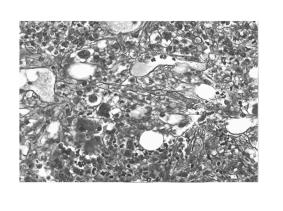


Answer:

Acute erythroid leukemia

Special features -

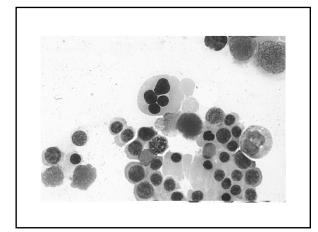
- Immature erythroblasts with:
 - Round nuclei
 - Basophilic cytoplasm
 - Large cytoplasmic vacuoles, PAS+
- Little or no myeloblastic component



Answer:

Acute megakaryoblastic leukemia

- Small to large megakaryoblasts, some exhibiting cytoplasmic blebs ("platelet budding")
- •Reticulin fibrosis in marrow

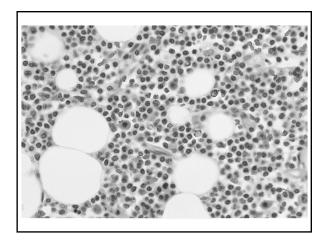


Myelodysplasia

Examples shown -

- Dwarf and multinucleated megakaryocytes
- Multinucleated and multilobulated erythrocytic precursors

Associated with MDS, post-chemo, HIV, etc.



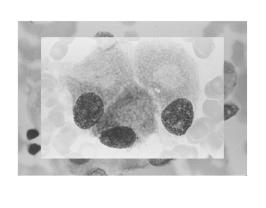
Answers:

Plasma cell myeloma

- · Increased osteoclasts [seen best on core biopsy]
- Plasma cell variants -
- Multinucleated
- Large eosinophilic cytoplasmic vacuole (Russell body)
- Mott or "morula" cell
- Flame cell
- Dutcher intranuclear pseudoinclusion

Lymphoplasmacytic lymphoma

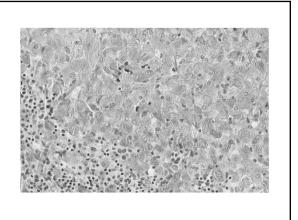
Lymphoplasmacytic infiltrate with Dutcher intranuclear pseudoinclusions



Answer:

Osteoblasts

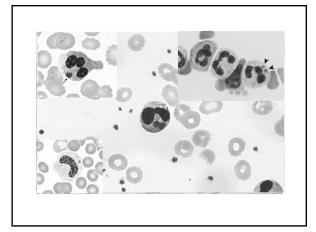
- May mimic plasma cells, but Golgi area distinct from nucleus (in plasma cells, adjacent to nucleus)
- Seen especially on bone marrow touch preps



Gaucher's disease

Special features -

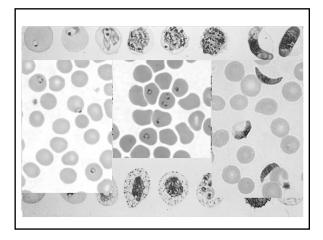
- On marrow aspirate smear: Histiocytes with "wrinkled tissue paper" appearance in cytoplasm
- On core biopsy: Histiocytes with PAS+ spindle shaped inclusions in cytoplasm



Answers:

White cell anomalies/inclusions

- Pelger-Huët anomaly
- May-Hegglin anomaly
- Alder-Reilly anomaly (associated with mucopolysaccharidoses)
- · Chédiak-Higashi syndrome
- Anaplasmosis (formerly called granulocytic Ehrlichiosis)



Answer:

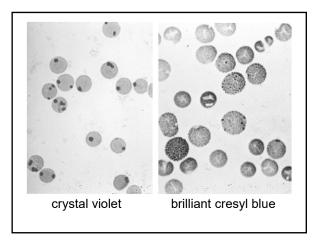
Intraerythrocytic microorganisms

Babesiosis -

- Multiple small ring forms, up to 4 per cell
- Tetrads or "Maltese cross" forms

Malaria -

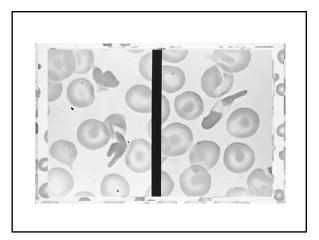
- Species characteristics
 - Ring size, ring number, RBC morphology, schizonts (number of merozoites), gametocytes (banana, other), etc.



Red cell inclusions associated with hemoglobin denaturation

With <u>crystal violet</u>: Heinz bodies (associated with G-6-PD deficiency, other enzyme deficiencies, alpha thalassemia)

With <u>brilliant cresyl blue</u>: "Golf ball" inclusions after 1 hour → Hemoglobin H and other unstable hemoglobins



Answer:

Red cell abnormalities

- Spherocytes
- Schistocytes
- Teardrop forms
- Acanthocytes (thorn cells)
- Sickle forms; target cells
- Pappenheimer bodies (in sickle cell smears)
- Howell-Jolly bodies
- Hybrid sickle forms in hemoglobin SC disease

