Hematology Syllabus

Session 1: Anemias, Bone Marrow Failure, and Sickle Cell Disease

Session 2: WBC Disorders and Coagulopathy

Session 3: Thrombocytopenias, Anemias and Myeloproliferative Disease

Course Organizers:

THE GEORGE WASHINGTON UNIVERSITY

WASHINGTON, DC

Biology of Hematopoiesis

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August 13, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

1 - Biology of Hematopoiesis

Jerry L. Spivack, MD, MACP

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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

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Requirements of Hematopoiesis

Cell Type	Life Span (days)	Turnover Rate (cells/day)
Erythrocytes	120	10^{12}
Granulocytes	0.5	10^{11}
Platelets	9	10^{11}

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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

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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) Axial	Enucleate Red cells	Adult

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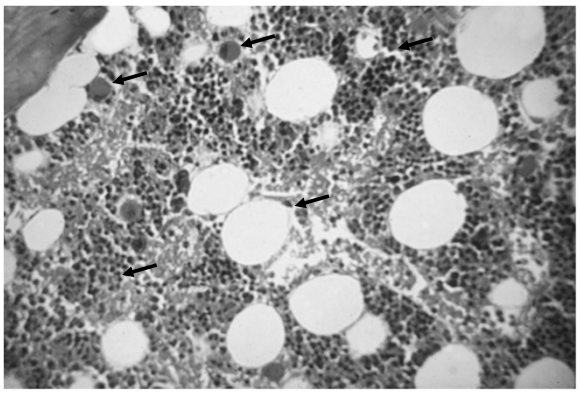
Normal Bone Marrow Biopsy (H&E)



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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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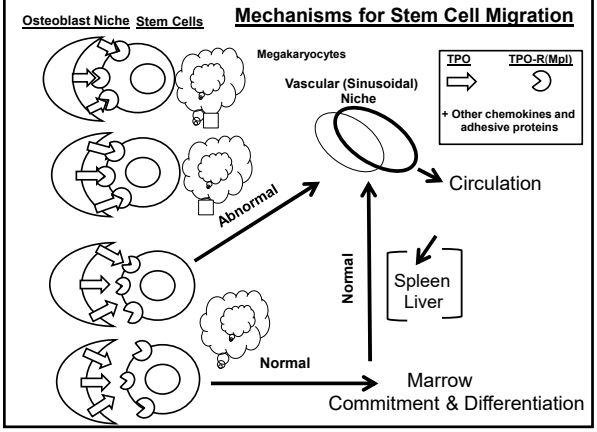
Mechanisms for Stem Cell Migration



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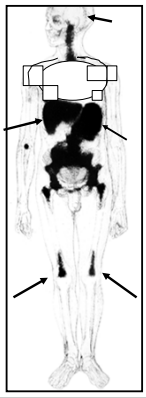
Expansion of Hematopoiesis with Increased Demand



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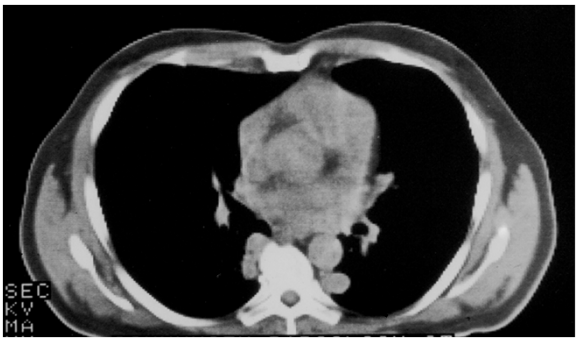
Paravertebral Extramedullary Hematopoiesis



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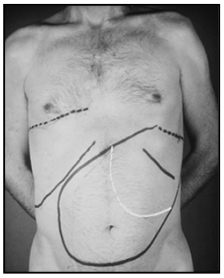
Polycythemia Vera: Extramedullary Hematopoiesis



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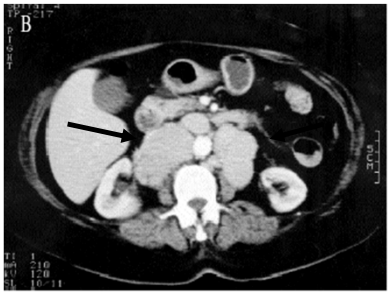
Primary Myelofibrosis: Extramedullary Hematopoiesis



Haematologica 86:784, 2001

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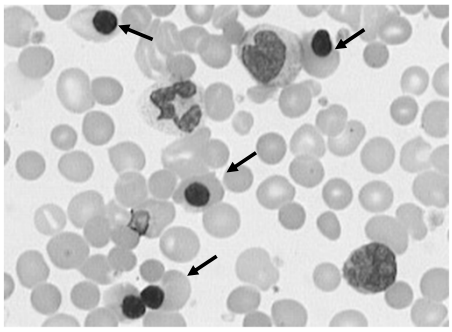
Leukoerythroblastic Reaction



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Causes of Extramedullary Hematopoiesis and Leukoerythroblastic Reactions

- Carcinoma metastatic to the bone marrow
  - (prostate, breast, lung, stomach)
- Lymphoma involving the bone marrow
  - (Hairy cell leukemia, CLL)
- Primary myelofibrosis
- Polycythemia vera
- Chronic myelogenous leukemia
- Myelodysplasia
- **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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Hematopoietic Growth Factors

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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Essential Factors in Erythropoiesis

- Intensity of the stimulus for red cell production
- Functional capacity of the bone marrow
- Available nutrients
- Red cell survival

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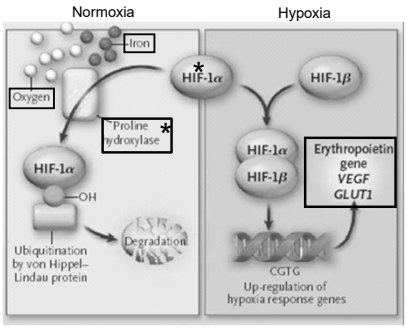
Hypoxic Regulation of Erythropoietin Production



N Engl J Med 348:1282, 2003

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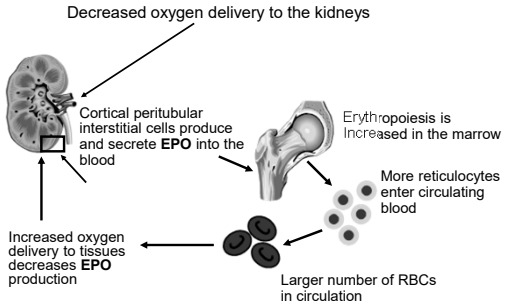
Erythropoietin Production



Semin Oncol. 2001;28(Suppl 8):19.

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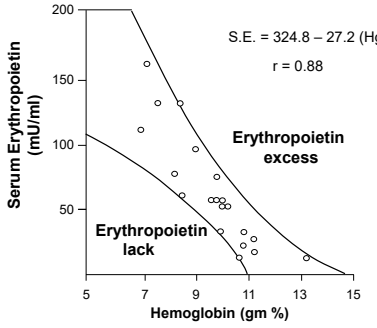
Confidence Intervals for Serum Immunoreactive Erythropoietin



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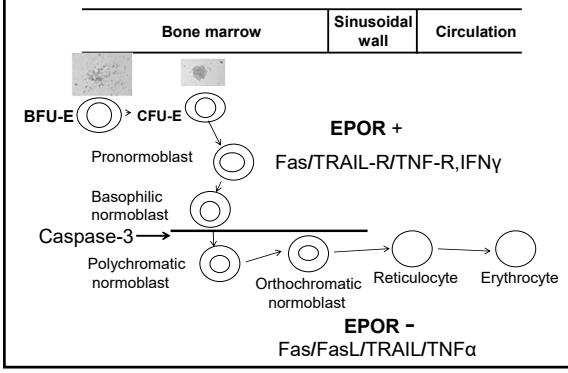
Erythropoiesis



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The Major Functions of EPO are Reflected in Its Plasma Level

	Production	Plasma Level
Erythroid cell viability factor	Constitutive	Constant
Erythroid cell mitogen	Inducible	Variable

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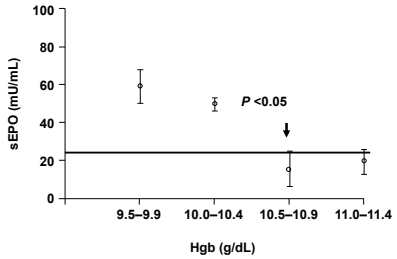
Serum Immunoreactive Erythropoietin in Iron Deficiency Anemia



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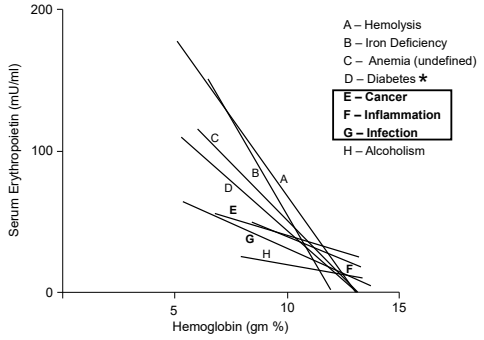
Association Between Hemoglobin and Serum Erythropoietin Levels in Various Disease States



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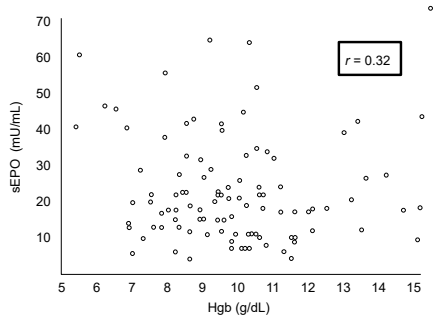
Loss of the Hgb-Serum EPO Correlation When the Serum Creatinine is Greater than 1.5 mg %



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Change in Risk with the Recombinant Erythropoietins Over Time

	RR	CI
2001		
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)
2007		
Risk of Thromboembolism	1.67	(1.35 - 2.06)
Overall Survival	1.08	(0.59 – 1.18)

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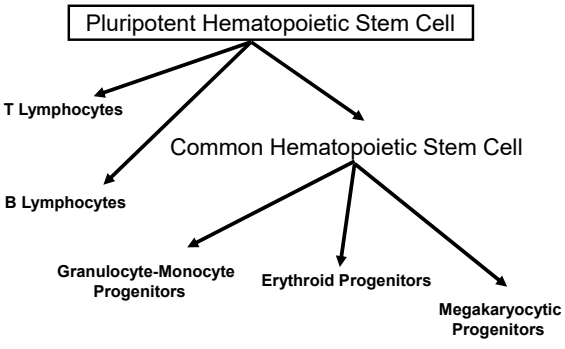
Pluripotent Hematopoietic Stem Cell



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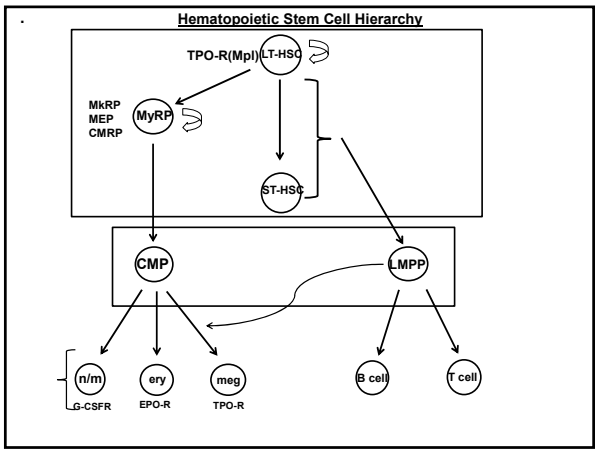

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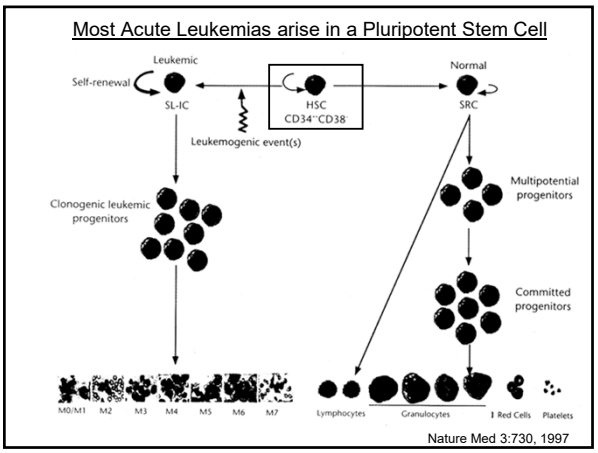

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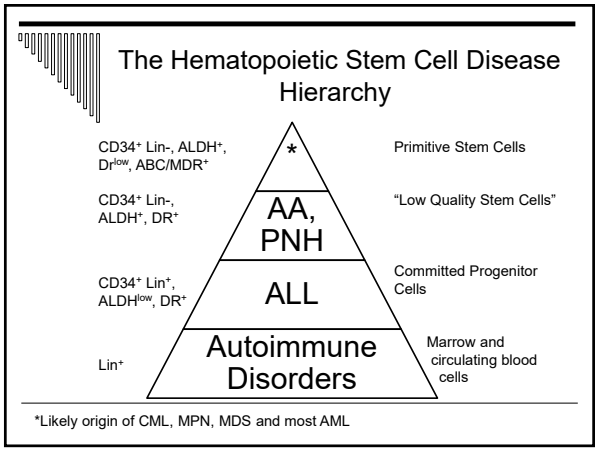

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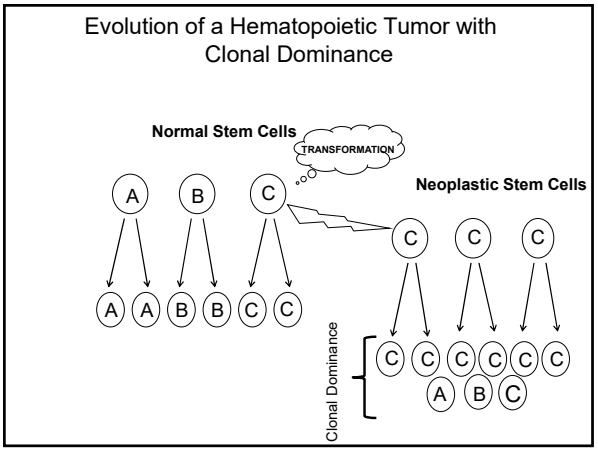

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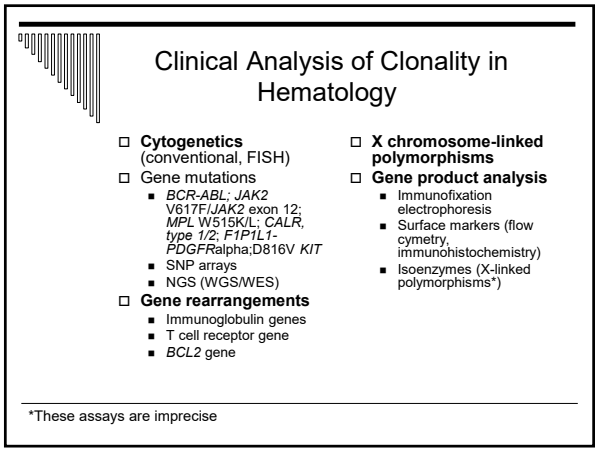

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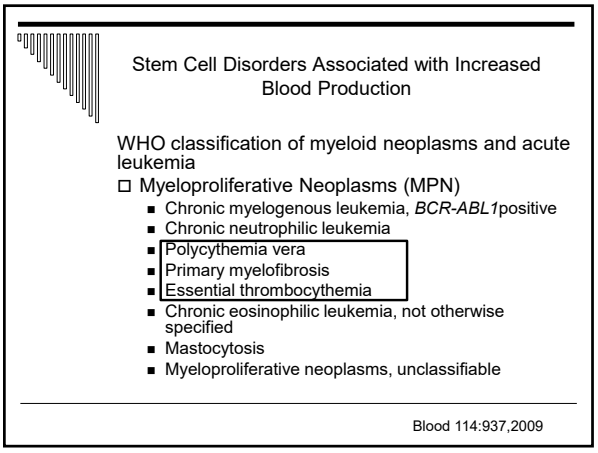

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Distribution of *JAK2*, *CALR* and *MPL* driver mutation in the MPN

	PV	PMF	ET
***JAK2* V617F**	92%	~ 55%	~ 50%
***JAK2* Exon12**	5%	0%	0%
CALR	~1%	~36%	~30%
MPL	0%	~ 4%	~ 8%
Unknown	3%	~ 5%	~ 12%

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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

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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, unclassifiable
    - Refractory anemia with ring sideroblasts and thrombocytosis (*SF3B1*)

Blood 114:937,2009

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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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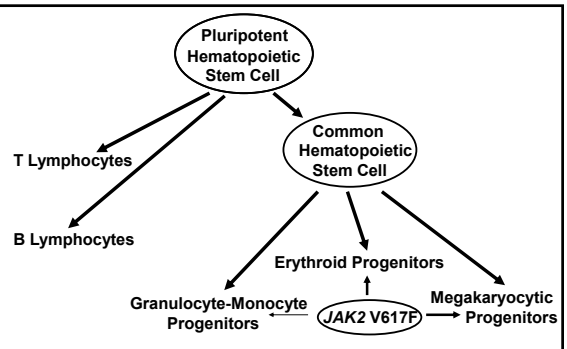

Polycythemia vera is the ultimate phenotypic consequence of the *JAK2* V617F mutation and most common MPN

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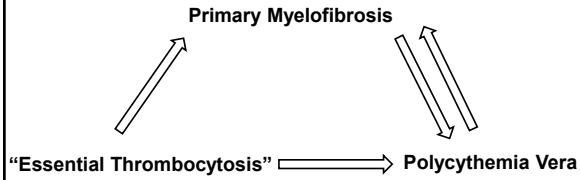
The Interrelationships Between the Chronic Myeloproliferative Disorders



"All pathways lead to polycythemia vera"

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Features "Unique" to Specific "Chronic Myeloproliferative Disorders"

Polycythemia vera	Erythrocytosis
Idiopathic Myelofibrosis	Elevated circulating CD34+ cells (early only)
"Essential Thrombocytosis"	None

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Splenomegaly has been omitted as a diagnostic criterion as have the red cell, leukocyte and platelet counts and the JAK2 V617F allele burden

		Polycythemia Vera (PV)	Essential Thrombocythemia (ET)
Major criteria	1	Hemoglobin (Hgb) >16.5 g/dL (men) >16 g/dL (women) or Hematocrit >49% (men) >48% (women) or ↑ red cell mass >25% above mean	1 Platelet count ≥450 x 10⁹/L
	2	Bone marrow (BM) tri-lineage myeloproliferation with pleomorphic mature megakaryocytes*	2 BM megakaryocyte proliferation with large and mature morphology and hyper-lobulated nuclei. Reticulin fibrosis grade should be ≤1
	3	Presence of *JAK2* mutation	3 Not meeting WHO criteria for other myeloid neoplasms
			4 Presence of *JAK2*, *CALR* or *MPL* mutation

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Microcytic erythrocytosis:a clue to polycythemia 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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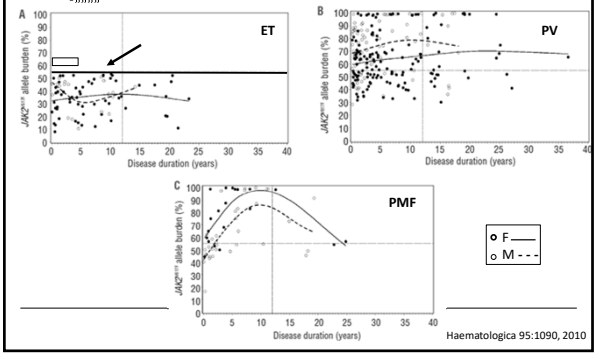
JAK2 V617F Quantitative Allele Burdens in the MPN



Haematologica 95:1090, 2010

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Causes of Absolute Erythrocytosis

- ☐ **Hypoxia**
  - Carbon monoxide intoxication (tobacco abuse, environmental)
  - High O_2 affinity hemoglobins
  - High altitude
  - Pulmonary disease
  - Right to left shunts
  - Sleep apnea
- ☐ **Neurologic Diseases**
- ☐ **Renal Disease**
  - Renal artery stenosis
  - Focal sclerosing or membranous glomerulonephritis
  - Renal transplantation

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Causes of Absolute Erythrocytosis (Continued)

- ☐ **Tumors**
  - Hypernephroma
  - Hepatoma
  - Cerebellar hemangioblastoma
  - Uterine fibromyoma
  - Adrenal tumors
  - Meningioma
  - Pheochromocytoma
- ☐ **Drugs**
  - Androgenic steroids
  - Recombinant EPO
- ☐ **Familial**
  - (with normal hemoglobin function; Chuvash(vHL mutations); EPO receptor mutations; 2, 3 BPG mutations;EPAS1(HIF2a) and EGLN1(PHD) mutations)
- ☐ **Polycythemia vera***
  - ***JAK2* V617F**
  - ***JAK2* exon 12 mutations**
  - **Rarely CALR**

*Only ~5-10% of erythrocytosis patients are likely to have polycythemia vera

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Causes of Relative Erythrocytosis

- **Loss of Fluid from the Vascular Space**
  - Emesis, diarrhea, diuretics, sweating, polyuria, hypodipsia, hypoalbuminemia, capillary leak syndromes, burns, peritonitis
- **Chronic Plasma Volume Contraction**
  - Hypoxia from any cause
  - Androgen therapy
  - Recombinant erythropoietin therapy
  - Hypertension
  - Tobacco use
  - Pheochromocytoma
  - Ethanol abuse
  - Sleep apnea

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Causes of Thrombocytosis

- Tissue Inflammation
  - Collagen vascular disease, inflammatory bowel disease
- Malignancy
- Infection
- Myeloproliferative Disorders
  Polycythemia vera, Primary myelofibrosis, Essential thrombocytosis, Chronic myelogenous leukemia
- Myelodysplastic Disorders
  5q-syndrome, Idiopathic refractory sideroblastic anemia

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Causes of Thrombocytosis (Continued)

- Postsplenectomy or hyposplenism
- Hemorrhage
- Iron deficiency anemia
- Surgery
- Rebound
  - Correction of vitamin B12 or folate deficiency, post ethanol abuse
- Hemolysis
- Familial
  - Thrombopoietin overproduction, constitutive Mpl activation(*MPL* S505N), *MPL* K39N, *MPL* P106L

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Causes of Myelofibrosis

Malignant
- Acute Leukemia
  - lymphocytic, myelogenous, megakaryocytic
- Chronic Myelogenous Leukemia
- Hairy Cell Leukemia
- Hodgkin's Disease
- **Primary Myelofibrosis**
- Lymphoma
- Multiple Myeloma
- Myelodysplasia
- Metastatic carcinoma
- Polycythemia Vera
- Systemic Mastocytosis

Non Malignant
- HIV infection
- Hyperparathyroidism
- Renal osteodystrophy
- Systemic Lupus Erythematosus
- Tuberculosis
- Vitamin D deficiency
- Thorium Dioxide exposure
- Gray Platelet Syndrome
- Drugs
  - Thrombopoietin analogs

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Differential Diagnosis of Primary Myelofibrosis

- Chronic myelogenous leukemia
- Polycythemia vera
- Acute myelofibrosis
- Myelodysplasia
- Hairy cell leukemia
- Primary bone marrow lymphoma
- Systemic mastocytosis
- Metastatic carcinoma

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Causes of Leukocytosis

- Infection
- Inflammation
- Chronic myeloproliferative disorders (clonal)
  Chronic myelogenous leukemia
  Polycythemia vera
  Primary myelofibrosis
  Hypereosinophilia
  Myelodysplasia
  CMMoL
- Acute leukemias (clonal)

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Causes of Leukocytosis (Continued)

- Drugs
  - Corticosteroids
  - Lithium
  - G-CSF, GM-CSF
- Tobacco
- Obesity
- Exercise/Seizures
- Postsplenectomy/hyposplenism
- Rebound from myelosuppression
- Sweet's syndrome
- Heat stroke
- Artifact
  - Cryoproteins

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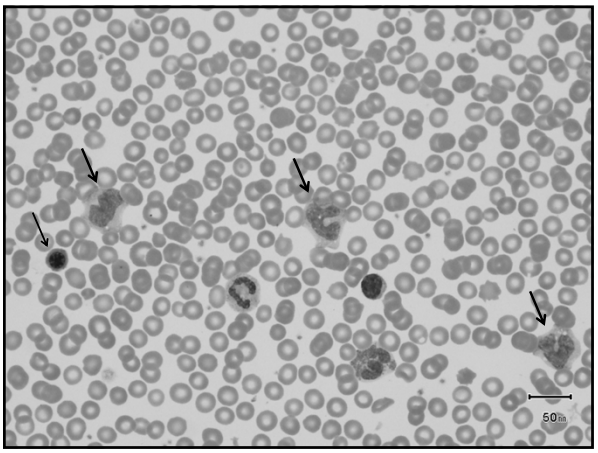

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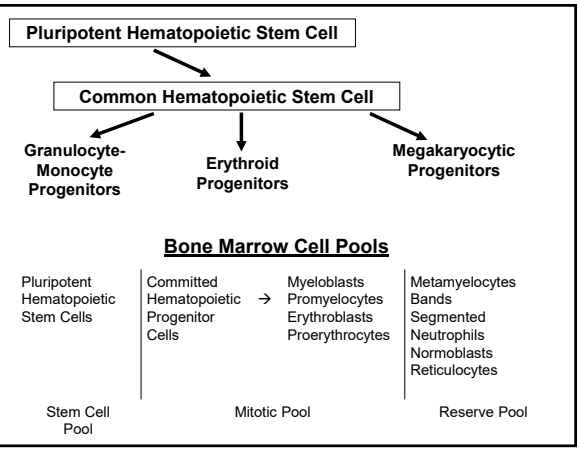

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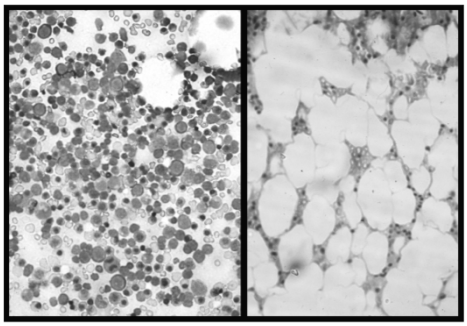
Severe Aplastic Anemia: Marrow Aspirate and Biopsy



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Diseases Causing Bone Marrow Aplasia or Hypoplasia

Inherited

- ☐ Fanconi Anemia
- ☐ Schachman-Diamond syndrome
- ☐ Dyskeratosis Congenita
- ☐ Amegakaryocytic thrombocytopenia

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Diseases Causing Bone Marrow Aplasia or Hypoplasia (Continued)

Acquired

- ☐ Idiopathic Aplastic Anemia*
- ☐ Drug-induced Aplastic Anemia
- ☐ Direct toxicity or idiosyncratic reaction
- ☐ Myelodysplasia*
- ☐ Paroxysmal Nocturnal Hemoglobinuria*
- ☐ Large granular lymphocyte syndrome (neutropenia, red cell aplasia, thrombocytopenia, aplastic anemia)
- ☐ Thymoma (red cell aplasia, aplastic anemia)
- ☐ Pregnancy (red cell aplasia, aplastic anemia)
- ☐ Thiopurine S-Methyltransferase deficiency (pancytopenia)

*Acquired clonal disorders

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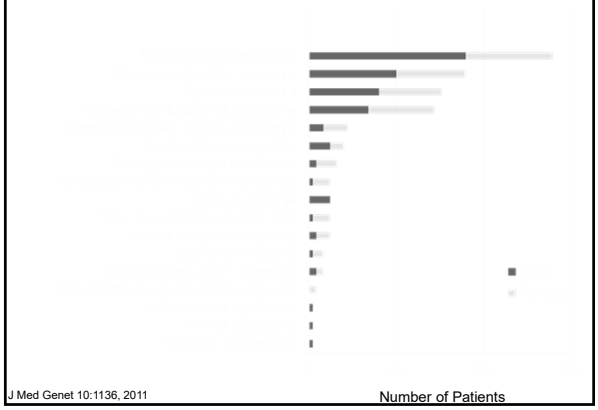
Distribution of Primary Inherited Bone Marrow Failure Syndromes



J Med Genet 10:1136, 2011

Number of Patients

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Inherited Marrow Failure Syndromes in Adults

Fanconi Anemia	Dyskeratosis Congenita	Diamond-Blackfan
Pancytopenia	Pancytopenia	Anemia
Aplastic Anemia	Aplastic Anemia	-
Leukemia/MDS	Leukemia/MDS	Leukemia/MDS
Cancer (HN, Gyn, Brain)	Cancer (HN)	Osteosarcoma
Café au Lait spots	Pigmentation, Gray hair Oral leukoplakia	-
Skeletal abnormalities	Nail dysplasia Pulmonary fibrosis	Short neck
FANC gene mutations	**Telomerase gene mutations** **Dyskerin gene mutations**	**RP S17, 19 and 24 loss**

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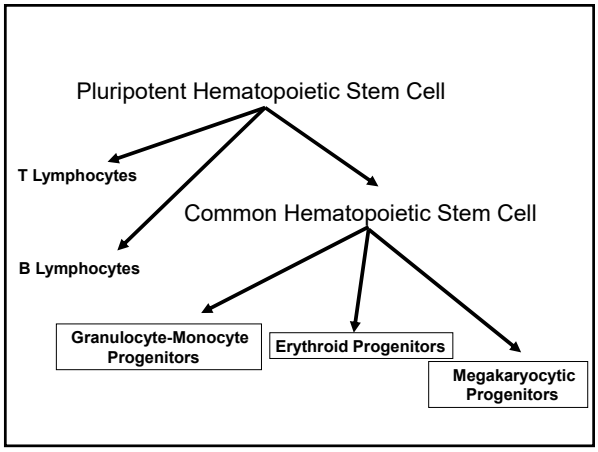

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Conditions Causing Single Lineage Bone Marrow Aplasia

- Pure Red Cell Aplasia or Hypoplasia
  - Congenital
    - Diamond Black-Fan Syndrome*
  - Acquired
    - Autoimmune
    - Thymoma, T-cell mediated (LGL)
    - Drug-induced
    - Solid tumors
    - **Hematological malignancies***
      (Myelodysplasia, CML lymphoma)
    - Infection (Parovirus B19)
    - Collagen-vascular disease
    - Pregnancy
    - Drugs
    - Erythropoietin antibodies

*Clonal disorders

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Conditions Causing Single Lineage Bone Marrow Aplasia (Continued)

- 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

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Stem Cell Defects Causing Monocytopenias

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

66

A 42-Year-Old Man

July	• Resection of brain tumor • Postoperative seizures	• Dilantin • Phenobarbital Carbamazepine • Steroids • 4 blood transfusions • HCT 42
Aug	• Abnormal liver function tests	• Dilantin • Carbamazepine • Steroids discontinued • HCT 37
Sept	• Diffuse skin rash • Abnormal liver function tests • Fever	• Phenobarbital discontinued • HCT 26 • WBC 11,500 • Eos 31% • PL 634,000 • Retic 0.2%

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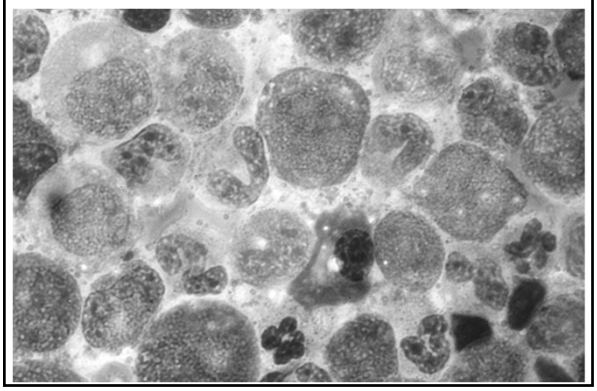
Bone Marrow Aspirate in Pure Red Cell Aplasia



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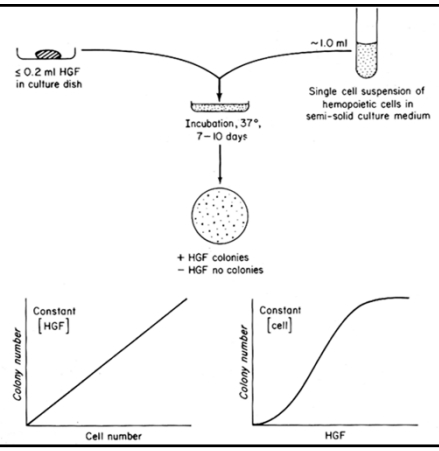

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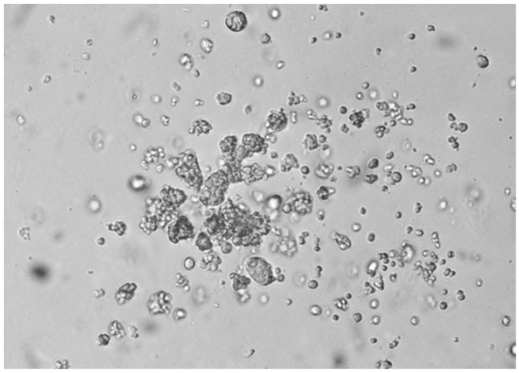
Erythroid Colony Grown In Vitro



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Erythroid Colony Growth in PRCA

Category	Colony Growth	Response to Therapy		
		C.R.	P.R.	None
I	Normal	70%	30%	-
II	Reduced	25%	-	75%
III	Undetectable	-	-	100%

Adapted from Blood 64:71, 1984

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Classification of Red Cell Aplasia or Hypoplasia

Congenital
- Diamond-Blackfan syndrome

Acquired
- Idiopathic
- Secondary
  - **Hematologic Malignancies**
    - (AL, MDS, CLL, NHL, HD, AILD, CML, PMF)
  - Solid Tumors
    - (Thymona, Lung, Stomach, Breast)
- Immunologic Disorders
  - (LGL syndrome, SLE, RA, AIHA, Pregnancy, BMT, HIV, Polyglandular syndromes I and II)
- Infectious Diseases
  - (Parvovirus B19, EBV, Hepatitis A, B, C)
- Drugs
- Anti-erythropoietin antibodies

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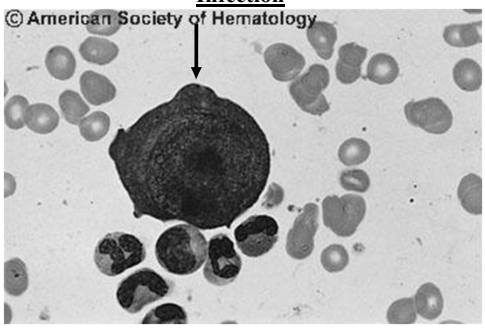
Giant Pronormoblast Seen in Parvovirus B19 Infection



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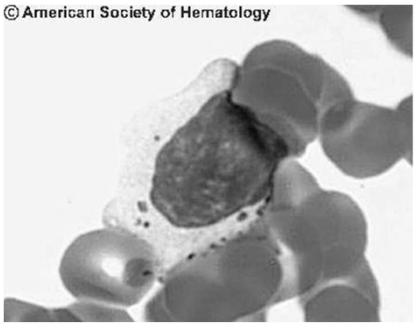
Large Granular Lymphocyte



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Drugs Associated with Red Cell Aplasia

Confirmed
- Phenytoin
- Azathioprine
- Isoniazid
- Mycophenolate mofetil
- Recombinant erythropoietin

Suspected
- Allopurinol
- D-penicillamine
- Interferon alpha
- FK506
- Lamivuudine
- Rifampicin
- Valproate
- Sulfonamide derivatives
- Halothane
- Rituximab*
- Fludarbine*
- Chloramphenicol**

*probably secondary to immunosuppression leading to B19 infection
**The effect is dose-dependent

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Classification of Adult Hematopoietic Disorders

	Clonal	Nonclonal
Decreased Production	Aplastic anemia Red cell aplasia Megakaryocytic aplasia MDS PNH Sideroblastic anemia*	Aplastic anemia Red cell aplasia White cell aplasia Megakaryocytic aplasia Anemia due to renal disease
Increased Production	Polycythemia vera* Essential thrombocytosis* Primary myelofibrosis* MDS (thrombocytosis; *JAK2* V617F) CML CMMoL; CNL	2^0 Erythrocytosis 2^0 Thrombocytosis Leukemoid reactions
Increased Destruction	PNH	Hemolytic anemia Immune thrombocytopenia Agranulocytosis

*Can be *JAK2* V617F+

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Summary

- Hematopoiesis is hierarchical
- Hematopoiesis is clonal but stem cell defects can mimic polyclonal (single cell line) disorders
- Hematopoiesis is governed by both intrinsic and extrinsic signals and thus its behavior is both nonrandom and random
- An explanation for the molecular basis of both the acute leukemias and the chronic myeloproliferative disorders will be found at the level of the hematopoietic stem cell
- Clonal disorders of hematopoiesis are often phenotypically similar to nonclonal disorders of hematopoiesis

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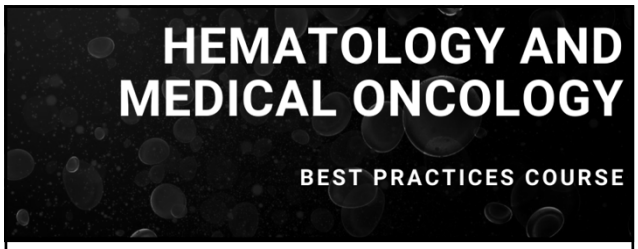
2

Iron Deficiency and Overload

Victor R. Gordeuk, MD

August 13, 2020



2 - Iron Deficiency and Iron Overload

Victor R. Gordeuk, MD

1

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**

3

Iron

- **Essential nutrient for all living organisms.**
  - **Reversible binding of O_2: Hb, myoglobin**
  - **Enzyme systems:**
    - **heme (cytochromes, catalase, glutathione peroxidase, NO synthase)**
    - **non-heme (RNR, aconitase)**
  - **Immunity: free radicals to destroy microbes**
- **Highly reactive with O_2; 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**

5

Iron Requirements

	Men	Women
Obligatory losses	**1.0 mg/d**	**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**

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Body Iron Compartments

	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**

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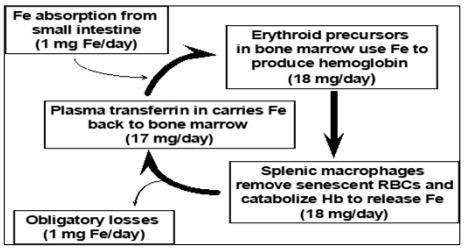
Simplified Diagram of Iron Movement in the Body



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Dietary Iron

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

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Iron absorption- proximal small bowel

Hepcidin- 25 aa peptide produced by liver that suppresses iron absorption

Enhanced absorption
- Low hepcidin:
  - Iron deficiency
  - ↑ erythropoiesis
- Dietary factors:
  - ascorbic acid (Fe^{+2} valance absorbed)
  - Heme vs non-heme Fe

Inhibited absorption
- High hepcidin
  - ↑ iron stores
  - Inflammation
- Dietary factors:
  - tannins (tea)
  - phytates (bran)

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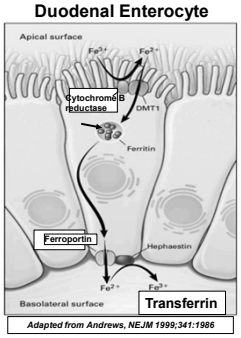
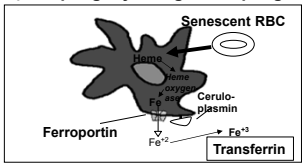
Duodenal Enterocyte



Adapted from Andrews, NEJM 1999;341:1986

Iron Transport into Plasma

Erythro-phagocytosing Macrophage



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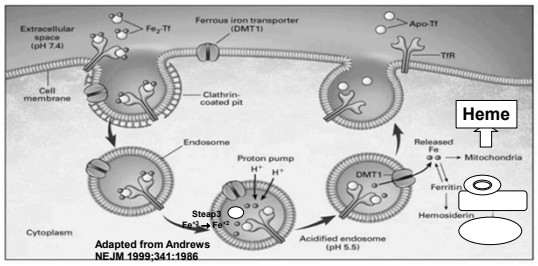
Iron Entry into Erythroid Precursors



Adapted from Andrews NEJM 1999;341:1986

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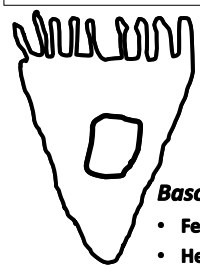
Molecules of Fe Metabolism

Duodenal Enterocyte: Fe Absorption



Brush Border

- **DMT-1 transports Fe^{+2} from lumen into enterocyte**
- **Cytochrome b ferri-reductase maintains Fe^{+2} valence**

Basolateral surface

- **Ferroportin exports Fe^{+2} to portal plasma**
- **Hephaestin converts Fe^{+2} released by fptn to Fe^{+3} for binding by Tf**

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Molecules of Fe Metabolism:

Iron Transport in Plasma

Transferrin

- **Dimer with two binding sites for Fe**
- **Normally ¼ to ⅓ of binding sites occupied by iron**
- **Delivers Fe released by macrophages and enterocytes to normoblasts in bone marrow**

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Molecules of Fe Metabolism:

Erythroid Progenitor: Hb production

Membrane

- **TfR1-mediated endocytosis of Tf-Fe**

Endosome

- **Acidification releases Fe from Tf**
- **Steap3 converts Fe^{+3} to Fe^{+2}**
- **DMT-1 exports Fe^{+2} to cytosol**

Cytosol

- **Ferritin stores excess Fe**

Mitochondrion

- **Mitoferrin-1 imports Fe^{+2}**
- **Ferrochelatase inserts Fe^{+2} into protoporphyrin to form heme**

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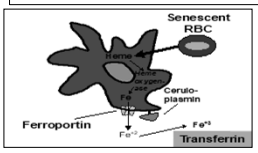
Molecules of Fe Metabolism:

Macrophage: phagocytosis; Fe processing



Cytosol

- **Heme oxygenase liberates Fe from heme**
- **Ferritin stores Fe for short-term**
- **Hemosiderin stores Fe for long-term**

Membrane

- **Ferroportin exports Fe^{+2} to plasma**
- **Ceruloplasmin- plasma and GPI-anchored ferrioxidase; converts Fe^{+2} (released by fpn) to Fe^{+3} (for binding by Tf)**

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Molecules of Fe Metabolism:

Hepatocyte: Systemic Iron Regulation

Hepcidin

- stimulated by ↑ Fe and inflammation
- suppressed by ↑ erythropoiesis
- binds ferroportin; ↓ Fe export (enterocyte, macrophage)

Up-regulation of hepcidin

- HFE, TfR2, Hemojuvelin, BMP6, BMPR, SMAD4

Down-regulation of hepcidin

- TMPRSS6 (matriptase-2)
- Erythroblast-derived: erythroferrone, GDF-15, GDF-11

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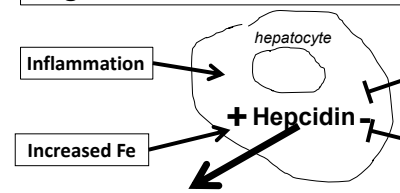
Regulation of Iron Metabolism



1. **Binds to ferroportin on enterocytes, macrophages**
2. **Hepcidin-ferroportin internalized and ferroportin degraded**

→

- **↓ Fe absorbed by enterocytes**
- **↓ Fe released by macrophages**
- **↑ Fe stored in macrophages**

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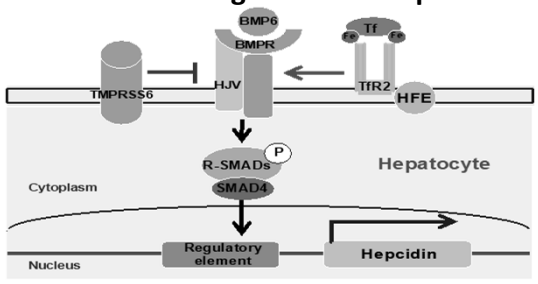
Iron-Mediated Regulation of Hepcidin



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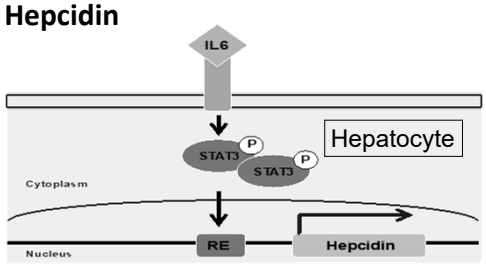
Inflammation-Mediated Regulation of Hepcidin



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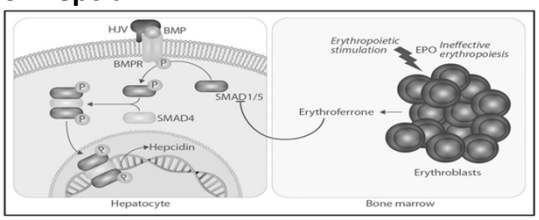
Erythropoiesis-Mediated Regulation of Hepcidin



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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

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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

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Changes in Indirect Indicators of Fe Status

↑ Serum Fe & Tf Sat	↑ Serum Ferritin
• Iron overload • Hepatocellular damage • BM suppression • Recent ingestion of Fe preparation	• Iron overload • Hepatocellular damage • Inflammation • Hyperferritinemia/cataract syndrome – autosomal dominant – ↑ftn but no Fe-loading – mutation IRE L-ferritin mRNA

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Changes in Indirect Indicators of Fe Status

↓ Serum Fe & Tf Sat	↓ Serum Ferritin
• Iron deficiency • Inflammation • Diurnal variation – blood drawn afternoon or evening	• Iron deficiency

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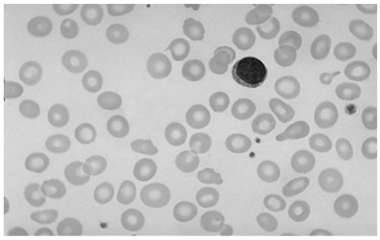
Fe deficiency anemia

- microcytosis
- central pallor



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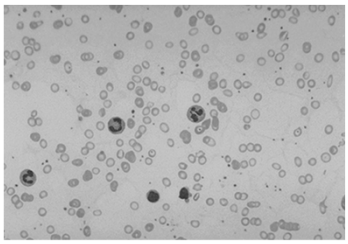
Fe deficiency anemia

- Marked microcytosis
- Marked central pallor



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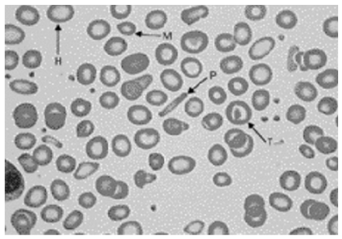
Fe deficiency anemia:

H- hypochromia
P- pencil RBC
T- targeting;
M- microcytosis
Lancet 2000;355:1260



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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)

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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

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Fe Deficiency: Causes

4. Hemoglobinuria (intravascular hemolysis)

- PNH
- Runners
- Damaged heart valves
- Microangiopathic hemolysis

5. Sequestration

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

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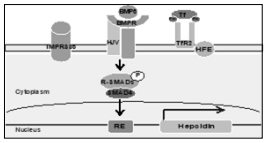
Fe Deficiency: Causes

6. Genetic predisposition

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



TMPRSS6 down-regulates hepcidin

- Inactivating mutations lead to ↑ hepcidin

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Iron-Refractory Iron Deficiency Anemia

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

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Fe Deficiency: Clinical Manifestations

- Impaired psychomotor development
- Fatigue, irritability, ↓ work productivity
- Pica
- Koilonychiae, glossitis, angular stomatitis
- Dysphagia 2° to esophageal web (Plummer-Vinson or Patterson-Kelly Sx)
- Increased risk of thrombosis

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Fe Deficiency: Lab Findings

1. Peripheral blood cells

- ↑ RDW, platelets
- ↓ MCV, MCH, MCHC, RBC, Hb, Hct
- Retics not increased
- ↑ RBC protoporphyrin

2. Serum tests

- ↓ Fe , Tf Sat, ferritin (< 12 µg/L)
- ↑TIBC, transferrin, transferrin receptor

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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 +

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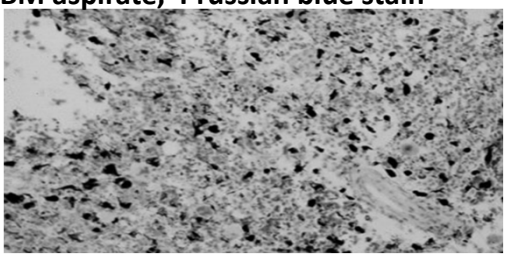
BM aspirate, Prussian blue stain



↑ Fe stores (sideroblastic anemia)

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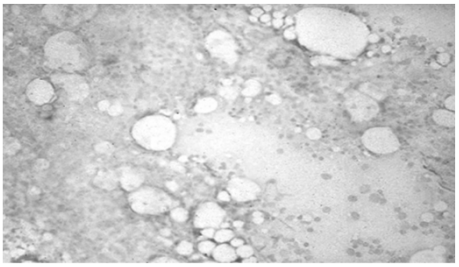
BM aspirate, Prussian blue stain



Absent Fe stores (Fe deficiency)

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Fe Deficiency: Therapy

1. 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 2017; 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

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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

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
Hb CC	10	74
Hb SC	11	78
Hereditary sideroblastic anemia	6	77

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Ftn cutoffs of absent BM Fe in anemia associated with inflammation

Ftn (ng/ml)	Sensitivity	Specificity	Positive predictive 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

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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 g**
  - **marked: 10-20 g; severe: >20 g**

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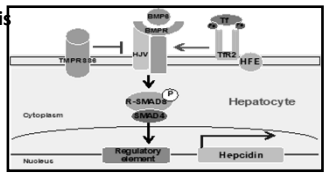
Iron Overload: Classification

1. **Hereditary conditions causing ↓ hepcidin**
   - ***HFE* hemochromatosis**
   - ***TFR2* hemochromatosis**
   - **Hemojuvelin hemochromatosis**
   - **Hepcidin hemochromatosis**



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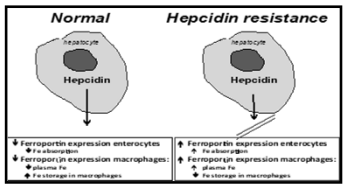
Iron Overload: Classification

2. **Hereditary conditions causing resistance of ferroportin to hepcidin**
   - **Ferroportin disease**



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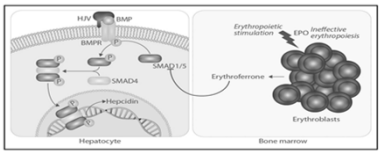
Iron Overload: Classification

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



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Iron Overload: Classification

4. **Multiple blood transfusions**
   - **Diamond Blackfan anemia**
   - **aplastic anemia**
   - **thalassemia major**
   - **sickle cell anemia**
   - **myelodysplasia**

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Iron Overload: Classification

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

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Fe Overload: Clinical Manifestations

1. Fatigue, abd. pain
2. Liver
   - ↑ ALT
   - fibrosis and cirrhosis
   - Hepatoma
3. Heart
   - CHF (restrictive or dilated)
   - arrhythmias
4. Endocrine
   - diabetes mellitus
   - secondary amenorrhea
   - Impotence
5. Hyperpigmentation
6. Certain infections
7. Arthritis
   - esp. 1st and 2nd MP joints (HFE hemochromatosis)

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Screening for hemochromatosis or increased iron stores

1. Screen pts
   - 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

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Diagnosis of hemochromatosis or increased iron stores

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

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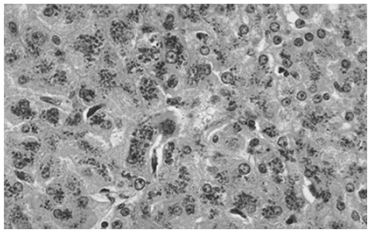
Liver Bx, *HFE* hemochromatosis (H&E):



Golden brown hemosiderin pigment predominantly in hepatocytes

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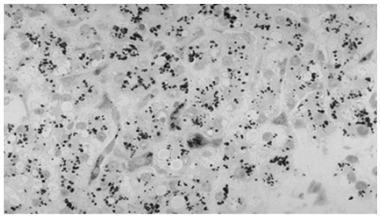
Liver Bx, *HFE* hemochromatosis Prussian blue stain:



Granules predominantly in hepatocytes

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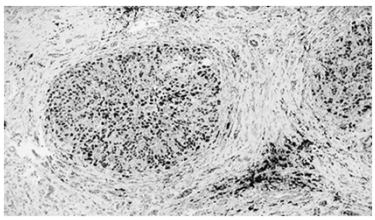
Liver Bx, *HFE* hemochromatosis Prussian blue stain:



Cirrhosis; hepatocytes in regenerating nodule heavily laden with iron

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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

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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
   - ↓ hepcidin production
     - ↑ Fe absorption
     - ↑ serum Fe and transferrin saturation
     - ↓ storage of Fe in macrophages
     - Fe-loading of parenchymal cells

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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*

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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

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Management of *HFE* hemochromatosis

1. Nl life expectancy if phlebotomy Rx started before cirrhosis or diabetes
2. If ferritin <1000 µg/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

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Phlebotomy Therapy

1. <u>Initial program to remove excess body Fe</u>
   - 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

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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.

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Other Forms of Genetic Hemochromatosis

- *TFR2* hemochromatosis
  - autosomal recessive; intermediate phenotype
  - TfR2 mutation 7q22
- Juvenile hemochromatosis
  - autosomal recessive; severe phenotype
  - hemojuvelin mutation 1q21.1
  - *HAMP* (hepcidin) mutation 19q13.1

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Other Forms of Genetic Hemochromatosis

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

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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, scurvy
   - infections
   - esophageal carcinoma
6. Management
   - prevention
   - phlebotomy therapy

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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

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Transfusional Iron Overload Quantitative Considerations

- One unit blood contains about 225 mg Fe as Hb
- Hepatic Fe concentration
  - normal <30 µmol/g dry wt. 0 units
  - worrisome 180 µmol/g dry wt. 50 units
  - Toxic 360 µmol/g dry wt. 100 units
  - highly toxic 720 µmol/g dry wt. 200 units
- At 2 units/month: 50 units in two yrs

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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

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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

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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
4. 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

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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

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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)

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Hemoglobinopathies

Victor R. Gordeuk, MD

August 13, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

3 - Hemoglobinopathies and Thalassemias

Victor R. Gordeuk, MD

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

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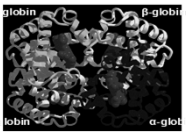
Ribbon structure of hemoglobin A



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 O_2

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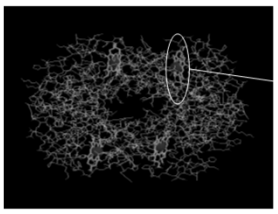
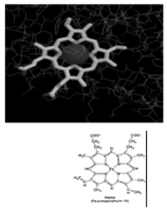
Stick Structure of Hemoglobin A



Close-up view of heme with Fe at center



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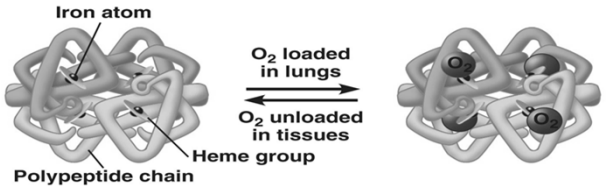

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Normal Hbs found in Adults

Hb A: $\alpha_2\beta_2$ 97%

Hb A_2: $\alpha_2\delta_2$ 3%

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

Primary structure

- Alpha globin- 141 amino acids
- Beta globin- 146 amino acids

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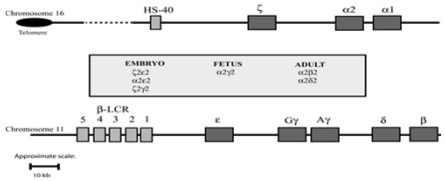
Human Globin Genes



DNA hypersensitive sites:
- β-globin LCR [locus control region]
- α-globin HS-40 [hypersensitive site-40]

(Weatherall and Proven, Lancet 2000;355:1169-1175)

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Chromosome 16

--------α--------- α---------

--------α--------- α---------

Chromosome 11

-----$^{G}\gamma$-- $^{A}\gamma$ ----- δ-- β----

-----$^{G}\gamma$-- $^{A}\gamma$ ----- 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

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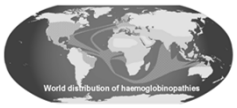
Geographical overlap in distribution

Distribution of hemoglobinopathies



Distribution of malaria

Evidence that these red cell disorders protect against malarial infection.

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Hemoglobinopathies and Thalassemias

Thalassemias:

reduced amounts or absence of structurally nl globin chain

- α-thalassemia
- β-thalassemia

Hemoglobinopathies:

amino acid substitutions; structurally abnl globin

- Hb S, Hb C, Hb G-Philadelphia, Hb D, Hb O-Arabia
- Hb E
- Unstable Hbs
- Altered O_2 affinity
- Hb M

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Hemoglobinopathies and Thalassemias

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

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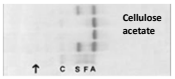
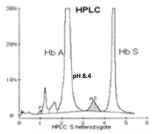
Hemoglobinopathies: Laboratory Dx

- 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 γ- to β-globin
- mostly caused by point mutations

(Steensma, Blood, 2005)

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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

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Membrane Defects in β-Thalassemia

Excess cellular Fe and unstable unpaired α-globin chains cause

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

Membrane damage leads to PS exposure and hypercoagulability

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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 Mutations

β^0-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

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Clinical Classification of β-Thalassemia*

β-Thalassemia trait

- uncomplicated heterozygous β-thalassemia
- β-thalassemia minor

β-Thalassemia intermedia

- no firm definition; many different genotypes

β-Thalassemia major

- Cooley's anemia
- homozygous or compound heterozygous β-thalassemia

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

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Clinical Diagnosis of β-Thalassemia

β-Thal trait

- microcytosis
- hypochromia
- +/- mild anemia
- elevated level of HbA_2 (>3.5%)

β-Thal intermedia

- microcytic anemia
- +/- Tx requirement
- high Hb F
- bone disease, iron loading, splenomegaly, pulmonary hypertension
- many different genotypes

β-Thalassemia major

- transfusion-dependent microcytic anemia
- very high Hb F (approaching 100%)
- bone disease, iron loading, splenomegaly, pulmonary hypertension
- many different genotypes

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Beta-Thalassemias

Genotype	Phenotype	Hematologic Findings
Heterozygote [illegible]	Silent carrier/ thal minima	Normal
Heterozygote [illegible]	thal minor	Mild hypochromic anemia
Homozygote or compd hetero, [illegible]	thal intermedia	Moderate hemolysis & ineffective erythropoiesis
Homozygote or compd hetero, [illegible]	thal major	Severe hemolysis & ineffective erythropoiesis

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Clinical Features of β-Thal Syndromes

	Major	Intermedia	Minor
Severity	4+	2+	+
Splenomegaly	4+	2-3+	0
Skeletal changes	2-4+	0-1+	0
Hemoglobin	<7 g/dL	7-10 g/dL	>10 g/dL
Hypochromia	4+	3+	2+
Microcytosis	3+	2+	1+
Nucleated RBC's	3+	0-1+	0

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Hb Fractions in β-Thal Syndromes

	Nl	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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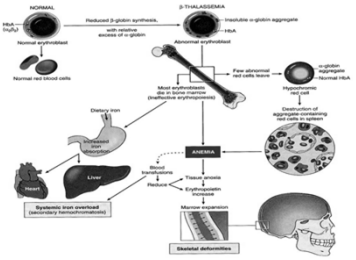
Pathophysiology of β-Thalassemia



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β-Thalassemia Major: Clinical Features

Hematologic
- Severe microcytic anemia
- Splenomegaly
- Extramedullary hematopoiesis

Thromboembolism

Skeletal changes
- Expanded marrow cavity
- Thalassemic facies
- Osteopenia,
- Thin cortex

Growth retardation

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β-Thalassemia Major: Clinical Features

Cardiopulmonary
- Myocardial Fe overload with arrhythmia; CHF
- Hemolytic PHT

Liver
- Hepatic iron-loading with fibrosis, cirrhosis
- Pigmented gall stones

Endocrinopathies
- diabetes mellitus
- hypoparathyroidism
- hypogonadism and delayed puberty

Transfusion related
- Infection
- alloimmunization

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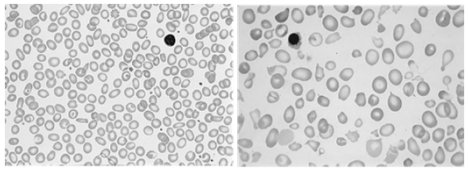
Peripheral Smear in β-Thalassemia



Thalassemia minor **Thalassemia major**

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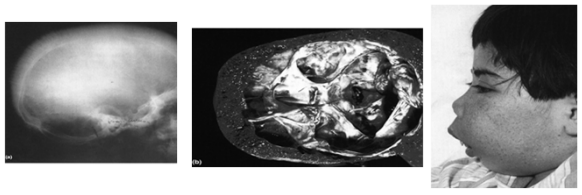
Skull and Face in Poorly Treated β-Thalassemia



(Weatherall & Clegg, The Thalassaemia Syndromes, 2001)

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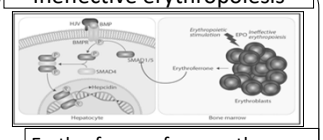
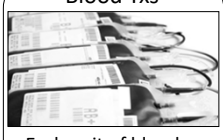
Iron Overload in Thalassemia Major

Ineffective erythropoiesis



Erythroferrone from erythro-blasts suppresses hepcidin

Blood Txs



Each unit of blood contains ≈ 225 mg Fe

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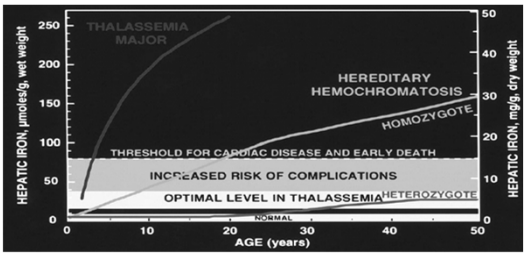
Hepatic Iron and Organ Damage



(Olivieri & Brittenham, Blood 89: 739, 1997)

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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

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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

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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

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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

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Potential Toxicity of Iron Chelation

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

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Alpha Thalassemia

- Decreased synthesis of α-globin chains
- Excess of beta-like globin chains
- Potential formation of abnl Hbs:
  - Hemoglobin Barts: γ_4
  - Hemoglobin H: β_4

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Gene Deletion α-Thalassemia*

α^+-thalassemia

- deletion of a single gene on one chromosome 16 allele

α^0 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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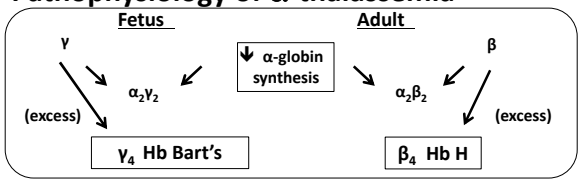
Pathophysiology of α-thalassemia



- High O_2 affinity- tissue hypoxia
- Instability of homotetramers
- Inclusion bodies; membrane damage
- Shortened RBC survival- hemolysis
- Splenomegarly- hypersplenism.

(Weatherall and Proven, Lancet 2000;355:1169-1175)

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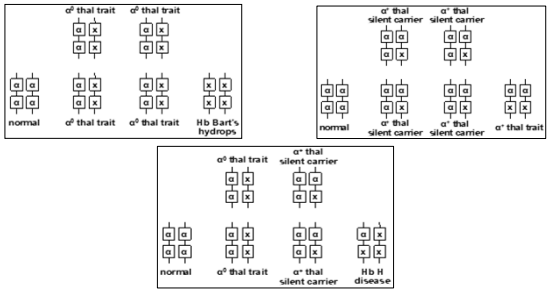
Genetics of α-Thalassemia: SE Asia



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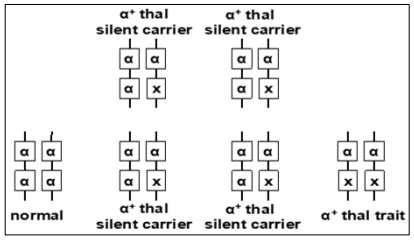
Genetics of α-Thalassemia: Africa



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Alpha-Thalassemias

Geno-type	Pheno-type	Hb Barts (γ_4)	Hb H (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 blood	---	Anemic stillborn

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α-Thalassemia 'Silent Carrier'

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

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Alpha-Thalassemia Trait

2 of 4 alpha genes present and functional
- Homozygous α^+ thal (α-/α-): ~7% of Africans
- Heterozyg. α^0 thal ($\alpha\alpha$/--): common SE Asia

Clinical features:
- +/- mild anemia
- MCV <78 fL
- Hb Barts (γ_4) 2-10% in newborns

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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

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Hemoglobin H Disease

- Genotype α-/-- (SE Asia)
  - α^+-thal one allele
  - α^0-thal other allele
- 20-40% Hb Barts (γ_4) in newborn
- 5-40% Hb H (β_4) in adults
  - visualized by brilliant cresyl blue
  - Hb electrophoresis
  - HPLC

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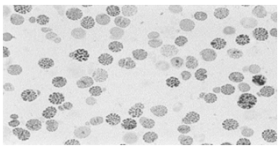
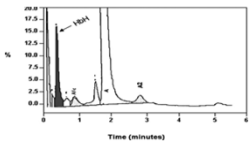
Diagnosis of Hemoglobin H Disease



RBC inclusions generated by brilliant cresyl blue



Fast moving peak on HPLC

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Hemoglobin H Disease

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

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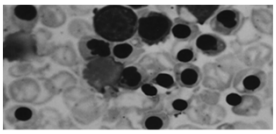
Hemoglobin Bart's Hydrops Fetalis

- Homozygous α^0-thalassemia (- -/- -)
- No functional α-globin genes: Hb Barts (γ_4)
- Eclampsia in mother
- Stillbirth
- Erythroblastosis in infant



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Perspective
blood
Blood. 2017;129(10):1251-1259

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

Duantida Songdej,[1,2] Christian Babbs,[1] and Douglas R. Higgs,[1] in collaboration with the BHFS International Consortium
[1]Medical Research Council Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom; and [2]Division of Hematology/Oncology, Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

Intra-uterine Rx

- RBC transfusion
- Hematopoietic stem cell transplant
- Improved perinatal care

Long-term complications in survivors

- Growth retardation and neurodevelopmental delay
- Lifelong transfusion
- Associated congenital abnormalities

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RBC Indices in Alpha-Thalassemia

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

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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

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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

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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

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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

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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
    - ↓ expression of KLF1 transcription factor that activates BCL11A Hb F suppressor

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Hemoglobin E (β26 glu→lys)

- Second most prevalent Hb variant: 30,000,000 worldwide; >80% in SE Asia
- Beta-thalassemia-like hemoglobinopathy (decreased β^E-mRNA production)
- RBC cytoplasm: precipitated α-chains, increased oxidant stress
- Carriers clinically silent; low MCV

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Hb E Disorders

Condition	*Genotype*	*Clinical*
Hb E Trait	A/E	30% Hb E ± ↓ MCV
Hb E Disease	E/E	90% Hb E, ↓ MCV
Hb E-β-thal	E/beta$^{0,+}$	Hb E 40-85%, Hb F 10-60%, ↓ 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

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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

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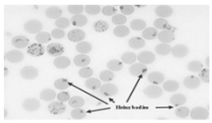
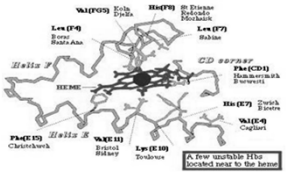
Unstable Hemoglobins





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

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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

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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

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Unstable Hemoglobins

Treatment

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

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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

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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

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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

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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

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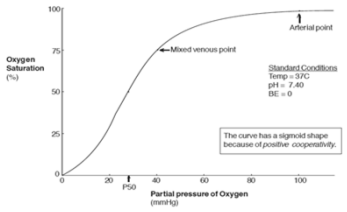
Hemoglobin O_2 Dissociation Curve



Arterial point:
pO_2 100, SaO_2 98%

Mixed venous:
pO_2 40, SaO_2 75%

p50:
pO_2 26, SaO_2 50%

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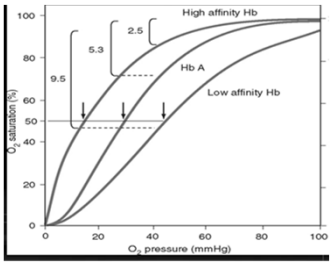
Hemoglobins with Altered O_2 Affinity



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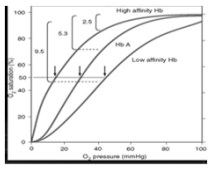
Hemoglobins with High O_2 Affinity

- Familial erythrocytosis; autosomal dominant
- α or β-chain can be affected
- ± distinct electrophoretic pattern
- Left shift O_2 dissociation curve (low P50)



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Hemoglobins with High O_2 Affinity

- Normal 2,3-DPG levels
- Diagnosis
  - Erythrocytosis in familial pattern
  - low P_{50}
  - Hb electrophoresis or HPLC
  - PCR or gene sequencing
- Treatment
  - Polycythemia mild; phlebotomy not necessary

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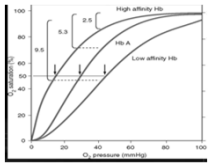
Hemoglobins with Low O_2 Affinity

- Asymptomatic cyanosis
- Right shift in O_2 dissociation curve (high P50)
- Hb electrophoresis, HPLC
- No Rx required



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Anemia of Chronic Illness

Vera Malkovska, MD

August 13, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

4 - Anemia of Chronic Illness

Vera Malkovska, MD, FRCPath

1

Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

- None

2

Anemia of Chronic Disease (ACD) = Anemia of Inflammation

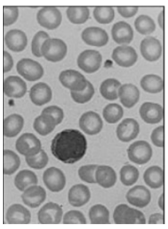
- Second most common anemia worldwide
- Most frequent anemia in hospitalized and chronically ill patients

Weiss G et al. Blood 2019

3

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



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ACD is caused by diverse disorders

Infectious
Tuberculosis
Endocarditis
HIV

Malignant
Metastatic cancers
Lymphomas
Myeloma

Inflammatory
Rheumatoid arthritis
Systemic lupus
Vasculitis

Miscelaneous
Chronic kidney disease
Congestive heart failure
Advanced atherosclerosis
Anemia of critical illness

5

Common features of ACD

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

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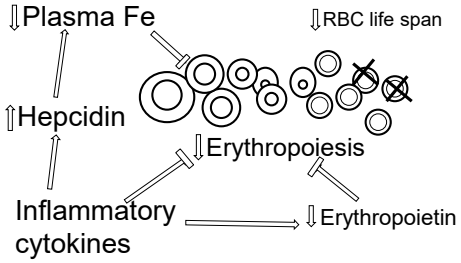
Pathogenesis of ACD

7

Mechanisms of ACD



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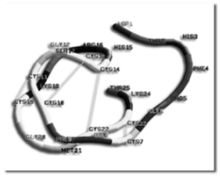
Hepcidin: the key iron regulator

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



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Ferroportin: the iron exporter

- Molecular target of hepcidin
- Transports Fe from cells
- Large transmembrane protein
- Expressed on
  - macrophages
  - enterocytes
  - hepatocytes
  - placental cells

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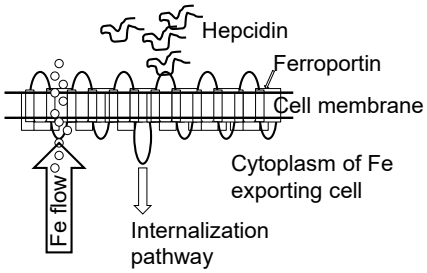
Ferroportin-hepcidin regulate iron transport to plasma



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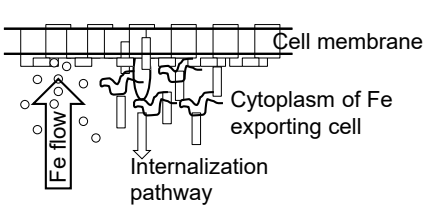
Ferroportin-hepcidin regulate iron transport to plasma



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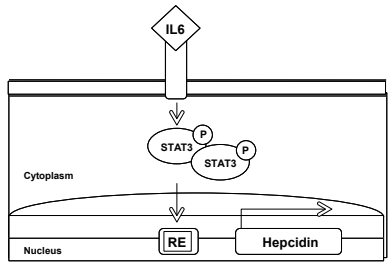
IL-6 regulates hepcidin via STAT3



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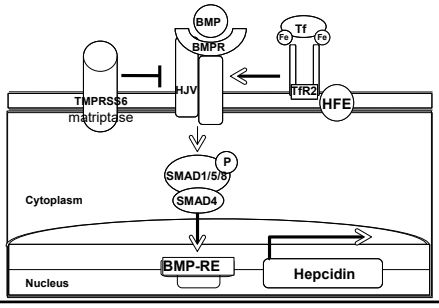
Iron regulates hepcidin via bone morphogenic proteins (BMP)



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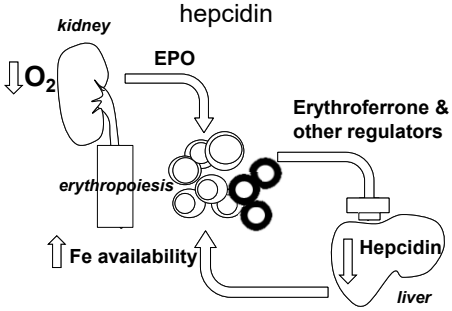
Increased erythropoiesis suppresses hepcidin



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Clinical conditions that impact hepcidin

⇧ Hepcidin	⇩ Hepcidin
Infection	↑ Erythropoiesis
Inflammation	Anemia/hypoxia
↑ iron stores	Liver disease
Iron therapy	Alcoholism
Transfusion	Chronic hepatitis C
Renal failure	Sex hormones
Genetic factors	Genetic factors

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Iron regulation in ACD in macrophages

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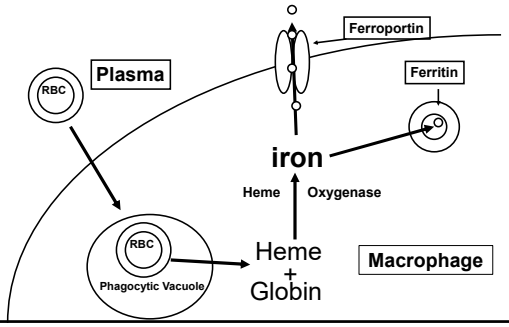
Iron flow in macrophages



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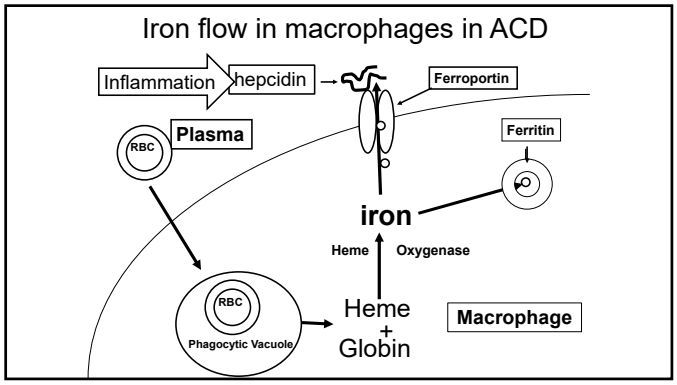

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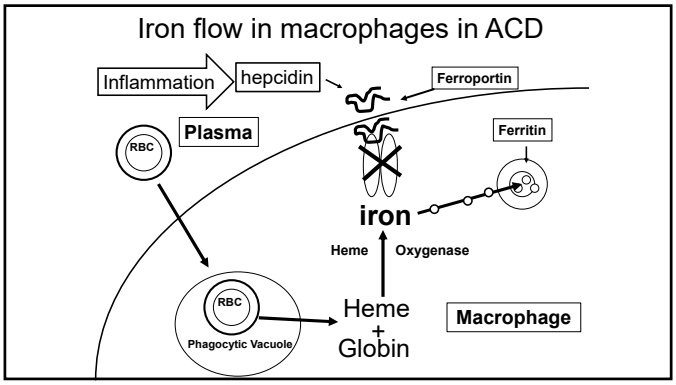

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Systemic iron regulation in ACD

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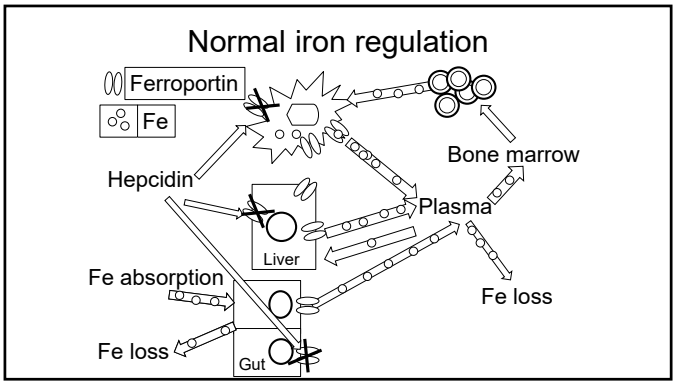

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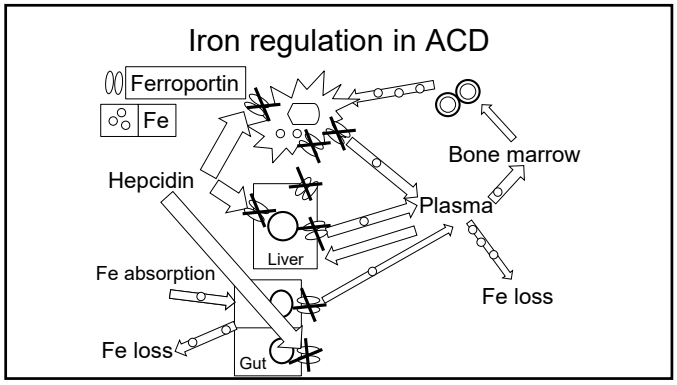

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Laboratory findings

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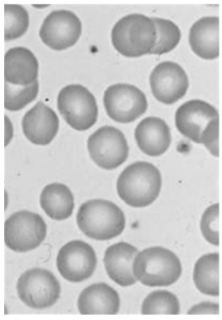
Blood smear

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



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Other findings

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

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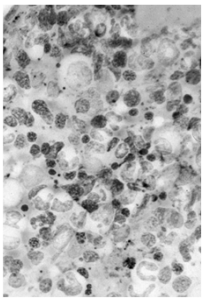
Bone marrow Prussian Blue stain

- Normal or ↑ macropage iron
- Decreased siderotic granules in normoblasts



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Iron studies in ACD and Fe deficiency

Anemia	Serum Fe	TIBC	IBC sat.	ferritin
Fe deficiency	↓	↑	↓	↓
ACD	↓	↓or N	N or↓	↑ or N

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Iron studies in ACD and Fe deficiency

Anemia	Serum Fe	TIBC	IBC sat.	ferritin
Fe deficiency	↓	(↑)	↓	(↓)
ACD	↓	(↓or N)	N or↓	(↑ or N)

Soluble transferrin receptor ↑ in iron deficiency

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Clinical findings and differential diagnosis of ACD

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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

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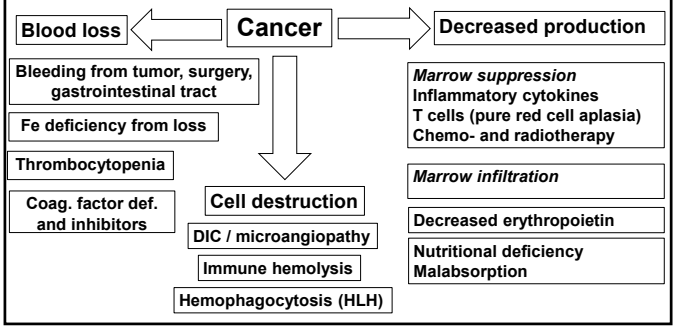
Causes of cancer-associated anemia



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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)

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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

- ↓ 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

35

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

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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

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ACD vs. ACD combined with iron deficiency

39

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

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

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Differential diagnosis of ACD

1. Exclude hyperproliferative anemias
   - Hemolysis
     - ↑ reticulocytes
     - ↓ haptoglobin, ↑ indirect bili and LDH
   - Ineffective erythropoiesis
     - reticulocytes not increased
     - ↓ haptoglobin, ↑ indirect bili and LDH

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

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

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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

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Useful tips for differential diagnosis

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

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Therapy of ACD

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Therapy

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

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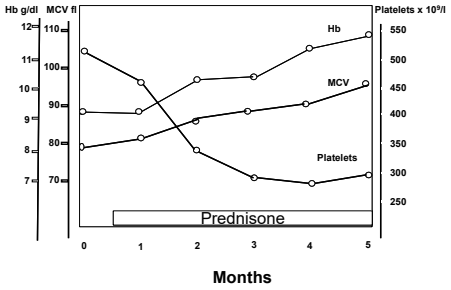
Anemia in polymyalgia rheumatica responds to prednisone



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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!

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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

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- ↓Epo 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

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

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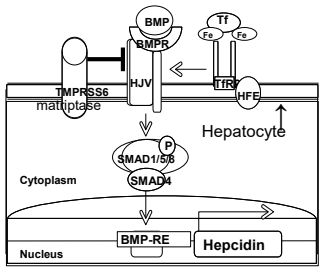
TMPRSS6 defect increases hepcidin in IRIDA



- Matriptase/TMPRSS6 decreases hepcidin expression and ↑ iron absorption
- TMPRSS6-inactivating mutations ↑ hepcidin

55

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

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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

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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

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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

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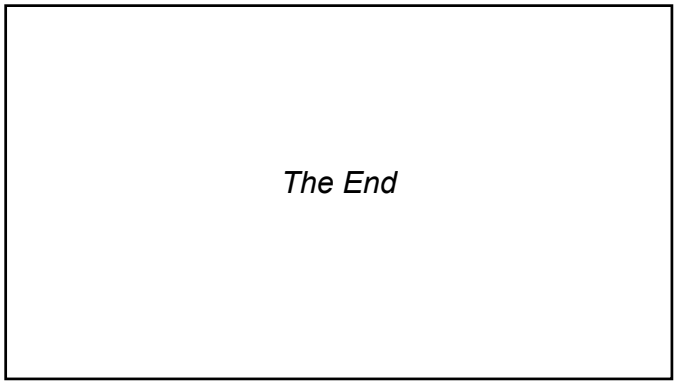

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5

Porphyria

Victor R. Gordeuk, MD

August 13, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

5 - Porphyria

Victor R. Gordeuk, MD

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Outline

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

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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

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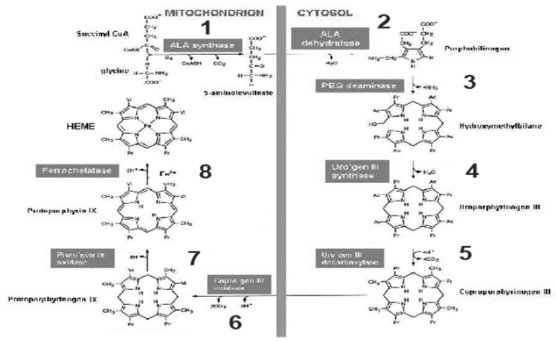

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Enzymes of Heme Biosynthesis

	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

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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

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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

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Acute Porphyrias

1. **Autosomal dominant**
   - But rare ALA dehydratase deficiency is recessive
2. **Heterogeneous mutations**
   - Deletion, alternative splicing, ↓ 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)

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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

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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.

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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.

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Acute Porphyrias- Precipitating Factors

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

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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	

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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 safe
   - narcotics for pain
   - phenothiazines for nausea or vomiting
   - beta blockers for hypertension, tachycardia

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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
   - 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.

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Acute Hepatic Porphyrias

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

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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

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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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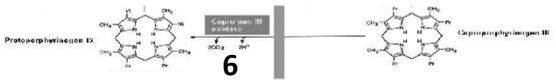
Acute Hepatic Porphyrias

Hereditary Coproporphyria



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

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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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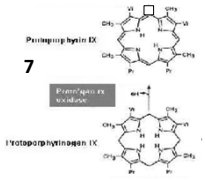
Acute Hepatic Porphyrias

Variegate Porphyria

- Autosomal dominant deficiency protopor-phyrinogen oxidase; >160 mutations
- 1/400 white South Africans
- AIP sx's plus photosensitivity, scarring
- Neurovisceral attacks and skin lesions may occur separately



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Acute Hepatic Porphyrias

Variegate Porphyria

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

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Acute Hepatic Porphyrias

ALA Dehydratase Deficiency

- Rare homozygous defect; 12 mutations
- Diagnosis
  - Urine ALA elevated
  - Urine PBG not increased
- Differential Diagnosis
  - Tyrosinemia; plumbism (lead posoning)

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Acute Hepatic Porphyrias

Acute Porphyria Management Summary

1. Spot urine PBG level for rapid Dx if possible
2. Carbohydrates to ↓ porphyrin synthesis
   - PO 1500-2000 kcal/24 h
   - IV 10- 20% dextrose
3. IV hematin (early)
   - 3-4 mg/kg IV qd
4. Avoid precipitating factors
   - Suppress ovulation with LHRH agonists
5. Consider IV hematin or SC givosiran
6. Mutation analysis in pts and family

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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

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Cutaneous Porphyrias

Cutaneous Porphyrias

Type	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

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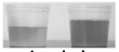
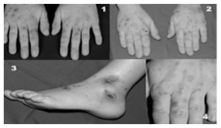
Cutaneous Porphyrias

Porphyria Cutanea Tarda

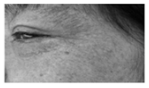


urine darker than control

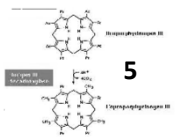
Fluorescence after acidification



Blistering and scarring of sun-exposed skin



Hyper-trichosis



Cloeren and Craig. NEJM 2001;245 no. 14

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Cutaneous Porphyrias

Porphyria Cutanea Tarda

1. Sx's when hepatic uroporphyrinogen decarboxylase activity ≤20% of nl
2. Type 1 acquired form is most common
   - Nl 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

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Cutaneous Porphyrias

Porphyria Cutanea Tarda

5. Most common porphyria
   - 1-5 cases/25,000
6. Precipitating factors oxidize uroporphyrinogen, which inhibits URO-D
   - ↑ iron stores; *HFE*, African iron overload
   - Hepatitis C (>50% of pts +), HIV
   - Alcohol, estrogens
   - Exposure to fungicide hexachlorobenzene (Turkey, 1956)

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Cutaneous Porphyrias

Porphyria Cutanea Tarda

7. Down-regulation of hepcidin may underlie increase in iron stores
8. Manifestations
   - Bullous dermatosis (blistering skin lesions)
   - Scarring
   - Hyperpigmentation
   - Hypertrichosis

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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 overload
   - Evaluate for viral hepatitis/other liver disease; R/O hepatocellular carcinoma

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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 in childhood
  - ↑ uroporphyrin and heptacarboxylporphyrin in urine
  - ↑ 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

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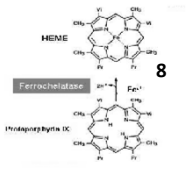
Cutaneous Porphyrias

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 allele
- More common than congenital erythropoietic protoporphyria



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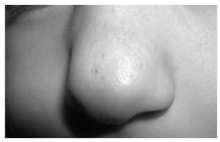
Cutaneous Porphyrias

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 be fatal
- **Diagnosis**
  - Massive increase erythrocyte protoporphyrin



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Cutaneous Porphyrias

Erythropoietic Protoporphyria

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

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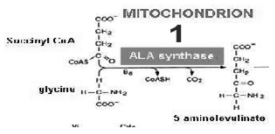
Cutaneous Porphyrias

X-LInked Protoporphyria

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



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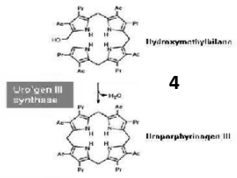
Cutaneous Porphyrias

Congenital Erythropoietic Porphyria

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



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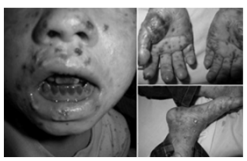
Cutaneous Porphyrias

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
- **Hemolytic anemia, splenomegaly**



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Cutaneous Porphyrias

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

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Porphyria: Clinical Approach Summary

1. General principles
   - Symptomatic porphyria always has ↑ heme 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

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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.

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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.

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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. Nl; RBC protoporphyrin 18,900 ug/dL (nl <90).

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Clinical Case 2:

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

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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.

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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.

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6

Megaloblastic and Sideroblastic Anemias

Vera Malkovska, MD

August 13, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

6 - Megaloblastic and Sideroblastic Anemias

Vera Malkovska, MD, FRCPath

1

Disclosures

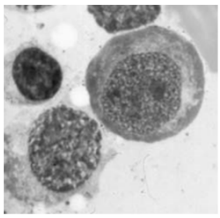
Disclosures of Financial Relationships with Relevant Commercial Interests

- None

2

Megaloblastic Anemias: Definition

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



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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

4

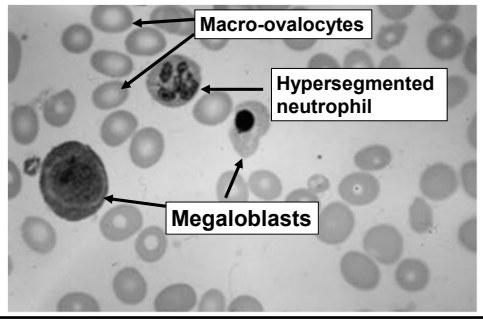
Morphology

Peripheral blood
- Macro-ovalocytes
- Hypersegmented neutrophils

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

5

Bone marrow morphology



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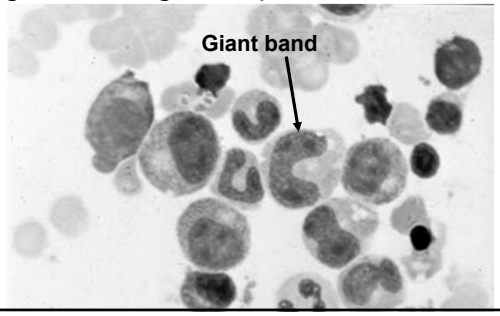
Megaloblastic granulopoiesis



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Labs reflect ineffective erythropoiesis

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

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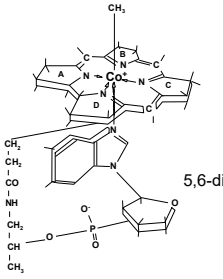
Cobalamine (CBL) deficiency

9

Structure of methyl-CBL



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

Nucleotide: 5,6-dimethyl-benzimidazolyl

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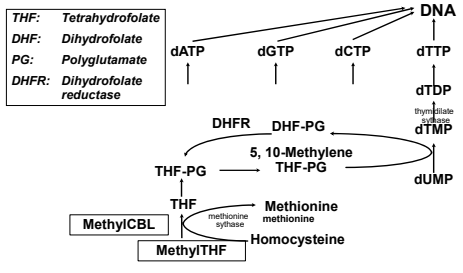
Biochemistry of CBL & folate deficiency



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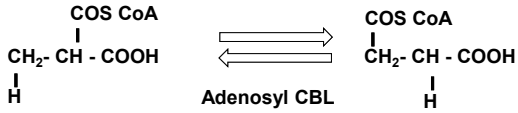
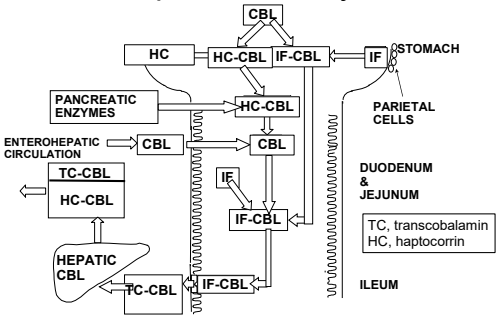
Adenosyl CBL-dependent reaction in mitochondria



Methylmalonyl CoA — Succinyl CoA

12

Absorption of dietary CBL



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Absorption of oral CBL

	Normal	Malabsorption
Daily loss	**1 µg**	**2 µg**
Body stores	**~ 2,500 µg**	**variable**
% absorbed from 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**

Adapted form Carmel R, Blood 2008,112:2214

14

CBL retention from injection

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

*Adapted form Chanarin I. The Megaloblastic Anemias,1979

15

Causes of clinical CBL deficiency

- Dietary deficiency (vegans)
- Inadequate proteolysis of food-CBL (gastric surgery)
- Intrinsic factor (IF) deficiency - pernicious anemia (PA)
- 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

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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

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PA: Clinical manifestations

Hematological
- Symptoms of anemia
- Pallor, lemon yellow skin

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

Other: Glossitis, other epithelial changes

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PA: Clinical manifestations

Associated autoimmune disorders
- Hypothyroidism, Hashimoto's
- Vitiligo, rarely hyperpigmentation
- Diabetes mellitus
- Addison's disease

Increased risk of gastric cancer and carcinoid

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Diagnosis and management of CBL deficiency

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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)

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Accurate diagnosis impacts therapy

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

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Low B12 levels without clinical deficiency

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

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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

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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

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Tests for the diagnosis of CBL and folate deficiency

Test	CBL deficiency	Folate deficiency
Serum CBL	↓	N or ↓
Serum folate*	N or ↑	↓
Red cell folate	↓ or N	↓
MMA*	↑	N
Plasma homocysteine	↑	↑
Deoxyuridine Suppression test*	Abnl.	Abnl.

*Best discriminators of CBL vs folate def.

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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

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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

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

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Folate deficiency

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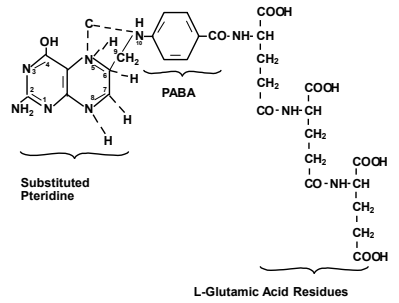
Structure of folates



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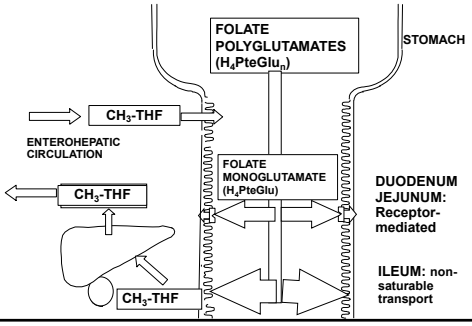
Absorption of Dietary Folates



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Folate in diet

- 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

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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

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Serum vs red cell (RBC) folate

Serum Folate	RBC Folate
• Unstable in vitro	• Better reflect body stores
• May normalize after one hospital meal	• Low in 60% CBL deficiency
• Increased with hemolysis	• Increased with hemolysis

36

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!

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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

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Sideroblastic anemias

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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

40

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

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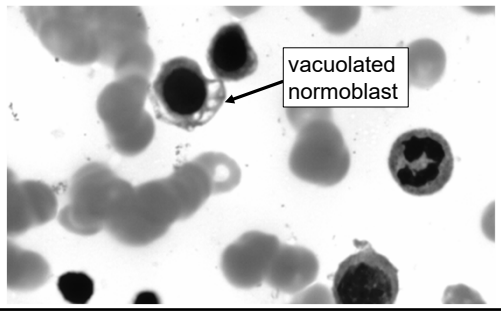
Bone marrow in SA



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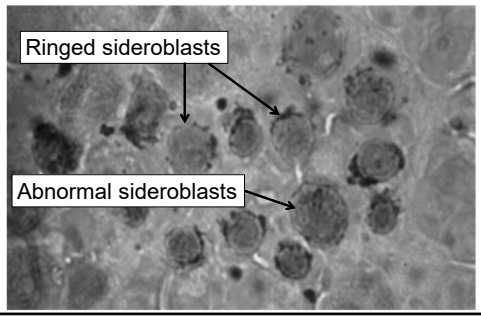
Bone marrow: Prussian blue stain



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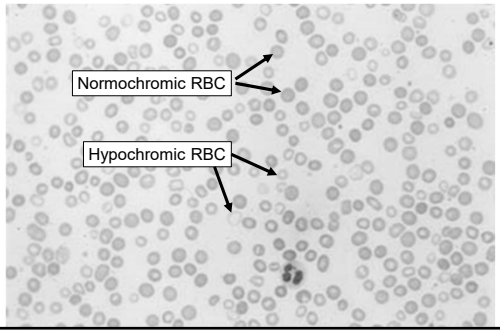
Laboratory findings

Signs of ineffective erythropoiesis

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

44

Dimorphic red cells in SA



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Laboratory findings

Signs of defective iron utilization

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

46

1. 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

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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*

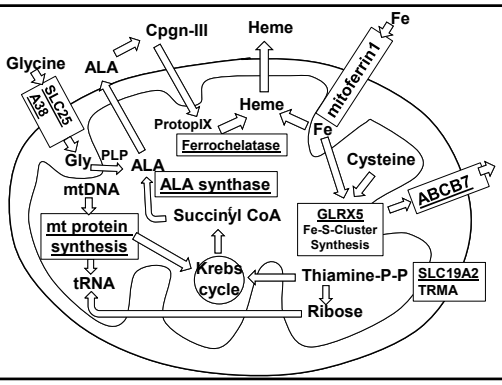
48

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*

49



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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

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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

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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

** The 2016 revision of the WHO classification of myeloid neoplasms and acute leukemia*

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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*

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3. Reversible acquired SA

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

Anemia corrects upon removal of precipitating cause

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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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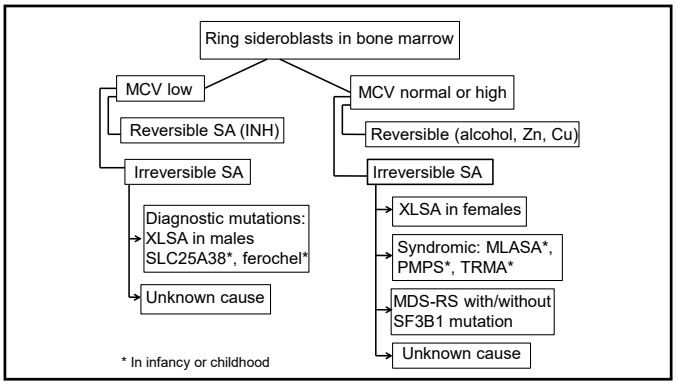

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Thank you

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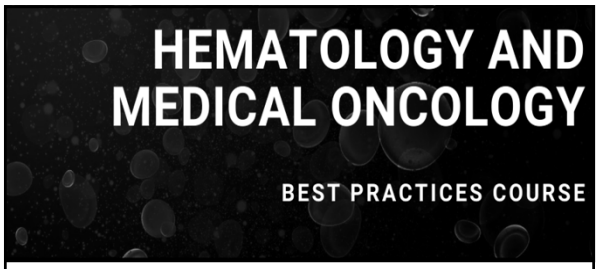
7

Hemolytic Anemias

Imad A. Tabbara, MD

August 13, 2020



7 - Hemolytic Anemias

Imad A. Tabbara, MD, FACP

1

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)

3

CLASSIFICATION

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

4

CLASSIFICATION

- **Acquired Hemolytic Disorders**
  * ***Immune hemolytic anemias***
  * Splenomegaly
  * Microangiopathic hemolytic anemia
  * PNH
  * Direct toxic effect (malaria, clostridial infections)
  * Spur cell anemia

5

IMMUNE HEMOLYTIC ANEMIAS

- Caused by:
  – ***WARM ANTIBODY***
  – OR
  – ***COLD ANTIBODY***

6

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

7

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)

8

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

9

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*

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IMMUNE HEMOLYTIC ANEMIAS Diagnosis

- **Direct Coombs test**
  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

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DAT-negative Hemolysis

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

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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)*

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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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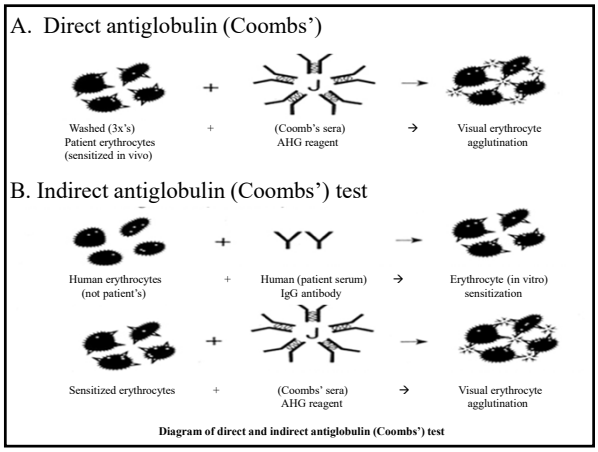

Diagram of direct and indirect antiglobulin (Coombs') test

17

IMMUNE HEMOLYTIC ANEMIAS Diagnosis

- **Cold Agglutinin Assay**
  - Detects serum Abs which induce clumping of O ⊕ 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
  - Physiologically significant cold agglutinins cause C3 fixation on RBCs (complement only ⊕ Coombs test)

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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 complement-mediated 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

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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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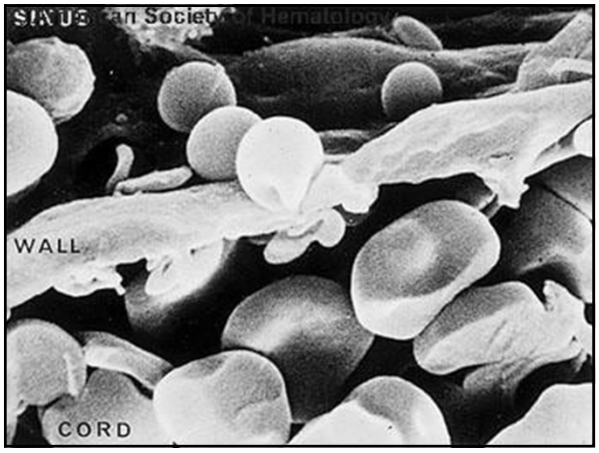

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IMMUNE HEMOLYTIC ANEMIAS
Warm-Reactive

- 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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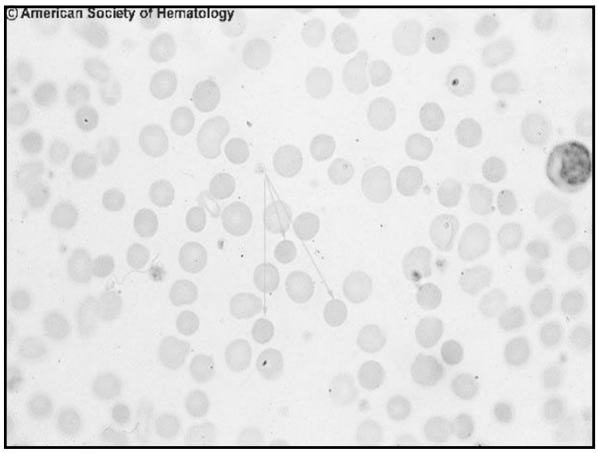

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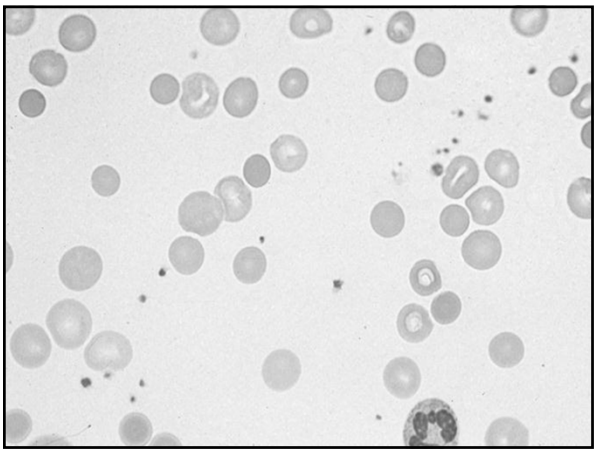

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IMMUNE HEMOLYTIC ANEMIAS
Warm-Reactive

- 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 re-treatment 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

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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

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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

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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***

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IMMUNE HEMOLYTIC ANEMIAS
Infections

- **CMV Infection**
  - Autoimmune hemolysis caused by warm-reactive IgG
- **Influenza A Infection**
  - Autoimmune hemolysis caused by high-titer complement-fixing Abs to virus-produced, RBC-bound polyribosome ribosylphosphate
- **HSV Infection**
  - Autoantibody is IgG with Rh (anti-c) specificity

33

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)

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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

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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

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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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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) & Sirolimus

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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

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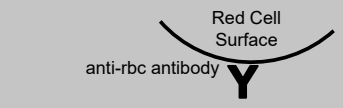
DRUG-INDUCED AUTOANTIBODY GENERATION



Key concept:
- Drug stimulates B cell production of anti-rbc autoantibodies

Examples
- α methyl Dopa
- L Dopa
- Fludarabine and Chloro-deoxyadenosine
- Procainamide
- Diclofenac

40

DRUG-INDUCED IMMUNE HEMOLYTIC ANEMIA

- **Innocent Bystander Type**
  - Least common
  - 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

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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

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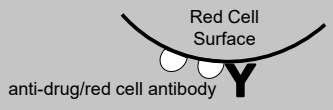
DRUG-INDUCED FORMATION OF ABS AGAINST THE RBC –Hapten COMPLEX



Key concept:
- 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**
  - The drug binds to the RBC membrane and becomes the target antigen
  - Caused by large IV doses of penicillin or penicillin-like antibiotics
  - Occurs 7-14 days after initiation of penicillin
  - Direct Coombs is positive for IgG during penicillin administration

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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

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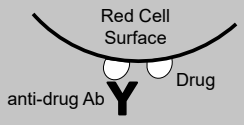
DRUG-INDUCED ANTIBODIES – PENICILLIN-LIKE MECHANISM



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

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AIHA /Warm-Reactive Treatment

- Treatment of underlying condition
- Removal of drug
- 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

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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

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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

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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

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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

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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

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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

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AIHA/ Warm-Reactive

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

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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

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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

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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

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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

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Cold Agglutinin Disease

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

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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

- Direct antiglobulin test detects C_3 since the bound IgM is released at 37°C
- Only red cells coated with C_3b are removed from the circulation by macrophages in liver
- Red cells coated with C_3d are not removed from the circulation and are protected from complement-mediated hemolysis because C_3d limits the sites available for C_3b activation

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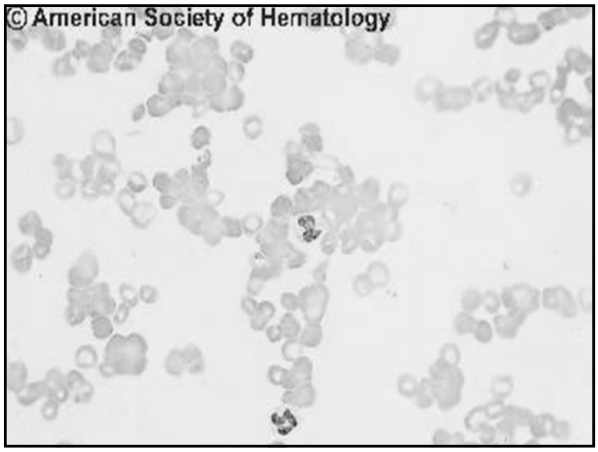

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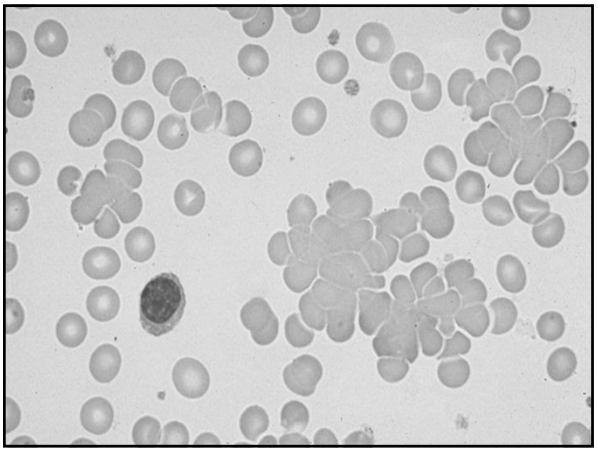

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Cold Agglutinin Disease

- **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 self-limited

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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

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Cold Agglutinin Disease

- CHRONIC COLD AGGLUTININ SYNDROME
  - Age >60
  - 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

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Secondary Cold Agglutinin Disease Treatment

Treat the Underlying disorder

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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

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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

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Cold Agglutinin Disease

- Special Precautions
  - 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 plasma-containing blood products
  - Use of warm intravenous solutions

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IMMUNE HEMOLYTIC ANEMIAS Cold-Reactive

- **Paroxysmal Cold Hemoglobinuria (PCH)**
  - Rare disorder
  - 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

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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

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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

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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

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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

NHL, non-Hodgkin lymphoma; SLE, systemic lupus erythematosus; CVID, common variable immune deficiency; ALPD, autoimmune lymphoproliferative disease; and SCT, stem cell transplantation.

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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 α	Withdrawal	Steroids	
Primary CAD	Protection from cold exposure	Rituximab, chlorambucil	
Paroxysmal cold hemoglobinuria	Supportive treatment	Rituximab	

MMF , mycophenolate mofetil; NHL, non-Hodgkin lymphoma; SMZL, splenic marginal zone lymphoma; SLE, systemic lupus erythematosus; SCT, stem cell transplantation; CVID, common variable immunodeficiency; ALPD, autoimmune lymphoproliferative disease; and CAD, cold agglutinin disease.

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Treatment of CLL-associated AIHA

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	

RCD indicates rituximab, cyclophosphamide, and dexamethasone; and R-CVP, rituximab, cyclophosphamide, vincristine, prednisone.

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IMMUNE HEMOLYTIC ANEMIAS Mechanisms of Hemolysis

	IgG	IgM
Fc Receptor mediated	Yes	No
Complement mediated	Yes	Yes
Cold-reaction dependent	No	Yes

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MECHANISMS OF ACTION OF CORTICOSTEROIDS

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

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IMMUNE HEMOLYTIC ANEMIAS THERAPY

	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 +	+	+	+

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Intravascular vs. Extravascular Hemolysis

	Intravascular Hemolysis	Extravascular Hemolysis
Pathophysiology	Complement-mediated lysis	Fc or C_3b 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

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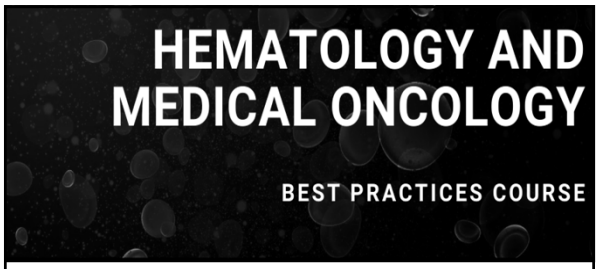
8

RBC Enzymes, Membranes, and Metabolism

Imad A. Tabbara, MD

August 13, 2020



8 - Red Cell Enzymes, Membranes, and Metabolism

Imad A. Tabbara, MD, FACP

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Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

2

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)

3

CLASSIFICATION OF HEMOLYTIC ANEMIAS

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

4

CLASSIFICATION OF HEMOLYTIC ANEMIAS

- Acquired Hemolytic Disorders
  - *Immune hemolytic anemias*
  - Splenomegaly
  - Microangiopathic hemolytic anemia
  - PNH
  - Direct toxic effect (malaria, clostridial infections)
  - Spur cell anemia

5

ERYTHROCYTE ENZYMOPATHIES

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

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ERYTHROCYTE ENZYMOPATHIES

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

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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.

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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

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ERYTHROCYTE ENZYMOPATHIES

DIAGNOSTIC TESTS

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

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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***
  - ***Nucleotide salvage pathways***

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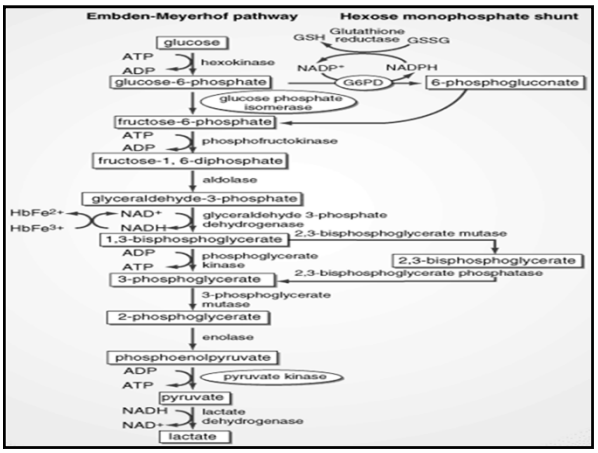

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ERYTHROCYTE ENZYMOPATHIES

- 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)

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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.

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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

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EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- **Hexokinase (HK) deficiency**
  - First enzyme in the glycolytic pathway
  - Least active of the glycolytic enzymes
  - Activity decreases as RBC matures
  - Decreased HK activity can be quantitative or qualitative
  - Activity increases with reticulocytosis

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EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- **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

- **Hexokinase (HK) deficiency**
  - ***Acquired*** HK deficiency occurs in Wilson's disease
  - Elevated copper levels inhibit HK intermittently leading to brisk intravascular hemolysis

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EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- **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

22

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- **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

23

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

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

24

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- **Pyruvate Kinase (PK) Deficiency**
  - 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

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EMBDEN- MEYERHOF (GLYCOLYTIC) PATHWAY

- **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

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

27

EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

- **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

- **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.

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EMBDEN-MEYERHOF (GLYCOLYTIC) PATHWAY

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

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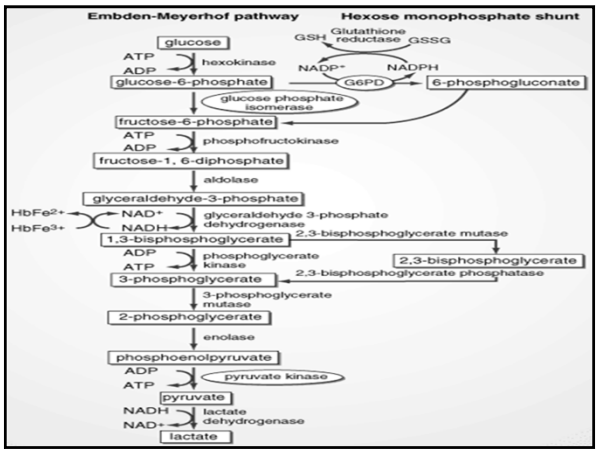

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HEXOSE MONOPHOSPHATE SHUNT

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

32

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 RBCs.

33

HEXOSE MONOPHOSPHATE SHUNT

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

34

HEXOSE MONOPHOSPHATE SHUNT

- Glucose-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).

35

G6PD DEFICIENCY

- Many 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)
- G6PD-A(+): is the normal African-American variant seen in 20% of this population. Has normal activity but increased electrophoretic mobility

36

G6PD DEFICIENCY

- G6PD-A(-) : 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.

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G6PD DEFICIENCY

- Classified 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)
  - Class 4 + 5: harmless

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G6PD DEFICIENCY

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

39

G6PD DEFICIENCY

- Neonatal jaundice and acute hemolytic anemia
- 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

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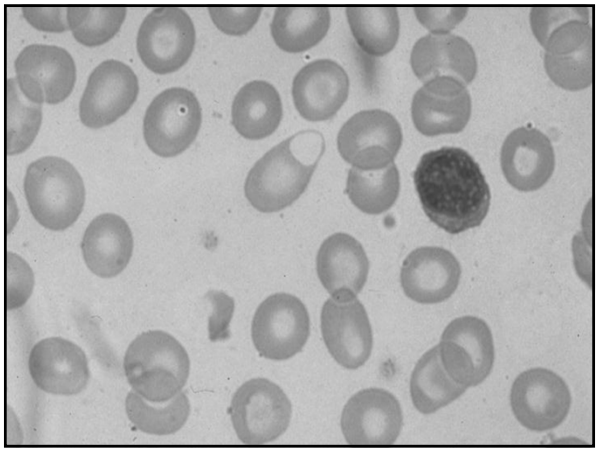

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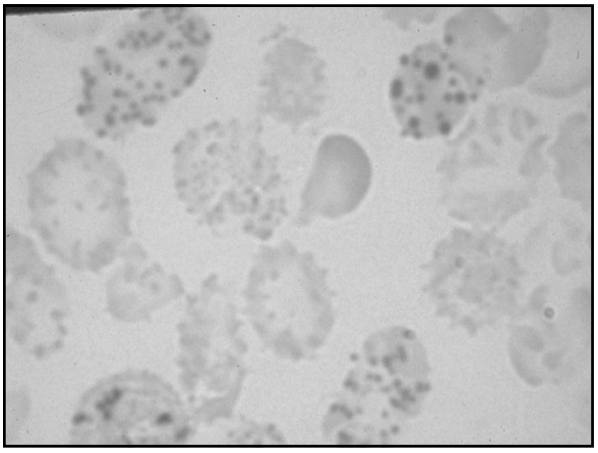

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HEXOSE MONOPHOSPHATE SHUNT

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

43

DEFECTS OF NUCLEOTIDE METABOLISM

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

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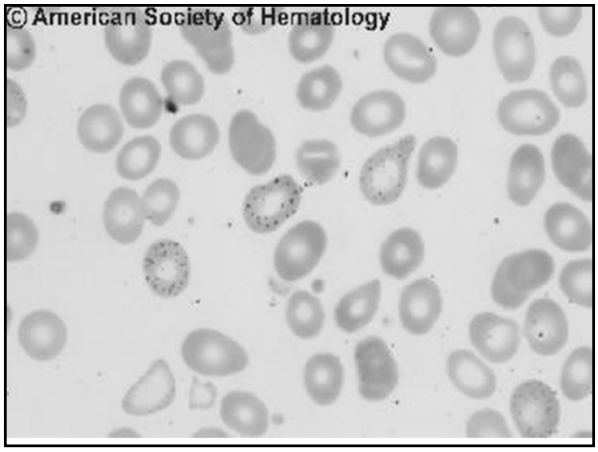

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DEFECTS OF NUCLEOTIDE METABOLISM

- Hyperactivity of Adenosine Deaminase (AD)
  - AD catalyzes deamination of adenosine to inosine
  - 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

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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

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RBC MEMBRANE DEFECTS

- RBC membrane consists of a phospholipid-cholesterol 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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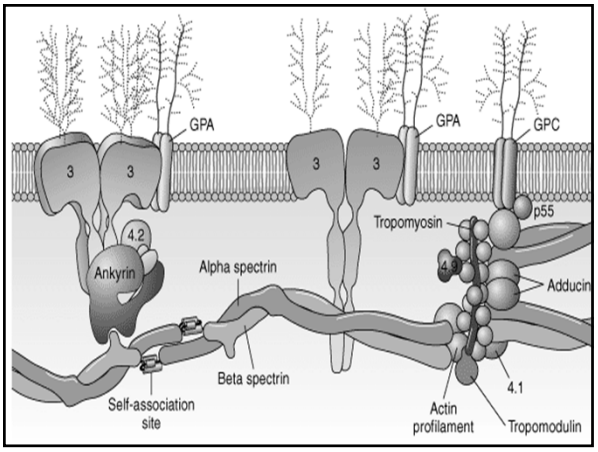

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RBC MEMBRANE DEFECTS

- Spherocytes and Stomatocytes
  - reduced surface-to-volume ratio
  - tolerate less osmotic swelling than normal cells before they lyse
- Target cells and dehydrated RBCs:
  - increased surface-to-volume ratio
  - osmotically resistant

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HEREDITARY SPHEROCYTOSIS

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

54

HEREDITARY SPHEROCYTOSIS Molecular Defects

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

55

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

56

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

57

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

58

CLINICAL FEATURES OF HS

- Anemia, Jaundice, Splenomegaly
- May present at any age
- Severe in Neonates
- Partially compensated hemolysis after neonatal period

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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)

60

DIAGNOSIS OF HS

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

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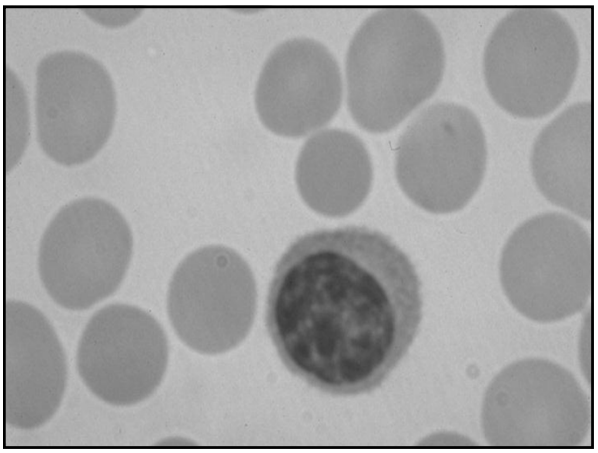

62

DIAGNOSIS of HS

- **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.

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Diagnosis of HS

- **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
  - 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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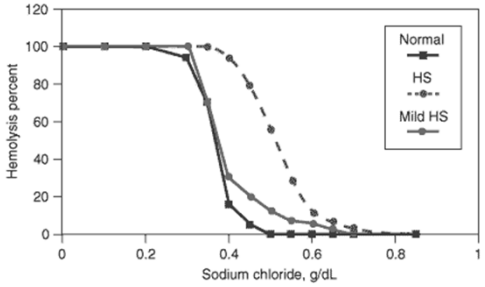
Osmotic fragility of RBC in hereditary spherocytosis (HS). The results from two patients are compared to those from a normal individual.



65

COMPLICATIONS OF HS

- Hyperhemolytic crises:
  - Most common
  - associated with infections
  - Accelerated hemolysis & splenic enlargement
- Aplastic crises:
  - Rare, severe, caused by parvovirus B19. Lasting immunity after 1st infection
- 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

67

TREATMENT OF HS

- Splenectomy
  - corrects 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

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HEREDITARY ELLIPTOCYTOSIS

- Three subtypes:
  - Common HE
  - Spherocytic HE
  - Southeast Asia Ovalocytosis

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HEREDITARY ELLIPTOCYTOSIS

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

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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

72

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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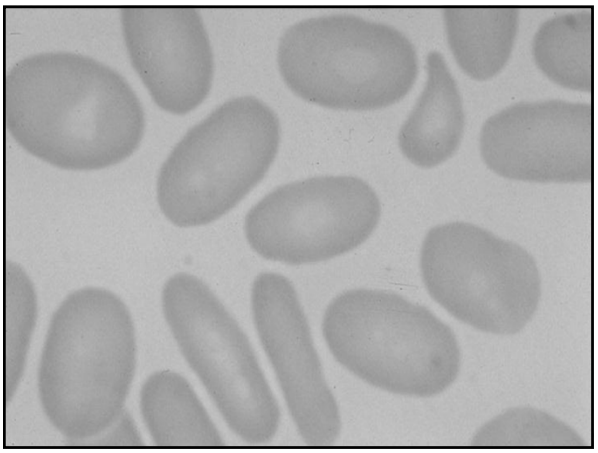

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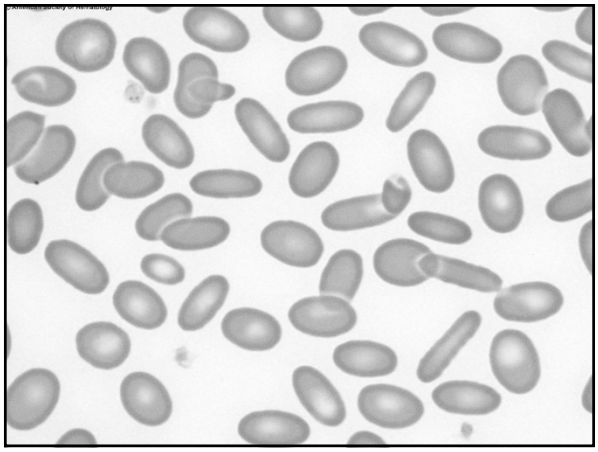

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SPHEROCYTIC ELLIPTOCYTOSIS

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

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SPHEROCYTIC ELLIPTOCYTOSIS

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

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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

78

SOUTHEAST ASIAN OVALOCYTOSIS

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

79

Disorders of Erythrocyte Hydration

- Heterogeneous disorders with abnormalities of RBC solute content
- 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
- Primary hydration defects include:
  - Hereditary xerocytosis syndromes (dehydrated phenotype)
  - Hereditary hydrocytosis syndromes (overhydrated phenotype)
  - And intermediate syndromes

80

Hereditary Xerocytosis

- 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 chelation

81

Hereditary Xerocytosis

- 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
- No specific treatment is needed or available
- Splenectomy of little or no benefit associated with life-threatening thrombotic episodes

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

83

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

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HEREDITARY STOMATCYTOSIS

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

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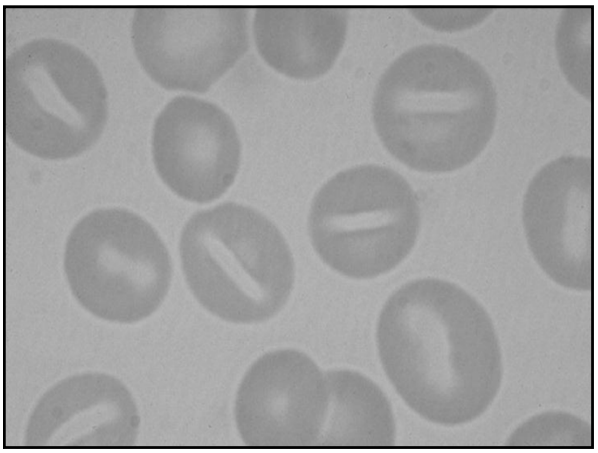

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Rh Deficiency Syndrome

- 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

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Rh Deficiency Syndrome

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

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HEREDITARY PYROPOIKILOCYTOSIS

- 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

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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

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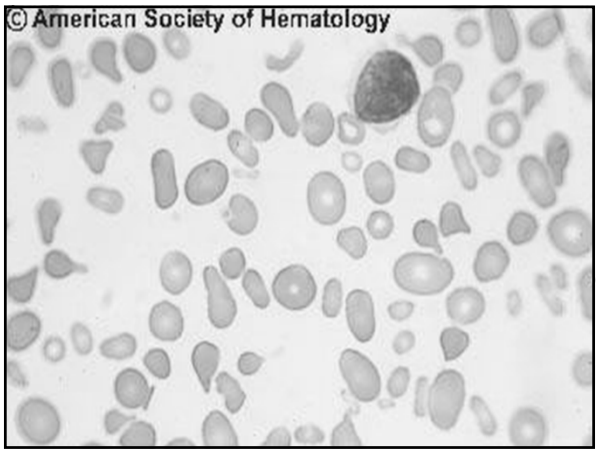

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ACANTHOCYTOSIS

- 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

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ACANTHOCYTOSIS

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

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ACANTHOCYTOSIS

- 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

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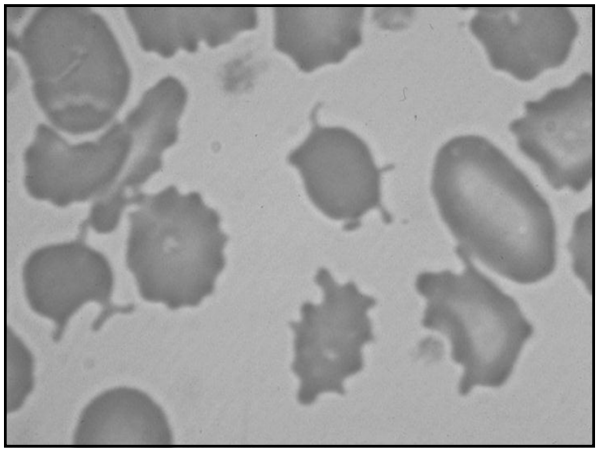

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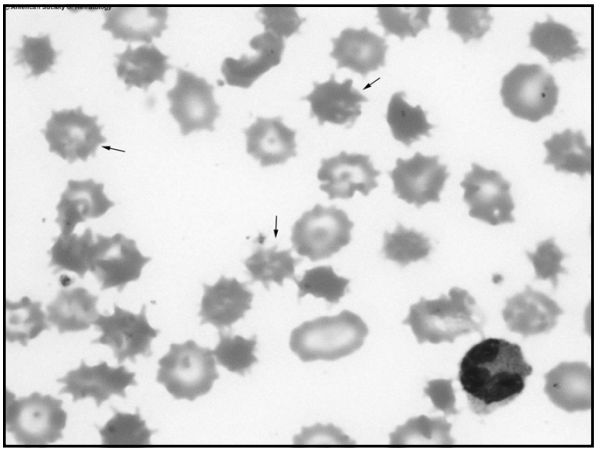

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Sickling Disorders

Santosh Saraf, MD

August 13, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

9 – Sickling Disorders

Santosh L. Saraf, MD

1

Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

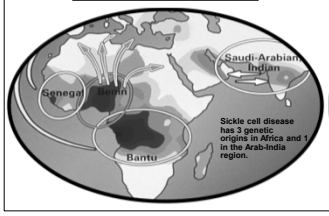
- Research Support – Pfizer, Novartis, Global blood Therapeutics
- Consulting – Novartis
- Advisory Board/Consulting – Global blood Therapeutics

2

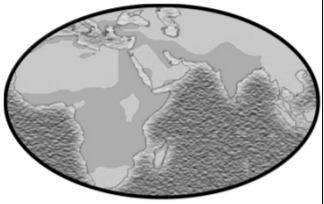
Epidemiology:

- Affects 1 in 365 African Americans
- World-wide: 300,000 births per year
  25 million people affected

Distribution of Hb S Mutation



Distribution of Malaria



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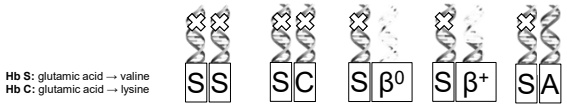
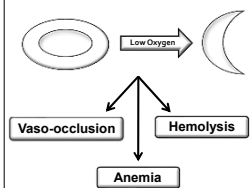
Sickle Cell Genotypes

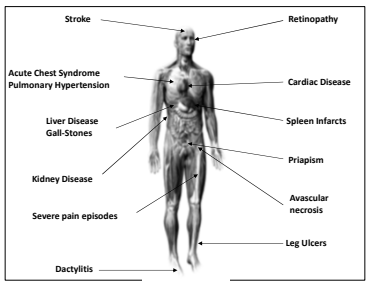


Hgb (g/dL)	**6 – 10**	**9 – 12**	**6 – 10**	**9 – 12**	**11 – 15**
Retic (%)	**5 – 20%**	**5 – 10%**	**5 – 20%**	**5 – 10%**	**1 – 2%**
MCV (fL)	**> 80**	**> 80**	**< 80**	**< 80**	**80 – 100**
HPLC	**HbS**: 90% **Hb A**: 0%	**Hb S**: 50% **Hb C**: 50%	**Hb S**: 90% **Hb A**: 0%	**Hb S**: 75% **Hb A**: 25%	**Hb S**: 40% **Hb A**: 60%
Clinical Complications	**Severe:** Hemolytic & Vaso-occlusive	**Mild:** Vaso-occlusive	**Severe:** Hemolytic & Vaso-occlusive	**Mild:** Vaso-occlusive	Renal & Thrombotic

4

Background





5

Endophenotypes

Hemolysis-
Endothelial Dysfunction

Higher
Hemolytic Rate

Pulmonary Hypertension
Kidney Disease
Leg Ulceration
Priapism
Stroke

Viscosity-
Vaso-occlusion

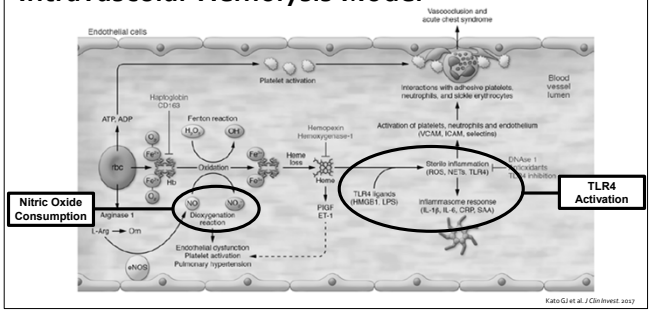
Lower
Hemolytic Rate

Pain crisis
Acute Chest Syndrome
Osteonecrosis

Kato et al. Blood Review 2007

6

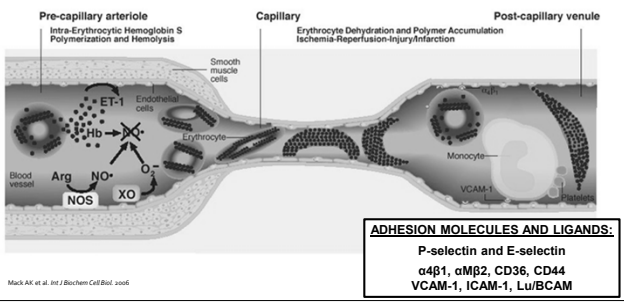
Intravascular Hemolysis Model



Kato GJ et al. *J Clin Invest.* 2017

7

Vaso-Occlusive Model



Mack AK et al. *Int J Biochem Cell Biol.* 2006

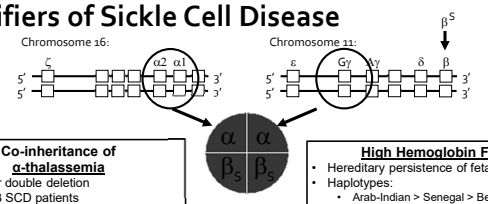
ADHESION MOLECULES AND LIGANDS:

P-selectin and E-selectin

α4β1, αMβ2, CD36, CD44
VCAM-1, ICAM-1, Lu/BCAM

8

Modifiers of Sickle Cell Disease



Co-inheritance of α-thalassemia

- Single or double deletion
  - ~1/3 SCD patients
- Lowers RBC density and MCHC
  - Higher Hb/Lower hemolytic markers
- Protective from some SCD complications
  - Kidney disease
  - Leg ulcers
  - Cardiopulmonary
- **Not protective for VOC**

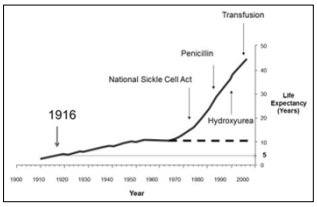
High Hemoglobin F%

- Hereditary persistence of fetal Hb
- Haplotypes:
  - Arab-Indian > Senegal > Benin > Bantu
- *BCL11A* rs1427407
  - Normally inhibits γ-globin
  - ~1/3 SCD patients
  - Target of gene therapy
- CSSCD: ↑ Hb F%: ↓ VOC and mortality
- Hb F > 10%: less stroke, AVN
- **Hb F > 20%: less VOC**

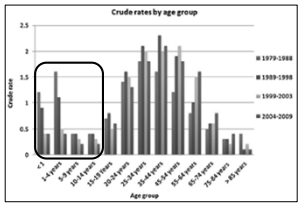
9

Morbidity & Mortality

≥ 95% of children with SCD survive into adulthood in high-income countries[1-3]



Wailoo K et al, NEJM 2017



Hamideh D et al, Pediatr Blood Cancer 2013

1. Telfer P et al, Haematologica 2007; 2. Quinn CT et al, Blood 2010; 3. Couque N et al, Br J Haematol 2016

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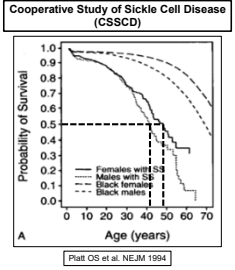
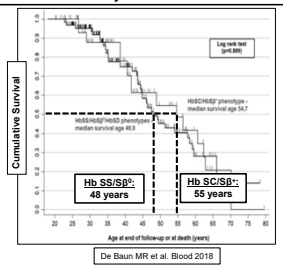
Median Survival

Cooperative Study of Sickle Cell Disease (CSSCD)



Platt OS et al. NEJM 1994

Contemporary estimates (high-income countries): 38 – 67 years in Hb SS[1-4]



De Baun MR et al. Blood 2018

1. Lanzkron S et al, Public Health Reports 2013; 2. Elmariah H et al, Am J Hematol 2014; 3. Gardner K et al, Blood 2016

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Predictors for Early Mortality

	CSSCD[1]	MSH[2]	USC[3]	NIH[4]	Brazil[5]	UK[6]	Meta-Analysis[7]
Frequent VOC		X		X	X	X	
Acute Chest Syndrome	X	X	X		X		
Stroke			X		X		
High TRJV				X			X
Kidney Disease	X		X	X		X	

VOC = vaso-occlusive crises
TRJV = tricuspid regurgitant jet velocity

1. Platt OS et al. NEJM 1994; 2. Powars DR et al. Medicine 2005; 3. Steinberg MH et al. JAMA 2003; 4. Darbari DS et al. PLOS One 2015; 5. Castro Lobo et al. Hematol Transfus Cell Ther 2018; 6. Gardner K et al. Blood 2016; 7. Madrid P et al. Haematologica 2017

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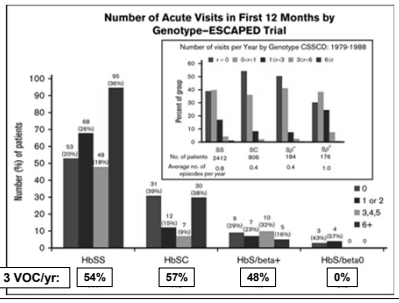
Predicting Risk for VOC



≥ 3 VOC/yr:	54%	57%	48%	0%

VOC Risk Factors:
- Lower Hb F%
- Nocturnal hypoxia
- Low vitamin D
- Higher hematocrit
- High WBC

Platt OS et al, NEJM 1991
Lanzkron S et al, Blood Adv 2018

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Exacerbation 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	1) Transfusion 2 – 14 days prior, positive direct antiglobulin test, alloantibodies, low Retic count 2) Elevated hemolysis markers (Indirect bilirubin, AST, Retic)	1) Steroids, IVIG 2) Exchange transfusion
Hepatic Sequestration	• Hepatomegaly • Elevated ALT	• Simple vs. Exchange transfusion
Splenic Sequestration	• Splenomegaly • Hb SC or Sβ⁺-thalassemia • Thrombocytopenia	• Simple vs. Exchange transfusion

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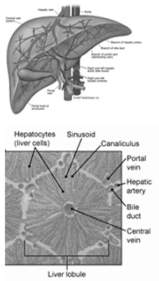
Sickle Hepatopathy

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



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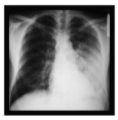
Acute Chest Syndrome



Definition: **Fever (T ≥ 38.5°C)** + **New Lung Infiltrate**
Hypoxia (↓O_2sat >2%)
Chest Pain
Cough, Wheezing, Tachypnea

Epidemiology:[1,2]
- 2nd most common cause of hospitalization
- Leading cause of death
- ½ of patients initially admitted for VOC
- Symptoms arise ~2.5 days after admission

1. Platt OS et al. NEJM 1994
2. Castro O et al. Blood 1994

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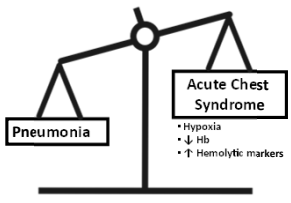
Acute Chest Syndrome

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



Vichinsky EP et al. NEJM 2000

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Acute Chest Syndrome

Prevention
Aggressive incentive spirometery • 10 breaths q2 hours
Avoid oversedation or excessive hydration
Immunizations: • S. pneumonia • H. influenzae • Influenza
SCD-specific Therapies: • Hydroxyurea • L-glutamine

Treatment	
Supplemental O_2	Minimum ≥ 92% Ideal ≥ 95%
Adequate pain control	
Empiric antibiotics	Cephalosporin + macrolide 4th generation quinolone
VTE Prophylaxis	Unfractionated Heparin LMWH
Bronchodilators	Wheezing or Asthma history
RBC transfusion	Simple – mild/moderate Exchange – severe

Glucocorticoids
- Not standard management
- May ↓ hospital duration in children
- Risk of rebound VOC

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Acute Chest Syndrome

Indications for RBC transfusion

Physical Exam:
- Unstable Vitals: Tachypnea, Tachycardia, Hypotension
- Worsening hypoxia (O_2sat < 92%)
- Altered mentation

Laboratory:
- PaO_2 < 70mmHgb
- Hb drop > 2 g/dL
- Evidence of other organ dysfunction (kidney, liver, CNS)

Radiographic
- Multilobe involvement

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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 → 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 2015

20

Acute Chest Syndrome: Summary

50% develop during hospitalization for VOC
- Vigilant for hypoxia, worsening hemolysis, drop in platelets

Treatment: Supplemental oxygen (goal O_2sat ≥ 95%)
Empiric antibiotics (cover atypical pathogens)

Common transfusion triggers:
Unstable vital signs
O_2sat < 92%
Drop in Hb > 2g/dL from baseline
Multi-lobe infiltrates

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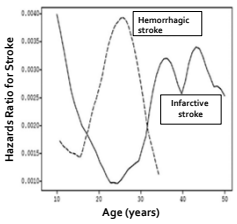
Stroke



Ohene-Frempong K et al, Blood 1998

Lifetime risk:[1] **30%** overt stroke
44% silent infarctions

Primary prevention: STOP Study[2] **(2 – 16 years)**

Transcranial Doppler Velocity > 200 cm/s → Chronic RBC transfusions reduce stroke risk by 92%

Secondary prevention:
- Untreated, 50% have repeat stroke within 2 years [3]
- Chronic RBC transfusions reduces risk

Goal: Hb 10g/dL & Hb S < 30%

1. WY Wong et al, Hematol Oncol Clin N Am 2005; 2. Adams RJ et al, Blood 2004; 3. Powars DR et al, Am J Med 1978

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Stroke: Acute Management

Initial Evaluation: CT of Head, MRI/MRV

Negative →
- Seizure
- Hemiplegic migraine
- TIA
- Post Reversible Encephalopathy Syndrome
- Central sinus venous thrombosis

Positive →
- **Exchange transfusion**
  - Hb 10g/dL
  - Hb S < 30%
- **Maintain O_2 sat ≥ 95%**
- **Adequate blood pressure control**

Role of thrombolytics (tPA)
- Not considered standard of care
- Risk of hemorrhagic stroke
- Retrospective claims database study[1]
  Intracranial hemorrhage (p = 0.45)
  - SCD: 4.9%
  - Non-SCD: 3.2%
- Used in a case-by-case situation

1. Adams RJ et al. Stroke 2017

23

Cardiovascular Disease

~26% of deaths in SCD adults attributed to cardiac disease[1]

Biomarkers	Mortality Risk Increase
TRJV > 2.5 m/s[2]	10-fold
Elevated NT-pro BNP[3]	5-fold
Low E/A ratio[4]	3.5-fold

TRJV = tricuspid regurgitant jet velocity

Pulmonary Hypertension[5]
- Present in ~10% of SCD patients
  - TRJV 2.5 – 2.9 m/s → 25 – 39% have mPAP ≥ 25 mmHg
  - TRJV ≥ 3.0 m/s → ~66% have mPAP ≥ 25 mmHg
- Associated with chronic intravascular hemolysis

Heart Failure w/ Preserved Ejection Fraction (HFpEF)/Diastolic Dysfunction[4,5]
- Present in ~20% of SCD patients
- Chronic effects of anemia, ischemia, reperfusion injury may result in diffuse myocardial fibrosis

1. Fitzhugh CD et al, Am J hematol 2009; 2. Gladwin MT et al, NEJM 2004; 3. Machado RF et al, JAMA 2006; 4. Sachdev V et al, J Am Coll Cardiol 2007; 5. Gladwin MET, ASH Education Program 2017

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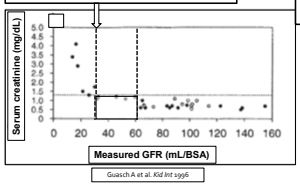
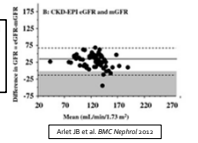
Kidney Function

Serum creatinine rose to standard definitions of abnormal after measured GFR < 30 mL/min/BSA



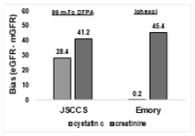
Guasch A et al. *Kid Int* 1996

Serum Creatinine-based CKD-EPI eGFR
- Lowest bias and greatest precision of serum creatinine-based formulas in SCD adults



Arlet JB et al. *BMC Nephrol* 2012

Cystatin C-based CKD-EPI eGFR



Asrani M et al. *Blood Cells Mol Dis* 2015
Yee MEM et al. *Am J Hematol* 2017

- Abnormal tubular secretion of creatinine into urine
- High cardiac output from anemia
- Serum creatinine levels affected by muscle mass

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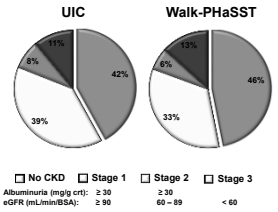
Kidney Disease

Serum creatinine ≥ 1.5 mg/dL → **Time to dialysis/kidney transplant or death averages 3.3 years**[1]



Albuminuria
- Early biomarker for kidney disease
- Associated with degree of hemolysis
- Predicts rapid eGFR decline[2]
- Measure annually
  - If abnormal, check q3 months
- BP < 130/80 mmHg
- Avoid chronic NSAIDs
- ASH Guidelines[3]: Consider ACE-i/ARB
  - Conditional with very low evidence

1. Powars DR et al. Ann Intern Med 1991; 2. Niss O et al. Blood Adv 2019; 3. Liem R et al. Blood Adv 2019

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Priapism

Chronic NO Depletion ⟹ **Down-regulation of Phosphodiesterase 5** ⊥

Stimulus ⟹ **cGMP Accumulation in Corpus Smooth Muscle** ⟹ **PRIAPISM**

Prevalence of 5 – 35% among adolescents and young men
- Mean age at first attack = 15 years
- Average duration 125 minutes

Management
- Self-help: moderate exercise, oral hydration, hot compresses
- Stuttering priapism (< 3 hours):
  - α-adrenergic agonists, hormonal analogues, smooth muscle regulators
- **Ischemic Priapism (≥ 4 hours)** – concern for compartment syndrome
  - Cavernous aspiration ± Irrigation

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Transfusion Therapy

NIH Expert Panel Report 2014

Evidence Based Recommendations

Indication	Strength
Symptomatic, severe Acute Chest Syndrome	Strong
Acute splenic sequestration + severe anemia	Strong
Stroke (Acute or Chronic)	Moderate

Additional Consensus-Based Recommendations

Indication	Method of Transfusion
Hepatic sequestration	Exchange or simple
Intrahepatic cholestasis	Exchange or simple
Multisystem organ failure	Exchange or simple
Aplastic crisis	Simple
Symptomatic anemia	Simple

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Pre-operative Transfusion

- 25 – 30% risk of SCD patients have post-operative complications
- RBC transfusions improve oxygen delivery and lower risk of vaso-occlusion

TAPS Study
- Randomized Hb SS/Sβ^0–thal patients
  - Transfusion (**goal Hb 10g/dL**) or no transfusion
  - Low-risk: Adenoidectomy, Herniorrhapy
  - Moderate-risk: Cholecystectomy, Joint replacement

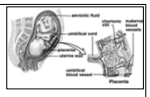
OR 3.8 for adverse events in no-transfusion arm

* Consider exchange transfusion for high risk surgeries (intracranial, cardiothoracic)

Howard J et al. Lancet 2013

29

Pregnancy



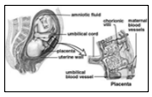
- Cardiopulmonary stressors (↑ cardiac output, plasma volume)
- Increased adhesion and coagulation proteins
- Reduction of endothelial NO synthase
- Maternal anemia

Maternal and Fetal Risks
• Miscarriage
• Placental abruption
• Pre-eclampsia
• Pre-term labor
• ↑ VOC, Acute chest syndrome
• Low birth weight

1. Koshy M et al. NEJM 1988
2. Malinowski AK et al. Blood 2015

30

Pregnancy



Randomized study in Hb SS[1]
- Emergent (Hb >6 g/dL) vs. Prophylactic transfusion (Hb ~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-eclampsia[3]

1. Koshy M et al. NEJM 1988; 2. Malinowski AK et al. Blood 2015; 3. NIH Guidelines 2004

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Transfusion Therapy

Red Blood Cell Alloimmunization
- Rh and K ~2/3 of RBC Antibodies
- Incidence[1]: **ABO/D** match → **18 – 76%**
  Full Rh/K match → **5 – 15%**
  Extended RBC match → **7%**

Disparate RBC antigens between donors (European ancestry) vs. SCD patients (African ancestry)[2]

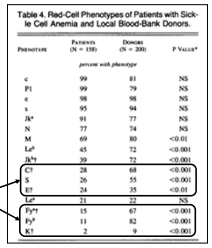

Table 4. Red-Cell Phenotypes of Patients with Sickle Cell Anemia and Local Blood-Bank Donors.

Phenotype	Patients (N = 150)	Donors (N = 200)	P Value*
	percent with phenotype		
c	99	81	NS
P_1	99	79	NS
e	98	98	NS
s	95	94	NS
Jk^a	91	77	NS
N	77	74	NS
M	69	80	<0.01
Le^b	45	72	<0.001
Jk^{a+}	39	72	<0.001
C^+	28	68	<0.001
S	26	55	<0.001
E^+	24	35	<0.01
Le^a	21	22	NS
Fy^{a+}	15	67	<0.001
Fy^b	11	82	<0.001
K^+	2	9	<0.001

1. Chou ST et al. ASH Education Book 2013; 2. Vichinsky E et al, NEJM 1990

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Transfusion Therapy

Delayed Hemolytic Transfusion Reaction
- **2 to 14 days** post-transfusion
- ~5% of transfused SCD patients experience DHTR[1]
- 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

1. Talano JA et al. Pediatrics 2003; de Montalembert M et al. Haematologica 2011

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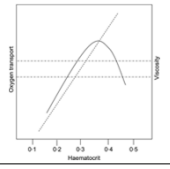
Transfusion Therapy

Iron Overload
- 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/g[1]
- Chelation: Subq/IV: Deferoxamine
  Oral: Deferasirox or Deferiprone

Hyperviscosity
- Avoid acute simple transfusion > 10 g/dL



1. Karam LB et al. Pediatr Blood Cancer 2008; 2. Jan K et al. Transfusion 1982

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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

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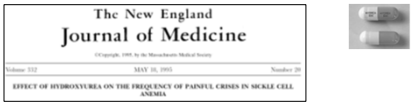
Hydroxyurea


The New England Journal of Medicine
EFFECT OF HYDROXYUREA ON THE FREQUENCY OF PAINFUL CRISES IN SICKLE CELL ANEMIA

Multicenter Study of Hydroxyurea (MSH) in Sickle Cell Anemia
- 299 Hb SS/Sβ^0-thal adults with ≥ 3 VOC/year
- Start 15 mg/kg, titrate up 5 mg/kg q12 weeks if no myelosuppression

Clinical Complication	HU	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	0.004

*** FDA-Approved for adults in 6/1998, children in 12/2017.**

Charache S et al. NEJM 1995

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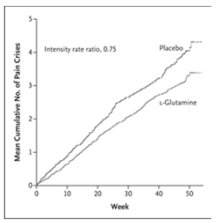
L-Glutamine

- Essential amino acid necessary for NAD production
- ↑ NAD redox ratio, ↓ Endothelial adhesion in sickle RBC
- 253 Hb SS/Sβ⁰-thal, Age ≥ 5 years, ≥ 2 VOC/year
- Randomized (2:1) to L-glutamine vs. placebo
  - 0.3 mg/kg given for 48 weeks
  - Stratified by HU use (~2/3 on HU in each group)



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

*** FDA-Approved in 7/2017**

Niihara Y et al. NEJM 2018

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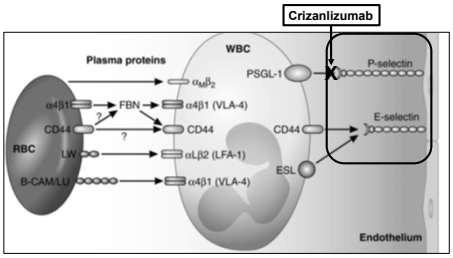
Selectin Mediates Vaso-Occlusion

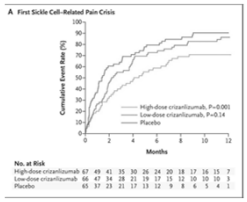


Telen MJ. Blood 2016

38

Crizanlizumab

- 198 SCD patients (all genotypes), Age 16 - 65 years, 2 -10 VOC/year
- Randomized (1:1:1) low (2.5mg/kg), high (5 mg/kg) dose or placebo
  - 52 week study
  - ~2/3 on HU in each group



Placebo: 3 VOC/yr
Crizanlizumab 2.5mg/kg: 2 VOC/yr
Crizanlizumab 5mg/kg: 1.6 VOC/yr

*** FDA-Approved in 11/2019**

Ataga KI et al. NEJM 2017

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FDA-Approved Therapies to Reduce VOC

Hydroxyurea[1]

Outcome	HU	Placebo	p-value
VOC	1.0 events	2.4 events	< 0.001
Acute Chest Syndrome	25%	51%	< 0.001

L-glutamine[2]

Outcome	L-glutamine	Placebo	p-value
VOC	3.0 events	4.0 events	0.005
Acute chest syndrome	8.6%	23.1%	0.003

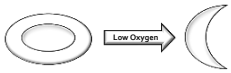
Crizanlizumab[3]

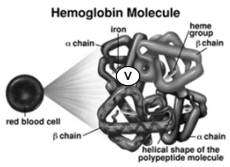
Outcome	Crizanlizumab	Placebo	p-value
VOC	1.6 events	3 events	0.005
Hospital days	4.0 days	6.9 days	0.02

1. Charache S et al, NEJM 1995; 2. Niihara et al., NEJM 2018; 3. Ataga KI et al, NEJM 2017

40

Deoxygenation Leads to Hb S Polymerization



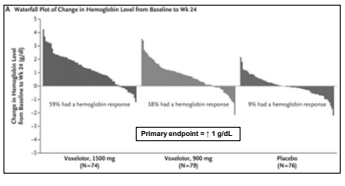


Voxelotor:
Small molecule that binds Hb stabilizing it in the oxygenated state

41

VOXELOTOR

- 274 SCD patients (all genotypes), Age 12 - 65 years
- Randomized (1:1:1) low (900mg), high (1500mg) dose or placebo
  - 1º endpoint assessed week 24
  - ~2/3 on HU in each group



Placebo: Δ -0.1 g/dL
Voxelotor 900mg: Δ +0.6 g/dL
Voxelotor 1500mg: Δ +1.1 g/dL

*** FDA-Approved in 11/2019**

Vichinsky et al. NEJM 2019

42

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

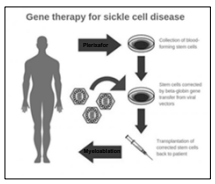
Haploidentical HSCT:[2]
- ~15% of eligible patients have HLA-matched sibling
- **Post-Cy:** **57 – 100% stable engraftment**
  0 – 25% aGVHD, 0 – 13% cGVHD

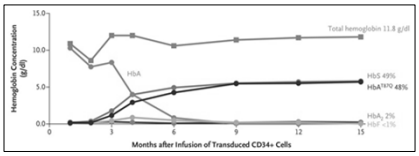
* Most common indications include stroke, recurrent VOC or acute chest syndrome despite HU therapy

1. Gluckman E et al, Blood 2017; 2. Saraf SL et al. J Clin Med 2019

43

Gene Therapy





6 gene therapy studies currently enrolling SCD patients on clinicaltrials.gov
- 4 targeting γ-globin/Hb F
- 2 targeting β-globin/Hb A

Ribeil JA et al. NEJM 2017

44

Therapy Summary

Hydroxyurea, L-Glutamine, Crizanlizumab → Reduce VOC
- L-glutamine & crizanlizumab improve VOC even in those on hydroxyrea

Voxelotor → 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

45

THANK YOU & QUESTIONS

46

46

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 2nd 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 O_2
2. Continued PCA for pain relief
3. Dexamethasone 4mg IV q12 hours
4. Red blood cell transfusion
5. Initiating antibiotics

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Question 1: Acute chest syndrome

1. Supplemental O_2: Maintain oxygen at least ≥ 92% (> 95% preferred)
2. Adequate pain management is essential
3. 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
5. Initiating antibiotics: Infection is most common identifiable cause of acute chest syndrome. Ensure that atypical bacteria are covered.

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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:

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

49

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).

50

Question 3

A 25 year old man with Hb Sβ^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

51

Question 3

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

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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?:

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

53

Question 4: FDA-approved therapies for SCD

Hydroxyurea

Outcome	HU	Placebo	p-value
VOC	1.0 events	2.4 events	< 0.001
Acute Chest Syndrome	25%	51%	< 0.001

L-glutamine

Outcome	L-glutamine	Placebo	p-value
VOC	3.0 events	4.0 events	0.005
Acute chest syndrome	8.6%	23.1%	0.003

Crizanlizumab

Outcome	Crizanlizumab	Placebo	p-value
VOC	1.6 events	3 events	0.005
Hospital days	4.0 days	6.9 days	0.02

Voxelotor

Outcome	Voxelotor	Placebo	p-value
Increase Hb > 1g/dL	51%	7%	< 0.001

54

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
3. Bicalutamide
4. IV hydration and clinical surveillance
5. Cavernosal artery embolization

55

Question 5: Ischemic Priapism

1. 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% of cases.
2. Exchange transfusion –There are case reports of ASPEN (**A**ssociation of **S**CD, **P**riapism, **E**xchange transfusion and **N**eurologic 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 to seizure or obtundation.
3. Bicalutamide – Preventative measure for future priapism episodes.
4. IV hydration and clinical surveillance – Not recommended for ischemic priapism.
5. Cavernosal artery embolization – Can be used for stuttering priapism but 50% risk of erectile dysfunction.

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10

Mechanisms for Clotting

Nigel S. Key, MD

August 14, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

10 - Mechanisms for Clotting

Nigel S. Key, MB, ChB, FRCP

1

Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

- None

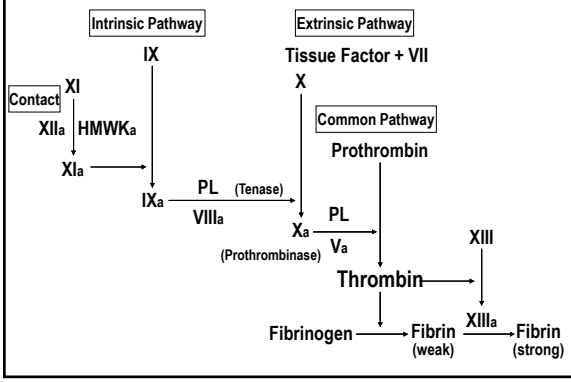
2

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

3

Coagulation Cascade 1960's-1980's



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")

5

Primary Hemostasis

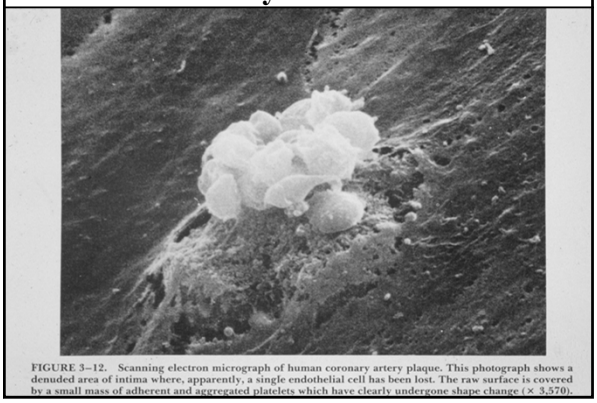
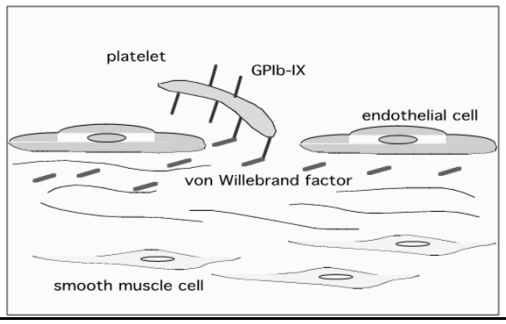


FIGURE 3–12. Scanning electron micrograph of human coronary artery plaque. This photograph shows a denuded area of intima where, apparently, a single endothelial cell has been lost. The raw surface is covered by a small mass of adherent and aggregated platelets which have clearly undergone shape change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

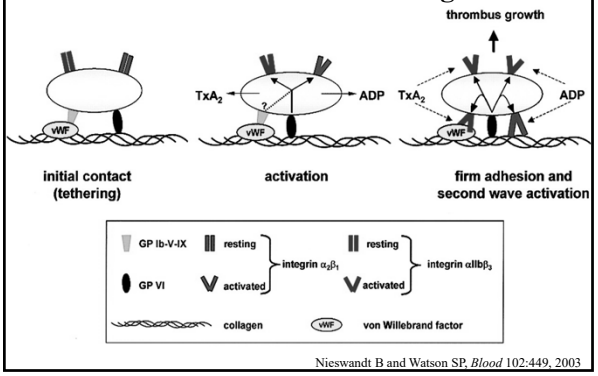
6

Sub-endothelial von Willebrand Factor Provides Initial Tethering of Platelets under Shear



7

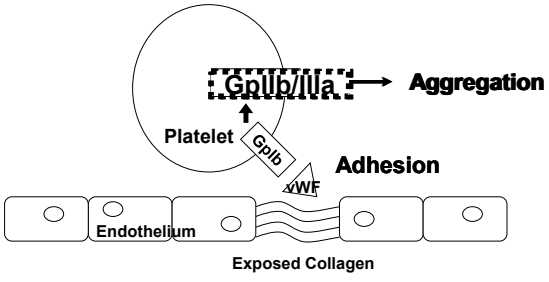
And….Other Mechanisms Consolidate Platelet Adhesion to Collagen



Nieswandt B and Watson SP, *Blood* 102:449, 2003

8

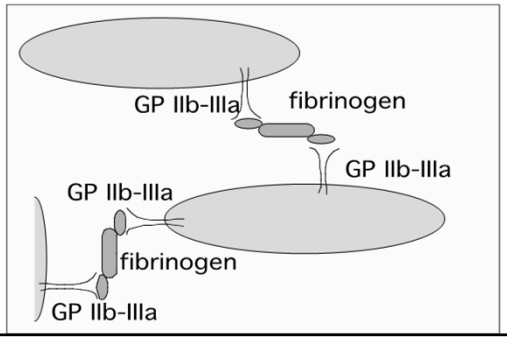
Primary Hemostasis: Transition From Platelet Adhesion to Activation



9

Platelet Plug Formation:

Platelet Aggregation is Mediated by GPIIb-IIIa in its Activated Conformation



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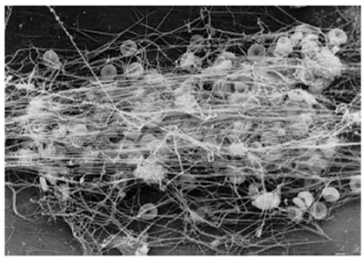
Secondary Hemostasis

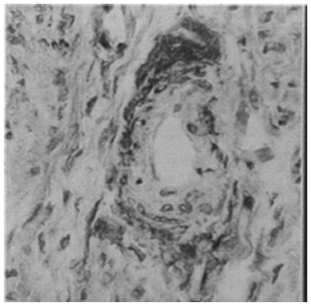


11

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

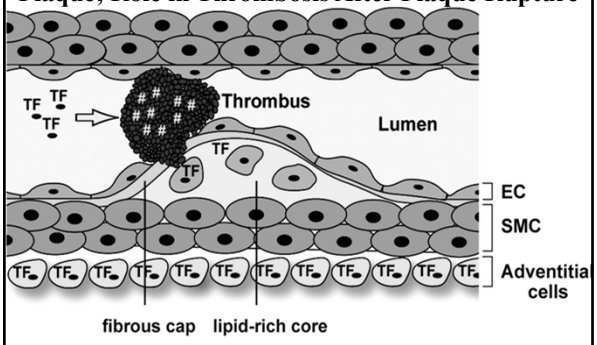
12



Tissue factor (rusty brown stain) in the adventitia; blood only contacts it in the event of injury.

13

Aberrant TF Expression by Foam Cells and Macrophages in the Intima of the Atherosclerotic Plaque; Role in Thrombosis After Plaque Rupture



Mackman, N. *Arterioscler Thromb Vasc Biol* 2004;24:1015-1022

14

Co-factor Functions of TF

TF + VIIa <=> TF.VIIa

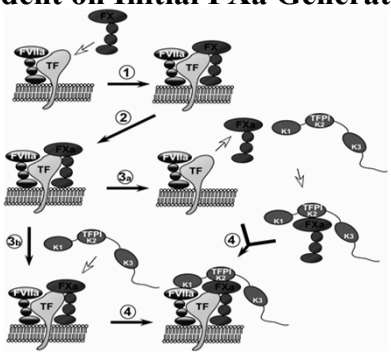
↓

X → Xa

IX → IXa

15

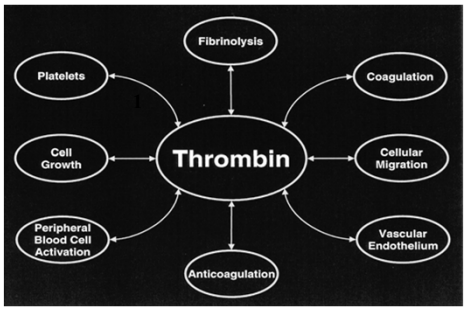
Inhibition of Coagulation by TFPI is Dependent on Initial FXa Generation



Crawley, J. et al. *Arterioscler Thromb Vasc Biol* 2008;28:233-242

16

Coagulation is All About Thrombin Generation…

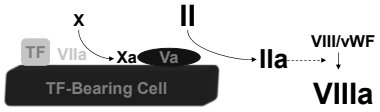


A schematic representation of the multiple functions of thrombin as a biological mediator. Note that many functions are antagonistic to one another, *eg*, both a procoagulant and anticoagulant.

Mann KG. Chest 2003;124:1S

17

Cell-Based Model of Hemostasis



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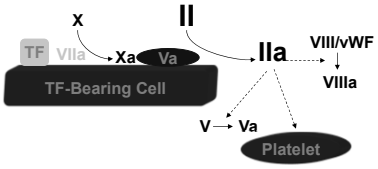
Cell-Based Model of Hemostasis



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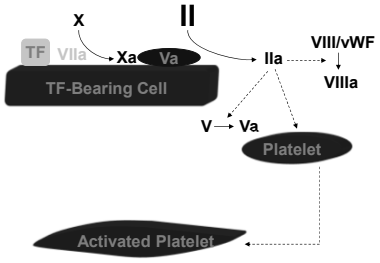
Cell-Based Model of Hemostasis



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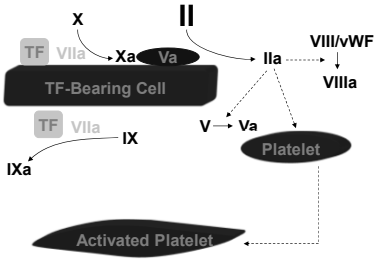
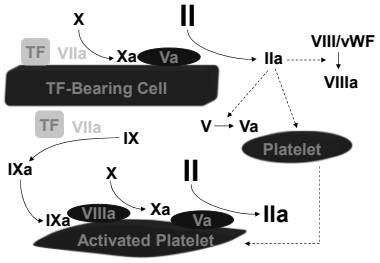
Cell-Based Model of Hemostasis



21

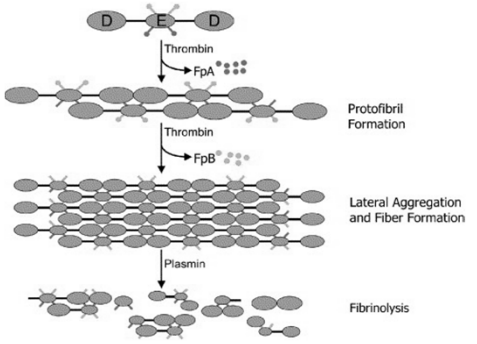
Cell-Based Model of Hemostasis



Hoffman et al. *Blood Coagul Fibrinolysis* 1998;9(suppl 1):S61.

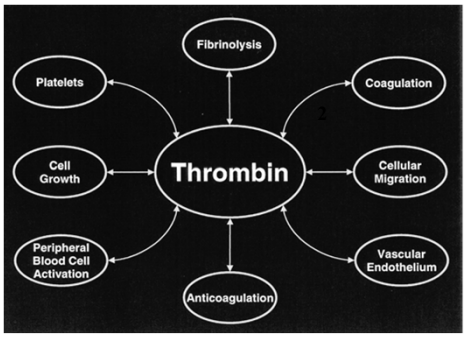
22

Conversion of Fibrinogen to Fibrin By Thrombin



Wolberg, AS. *Blood Rev* 2007;21:131

23

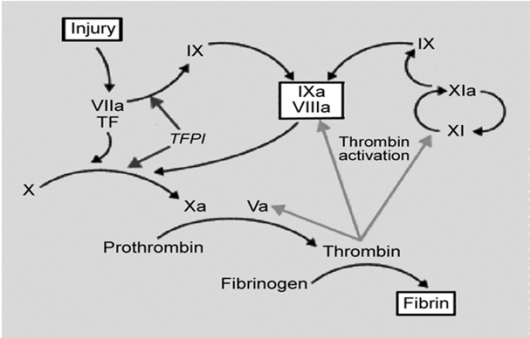


A schematic representation of the multiple functions of thrombin as a biological mediator. Note that many functions are antagonistic to one another, *eg*, both a procoagulant and anticoagulant.

Mann KG. Chest 2003;124:1S

24

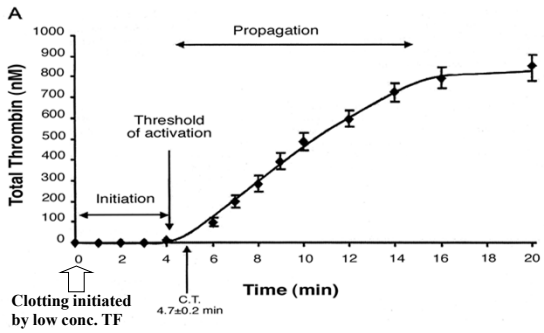
Revised Coagulation Schematic: 1990's and Beyond



Bolton-Maggs and Pasi, *Lancet* 361:1801, 2003

25

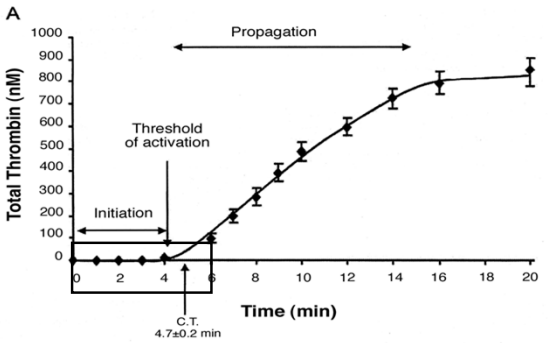
The Majority of Thrombin is Generated *After* Whole Blood Clots



Brummel KE. Blood 2002;100:148

26

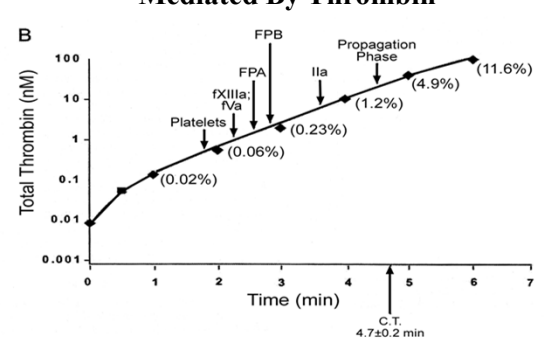
Focusing on the Early Events in Blood Clotting…



Brummel KE. Blood 2002;100:148

27

Early Coagulation Activation Events Mediated By Thrombin



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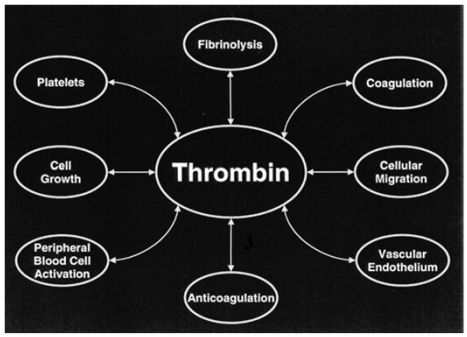

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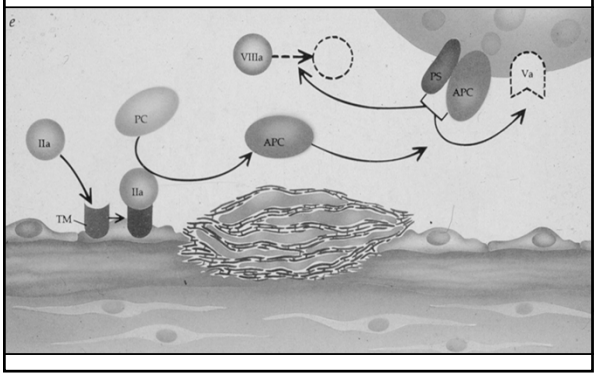
29

The Protein C/Protein S System



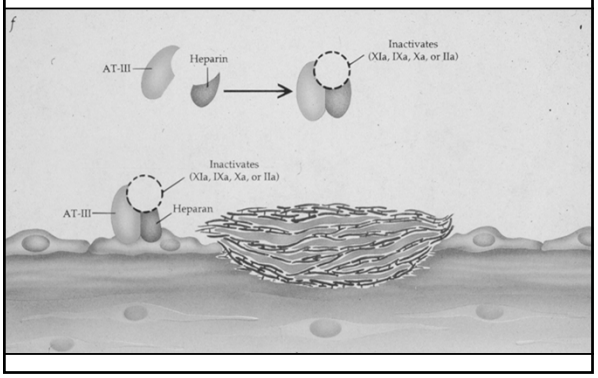
30

Anticoagulant Pathways

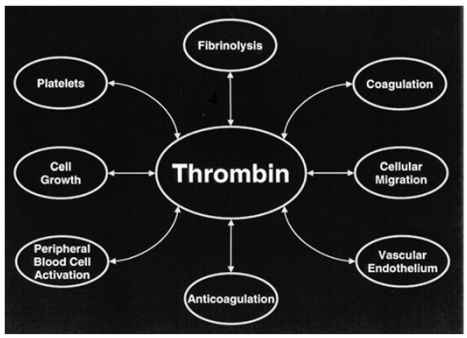
- Tissue factor pathway inhibitor
- Protein C/Protein S
- Antithrombin (III)

31

The Antithrombin (III) System



32



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Mann KG. Chest 2003;124:1S

33

The Fibrinolytic System

- Goal: generate plasmin
- Function: 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)

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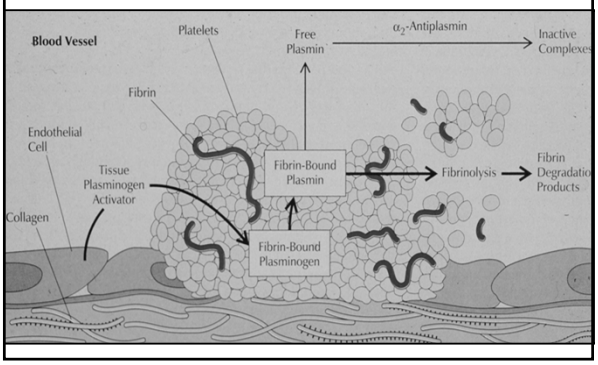
The Fibrinolytic System



35

Principal Inhibitors of Fibrinolysis

1. Plasminogen activator inhibitor-1 (PAI-1) ….inhibits t-Pa and urokinase
2. Alpha$_2$-antiplasmin……inhibits plasmin
3. Thrombin Activatable Fibrinolysis Inhibitor (TAFI)…..inhibits binding of plasminogen and tPa

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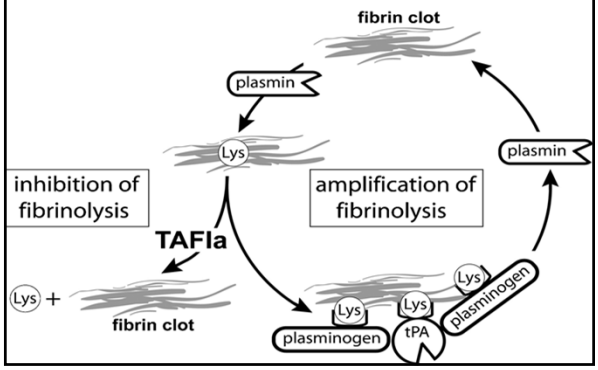
Activated TAFI (TAFIa) Inhibits Fibrinolysis by Cleaving Essential Lysine Residues on Fibrin



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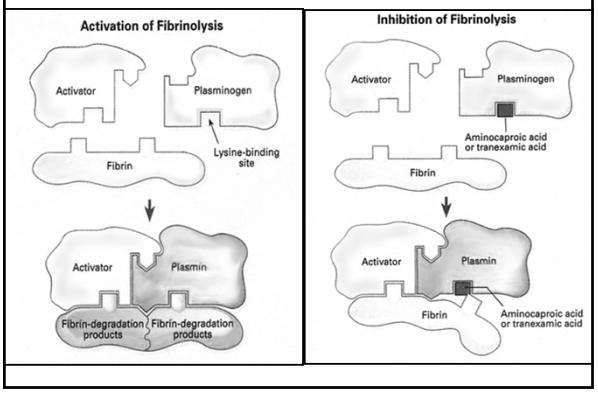
Anti-fibrinolytic Lysine Analogs



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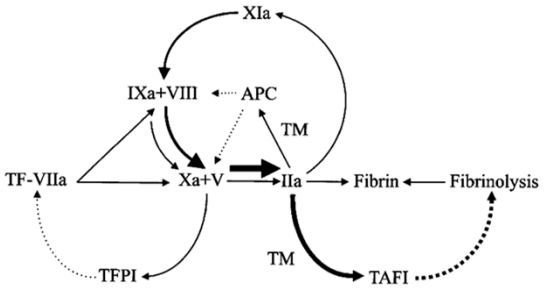
High Concentrations of Thrombin are Needed to Activate Thrombin-Activatable Fibrinolysis Inhibitor (TAFI)



Bouma et al. *Thromb Res* 101:329, 2001

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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

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Prothrombin Time (PT)

- "Thromboplastin" (TF + PL + Ca^{2+}) 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}*

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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)

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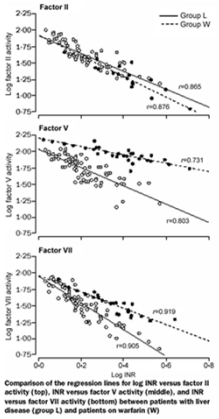
Relationship of INR to Plasma Levels of Factors II,V, and VII: Comparison of Liver Disease and Warfarin Therapy



Comparison of the regression lines for log INR versus factor II activity (top), INR versus factor V activity (middle), and INR versus factor VII activity (bottom) between patients with liver disease (group L) and patients on warfarin (W)

Deitcher SR, *Lancet* 359:47, 2002

44

Activated Partial Thromboplastin Time (APTT)

- Add to citrated plasma
  - Activator (celite, kaolin)
  - "Partial thromboplastin" (= phospholipid)
  - Ca^{2+}
- Variable sensitivity to lupus inhibitors, heparin
- No standardization between labs

45

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)

46

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)

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Anti-Phospholipid Antibody Detection

Lupus Inhibitor

- prolongs phospholipid-dependent 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)
- may be IgG or IgM

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Thank you for Your Attention

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11

The Hemophilias

Nigel S. Key, MD

August 14, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

11 - The Hemophilias

Nigel S. Key, MB, ChB, FRCP

1

Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

- None

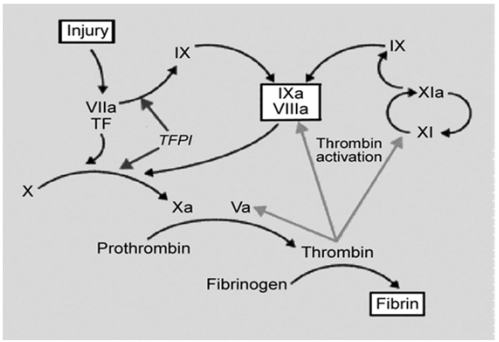
2

Prevalence of Inherited Factor Deficiency States

	Prevalence	Inheritance	Chromosome
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)

3

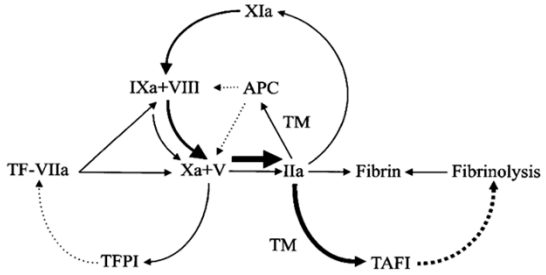
Hemophilia: A Defect In The Thrombin Propagation Phase of Coagulation



Bolton-Maggs and Pasi, *Lancet* 361:1801, 2003

4

FXI is Activated by Thrombin;
FXIa Provides "Over-drive" Thrombin Production That Activates TAFI



Bouma et al. *Thromb Res* 101:329, 2001

5

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.

6

X-Linked Hemophilias

- Hemophilia A and B are essentially indistinguishable on clinical grounds

7

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*)

8

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

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Hemophilia

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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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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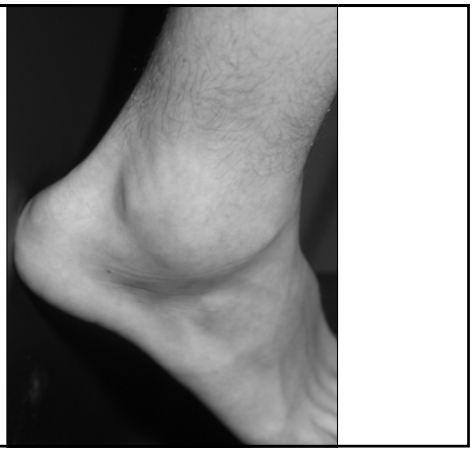

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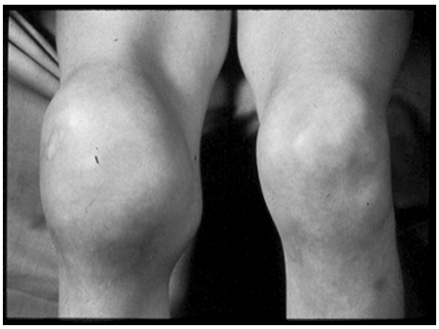

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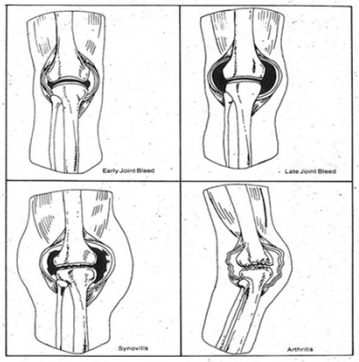
Progression of Hemophilic Arthropathy



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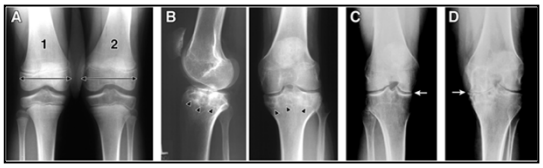
Progression of Hemophilic Arthropathy



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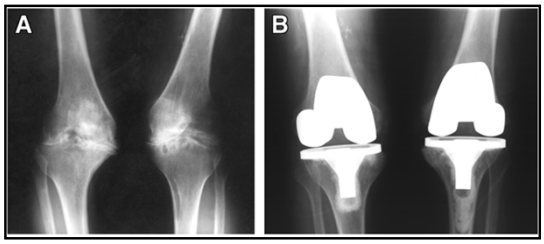
End Stage Hemophilic Arthropathy



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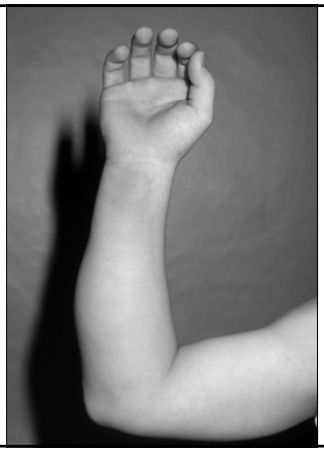
Hemophilia: Clinical Features (2)

- *Muscle Bleeds*

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

17



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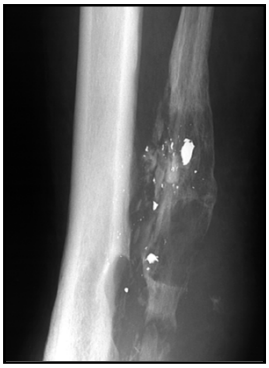
HEMOPHILIC PSEUDOTUMOR



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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

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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 (Amicar™) or tranexamic acid (Lysteda™)
  - 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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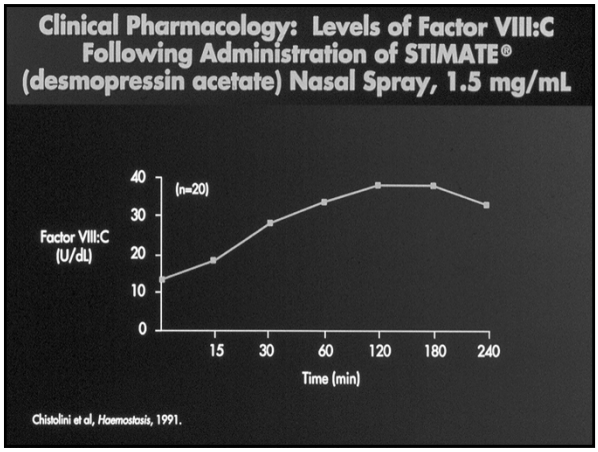

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Treatment of Hemophilia

<u>Spontaneous bleeds</u>:

- 1-2 doses of FVIII or FIX (≈ 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

<u>Considerations for surgery</u>:

- 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)
  → FVIII: 25-40 u/kg t.i.w.
  → FIX: 25-40 u/kg b.i.w.
- High expense, need for reliable i.v. access
- Prevents progressive arthropathy

30

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

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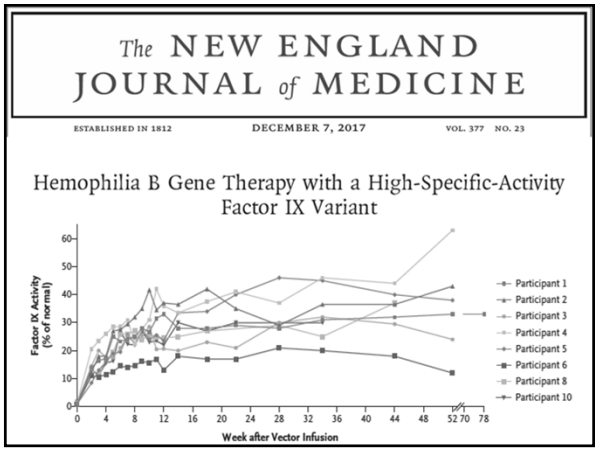

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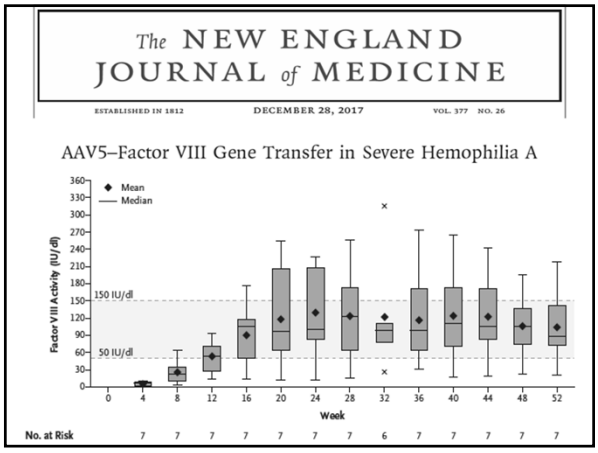

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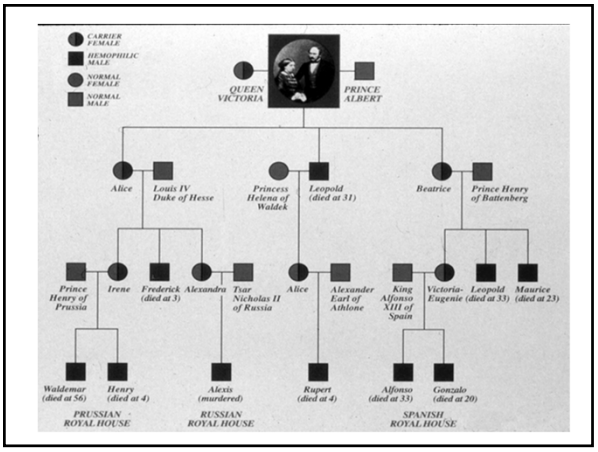
Hemophilia Carriers

"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"

Babylonian Talmud, 200 A.D.

34



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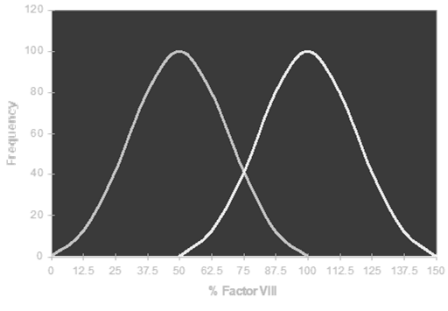
FVIII Levels in Women:
Hemophilia Carriers *vs.* Normals



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Hemophilia Carriers May be Symptomatic

Table 4. Risk of bleeding after medical interventions

	Carriers, event/total (%)	Noncarriers, event/total (%)	RR (CI)
Tooth extraction			
Prolonged bleeding; more than 3 h	61/228 (27)	26/219 (12)	2.3 (1.5-3.4)
Treatment after intervention	24/228 (11)	1/219 (0.5)	23.1 (3.1-169)
Tonsillectomy or adenotomy			
Prolonged bleeding; more than 3 h	29/123 (24)	16/122 (13)	1.8 (1.0-3.1)
Treatment after intervention	10/123 (8)	1/122 (0.8)	9.9 (1.3-76.3)
Operations			
Prolonged bleeding; more than 3 h	46/163 (28)	16/146 (11)	2.6 (1.5-4.3)
Treatment; ever	16/174 (9)	6/149 (4)	2.3 (0.9-5.7)
Blood transfusion	29/174 (17)	18/149 (12)	1.4 (0.8-2.4)

Participants who had been treated prior to the clinical intervention with clotting factor preparations, tranexamic acid, or desmopressin were excluded from the analysis.

Plug I, et al. *Blood* 108:52, 2006

37

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):*

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Hemophilia Carriers and Pregnancy

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

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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: 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
- → HIV: prior to 1983

Inhibitors….

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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

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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

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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)*

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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

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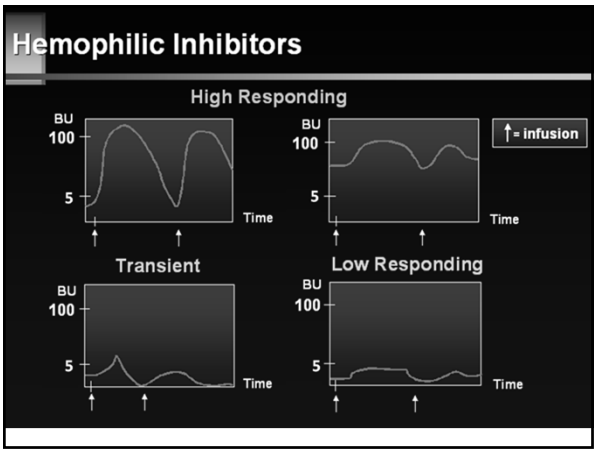

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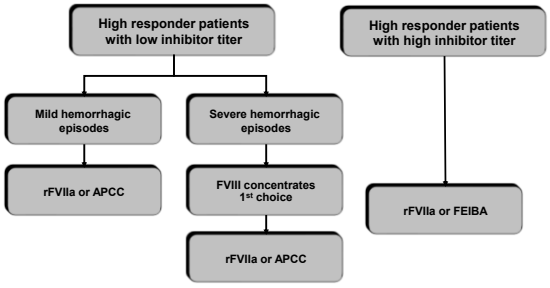
Treatment of Bleeding in FVIII Inhibitor Patients



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

47

Bypassing Agents

- FEIBA™
  - 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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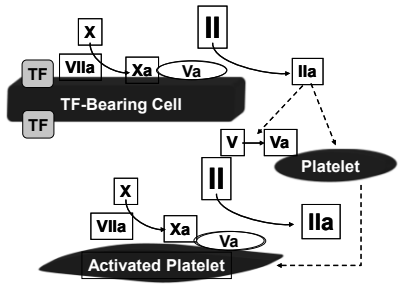
FVIIa in Hemophilia: Promotion of TF-Independent Activation of FX on Platelets



Hoffman M et al. *Blood Coagul Fibrinolysis.* 1998;9(suppl 1):S61.

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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 ~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 (≈ 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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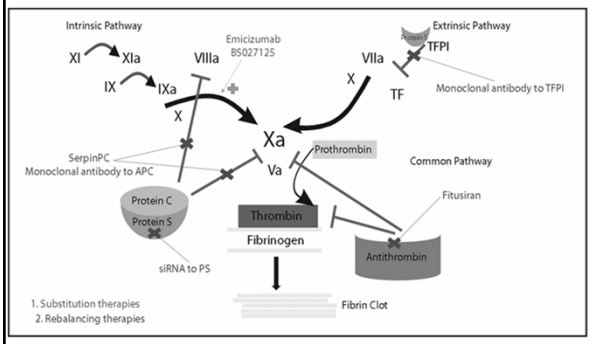
Non Factor Replacement Therapies



Weyand AC & Pipe, SW *Blood*, 2019:133(5);389

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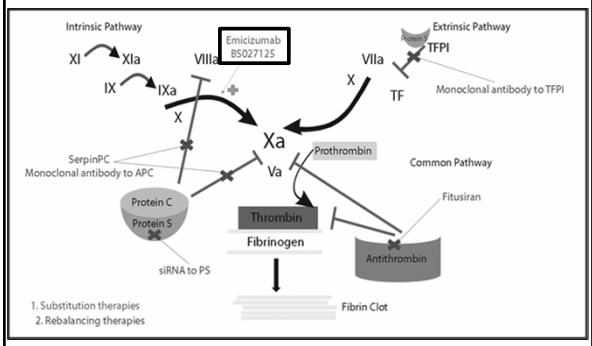
Non Factor Replacement Therapies



Weyand AC & Pipe, SW *Blood*, 2019:133(5);389

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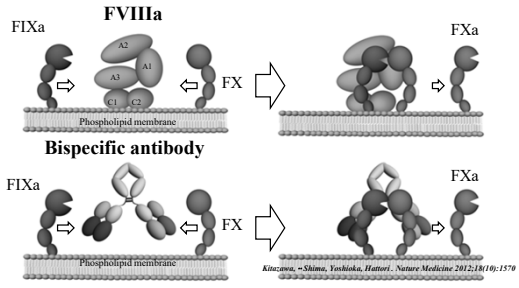
FVIIIa Mimetic Bi-specific Antibody (Emicizumab: Hemlibra™)



- **Emicizumab supports the interaction between FIXa and FX, thereby promoting FX activation by FIXa in the absence of FVIII.**

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The NEW ENGLAND JOURNAL of MEDICINE
ESTABLISHED IN 1812 AUGUST 31, 2017 VOL. 377 NO. 9

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).

55

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

57

Thank you for your attention!

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12

Von Willebrand's Disease

Alice Ma, MD

August 14, 2020

Acquired Disorders of Coagulation

Alice Ma, MD

August 14, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

13 – Acquired Disorders of Coagulation

Alice Ma, MD

1

DISCLOSURES

Off Label Usage
- Rituximab
- Profilnine
- 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

3

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

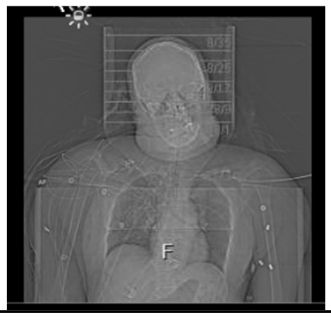
4

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.

5

Case 1



The pharynx and upper trachea are near completely opacified with blood.

Blood throughout the paranasal sinuses.

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

6

Acquired Disorders of Coagulation

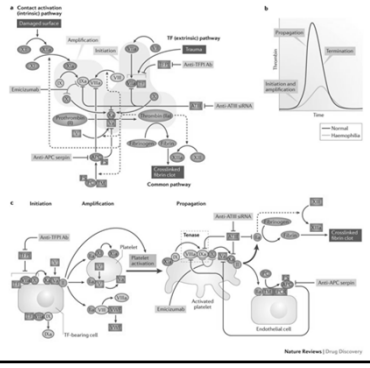
Alice Ma, MD

Case 1-Labs

- CBC Hgb 8 (baseline 10.5), nl WBC, plts 350.
- PT/INR normal, aPTT 90 sec (25-35),

7

Obligatory Confusing intimidating Coagulation Cascade



8

The PTT Pathway **The PT Pathway**

Rather than thinking about the intrinsic and the extrinsic pathways,
think about the PTT and the PT pathways

9

The PTT Pathway **The PT Pathway**

X

The PT and the PTT pathways meet at factor X, because "X" marks the spot

10

The PTT Pathway **The PT Pathway**

V
X

Factor V is a cofactor for factor X, and you can remember this because V fits into the notch of the X

11

The PTT Pathway **The PT Pathway**

V
X

Prothrombin (II) → **Thrombin**

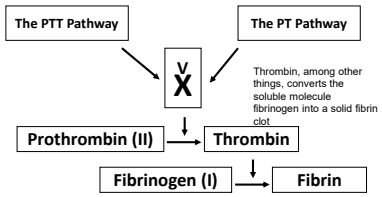
Factor Xa converts prothrombin (Factor II) into thrombin, the most important enzyme on the planet

12

Acquired Disorders of Coagulation

Alice Ma, MD

This is the Common Pathway



13

The Common Pathway = Small Bills

V + X

II = prothrombin

I = fibrinogen

You can remember the factors in the common pathway by remembering the bills in your wallet smaller than a $20. Don't forget the $2 bill !!

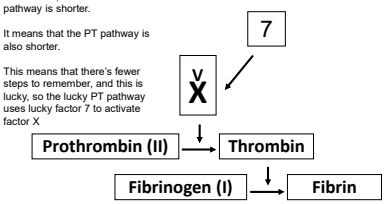
14

The PT Pathway

PT has one less letter than PTT, and PT values are shorter than PTT values, because the pathway is shorter.

It means that the PT pathway is also shorter.

This means that there's fewer steps to remember, and this is lucky, so the lucky PT pathway uses lucky factor 7 to activate factor X



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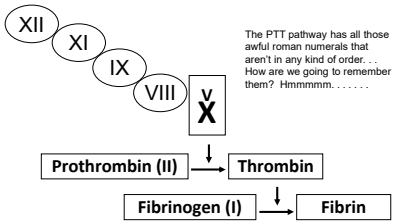
The PTT Pathway



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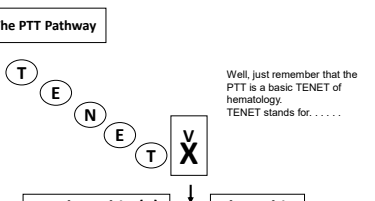
The PTT Pathway



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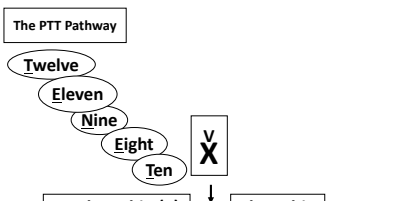
The PTT Pathway



18

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

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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

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

21

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

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

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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

24

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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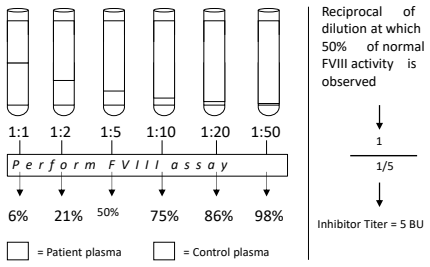
The Bethesda Assay

27



Reciprocal of dilution at which 50% of normal FVIII activity is observed

1

1/5

Inhibitor Titer = 5 BU

27

Laboratory Diagnosis of Long aPTT

	Acquired Inhibitor	Lupus Inhibitor	Heparin	FVIII Deficiency	Inhibitor in Congenital Hemophilia
aPTT	Prolonged	Prolonged	Prolonged	Prolonged	Prolonged

28

Laboratory Diagnosis of Long aPTT

	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

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Laboratory Diagnosis of Long aPTT

	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

30

Laboratory Diagnosis of Long aPTT

	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

31

Laboratory Diagnosis of Long aPTT

	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

32

Laboratory Diagnosis of Long aPTT

	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
Bethesda titer	Positive	0	0	0	Positive

33

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

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Increasing FVIII Levels

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

35

Bypassing agents

aPCC	rFVIIa
Plasma derived (virally inactivated)	Recombinant
Contains factors II, IX, X and activated VII	Contains activated factor VII
Dose 50-100 U/kg every 8-12 hours Maximum dose 200 U/kg/day	Dose 90 µg/kg (range 40-180) every 2-6 hours

Both given IV
Both effective at controlling bleeding
No comparison efficacy studies in AH
Difficult to monitor

36

Inhibitor Eradication in AH

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

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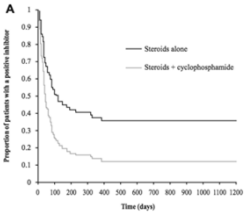
Immunosuppression: Steroid ± Cyclophosphamide

- EACH2 registry
- Prednisone + oral cyclophosphamide more likely to achieve stable CR than prednisone alone



Collins P, et al. *Blood*. 2012;120(1):47-55.

38

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

39

The Common Pathway = Small Bills

V + X

II = prothrombin

I = fibrinogen

You can remember the factors in the common pathway by remembering the bills in your wallet smaller than a $20. Don't forget the $2 bill !!

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.

41

Case 2

- 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

42

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

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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.

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!

45

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

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

47

Case 4

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

48

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

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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

50

Summary

- Acquired FVIII inhibitor
  - New onset of bleeding, no prior personal or family history of bleeding
  - Isolated prolonged aPTT
  - No correction with 1:1 mix
  - Fully diagnose with FVIII activity and Bethesda assay
  - Treat bleeding with bypassing agents or recombinant procine FVIII
    - rVIIa
    - FEIBA
    - Obizur (rpFVIII)
  - Eradicate the inhibitor
    - Prednisone and cyclophosphamide
    - Rituximab

51

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

52

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

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14

Hypercoagulable States

Kenneth A. Bauer, MD

August 14, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

14 – Hypercoagulable States

Kenneth A. Bauer, MD

1

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

3

Risk Factors for VTE

Transient/Provoked	Persistent	Idiopathic/ Unprovoked
▪ 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)	▪ Obesity ▪ Chronic Medical Illnesses ○ Cancer and its therapy ○ Inflammatory bowel disease ○ Nephrotic syndrome ○ Myeloproliferative neoplasms/PNH ▪ Paralysis	

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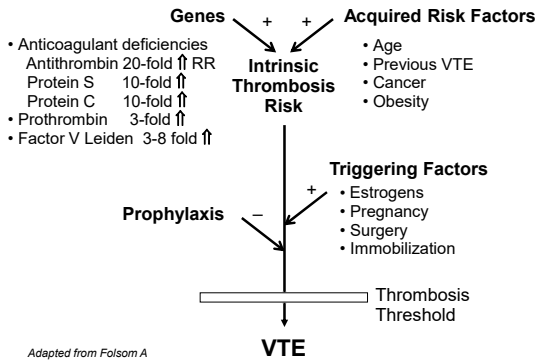
4

VTE Risk Factor Model



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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**

7

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)

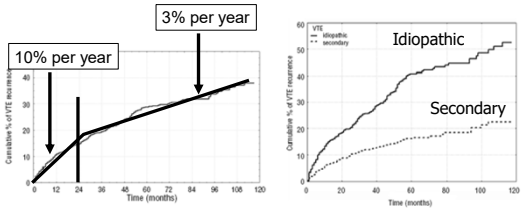
Highly "thrombosis-prone" patients (recurrent DVT or PE on therapeutic anticoagulation)

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

8

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

11

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

12

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)**
  - **β_2-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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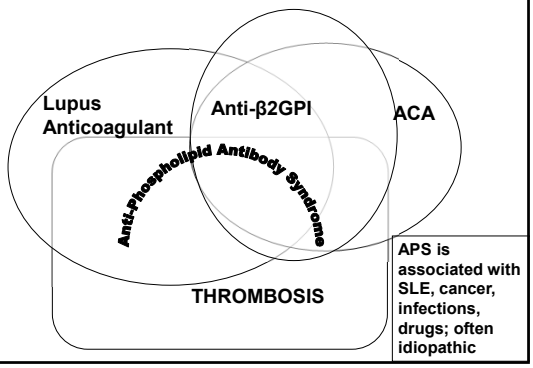
13

Antiphospholipid Antibody Syndrome



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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.**

15

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 Triple Positive 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.

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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 anti-beta2glycoprotein I IgM antibodies in the antiphospholipid antibody syndrome. J Thromb Haemost. 2019

18

Venous Thromboembolism in Cancer

Common (~20% of all patients with VTE)

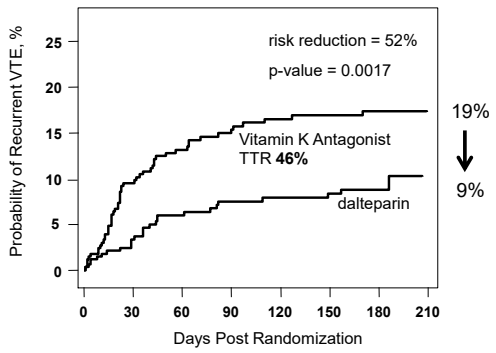
Increased risk of recurrent VTE on VKA
- Can occur with a therapeutic INR ("warfarin failure")

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).

19

CLOT in Cancer Trial: Recurrent VTE

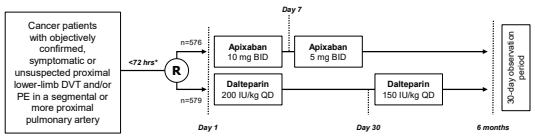
Lee A. New Eng J Med 2003



20

CARAVAGGIO: Study Design

- 119 sites in Europe, Israel, US; investigator-initiated, noninferiority trial with PROBE design
- Largest DOAC trial in patients with cancer-associated VTE to date (1155 patients in mITT analysis)



Randomization stratified by:
- Symptomatic or incidental† VTE
- Active or history† of cancer

†Each capped at 20%

*Max time allowed between diagnosis and randomization

Agnelli G et al. *N Engl J Med*. 2020;382:1599-1607.

21

CARAVAGGIO: Patient Demographics and Clinical Characteristics at Baseline

Characteristic	Apixaban (N = 576)	Dalteparin (N = 579)
Age – yr	67.2 ± 11.3	67.2 ± 10.9
Male sex – no. (%)	292 (50.7)	276 (47.7)
Weight – kg	75.7 ± 16.1	76.1 ± 16.7
Platelet count < 100,000/mm³ – no. (%)	21 (3.6)	22 (3.8)
Creatinine clearance ≤ 50 mL/min – no. (%)	51 (8.9)	61 (10.5)
Qualifying diagnosis of VTE - no. (%)		
PE ± DVT	304 (52.8)	334 (57.7)
DVT only	272 (47.2)	245 (42.3)
Symptomatic DVT or PE	460 (79.9)	465 (80.3)
Incidental DVT or PE	116 (20.1)	114 (19.7)
History of VTE before index event – no. (%)	45 (7.8)	61 (10.5)
Type of cancer – no. (%)		
Active	559 (97.0)	565 (97.6)
Recurrent locally advanced or metastatic	389 (67.5)	396 (68.4)
Cancer treatment – no. (%)		
At enrollment	350 (60.8)	367 (63.4)
Within previous 6 months	143 (24.8)	129 (22.3)
During trial period	344 (59.7)	346 (59.8)
ECOG performance-status score – no. (%)		
0	186 (32.3)	170 (29.4)
1	281 (48.8)	277 (47.8)
2	109 (18.9)	132 (22.8)

Plus–minus values are means ±SD.
Agnelli G et al. *N Engl J Med*. 2020;382:1599-1607.

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CARAVAGGIO: Cancer Type at Baseline

	Apixaban (N = 576)	Dalteparin (N = 579)
Solid 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)
Hematological malignancy – no (%)	33 (5.7)	52 (9.0)

~32% of patients had GI cancer at baseline.

Agnelli G et al. *N Engl J Med*. 2020; 382:1599-1607.

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CARAVAGGIO: Primary Efficacy Outcome

	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)	

Agnelli G et al. *N Engl J Med*. 2020;382:1599-1607.

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CARAVAGGIO: Primary Safety Outcome

	Apixaban N = 576	Dalteparin N = 579	Hazard Ratio (95% CI)	*P* Value
Major bleeding – no. (%)	22 (3.8)	23 (4.0)	0.82 (0.40-1.69)	0.60
Major GI bleeding	11 (1.9)	10 (1.7)	1.05 (0.44-2.50)	
Major non-GI bleeding	11 (1.9)	13 (2.2)	0.68 (0.21-2.20)	

Agnelli G et al. *N Engl J Med.* 2020;382:1599-1607.

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CARAVAGGIO: Secondary Outcomes

	Apixaban N = 576	Dalteparin N = 579	Hazard Ratio (95% CI)	*P* Value
Secondary outcomes – no. (%)				
Recurrent venous thromboembolism or major bleeding	51 (8.9)	66 (11.4)	0.70 (0.45-1.07)	
CRNM bleeding	52 (9.0)	35 (6.0)	1.42 (0.88-2.30)	
Major + CRNM bleeding*	70 (12.2)	56 (9.7)	1.16 (0.77-1.75)	
Death from any cause†	135 (23.4)	153 (26.4)	0.82 (0.62-1.09)	
Event-free survival‡	422 (73.3)	397 (68.6)	1.36 (1.05-1.76)	

*In patients who had more than one event, only the first event was counted.
†Death was assessed up to 210 days after randomization.
‡Event-free survival was defined as the absence of recurrent VTE, major bleeding, and death.

Agnelli G et al. *N Engl J Med.* 2020;382:1599-1607.

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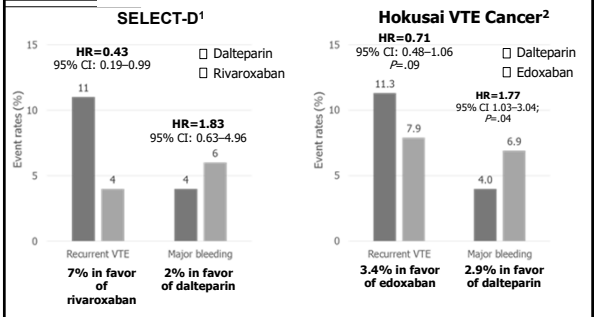
DOACs for Treatment of CAT: RCTs



1. Young A, et al. J Clin Oncol. 2018;36:2017-2023
2. Raskob GE, et al. N Engl J Med. 2018;378:615-624

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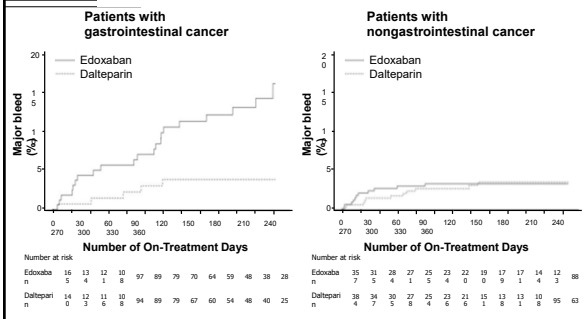
Hokusai VTE: Bleeding GI vs non-GI Cancers



Kraaijpoel N, et al. *Thromb Haemost.* 2018;118:1439-1449

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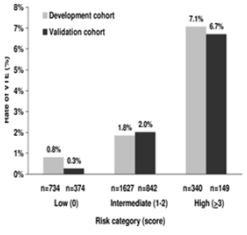
Cancer-Associated Thrombosis: Duration of Treatment?

- 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

29

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

Characteristic	Score
Site of Cancer	
Very high risk (stomach, pancreas)	2
High risk (lung, lymphoma, gynecologic, GU excluding prostate)	1
Platelet count ≥ 350,000/mm^3	1
Hb < 10g/dL or use of ESA	1
Leukocyte count > 11,000/mm^3	1
BMI ≥ 35 kg/m^2	1



30

Primary Prevention of Cancer-Associated Thrombosis with DOACs
Khorana A, et al. NEJM 2019; Carrier M, et al. NEJM 2019

	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

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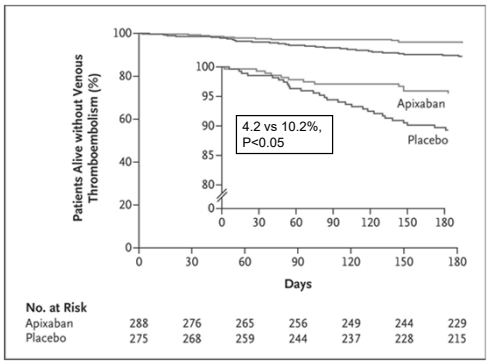

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Evaluation for occult malignancy in the patient presenting with unprovoked VTE

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

33

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).

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Sites of Thrombosis

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

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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 genome-wide screens – all common and weak (OR < 1.5) (Smith NL, JAMA 2007; Bezemer ID, JAMA 2008; Li Y, JTH 2009

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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 loss**
  - **One positive trial in thrombophilic women**
  - **Multiple negative trials in women with recurrent losses before 20 weeks including thrombophilic women (TIPPS, Lancet 2014)**

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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 assay)**
  - **Total Protein S Antigen**
  - **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 (<0.10th percentile):

Free protein S < 33 U/dL (OR 5.4, 95th 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
Estrogens		Estrogens

Caveats:

1. **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).**
2. **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**

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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)

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

PRO

Improve understanding of pathogenesis of VTE

Identify and counsel affected family members

CON

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
- **Duration of anticoagulation/Risk Stratification**

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

Indication	Duration of Anticoagulation
○ Proximal DVT/PE secondary to a transient risk factor	3 months
○ 1st isolated, unprovoked distal DVT	
○ Idiopathic proximal DVT or PE	3-6 months
○ 2nd Unprovoked DVT or PE	Long-term
○ VTE in setting of active cancer: LMWH for at least 3 months	

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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	**Death by major bleed**
80 VTE recurrences	20 to 60 bleeds
Case-fatality rate 4-12%	Case-fatality rate 10%
3 to 10 deaths	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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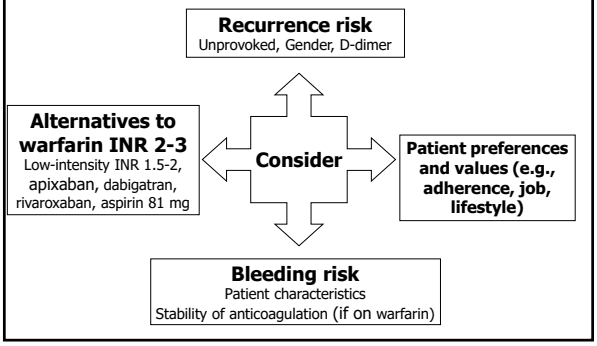
Extended (or Chronic) Treatment - an Individualized Management Decision



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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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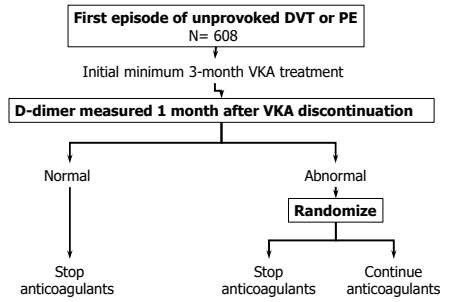
D-dimer to guide prolongation of anticoagulant treatment? PROLONG I Study

Palareti G, et al. NEJM 2006



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D-dimer for VTE: Risk Stratification

Palareti 2006 (Simplify)	Negative (<500 ng/mL) Positive (>500 ng/mL)	4.4% per year 10.9% per year	HR 2.49 (95% CI, 1.35-4.59)

D-Dimer < 500 ng/mL

Sex	Age < 65	Age > 65
Female	0.4% per year	6.6% per year
Male	5.1% per year	8.1% per year

Palareti G, et al. *N Engl J Med.* 2006;355:1780-1789.
Cosmi B, et al. *J Thromb Haemost.* 2010; 8:1933-1942.

50

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

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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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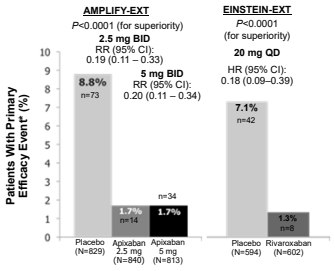
Recurrent VTE/VTE-Related Death* in DOAC Extended Treatment Trials



Low doses of oral DOACs vs standard doses did not show non inferiority with respect to efficacy or safety (i.e., underpowered to show this).

Bleeding rates with DOACs were very low with both doses and no different than placebo (for apixaban) or aspirin (for rivaroxaban).[1,26]

* Primary efficacy endpoint in AMPLIFY-EXT was recurrent VTE and all-cause death; in EINSTEIN-EXT, was recurrent VTE and VTE-related death

5

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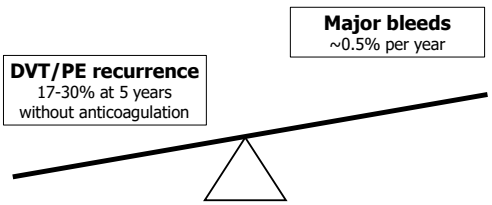
Should the superior safety profile of direct oral anticoagulants (DOACs) lead to the treatment of more, most patients with a 1st unprovoked VTE with extended therapy?

KAB9 KAB10

"Greater net clinical benefit with DOACs than VKAs"



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Slide 54

KAB9 KENNETH A BAUER, 10/1/2014

KAB10 KENNETH A BAUER, 10/1/2014

Other Considerations in Deciding on Long-Term Oral Anticoagulation

- **Site of thrombosis**
  - **PE more likely to recur as PE, DVT as DVT**
- **Severity of Thrombosis**
  - **Massive PE**
  - **Ilio-femoral DVT**
  - **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)**
  - **⇑ 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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15

ITP and Drug-Induced Thrombocytopenia

Robert S. Siegel, MD

August 14, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

15 – ITP and Drug-Induced Thrombocytopenia

Robert S. Siegel, MD

1

DISCLOSURES

Off-Label Usage:

•None

Interests:

•Consultant: CVS/Caremark (Formulary Review)

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

2

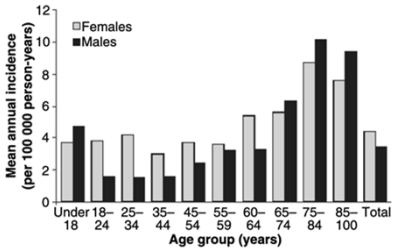
Primary Immune Thrombocytopenia

- 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

3

Age and Sex Distribution of ITP

Average annual ITP incidence by age group and gender (n=1145) diagnosed between 1990-2005 as reported by the General Practice Research Database, United Kingdom.



Marieke Schoonen W, Kucera G, Coalson J, et al. Epidemiology of immune thrombocytopenic purpura in the general practice research database. Br J Haematol. 2009;145(2):235-244.

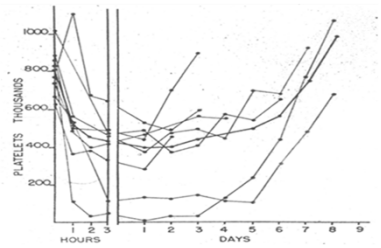
4

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

5

Idiopathic Thrombocytopenic Purpura: 8 Cases



Effect of infusing 250 ml of plasma from patients with chronic idiopathic thrombocytopenic purpura (ITP) on platelet levels in normal recipients. On each of eight occasions, a significant decrease in platelet levels was observed. Average change is denoted by the heavy line. High initial platelet concentrations are due to use of an indirect counting technique.

HARRINGTON WJ, MINNICH V, HOLLINGSWORTH JW, MOORE CV. Demonstration of a thrombocytopenic factor in the blood of patients with thrombocytopenic purpura. *J Lab Clin Med.* 1951;38(1):1-10.

6

Overview

- Autoantibodies to platelet membrane antigens
- Chronic ITP is typically seen in adults
  - Acute ITP is typically seen in children
- Prevalence: 9.5 to 23.6/100,000 (UK health registry)*
  - Lower in US**
- Most common in females 20-45 years
- Possible trend: in older patients, female to male ratio: 1
- Biggest risk - Intracranial bleed
- Spleen is normal size!

*Abrahamson PE, Hall SA, Feudjo-Tepie M, Mitrani-Gold FS, Logie J. The incidence of idiopathic thrombocytopenic purpura among adults: A population-based study and literature review. *Eur J Haematol*. 2009;83(2):83-89.
**Segal JB, Powe NR. Prevalence of immune thrombocytopenia: analyses of administrative data. *J Thromb Haemost*. 2006;4(11):2377-2383.

7

Immune Thrombocytopenia Findings

- Thrombocytopenia
- Increased megakaryocytes
- Increased PAIgG
- Increased platelet destruction
- Decreased platelet production

8

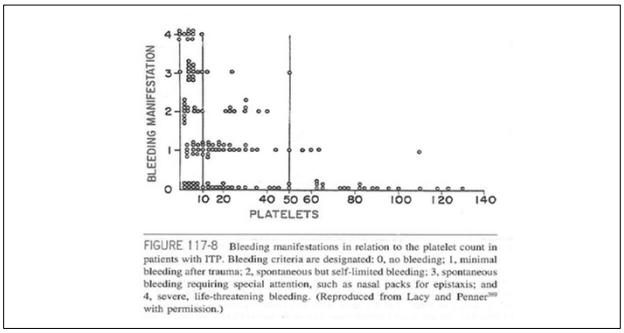


FIGURE 117-8 Bleeding manifestations in relation to the platelet count in patients with ITP. Bleeding criteria are designated: 0, no bleeding; 1, minimal bleeding after trauma; 2, spontaneous but self-limited bleeding; 3, spontaneous bleeding requiring special attention, such as nasal packs for epistaxis; and 4, severe, life-threatening bleeding. (Reproduced from Lacy and Penner[389] with permission.)

Lacey JV, Penner JA. Management of idiopathic thrombocytopenic purpura in the adult. *Semin Thromb Hemost*. 1977;3(3):160-174.

9

Immune Thrombocytopenia: Major Sections

- **PATHOPHYSIOLOGY**
- MAKING THE DIAGNOSIS
- TREATMENT & TREATMENT GUIDELINES

10

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

12

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

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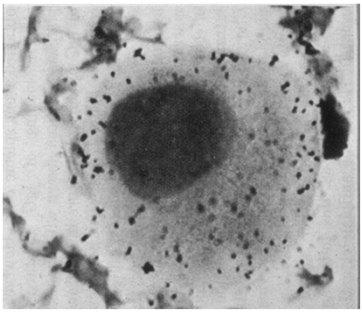

McMillan R, Luiken GA, Levy R, Yelenosky R, Longmire RL. Antibody against megakaryocytes in idiopathic thrombocytopenic purpura. JAMA. 1978;239(23):2460-2462.

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

15

Causes of Secondary ITP

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

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Causes of Secondary ITP (Cont.)

- Vaccination side effect
- Systemic lupus erythematosus
- Myelodysplasia
- AFB disease
- Other Granulomatous disease
- Drug-induced
  - Decreased production
  - More rapid destruction

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Drug-Induced Thrombocytopenia

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

18

Drug-Induced Thrombocytopenia

- Usually first diagnosed as ITP
- Correct diagnosis is essential to:
  - Avoid inappropriate treatment
  - Prevent recurrence

19

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 Haematol*. 2003;122(6):966-974.

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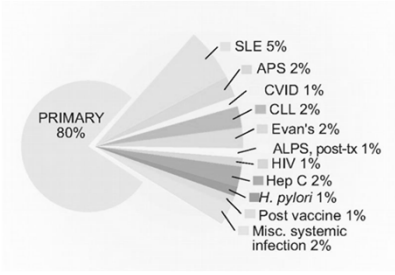
Estimated fraction of the various forms of secondary ITP based on clinical experience of the authors



Cines DB, Bussel JB, Liebman HA, Luning Prak ET. The ITP syndrome: pathogenic and clinical diversity. *Blood*. 2009;113(26):6511-6521.

21

Acute and Chronic ITP

	Acute	Chronic
Peak Age	2-6 Years	20-40 Years
Sex Predilection	None	Women 3:1
Prior Infection	Yes	No
Onset	Abrupt	Insidious
Platelet Count	<20,000	20,000-80,000
Duration	6-8 Weeks	Chronic
Spontaneous Remission	Usual	Unusual

22

Physiology of Thrombopoietin

- A constant amount is produced by the liver
- Binds to TPO receptors of:
  - 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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Immune Thrombocytopenia: Major Sections

- PATHOPHYSIOLOGY
- **MAKING THE DIAGNOSIS**
- TREATMENT & TREATMENT GUIDELINES

25

International Consensus Report: Recommendations 2019

- Diagnosis of exclusion
- H&P, CBC, peripheral smear, and blood group (Rh)
- HIV, HBV, and HCV testing in those at risk
- Bone marrow biopsy not required if physical examination and blood smear are normal

Provan D, Arnold DM, Bussel JB, et al. Updated international consensus report on the investigation and management of primary immune thrombocytopenia. *Blood Adv*. 2019;3(22):3780-3817.

26

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
- ANA
- 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 primary immune thrombocytopenia. *Blood Adv*. 2019;3(22):3780-3817.

27

International Consensus Report Recommendations 2019 (Cont.)

Tests of unproven or uncertain benefit:

- TPO level
- 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 primary immune thrombocytopenia. *Blood Adv*. 2019;3(22):3780-3817.

28

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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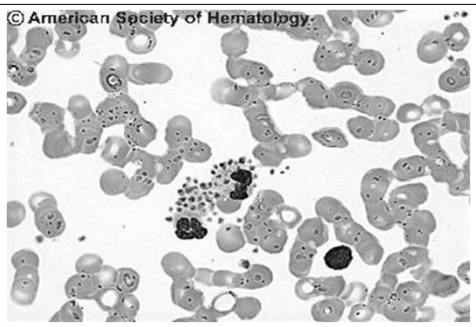

PSEUDOTHROMBOCYTOPENIA

30

Immune Thrombocytopenia: Major Sections

- PATHOPHYSIOLOGY
- MAKING THE DIAGNOSIS
- **TREATMENT & TREATMENT GUIDELINES**

31

Treatment Goals in AITP

- Maintain Hemostasis
- Minimize Toxicity

32

Recommendations for Surgery

SURGERY	RECOMMENDED PLATELET COUNT
Dental Prophylaxis (Cleaning/Scaling)	>20,000
Simple Dental Extraction	>30,000
Complex Dental Extraction	>50,000
Minor Surgery	>50,000
Major Surgery	>80,000
Major Neurosurgery	>100,000

Cuker A, Cines DB. Immune thrombocytopenia. *Hematology Am Soc Hematol Educ Program*. 2010;2010:377-384.

33

Immune Thrombocytopenia: Emergency Therapy

- Platelet Transfusion
- IVIG
- Corticosteroids

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)
- Use IVIg with steroids when rapid response is needed (2B)
- 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

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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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

36

High Dose Dexamethasone

- 125 patients with mean platelet count of 12,000
- Treated with Dexamethasone 40 mg x 4 days
- Major Results:
  - Initial response in 106/125 (85%)
  - Mean platelet count of 101,400 at 7 days
  - 50% had sustained response
  - Platelets <90,000 on day 10 was a significant risk factor for relapse within 3 months
  - Well tolerated
  - 45/125 (36%) required splenectomy or other treatment

Cheng Y, Wong RS, Soo YO, et al. Initial treatment of immune thrombocytopenic purpura with high-dose dexamethasone. *N Engl J Med*. 2003;349(9):831-836.

37

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**

*Liu Z, Wang M, Zhou S, et al. Pulsed high-dose dexamethasone modulates Th1-/Th2-chemokine imbalance in immune thrombocytopenia. *J Transl Med*. 2016;14(1):301. Published 2016 Oct 24.
**Mithoowani S, Gregory-Miller K, Goy J, et al. High-dose dexamethasone compared with prednisone for previously untreated primary immune thrombocytopenia: a systematic review and meta-analysis. *Lancet Haematol*. 2016;3(10):e489-e496.

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, Ji XB, 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.

39

IVIg in ITP

Mechanism

- Reticuloendothelial blockade
- Anti-idiotype antibodies

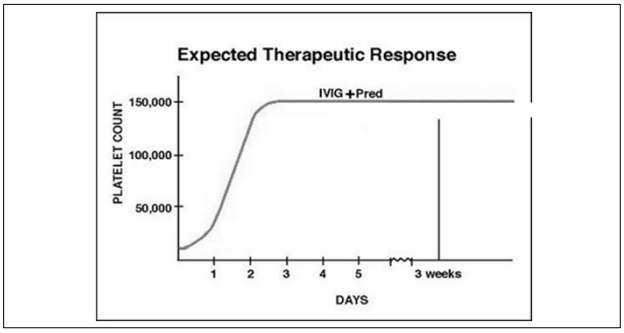
Advantages

- 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

40



ASH Online Teaching Program

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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

42

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
- Wait at least 1-2 years from diagnosis before considering splenectomy

*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.
**Provan D, Arnold DM, Bussel JB, et al. Updated international consensus report on the investigation and management of primary immune thrombocytopenia. *Blood Adv*. 2019;3(22):3780-3817.

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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

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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.

45

Rituximab + rhTPO vs. Rituximab alone

- Multicenter trial including115 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 randomized open-label study of rituximab plus rhTPO vs rituximab in corticosteroid-resistant or relapsed ITP. *Blood*. 2015;125(10):1541-1547.

46

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***

*Chaturvedi S, 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 DJ, 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.

47

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.*****

*Mahévas M, Fain O, Ebbo M, et al. The temporary use of thrombopoietin-receptor agonists may induce a prolonged remission in adult chronic immune thrombocytopenia. Results of a French observational study. *Br J Haematol*. 2014;165(6):865-869.
** Cervinek L, Mayer J, Doubek M. Sustained remission of chronic immune thrombocytopenia after discontinuation of treatment with thrombopoietin-receptor agonists in adults. *Int J Hematol*. 2015;102(1):7-11.
*** Ghadaki B, Nazi I, Kelton JG, Arnold DM. Sustained remissions of immune thrombocytopenia associated with the use of thrombopoietin receptor agonists. *Transfusion*. 2013;53(11):2807-2812.
**** Provan, D. Sustained responses following treatment with romiplostim in immune thrombocytopenia: a single-centre experience. *J Hematol Thromboembolic Dis*. 2014;2:147-149.
***** González-López TJ, Pascual C, Álvarez-Román MT, et al. Successful discontinuation of eltrombopag after complete remission in patients with primary immune thrombocytopenia. *Am J Hematol*. 2015;90(3):E40-E43.

48

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

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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

50

Romiplostim vs. Eltrombopag

- Systematic review and meta-analysis of nine randomized controlled trials
  - No significant difference in durable platelet response, clinically significant bleeding, and all bleeding events
  - Major conclusion: equivalent efficacy

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

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Avatrombopag

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

Jurczak W, Chojnowski K, Mayer J, et al. Phase 3 randomised study of avatrombopag, a novel thrombopoietin receptor agonist for the treatment of chronic immune thrombocytopenia. *Br J Haematol*. 2018;183(3):479-490.

52

Avatrombopag (Cont.)

- 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

Jurczak W, Chojnowski K, Mayer J, et al. Phase 3 randomised study of avatrombopag, a novel thrombopoietin receptor agonist for the treatment of chronic immune thrombocytopenia. *Br J Haematol*. 2018;183(3):479-490.

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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 Hematol*. 2018;93(7):921-930.

54

Splenectomy

Splenectomy is not indicated:
- When platelets >50,000, Dx > 6 months, No bleeding
- As initial therapy in patients without bleeding, minor purpura, or major bleeding risks

Splenectomy is indicated:
- Bleeding, platelet count <30,000, after medical treatment 4-6 weeks

Pre-Op therapy before splenectomy:
- IVIg or steroids, if platelets <50,000
- Transfusions only in platelets <10,000

George JN, Woolf SH, Raskob GE, et al. Idiopathic thrombocytopenic purpura: a practice guideline developed by explicit methods for the American Society of Hematology. *Blood*. 1996;88(1):3-40.
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.

55

Splenectomy: Definitive Therapy for ITP

- Remember immunizations for pneumococcus, H. flu, and N. meningitidis
- 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

Karpatkin S, Strick N, Karpatkin MB, Siskind GW. Cumulative experience in the detection of antiplatelet antibody in 234 patients with idiopathic thrombocytopenic purpura, systemic lupus erythematosus and other clinical disorders. *Am J Med*. 1972;52(6):776-785.
McMillan R, Longmire RL, Yelenosky R, Smith RS, Craddock CG. Immunoglobulin synthesis in vitro by splenic tissue in idiopathic thrombocytopenic purpura. *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
- Post-op platelet count > 500,000 plts/ul
- Good response to corticosteroids
- Good response to IVIg
- Patients < 60 y.o.

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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

Law C, Marcaccio M, Tam P, Heddle N, Kelton JG. High-dose intravenous immune globulin and the response to splenectomy in patients with idiopathic thrombocytopenic purpura. *N Engl J Med*. 1997;336(21):1494-1498.
Choi CW, Kim BS, Seo JH, et al. Response to high-dose intravenous immune globulin as a valuable factor predicting the effect of splenectomy in chronic idiopathic thrombocytopenic purpura patients. *Am J Hematol*. 2001;66(3):197-202.

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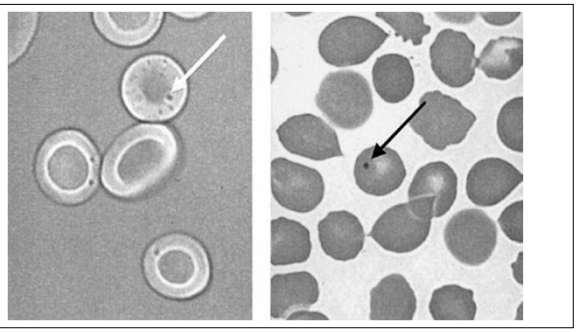

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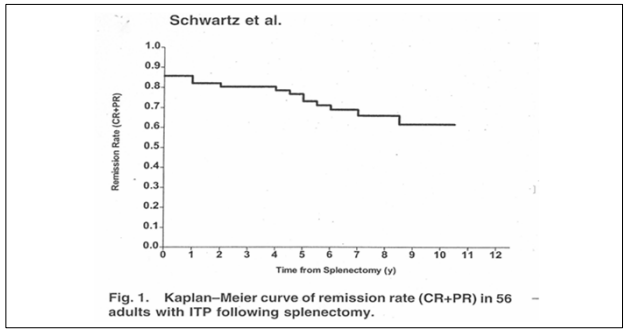

Fig. 1. Kaplan–Meier curve of remission rate (CR+PR) in 56 adults with ITP following splenectomy.

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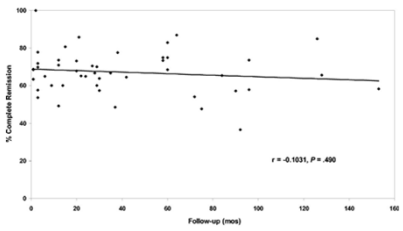
Frequency of complete remission in ITP following splenectomy



Kojouri K, Vesely SK, Terrell DR, George JN. Splenectomy for adult patients with idiopathic thrombocytopenic purpura: a systematic review to assess long-term platelet count responses, prediction of response, and surgical complications. *Blood.* 2004;104(9):2623-2634.

61

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)

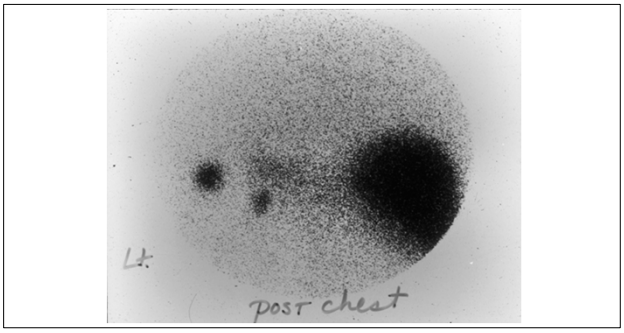
Neunert C, Lim W, Crowther M, et al. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. *Blood.* 2011;117(16):4190-4207.

63

Accessory Spleens

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

64



65

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

Calverley DC, Jones GW, Kelton JG. Splenic radiation for corticosteroid-resistant immune thrombocytopenia. Ann Intern Med. 1992;116(12 Pt 1):977-981.

66

Management of Secondary ITP, HCV

Suggestions:

- Antiviral therapy should be used, but platelet count should be closely monitored
- If treatment for ITP is required, initial treatment should be IVIg (2C)

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.

67

Management of Secondary ITP, H. pylori

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

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.

68

Management of Secondary ITP, HIV

- Steroids
- IVIg
- Anti-D (winRho)
- Splenectomy
  - Effective
  - No difference in CD4 decline

69

Refractory ITP

- Patients who do not respond to splenectomy
- Patients who recur after remission from splenectomy

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

Andersen JC. Response of resistant idiopathic thrombocytopenic purpura to pulsed high-dose dexamethasone therapy [published correction appears in N Engl J Med 1994 Jul 28;331(4):283]. *N Engl J Med.* 1994;330(22):1560-1564.

71

Danazol

- Dose: 50 mg qD to 800 mg qD
- Decreases number of monocyte receptors
- Alters clearance of Ig coated platelets

Ahn YS, Harrington WJ, Simon SR, Mylvaganam R, Pall LM, So AG. Danazol for the treatment of idiopathic thrombocytopenic purpura. *N Engl J Med.* 1983;308(23):1396-1399.
Schreiber AD, Chien P, Tomaski A, Cines DB. Effect of danazol in immune thrombocytopenic purpura. *N Engl J Med.* 1987;316(9):503-508.
Ahn YS, Mylvaganam R, Garcia RO, Kim CI, Palow D, Harrington WJ. Low-dose danazol therapy in idiopathic thrombocytopenic purpura. *Ann Intern Med.* 1987;107(2):177-181.

72

Danazol – Dose Related Problems

- Expensive
- Voice change
- Increased weight
- Increased LFTs
- Other androgen consequences: hirsutism

73

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 treatment of idiopathic thrombocytopenic purpura. Ann Intern Med. 1984;100(2):192-196.

74

Cytotoxic Agents

- Cyclophosphamide (Cytoxan)
- Azathioprine (Imuran)
- Advantages:
  - Salvage therapy
  - Usually no acute toxicity
- Disadvantages:
  - Up to 2-month lag period
  - Possible leukemia

75

Pulse Cyclophosphamide

- 20 refractory AITP patients
- Received 2-8 (mean 4.8) prior treatments
- Cytoxan 1.0-1.5 mg/m^2 (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

Reiner A, Gernsheimer T, Slichter SJ. Pulse cyclophosphamide therapy for refractory autoimmune thrombocytopenic purpura. *Blood*. 1995;85(2):351-358.

76

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 patients enrolled and given 4, 7, 10, 15, or 20 mg/kg SQ infusion
  - 30% of patients previously received TPO-Ras
  - Dose-dependent increases in platelet count were observed with peak median counts >100x10^9/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

Tadeusz Robak, Maciej Kaźmierczak, Isidro Jarque, Vasile Musteata, Jacek Trelinski, Nichola Cooper, Peter Kiessling, Ute Massow, Franz Woltering, Rose Snipes, Juan Ke, Grant Langdon, Birgit Haier, James B. Bussel, Stephen Jolles; Rozanolixizumab, an Anti-FcRn Antibody: Final Results from a Phase II, Multiple-Dose Study in Patients with Primary Immune Thrombocytopenia. *Blood* 2019; 134

77

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 ≥ 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

Newland AC, Sánchez-González B, Rejtő L, et al. Phase 2 study of efgartigimod, a novel FcRn antagonist, in adult patients with primary immune thrombocytopenia. Am J Hematol. 2020;95(2):178-187.

78

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

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

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):730-742. doi:10.1182/blood-2018-05-847624

81

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 Trněný, Milan Kostal, Roman Hajek, Vickie McDonald, Olga Bandman, Regan Burns, Ann Neale, Dalca Thomas, Nichola Cooper; Phase I/II, Open-Label, Adaptive Study of Oral Bruton Tyrosine Kinase Inhibitor PRN1008 in Patients with Relapsed/Refractory Primary or Secondary Immune Thrombocytopenia. *Blood* 2019; 134 (Supplement_1): 87.

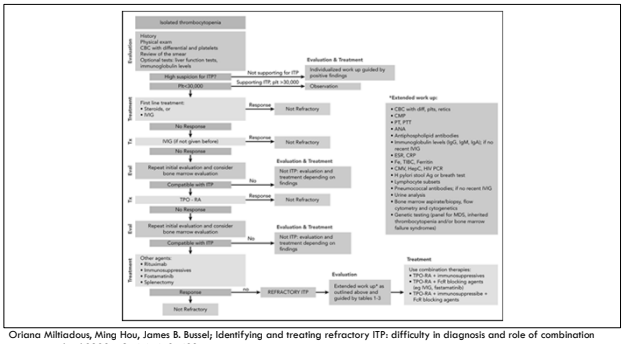
82

Time to Platelet Count Response for ITP Treatments

Treatment	Initial response (days)	Peak response (days)
IVIG	1 to 3	2 to 7
Anti-D	1 to 3	3 to 7
Splenectomy	1 to 56	7 to 56
Dexamethasone	2 to 14	4 to 28
Prednisone	4 to 14	7 to 28
Romiplostim	5 to 14	14 to 60
Rituximab	7 to 56	14 to 180
Eltrombopag	7 to 28	14 to 90
Vinblastine	7 to 14	7 to 42
Vincristine	7 to 14	7 to 42
Danazol	14 to 90	28 to 180
Azathioprine	30 to 90	30 to 180

Adapted from: Rodeghiero, F. F. (03/2009). *Blood: Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: Report from an international working group.* American Society of Hematology. doi:10.1182/blood-2008-07-162503

83



Oriana Miltiadous, Ming Hou, James B. Bussel; Identifying and treating refractory ITP: difficulty in diagnosis and role of combination treatment. *Blood* 2020; 135 (7): 472–490.

84

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

85

86

16

Qualitative Platelet Defects

A. Koneti Rao, MD

August 14, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

16 – Qualitative Platelet Defects

A. Koneti Rao, MD

1

DISCLOSURES

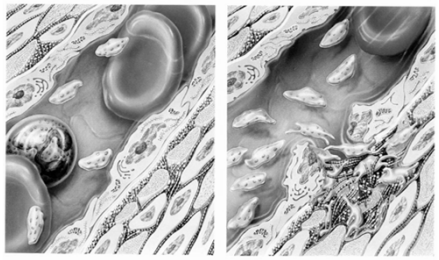
Off Label Usage

•DDAVP – Inherited platelet defects

Interests

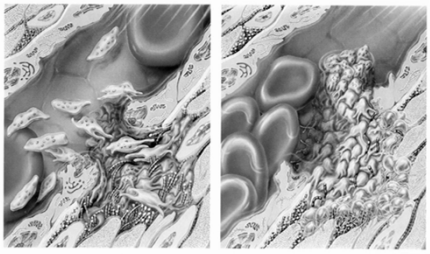
•None

2



Akkerman JW, Bouma BN, Sixma JJ. Atlas of Hemostasis

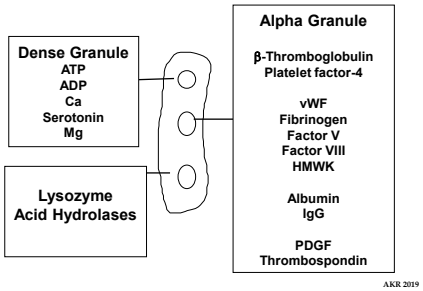
3



Akkerman JW, Bouma BN, Sixma JJ. Atlas of Hemostasis.

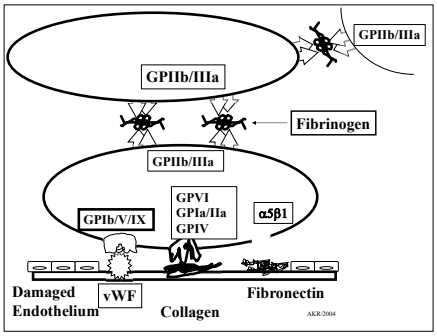
4

Platelet Granule Constituents

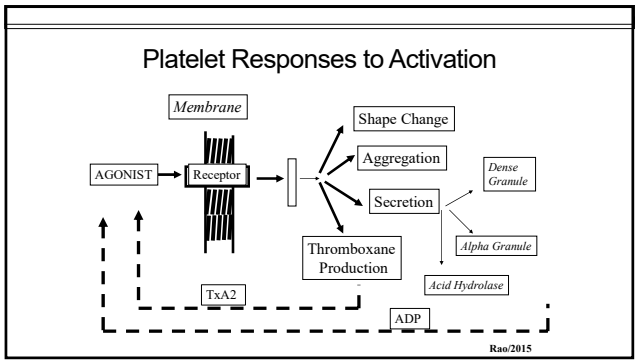


AKR 2019

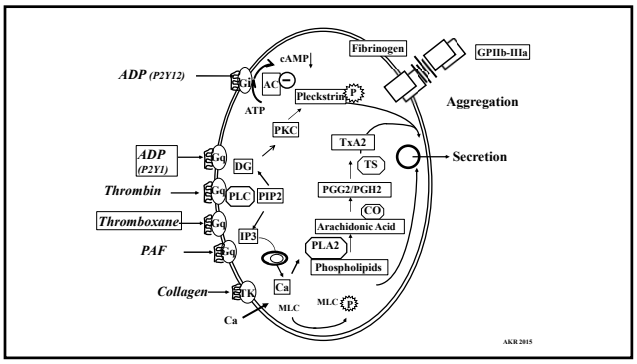
5



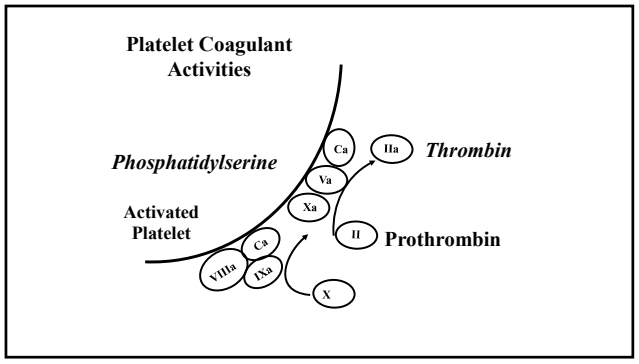
6


Platelet Responses to Activation
Membrane
AGONIST
Receptor
Shape Change
Aggregation
Secretion
Dense Granule
Alpha Granule
Acid Hydrolase
Thromboxane Production
TxA2
ADP
Rao/2015

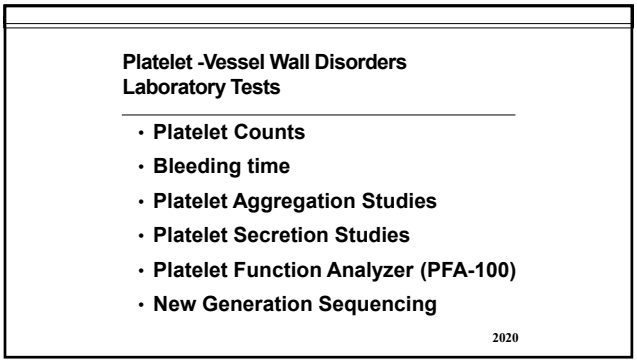
7


ADP (P2Y12)
cAMP
AC
Fibrinogen
GPIIb-IIIa
Pleckstrin
P
ATP
Aggregation
PKC
TxA2
ADP (P2Y1)
DG
TS
Secretion
Thrombin
PLC
PIP2
PGG2/PGH2
CO
Thromboxane
IP3
Arachidonic Acid
PLA2
PAF
Phospholipids
Collagen
Ca
MLC
MLC
Ca
AKR 2015

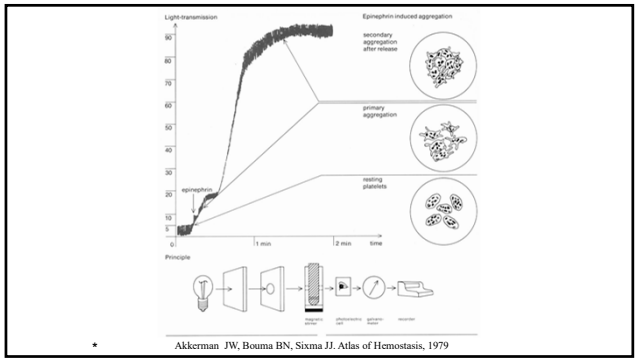
8


Platelet Coagulant Activities
Phosphatidylserine
Activated Platelet
Ca
IIa
Thrombin
Va
Xa
II
Prothrombin
Ca
VIIIa
IXa
X

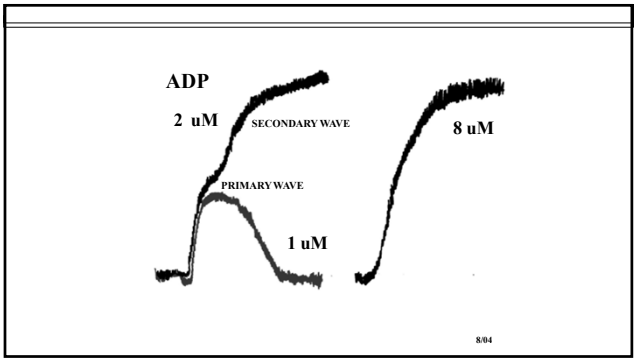
9


Platelet -Vessel Wall Disorders
Laboratory Tests
• Platelet Counts
• Bleeding time
• Platelet Aggregation Studies
• Platelet Secretion Studies
• Platelet Function Analyzer (PFA-100)
• New Generation Sequencing
2020

10


Light-transmission
Epinephrin induced aggregation
secondary aggregation after release
primary aggregation
resting platelets
epinephrin
1 min
2 min
time
Principle
Akkerman JW, Bouma BN, Sixma JJ. Atlas of Hemostasis, 1979

11

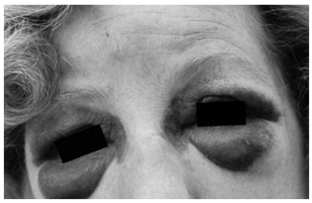

ADP
2 uM
SECONDARY WAVE
8 uM
PRIMARY WAVE
1 uM
8/04

12

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

13



Akkerman JW, Bouma BN, Sixma JJ. Atlas of Hemostasis, 1979

14

Congenital Disorders of Platelet Function

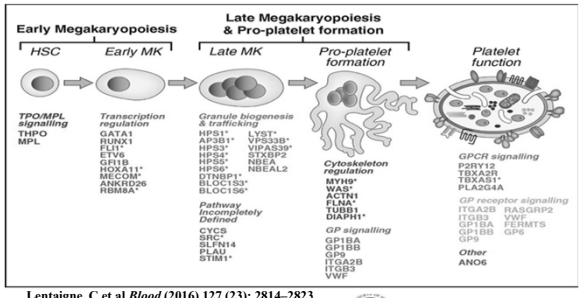
- Studies using new generation sequencing and whole genome sequencing show association with mutations in numerous genes in patients with impaired platelet number and function.
- These studies provide important new information into the pathogenesis of inherited platelet defects and platelet/megakaryocyte 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

AKR 8/2020

15

Inherited platelet disorders: toward DNA-based diagnosis



Lentaigne, C et al *Blood* (2016) 127 (23): 2814–2823

American Society of Hematology
Helping hematologists conquer blood diseases worldwide

16

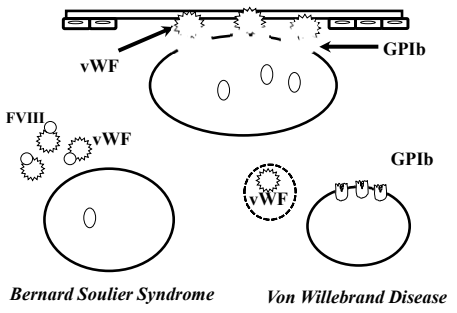
Congenital Disorders of Platelet Function

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

AKR 8/2014

17

Disorders of Adhesion



18

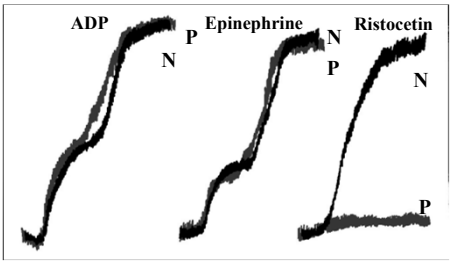
Bernard Soulier Syndrome

- 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

AKR 2012

19

von Willebrand Disease/ Bernard Soulier Syndrome



20

Bernard-Soulier Syndrome and vWD

	ADP	Epi	Collagen	AA	Ristocetin
	NL	NL	NL	NL	
Aggregation	Both Primary and Secondary Waves Present				Decreased

21

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
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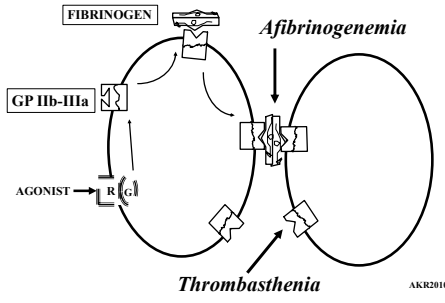
   - Signal Transduction Defects
   - Abnormalities in Arachidonate Pathways and Thromboxane synthesis
4. Disorders of Platelet Coagulant-Protein Interactions
5. Miscellaneous Disorders

AKR 8/2014

22

Disorders of Aggregation



AKR2010

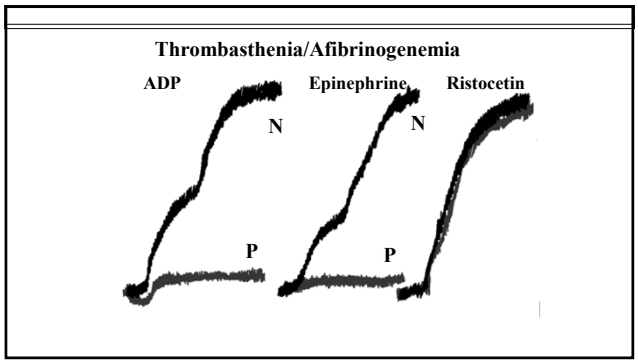
23

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

24



25

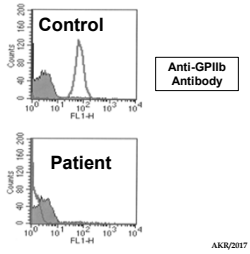
Thrombasthenia / Afibrinogenemia

	ADP Absent	Epi Absent	Collagen Absent	AA Absent	Ristocetin
Aggregation	*No Primary Wave or Secondary Wave*				Normal

26

Diagnosis of Glanzmann Thrombasthenia using Flow Cytometry

Thrombasthenia



Anti-GPIIb Antibody

AKR/2017

27

Congenital Disorders of Platelet Function

3. Disorders of Platelet Secretion/Signal Transduction

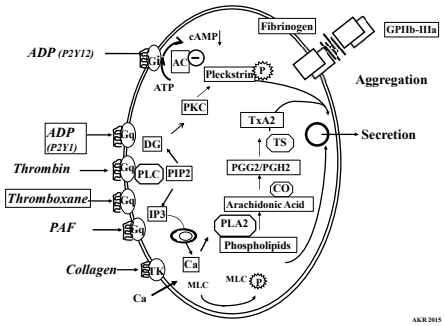
   a. Abnormalities of Granules
      Storage Pool Deficiency (δ, α, αδ)
      Gray Platelet Syndrome (α)
      Quebec Platelet Disorder

   b. Signal Transduction Defects (Activation Defects)

   c. Abnormalities in Arachidonic Acid Pathways and in Thromboxane Synthesis

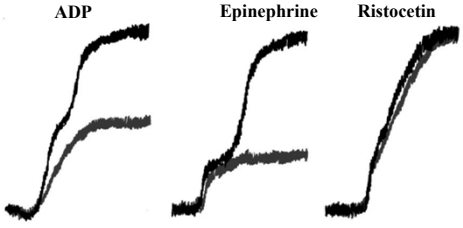
AKR/2014

28



29

Storage Pool Disease/ Secretion Defects



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Congenital Disorders Of Platelet Function

3. Disorders of Platelet Secretion/ Signal Transduction

a. 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/2011

31

Disorders of Platelet Granules
Storage Pool Deficiency

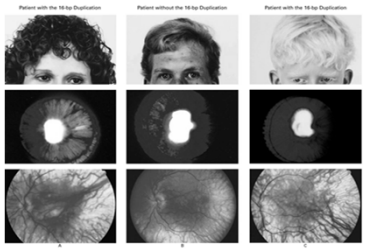
- Platelets may be deficient in dense granules (DG), alpha granules or both types
- Gray Platelet syndrome: deficiency of only alpha granules
- DG deficiency – syndromic or non-syndromic

Hermansky-Pudlak syndrome (HPS)
- DG-SPD with Oculocutaneous albinism, nystagmus.
- Autosomal recessive; Pocket in Puerto Rico
- Colitis and pulmonary fibrosis

AKR/2017

32

Variability in Pigmentation in Three Patients with Hermansky–Pudlak Syndrome.



Gahl, WA et al.N Engl J Med 1998; 338:1258-1265.

33

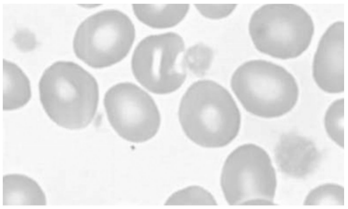
Disorders of Platelet Granules
Gray Platelet Syndrome

- 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

34

Gray Platelet Syndrome: A 5-year-old girl was treated for 4 years based on a diagnosis of immune thrombocytopenia



Alan D. Michelson Blood 2013;121:250

blood

35

SPD/Secretion Defects/Activation Defects

	ADP	Epi	Collagen	AA	Ristocetin
Aggregation	*Only Primary Wave Present*		*Decreased*		Normal

Secretion of Dense Granule Contents Decreased

36

Disorders of Platelet Granules
Quebec Platelet Disorder

- Autosomal dominant disorder of α granules
- Normal to reduced platelet counts
- Decreased platelet Factor V, multimerin, vWF, fibrinogen, fibronectin, thrombospondin
- 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

AKR 7/2017

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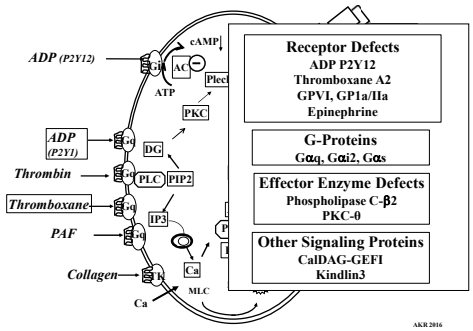

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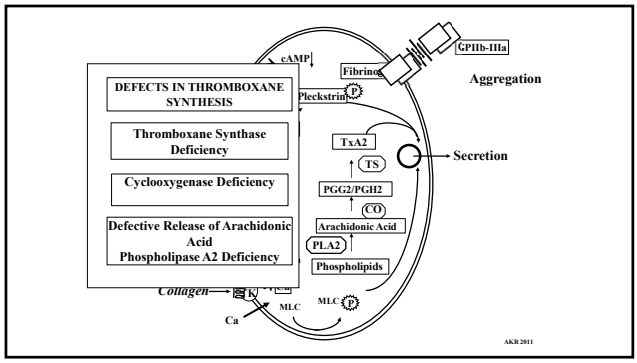

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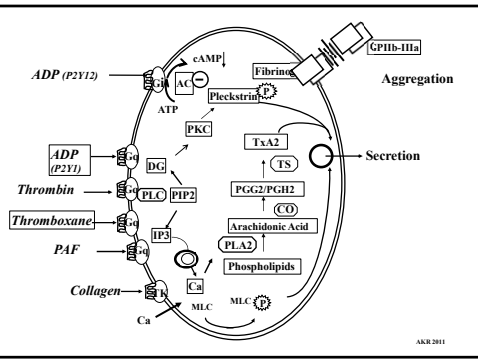

40

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

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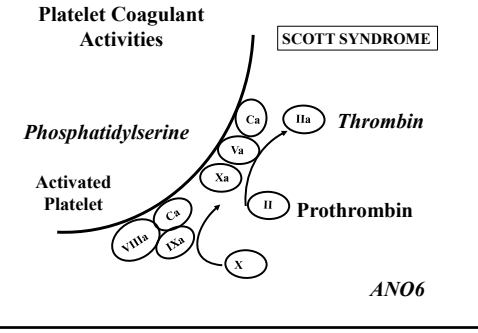

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Congenital Disorders of Platelet Function

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5. Miscellaneous Disorders

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Congenital Disorders Of Platelet Function
Miscellaneous Disorders

1. Abnormalities of Cytoskeletal/ Structural proteins
   - Wiskott-Aldrich Syndrome
   - β1-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

44

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
- GATA-1
- FLI1: Dysmorphic platelets- giant α-granules
- GFI1B

Songdej, N and Rao, AK, Blood, 2017;129:2873-2881

45

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

46

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

AKR/2014

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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

48

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

49

Pharmacologic Agents Effecting Platelet Function

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)**

Anesthetics
- **Local (e.g., Dibucaine); General (e.g., Halothane)**

Chemotherapeutic Agents
- **Mithramycin, BCNU, Daunorubicin**

Dextrans
Ethanol
Vitamin E
Radiographic Contrast Media

AKR/2015

50

Antibiotics and Platelet Function

β-Lactam Antibiotics (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**

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Pharmacologic Agents Effecting Platelet Function

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Dextrans
Ethanol
Vitamin E
Radiographic Contrast Media

Adapted from Rao, 2006
AKR/2012

52

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

53

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 acid)**
- **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**

AKR 2014

54

Chronic Renal Failure: Therapeutic Approaches

- Dialysis (hemodialysis and peritoneal) effective in correcting the BT and platelet aggregation defect
- Platelet transfusions
- DDAVP shortens BT in 50-75% patients
- Cryoprecipitate shortens the BT in some studies
- Increase in Hct by RBC transfusions and erythropoietin is associated with correction of BT and diminished clinical bleeding
- Conjugated estrogens (IV or oral) reported to shorten prolonged BT

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Acquired Disorders of Platelet Function

- Drugs
  - Aspirin
  - Nonsteroidal Anti-inflammatory Agents
  - Antibiotics
  - Other Drugs
- Chronic Renal Failure
- Bone Marrow Disorders
  - Myeloproliferative Neoplasms
  - Dysproteinemias
  - Leukemias; Preleukemia
- Cardiopulmonary Bypass
- Liver Disease
- Antiplatelet Antibodies
- Acquired Storage Pool Deficiency

AKR/2012

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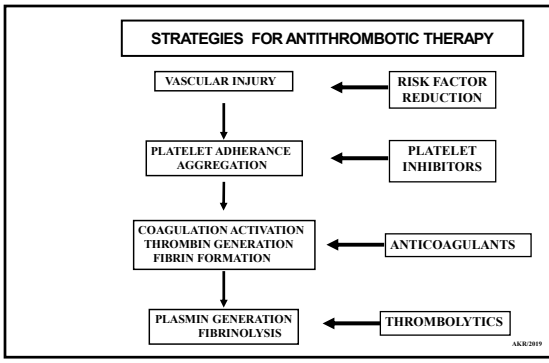
2012/AKR

57



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Platelet Inhibiting Drugs

- Aspirin
- 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*)

AKR-2017

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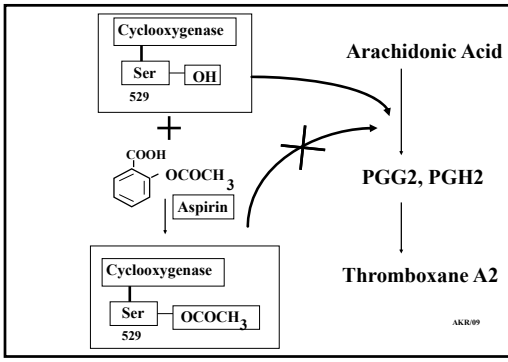


60

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

61

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)

AKR/2010

62

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:

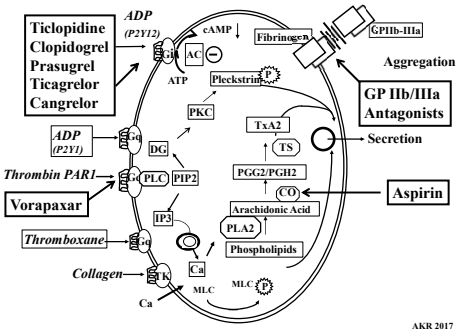
- 6602 patients with stroke or TIA
- Randomized - placebo, aspirin, Aggrenox

2 yr stroke rates: Placebo 15.2%

Aspirin 12.5% (Reduction 19%; p = 0.0009)
Aggrenox 9.5% (Reduction 37%; p < 0.001)

AKR/2017

63



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P2Y12 Antagonists: Thienopyridines Ticlopidine, Clopidogrel, Prasugrel

- Prodrugs
- Converted to active metabolites by liver cytochrome P450 isoenzymes
- Irreversible P2Y12 Inhibitors

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65

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

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Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events

CAPRIE Study: Design

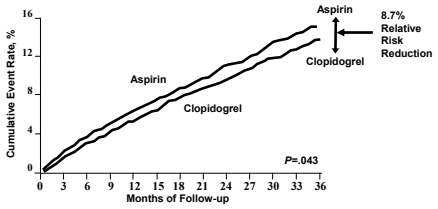
Study Design	Prospective, randomized, blinded
Number of Patients	19,185 patients with atherosclerotic vascular disease
Inclusion Criteria	Recent ischemic stroke (≤6 mo) Recent MI (≤35 d) Established peripheral arterial disease
Study Drugs	Clopidogrel: 75 mg qd Aspirin: 325 mg qd
Treatment Duration	Up to 3 y (mean, 1.6 y)
Investigational Centers	304 in 16 countries, including the US

Lancet. 1996; 348:1329-1339

67

CAPRIE Study

Efficacy of Clopidogrel in Primary Analysis of MI, Ischemic Stroke, or Vascular Death



ITT analysis.
CAPRIE Steering Committee. *Lancet.* 1996;348:1329-1339.

68

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

69

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

70

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

Cangrelor:

- ATP analog; Not a thienopyridine
- Direct acting; rapid onset of action
- Given IV
- PCI

AKR/2019

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Clopidogrel: Adverse Effects

- Bleeding events
- Thrombotic thrombocytopenia purpura

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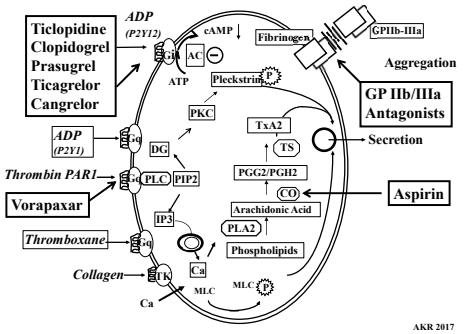

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GPIIb/IIIa Inhibitors

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

AKR-2019

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GPIIb-IIIa Antagonists

- Indications: Acute coronary syndromes and in conjunction with percutaneous coronary interventions
- Major side effects:
  - Hemorrhage
  - Thrombocytopenia

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GPIIb-IIIa Antagonists and Thrombocytopenia

- Occurs with all GPIIb-IIIa 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

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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

78

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.

79

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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17

Oral Anticoagulants and Antithrombotic Therapy

B. Gail Macik, MD

August 14, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

17 – Oral Anticoagulants and Antithrombotic Therapy

B. Gail Macik, MD

1

DISCLOSURES

Off-Label Usage

•Novel dosing for anticoagulants for which labelled indications are not available

Interests

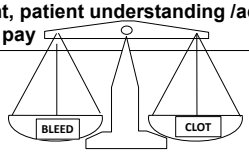
•Pfizer Speaker bureau for apixaban

•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**
- **Guidelines lag behind current needs and do not address individual patient profile**
- **Treatment decisions require patient specific risk assessment, patient understanding /acceptance & ability to pay**



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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**

4

ANTICOAGULANTS– Approved US

DRUG	PARENTERAL	ORAL
Vitamin K antagonist (Inhibits II, VII, IX, X, & PC,PS)	Warfarin – All indications	Warfarin – All indications (grandfathered)
Indirect inhibitors of IIa and / or Xa (require ATIII)	Heparin – All indications anti-IIa = anti-Xa (grandfathered) LMWHs – varies by drug anti-Xa > anti-IIa Fondaparinux– anti-Xa only 1. Prevent DVT/PE TKA/THA 2. Treatment DVT/PE	NONE

5

ANTICOAGULANTS– Approved US

DRUG	PARENTERAL	ORAL
Direct inhibitors Xa	NONE	*RIVAROXABAN* – 1. Prophylaxis of DVT /PE - TKA/THA 2. Reduce risk of stroke/systemic embolism in nonvalve afib 3. Treatment of DVT/PE 4. Reduce risk recurrent DVT/PE 5. Prophylaxis of VTE in acutely ill medical patients 6. Reduce risk major CV event in CAD/PAD *APIXABAN* – 1. Prevent stroke/embolism in nonvalve afib 2. Reduce risk DVT/PE – TKA/THA 3. Treatment of DVT/PE 4. Reduce risk recurrent DVT/PE

6

ANTICOAGULANTS– Approved US

DRUG	PARENTERAL	ORAL
Direct inhibitors Xa (cont)		*EDOXABAN* – 1. Treatment of DVT/PE after 5-10 days of parenteral anticoagulant 2. 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.

7

ANTICOAGULANTS– Approved US

DRUG	PARENTERAL	ORAL
Direct inhibitors IIa	Argatroban Bivalirudin	*DABIGATRAN*– 1. Prevent stroke/emboli in nonvalve afib 2. Treatment of DVT/PE after 5-7 days of parenteral anticoagulant 3. Reduce Risk of recurrent DVT/PE 4. Prevent DVT hip surgery

8

WARFARIN

- Began use in the US in 1951 (grandfathered by FDA)
- Half-life of 36-42 hours
- 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

9

WARFARIN

- Mixture of two active isomers, (R) and (S)
- (S) is 3-5 fold more potent than R
- 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

10

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)

11

Factors That Influence Warfarin Dose Effect

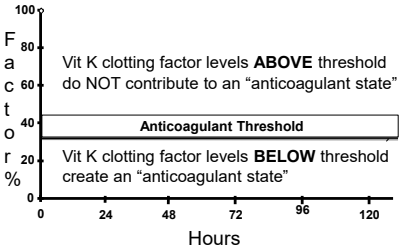
- Altered vitamin K balance due to
  - ✓ Diet
  - ✓ Biliary obstruction (no bile to absorb fat soluble vitamin)
  - ✓ Change in gut flora (prevents conversion of dietary vit K)
- Impaired liver function – decreased clotting factor production
- Hypermetabolic states - fever or hyperthyroid

12

WHY GIVE 4-5 DAYS OF HEPARIN IF THE INR IS OK THE SECOND DAY?

13

WARFARIN EFFECT



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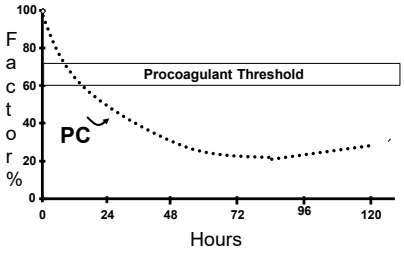
Vitamin K Factors - Warfarin Effect



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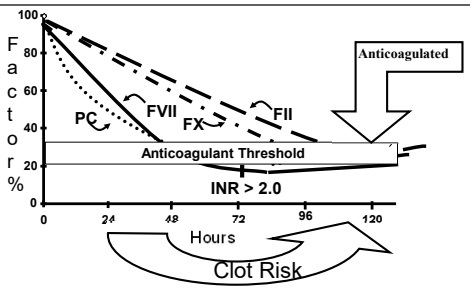
Full Anticoagulant Effect when ALL vit K Factors BELOW threshold – t ½ differ for each factor



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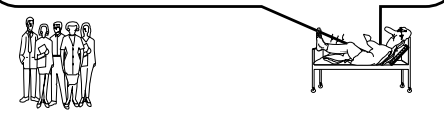
INITIATION OF WARFARIN RULE 1:

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)

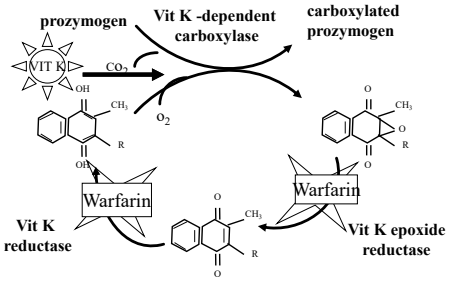


17

WHAT DO I DO IF THE PATIENT'S INR INCREASES TO >4.5 or bleeding?

18

THE VITAMIN K PATHWAY AND WARFARIN



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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
  - 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.

20

WHAT ABOUT THE DIRECT ACTING ORAL ANTICOAGULANT DRUGS?

21

DOAC Characteristics

	Apixaban Anti-Xa	Rivaroxaban 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

DOAC Characteristics

	Edoxaban (anti-Xa)	Dabigatran (anti-IIa)
Effective life **Half-life**	**24 hours** **6-11 hours**	**12-17 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**

23

DOAC Characteristics

	Betrixaban ?? (discontinued 2020)
	(anti-Xa)
Effective life	19 to 27 hours
Elimination	**Mostly gut,**
Reversible	**Yes**
Monitor	**Not Yet**
Peak effect	**3-4 hours**
Absorption	**Effected by fatty food**

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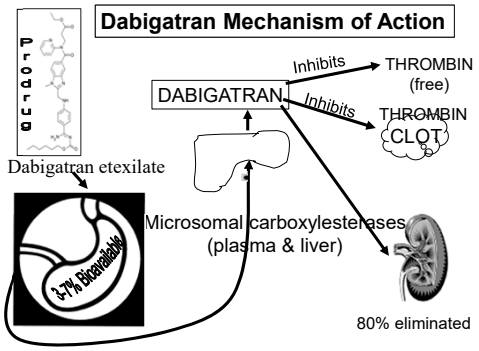
Dabigatran Mechanism of Action



25

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 with acid

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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 GERD, 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**

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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)
  - Renal impairment and co-administration with P-gp inhibitors
  - 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 renal)
  - CrCl <50 mL/min with concomitant use of P-gp inhibitors: Avoid coadministration
- Tablets available 75mg, 110 mg (for prevention), 150 (for treatment)

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Dabigatran (Pradaxa)–Oral Antithrombin

Gastrointestinal Adverse Reactions

TAKE HOME
PPI/antacid may interfere doesn't help

30

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 mg dose
- No dosage adjustment with mild liver impairment but not recommend for moderate / severe liver impairment

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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
2. Treatment of DVT and/or PE
   - CrCl> 15: 15mg TWICE daily X 21 days then 20 mg DAILY + food
   - CrCl< 15: Avoid use
3. 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
   - CrCl< 15: Avoid Use
4. Hip Replacement Surgery
   - CrCl > 15: 10 mg daily X 35 days +/- food, start 6-10 hrs post surg
   - CrCl < Avoid Use
5. 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 events
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 fatal
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

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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

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APIXABAN DOSING

1. 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

     { ADJUSTMENT ONLY FOR AFIB NOT VTE
2. 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
3. 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
4. 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

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APIXABAN ADVERSE EVENTS

- Bleeding most common but less than comparator
- Other reported side effects
  - ✓ GI symptoms: nausea (3%), increase LFTs (<1%)
  - ✓ Reported at < 1%: syncope, rash, anaphylaxis

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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

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EDOXABAN ADVERSE EVENTS

- Bleeding most common, similar to warfarin
- Other reported side effects
  - ✓ Rash 3.6%
  - ✓ Increase LFTs 7.8%
  - ✓ Anemia 1.7%

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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

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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%

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MONITORING OF DOACs

- Dabigatran my be monitored by ECT or dilute Thrombin Time but difficult to acquire
- 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

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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

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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 warfarin
- ✓ 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

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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)

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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
- Re-elevation or incomplete reversal can occur
- Monitor for thromboembolic events and start anticoagulation when medically appropriate

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ANDEXXA DOSING
Bolus Followed by Infusion

DOSE	Initial IV Bolus	IV Infusion post Bolus	# of 200mg Vials
Low Dose	400 mg @ 30 mg/min	4 mg/min up to 120 min (480 mg)	5 vials (2 bolus + 3 infusion)
High Dose	800 mg @ 30 mg/min	8 mg/min up to 120 min (960 mg)	9 vials (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

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ANDEXANET ALFA – DOSING
(revised 3/2020)

- Dose determined by
  - Drug patient taking
  - Dose 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	Low Dose
	> 10 mg or unknown	High Dose	
Apixaban	< 5 mg	Low Dose	
	> 5 mg or unknown	High Dose	

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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

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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)

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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

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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

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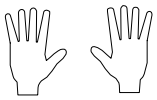

Tune in again for more - Cascade of Caveats - during the next COAG HOUR

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18

Parenteral Antithrombotics and Thrombolytic Therapy

B. Gail Macik, MD

August 14, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

18 – Parenteral Antithrombotics and Thrombolytic Therapy

B. Gail Macik, MD

1

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

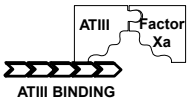
Mechanism of Action for Heparin Family
Indirect Inhibition - Requires ATIII

Anti-Thrombin Activity
Heparin > LMWH

3

Mechanism of Action for Heparin Family
Indirect Inhibition - Requires ATIII

Anti- Xa Activity
Fondaparinux > LMWH > Heparin



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-IIa + anti-Fxa

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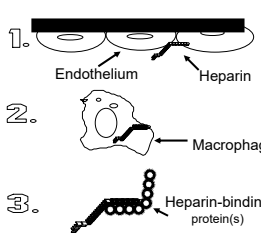
Source Of Heparin

- 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

6

Saturable Heparin Clearance Or Why Is a Heparin Loading Dose Needed



- Binding to endothelium, macrophages, plasma proteins
- Patient size correlates with endothelial binding
- Physiologic conditions effect macrophages -- acute phase reactants
- Sub-saturating dose RAPIDLY cleared by binding
- ONLY FREE HEPARIN IS ACTIVE

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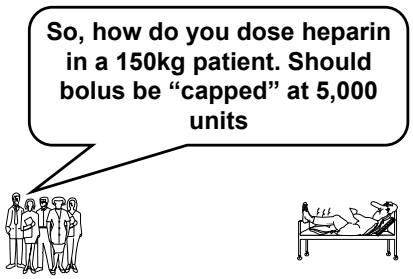

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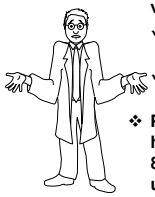
Tidbits Regarding Heparin Therapy



- **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**

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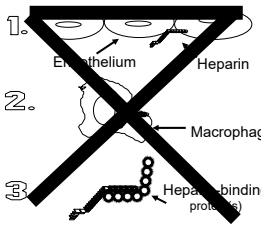
LMW Heparin Clearance or Why Loading Dose NOT needed



- **Weak Binding to endothelial cells, macrophages, and other plasma proteins- NO BINDING SITES TO SATURATE / NO LOADING DOSE**
- **Limited removal of drug by rapid binding – slower clearance by Kidney**

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**
- **Cross reacts with antibodies caused by UFH**

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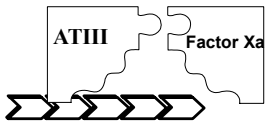
Fondaparinux (ARIXTRA) Indirect Inhibition Factor Xa - Requires ATIII

Pure Anti- Xa Activity



Synthetic Pentasaccharide
Antithrombin Binding Site Only

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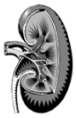
FONDAPARINUX CLEARANCE



- Highly cleared by Kidney
- Must have normal Kidney function to use the drug

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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

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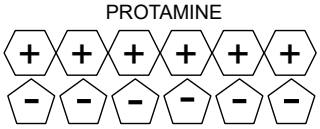
How Does Protamine Work



Charge interaction creates complex and heparin no longer free to bind other proteins

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HEPARIN (Like) FAMILY

PROTAMINE REVERSAL		ANTI IIa:ANTIXa
Excellent	UNFRACTIONATED HEPARIN (-)(-)(-)(-)(-)(-)(-)(-)(-)(-)(-)(-)	1:1
Some	DALTEPARIN (-)(-)(-)(-)(-)(-)	1:2
Minimal	ENOXAPARIN (-)(-)(-)(-)	1:4
FORGET IT	FONDAPARINUX ( )	ALL Anti-Xa

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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

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Indirect Thrombin and/or FXa Inhibitors

	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**

*Indications/characteristics/reversibility vary by product

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Indirect Thrombin and/or FXa Inhibitors

	Heparin	LMWH*	Fondaparinux (Arixtra)
Monitor	aPTT /Anti-Xa	Anti-Xa	Anti-Xa
Bleeding	+++	++	++
HITT Binds PF4	**YES**	Yes-less	Maybe a little

*Indications and characteristics vary by product

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WHAT OTHER DRUGS ARE APPROVED FOR ANTICOAGULATION

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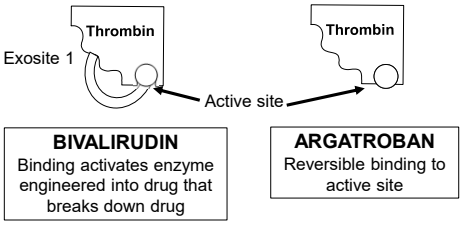
Direct Thrombin Inhibitors



BIVALIRUDIN
Binding activates enzyme engineered into drug that breaks down drug

ARGATROBAN
Reversible binding to active site

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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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Direct Thrombin Inhibitors (DTI)

	Argatroban	**Bivalirudin**
Product	Synthetic arginine analogue	Synthetic peptide
FDA	Approved for HITT	Approved for PCI
MW	506	2180
Clinical Effect	Direct anti-IIa Irreversible	Direct anti-IIa Self-destructs

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Direct Thrombin Inhibitors (DTI)

	Argatroban	**Bivalirudin**
Monitor	**aPTT 1.5-3.0**	**ACT 250-350**
Effects PT	**YES**	**Yes**
Bleeding	**++**	**++**

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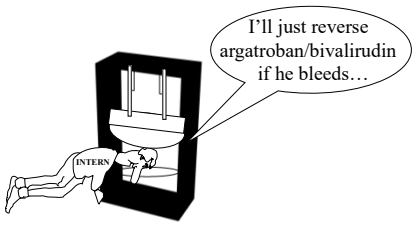
Direct Thrombin Inhibitors

	Argatroban	Bivalirudin
T ½	39-51 min	25 min
Excretion		
Change T ½	Liver disease	Kidney disease 3.5 hours
Reversible	NO	NO – self destructs

25

Treatment Pitfalls - Famous Last Words!



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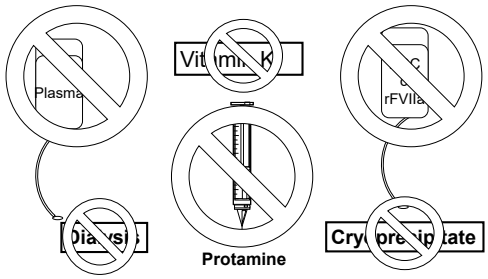
Are Direct Thrombin Inhibitors Reversible?



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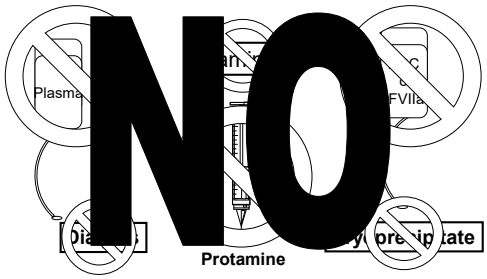
Are Direct Thrombin Inhibitors Reversible?



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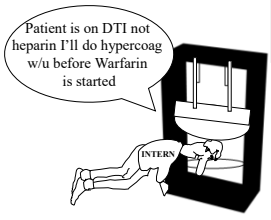
Treatment Pitfalls - Famous Last Words!



DTIs EFFECT ALL CLOT BASED ASSAYS – Beware False Results

- ✓ FIBRINOGEN
- ✓ PT/PTT
- ✓ RVVT
- ✓ Silica Clot time
- ✓ StaClot
- ✓ Protein C activity
- ✓ Protein S activity
- ✓ Reptilase
- ✓ Thrombin clot time

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Guidelines for Conversion To Warfarin From Argatroban

Initiate warfarin using expected daily dose and continue Argatroban. NO WARFARIN LOADING DOSE.

Measure INR daily

If INR is ≤4.0 continue both drugs

If INR is >4.0, stop Argatroban

Repeat INR 4-6 hours later

If INR is in therapeutic range on warfarin, continue warfarin monotherapy

If INR is not in therapeutic range on warfarin, resume DTI

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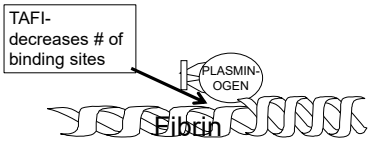
FIBRINOLYTIC CASCADE

- **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**



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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**

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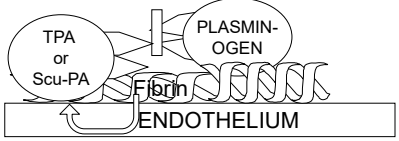
FIBRINOLYTIC CASCADE

- Fibrin provokes release of tissue Plasminogen Activator (tPA) and Single Chain Urokinase (Scu-PA) from intact endothelium
- Fibrin catalysis its own destruction.



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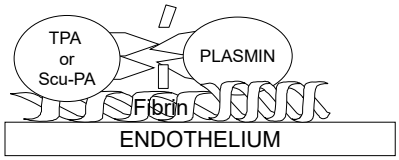
FIBRINOLYTIC CASCADE

- tPA and/or Scu-PA "clips" plasminogen and creates the active enzyme Plasmin.



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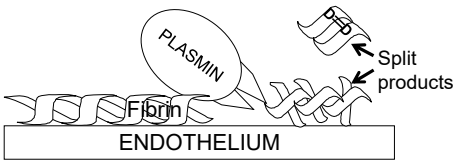
FIBRINOLYTIC CASCADE

- Plasmin clips off pieces of fibrin
- The smallest subunit that cannot by broken is the d-dimer.



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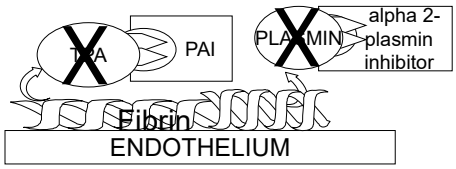
FIBRINOLYTIC CASCADE

- **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)**



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THROMBOLYTIC AGENTS

- Streptokinase and Urokinase no longer marketed in the US
- Tissue Plasminogen Activator derivative drugs available US
  - Alteplase
  - Reteplase
  - Tenecteplase

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THROMBOLYTIC AGENTS

- Alteplase (Activase®; rtPA)
  - recombinant form of human tPA – rare immune response
  - 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

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THROMBOLYTIC AGENTS (continued)

- Retaplase (Retavase®)
  - Less fibrin specific than alteplase
  - Genetically engineered smaller derivative of recombinant tPA
  - Increased potency and is faster acting than rtPA
  - Indicated for acute STEMI only
  - Administered as IV bolus injections

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THROMBOLYTIC AGENTS (continued)

- **Tenecteplase (TNK-tPA)**
  - **Mutant form of rtPA**
  - **Indication: acute myocardial infarction only**
  - **Longer half-life, initial 20-24 min, terminal phase 90-130 min**
  - **Greater binding affinity for fibrin and greater resistance to PAI -1 than rtPA**
  - **Administered by IV bolus**

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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

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INDICATIONS FOR THROMBOLYTIC THERAPY

QUESTION

Does therapy do more than provide accelerated clot lysis and short-term physiologic improvement?

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THROMBOLYTIC THERAPY CONTRAINDICATIONS

ABSOLUTE?

- **History of hemorrhagic stroke**
- **Major internal bleeding in previous 6 months**
- **Intracranial or intraspinal neoplasm**
- **Recent (<2mon) intracranial surgery / trauma**

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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**

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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**

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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

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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 responsive**
- **Premature termination of therapy - monitor progress**
  - ✓**Stop therapy at 24 hrs if no lysis has occurred**
  - ✓**Continue therapy after 24 hours if partial lysis**

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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

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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**

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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

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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**

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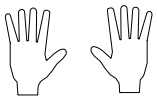

Tune in again for more - Cascade of Caveats - during the next COAG HOUR

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Transfusion Medicine

Thomas S. Kickler, MD

August 15, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

19 - Transfusion Medicine

Thomas Kickler, MD

Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

- None

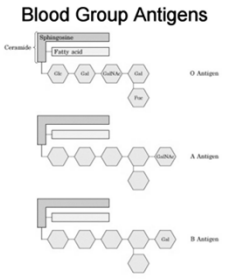
Topics

- 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

Biochemistry of ABH Antigens



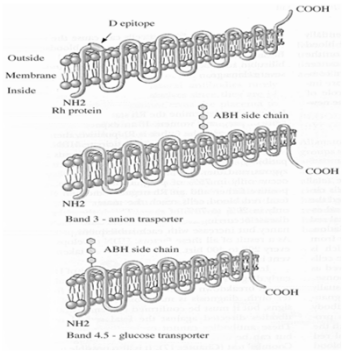
ABO Immunology

Cell Type	Isoagglutin Abs in serum	Class of Ab	All ABO Abs
A	Anti-B	IgM & IgG	Fix complement
B	Anti - A	IgM & IgG	Agglutinate RBCs
O	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

Biochemistry of ABH, Rh(D)



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

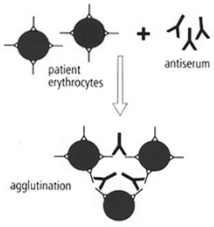
Blood Groups & Disease Associations

- 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

Direct Antiglobulin Test (Coombs Test)

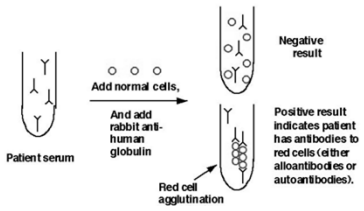


Detects about 100 molecules of IGG per red cell!!

Test done using anti-IGG and third component of complement

Antiglobulin Testing detects nonagglutinating Abs --IgG or complement

Indirect Coombs' test

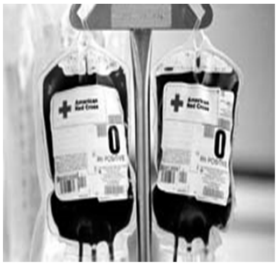


Used to detect Abs in the serum
Most Alloantibodies are non-agglutinating (incomplete)
A technique used to do compatibility testing
Also used to type for minor antigens (Rh, Kell, Duffy)

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 X 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	Characteristics	Indications
Packed Cells, stored up to 42 days	250 ml with Hct of 80%	Red Cell Deficit
Leucocyte Reduced	< 10,000,000 WBC	Prevent Febrile reactions ;HLA alloimmunization Reduces CMV transmission
Washed Red Cells	Plasma depleted	Prevent severe allergic reactions
Frozen Red Cells	150 ml of RBC;plasma and WBCs depleted	Rare donor or autologous storage
Whole Blood	450 ml	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 Transfusion Dependent Patients, for example myelodysplastic patient or sickle cell anemia *How far to match for antigens other than ABO & Rh(D)?*
- 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--- *Rationale- 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

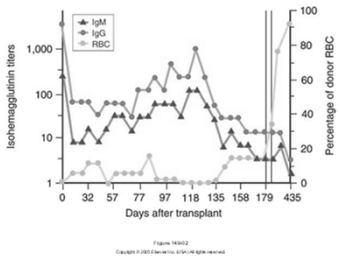
Transfusion Support in Hematopoietic Stem Cell Transplant

- 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, <u>to avoid confusion we use Group O</u>

Delayed RBC engraftment in the recipient of a Major ABO incompatible SCT

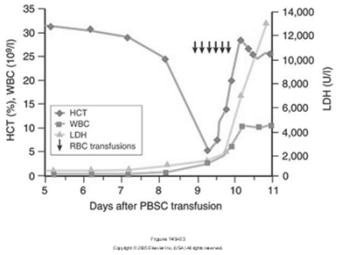


The appearance of mature RBC corresponded to the disappearance of isoagglutins

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

Severe Hemolysis in a Minor Incompatible SCT: The Donor was Group O, and Recipient Group A



Serologically the Direct Antiglobulin Test is Positive with IgG, C3d and an eluate from the RBCs showed Anti - A

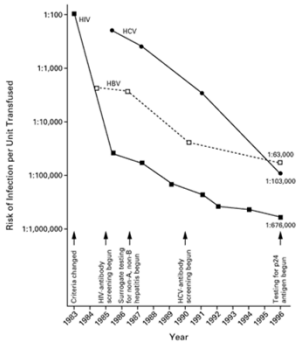
Adverse Effects of Transfusion

- Infectious
- Immunological
- Metabolic
- Volume

Warning Transfusions are Hazardous Package Insert

- Disease transmission
- Hemolysis
- Febrile reactions
- Allergies
- Metabolic abnormalities
- Volume overload
- Death
- AIDS
- Malaria
- Immune suppression
- Sepsis
- GVHD
- Dementia (?)
- 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)

HTLV-I and HTLV-II - Test for antibodies to HTLV-I and HTLV-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 Parvovirus	**1:40,000**	**low**

West Nile virus- estimated risk- depends upon location

Infectious Risk Due to Bacteria

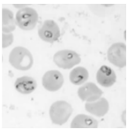
Blood Product	Estimated Risk/Unit	**Estimated % if infected units that transmit**
RBCs	**1:1,000**	**1:10,000,000 fatal**
Platelets	**1:2,000**	**1:2,500 result in sepsis**
Platelets Pheresis	**1:2,000**	**1:13,400 result in sepsis**

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
Trypanosoma Cruzi	**1:42,000**	<10



Transfused patient with fevers and babesia

Pathogen-Reduction Technologies Approved and in Development in the United States and Europe.[a]

Component and Source	Manufacturer and Technology	Treatment Process	Manner of Inhibiting Replication	Regulatory Status
Platelets				
Individual volunteer donors	Cerus Intercept Blood System	Psoralen (amotosalen) and UVA light exposure	Formation of DNA and RNA monoadducts and cross-linkage	FDA approved; CE marked
	Terumo BCT Mirasol Pathogen Reduction Technology (PRT) System	Riboflavin and ultraviolet light exposure	Direct DNA and RNA damage and guanine modification	Phase 3 study planned in the United States; CE marked
	Macopharma Theraflex ultraviolet platelets	UVC light exposure	Direct DNA and RNA damage and thymine dimer formation	CE marked
Plasma				
Pools of volunteer and paid donors	Octapharma Octaplas	Plasma pools treated with solvent, tri-n-butyl phosphate and detergent (octoxynol)	Lipid membrane disruption of enveloped viruses	FDA approved; CE marked
Individual and minipools of volunteer donors	Cerus Intercept Blood System	Psoralen (amotosalen) and UVA light exposure	Formation of DNA and RNA monoadducts and cross-linkage	FDA approved; CE marked
Individual volunteer donors	Macopharma Theraflex MB Plasma System	Filtration, methylene blue treatment and visible light exposure	DNA and RNA damage by type I and type II redox reactions	CE marked
	Terumo BCT Mirasol PRT System	Riboflavin and ultraviolet light exposure	Direct DNA and RNA damage and guanine modification	CE marked
Whole blood				
Individual volunteer donors	Terumo BCT Mirasol PRT System	Riboflavin and ultraviolet light exposure	Direct DNA and RNA damage and guanine modification	Phase 3 studies planned in the United States, completed in Africa
Red cells				
Individual volunteer donors	Cerus Intercept Blood System	Frangible Anchor-Linker Effector (S303) and glutathione	Formation of DNA and RNA monoadducts and cross-linkage	U.S. phase 2 and European phase 3 studies complete

NEJM, May 14 2015
$$$$$$
Is there a will to do this?

Immunologic Transfusion Reactions

Immunologic Reactions

- Hemolytic
- Allergic
- WBC & Cytokine mediated
- GVHD
- Transfusion Related Acute Lung Injury
- Anaphylactic
- Immune suppression

Hemolytic Transfusion Reactions

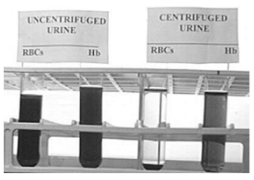
- Acute Hemolytic: ABO mismatched blood, and some IgG antibodies to other Alloantigens, such as E, C, e, Fy
- Delayed Hemolytic Reactions: IgG antibodies form anamestically in previously immunized people
- Passive administration of isoagglutins in FFP and platelets

ABO Hemolytic Reactions

- Acute pain, arm, back
- Fever, chills
- DIC, in O.R. unexplained bleeding
- hemoglobinuria, and hemoglobinemia
- DAT positive for Complement

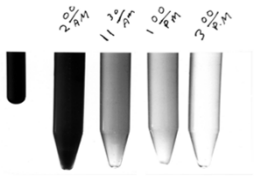
Intravascular Hemolysis

HEMOGLOBINURIA



Urine, red
Centrifuge , if RBC all sediment; if hemoglobinuria, supernant red

HEMOGLOBINEMIA



Intensely pigmented plasma, after centrifugation, hemoglobin cleared in several hours

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

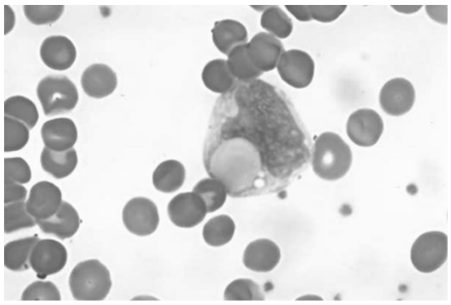
Lab Findings of A Hemolytic Transfusion Reaction

- 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

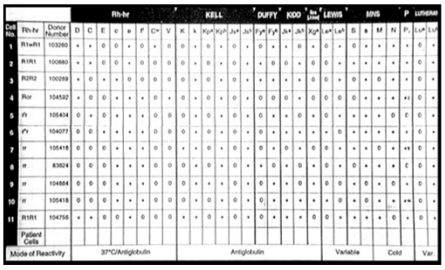
Spherocytes in Delayed Transfusion Reactions



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

Usual Urgency	Reaction Type	Approx Incidence	Clinical significance/ Burn points
++++	**Acute Hemolytic**	1:12,000-35,000	**~50-75% of Txn fatalities** (1:100,000 – 600,000)
+	**Delayed Hemolytic**	1: 1000-12,000	• Severe symptomatic hemolysis: 1: 250,000; death: rare; •↑ risk sickle cell
+/- to ++	**Febrile Non-Hemolytic**	0.5-1.4% 15% recur	• **Frequent: 43- 75% of Txn Rxns** • Frightening to patient • Specters of AHTR + bacterial sepsis

Allergic Reactions

- Hives
- 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

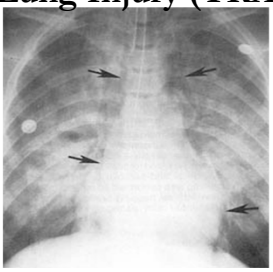
Transfusion Related Acute Lung Injury(TRALI)

- **severe respiratory compromise (clinically ARDS: severe hypoxemia + non-cardiogenic pulmonary edema)**
- **Occurs within 4-6 hrs of txn; no other cause evident**

<u>Other usual clinical concomitants</u>:

- **Rapid onset: 15-20 mins**
- **Fever almost invariable**
- **↑ bp initially; most severe → ↓ bp**
- **↑ pulmonary artery pressures**
- **Resolution usually rapid (hrs) ≠ ARDS**

Transfusion Related Acute Lung Injury (TRALI)



Caused by antigranulocyte/HLA antibodies being passively administered in blood products

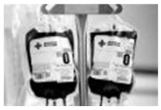
Donor usually Multiparous female

Donor anti-WBC antibodies (abs) definitely seem to be a significant cause of TRALI:
Other factors, cytokines, biologically active lipids

Pathogenesis:

Passive donor anti-WBC abs → **React with recipient's WBCs**

- **C' activation**
- **Pulmonary leukosequestration**
- **Non-cardiogenic pulmonary edema**



Prevention, limit plasma production from mulitparous women??

Transfusion Associated GVHD

- Seen in severe immune suppressed patients
- Patients with congenital cellular immunodeficencies
- Premature babies
- Give irradiated blood to these patients
- Patient groups, BMT, Lymphoma, leukemics undergoing induction therapy

---

Other Adverse Effects of Transfusion

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 imbalances**		
	• ↑ K	Rare	Rapid infusion of old RBCs; renal insufficiency
	• Acidosis	Rare	
	• ↓ Ca^{++} (Mg^{++})	Rare	• Massive transfusion Special attn: young children

---

Other Adverse Effects of Transfusion

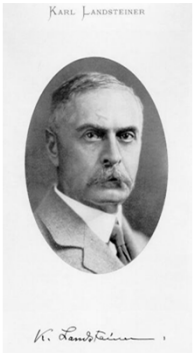
Usual Urgency	Reaction Type	Approx Incidence	Clinical significance
+++	**Hypothermia**	-	• Massive Txn; trauma • coagulopathy
	Fe++ overload	-	Chronic Txn -250 mg/unit

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



The Discoverer of the ABO Blood Group

Nobel Laureate

Karl Landsteiner

20

Platelet Alloimmunization

Thomas S. Kickler, MD

August 15, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

20 - Platelet Alloimmunization

Thomas Kickler, MD

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

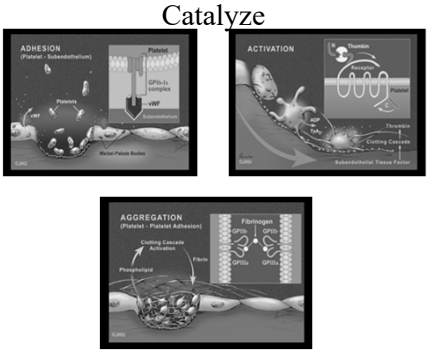
Immune Cytopenias

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

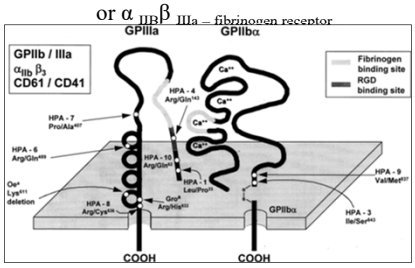
- HLA CLASS I ANTIGENS – Transfusion Refractoriness
- HUMAN PLATELET ALLOANTIGENS- cause Post Tranfusion Purpura, Neonatal Alloimmune Thrombocytopenia, Link to platelet hyper-reactivity??
- ABO BLOOD GROUPS

Platelets – Adhere, Secrete, Aggregate, Catalyze



PLT-GP IIB-IIIA

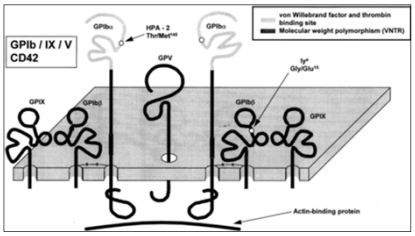
or $\alpha_{IIB}\beta_{IIIa}$ – fibrinogen receptor



More variants than other PLT Glycoproteins

These variants give rise to diallelic platelet antigen systems

PLT-GP IB/IX/V – von Willebrand receptor



1 known polymorphism of Ib alpha

Ab block function

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 names	Plt Glyco-protein	Amino Acid Substitution	DNA substitution
1a 1b	Pl(A1) Pl(A2)	IIIa	Leu33 Pro33	T196 C196
2a 2b	Ko(b) Ko(a)	Ib	Thr145 Met145	C524 T524
3a 3b	Bak(a) Bak(b)	IIb	Ile843 Ser843	T2622 G2622
4a 4b	Pen(a) Pen(b)	IIIa	Arg143 Gln143	G526 G526
5a 5b	Br(b) Br(a)	Ia	Glu505 Lys505	G1648 A1648

+ 10 other less common

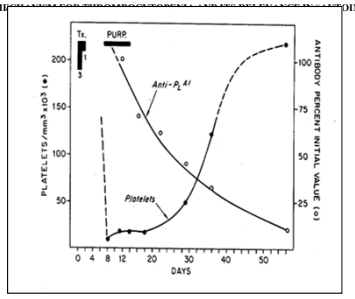
Immunogenetic Studies on 5 Human Platelet Antigens Most Frequently Associated with Alloimmune Disorders

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

Kickler, et al. Blood 1993

Schulman NR, et al.
J Clin Invest. 40(9): 1597–1620.

IMMUNOREACTIONS INVOLVING PLATELETS. V. POST-TRANSFUSION PURPURA DUE TO A COMPLEMENT-FIXING ANTIBODY AGAINST A GENETICALLY CONTROLLED PLATELET ANTIGEN. A PROPOSED MECHANISM FOR AUTOIMMUNITY



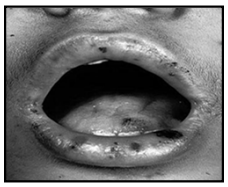
Note: important ground work for characterization of first important integrin- fibrinogen receptor, relevancy to Autoimmunity – target of autoAB

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

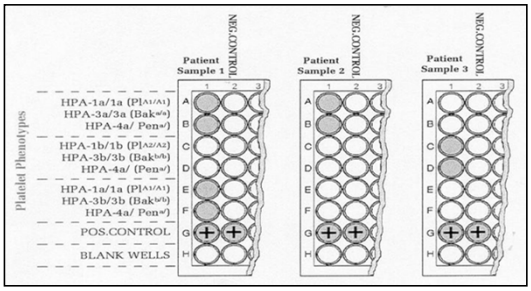


JHH 45 cases from 1981-1995

- 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

Antibody Testing for
HPA Testing
(uses monoclonal captured platelet glycoprotein variants)

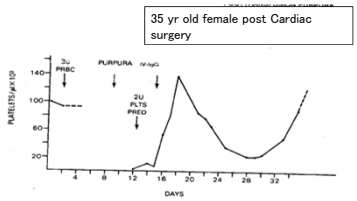


Kickler, Herman, Kunicki, Aster. Blood v. 73

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?

IV-IgG Treatment



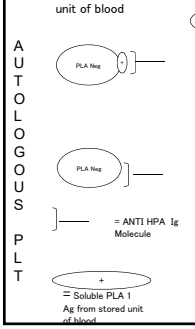
Rothko, Kickler Role of IVGG in Immune Thrombocytopenia. Blood v.

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

Pathogenesis of PTP- Why Enigmatic Destruction of Autologous Platelets By an Alloantibody?

Anamnestic immune response to make anti HPA + Soluble PLA 1 Ag from stored unit of blood



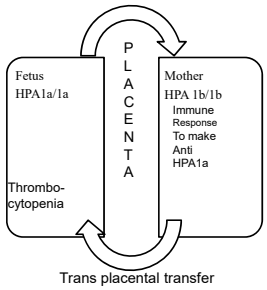
Mechanism One –Quick Recovery
- Hapten Like Mechanism: Incubating Ultracentrifuged plasma from (+) unit of stored blood permits binding of PLA 1 Ab to PLA 1 (-) Platelets
- Patient's plasma has PLA 1 antigen and it disappears with platelet recovery

Mechanism Two – Delayed Recovery
- Autoimmune Process: Display phage cloning shows autoreactive clones
- also serologic data
- HPA1a inhibits thrombopoiesis in culture

Kickler. Et al. Blood v. 75

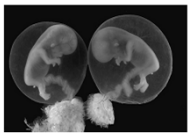
Fetal/Neonatal Alloimmune Thrombocytopenia
Due to Fetal- Maternal Incompatibility

HPA1a antigen



Trans placental transfer of Anti HPA 1a

Fetal/Neonatal Alloimmune Thrombocytopenia



Twins at 9 weeks gestational age in separate amniotic cavities. Placental villi extend from chorionic plate into maternal decidua (not present).

- Antibody formation by mother, cross placenta
- Occurs in first pregnancy- early contrast to Rh Disease
- Antigen stimulus- shed, circulating syncytiophoblast that express $\alpha_{IIB}\beta_{IIIa\ PRO\ 33}$- detect in maternal blood by 9 weeks

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

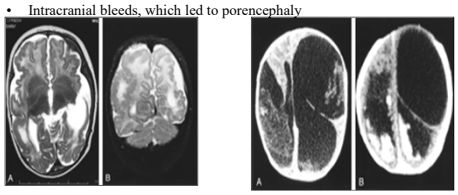
- Serious fetal consequences are common

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

CNS Bleeding In Utero

- Intracranial bleeds, which led to porencephaly



Herman, Kickler American Journal of Pediatric Hematology and Oncology 8(4):312-317, 1986.

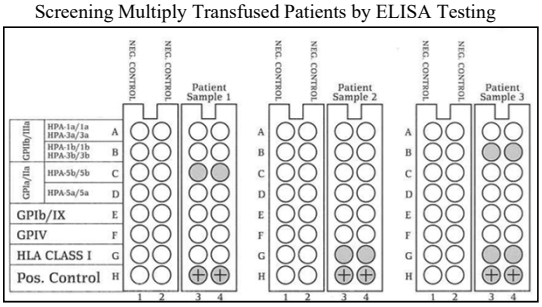
FMAIT, contd

- 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

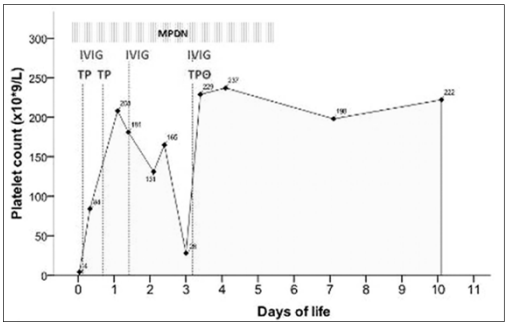
Screening Multiply Transfused Patients by ELISA Testing



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

Treatment and platelet count response.



TP – unmatched Plt TRX

TPθ – HPA matched Plt TRX

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
- Massive Transfusion
- Cardiac Surgery
- Aim for count > 50,000/ul
- Keep count >50,000
- 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 platelets-increase 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

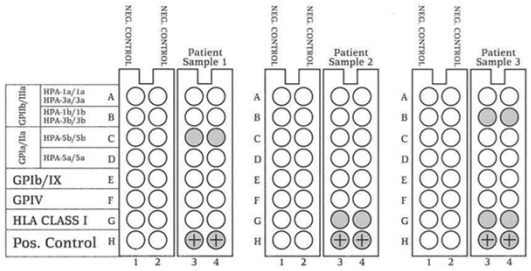
Alloimmune Platelet Transfusion Refractoriness

- 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 ***

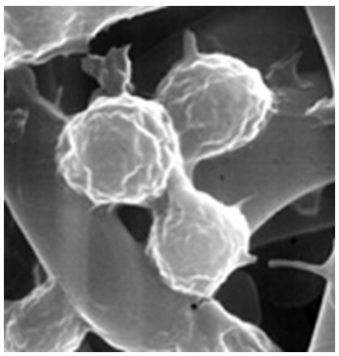
Screening Multiply Transfused Patients by ELISA Testing



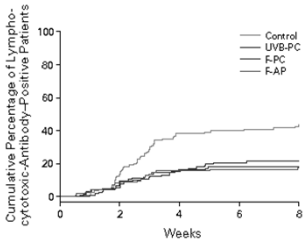
Screen weekly during extended transfusion, antibodies develop after 1-2 weeks in induction chemo of leukemic patients, may disappear– good news

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!



PREVENTION OF ALLOIMMUNIZATION



TRAP STUDY
NEJM:337:1861, 1998

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

21

White Cell Disorders

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August 15, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

21 - White Cell Disorders

Amy E. DeZern, MD, MHS

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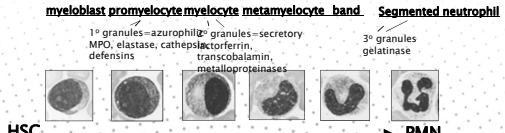
- None

MYELOPOIESIS

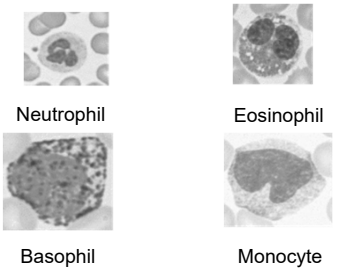


- phagocytosis
- chemotaxis
- acquisition of respiratory burst
- O_2 independent killing

Daily production = $.10^{10}$ neutrophils
Primary (classic) role = bacterial killing
Heterogeneity due to activation states/discrete subpopulations
Susceptible to priming by GSCF, LPS, TNF

HEMONC BEST PRACTICES

MATURE MYELOID CELLS



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DISORDERS OF WHITE BLOOD CELLS: TOO LOW, TOO HIGH, OR NOT FUNCTIONING PROPERLY

TOO LOW

- Neutropenia: congenital or acquired
- Shwachman–Diamond syndrome: inherited disease marked by pancreas dysfunction and congenital neutropenia
- Kostmann syndrome: severe congenital neutropenia from poor maturation

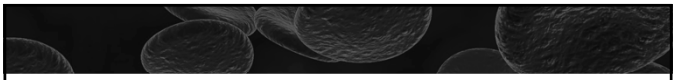
TOO HIGH

- Neutrophilia: most often acquired and occurs in response to infections or drugs
- Eosinophilia– so many causes

MALFUNCTION

- Chronic granulomatous disease– inherited disorder in which neutrophils, monocytes and macrophages cannot fight bacteria and fungal infections
- Leukocyte adhesion deficiency: inherited problem when WBCs are unable to produce the proteins they need in order to travel to the site of an infection
- Myeloperoxidase deficiency: the enzymes that help neutrophils fight bacteria are missing
- Chediak Higashi syndrome: VERY rare inherited disorder–the immune system can't properly store and release important enzyme

HEMONC



TOO LOW

HEMONC HEMATOLOGY AND MEDICAL ONCOLOGY BEST PRACTICES

MANAGEMENT OF NEUTROPENIA

Diagnosis

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

Treatment

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 BEST PRACTICES

ACQUIRED CAUSES OF NEUTROPENIA

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

8

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
- 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 BEST PRACTICES

Hsieh MM et al. Ann Intern Med 2007; 146:486.

NEUTROPENIA CAUSED BY TOXINS/ DRUGS

- Antibiotics
  - Macrolides
  - Chloramphenicol
  - Anti-malarials
  - Anti-virals
  - Dapsone
- Psychotropics
  - Clozapine
  - TCAs
- Anti-convulsants
  - Carbamazepine
  - Phenytoin
- H2 blockers
- Diuretics
- Cardiovascular drugs
  - Flecainide
- Deferiprone
- Rituximab

Bone Marrow Failure

Amy DeZern, MD

August 15, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

22 - Bone Marrow Failure

Amy E. DeZern, MD, MHS

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 therapies

Clonal evolution and transfusional iron overload cause severe morbidity and mortality

APPROACH TO DIFFERENTIAL DIAGNOSIS VARIES

Acute versus Chronic

Inherited versus Acquired (Younger vs Older)

Neoplastic versus not

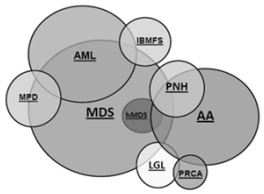
Immune mediated versus non immune

Cellular versus *hypo*cellular marrow

Primary stem cell disorder versus stem cell toxic

HEMONC BEST PRACTICES

BONE MARROW FAILURE



- **Myelodysplastic syndrome (MDS)**
- **Hypoplastic MDS**
- **Aplastic anemia (AA)**
- **Large granular lymphocytosis (LGL)**
- **Pure Red Cell Aplasia (PRCA)**
- **Paroxysmal nocturnal hemoglobinuria (PNH)**
- **Inherited syndromes**
- **Nutritional deficiencies**

DIFFERENTIAL DIAGNOSIS OF PANCYTOPENIA

With hypocellular BM

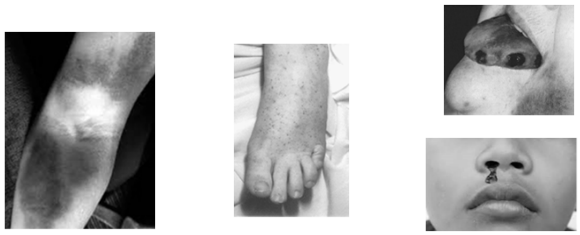
- Aplastic anemia
  - acquired versus constitutional (FA, DKC)
- Myelodysplasia (<15% cases)
- Myelofibrosis ("dry tap")
- Rarely: ALL (children), AML (elderly) as aleukemic leukemia), lymphoma

With normo- or hypercellular BM

- **Primary marrow disease**
  - MDS
  - PNH
  - Myelofibrosis
  - Leukemias and lymphomas
  - Hairy cell leukemia
  - Large granular lymphocytosis
- **Secondary**
  - Hypersplenism
  - Systemic lupus erythematosus
  - Vitamin deficiency (B12, folate)
  - Alcohol
  - Tuberculosis and other mycobacteria; brucellosis; L
  - Sarcoidosis

HEMONC BEST PRACTICES

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
- Fanconi Anemia
- Dyskeratosis congenita

Nonclonal disorders
- Vitamin deficiencies
- Toxins

HEMONC BEST PRACTICES

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 post surgery

HEMONC BEST PRACTICES

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

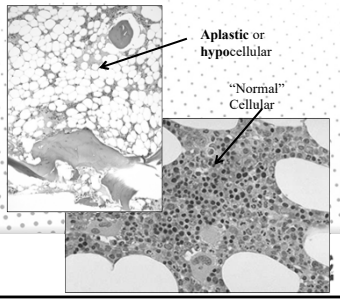
HEMONC BEST PRACTICES

APLASTIC ANEMIA

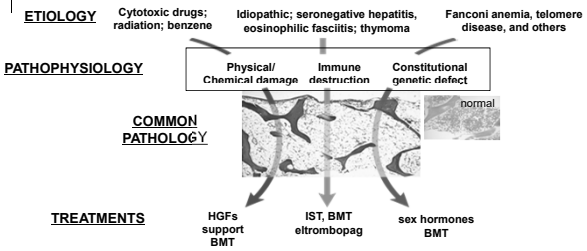
HEMONC
HEMATOLOGY AND MEDICAL ONCOLOGY
BEST PRACTICES

APLASTIC ANEMIA IS BONE MARROW FAILURE

- The bone marrow is the spongy stem cell tissue that produces the blood:
  - Red cells
  - White cells (neutrophils)
  - Platelets
- When all three cell lines are low→ Pancytopenia



DEFINING "APLASTIC ANEMIA"



DRUG/ TOXIN CAUSES

Carbamazepine
Phenytoin
Hydantoins
Sulfonamidse
Chloramphenicol
Phenylbutazeon
Indomethacin
Methimazole
Propylthiouracil
Gold
Arsenicals
Benzene

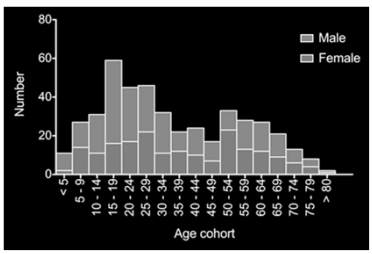
EPIDEMIOLOGY

Precise estimates of number of patients with AA are confounded by imprecision in diagnosis
- In Europe, Israel, USA ~2-4 cases per 1 million people
- Higher in Asia ~5-8 cases per 1 million people

Two age groups for presentation
- Ages 15-25 years
- Age >60 years

Acquired and inherited cases
- Most acquired are idiopathic
- Drug or toxins cause are minority

AGE AT DIAGNOSIS OF SEVERE APLASTIC ANEMIA



DISTINGUISHING ACQUIRED FROM CONSTITUTIONAL AA

- Age
- Family history
- Physical anomalies

Fanconi
Stature; thumb anomalies, facies, urogenital structural abnormalities, infertility.
Dx: FA chromosome stress (PB), focused genetic testing

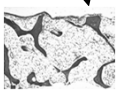
Short telomere syndroms
Early greying of hair; history or family history low blood counts, AML, liver/lung disease; pregnancy complications (miscarriages). Often gradual onset, ANC spared.
Dx: telomere length by flow-FISH (CLIA certified), focused genetic testing

GATA2 deficiency
Personal and family history of warts, severe infections; AML in family.
Dx: focused genetic testing

CONSTITUTIONAL MARROW FAILURE

PATHOPHYSIOLOGY

Constitutional genetic defect



Component of defined genetic syndromes

- often pediatric (not always)
- usually family history
- multi-organ involvement typical
- may be physical anomalies
- rare in acute and severe pancytopenia

SCREENING FOR GERMLINE MUTATIONS IN HUMAN BONE MARROW FAILURE

Syndrome	Gene(s)	Inheritance, mechanism Mutation types	Predominant age of reported MDS/AML onset (range)	Heme features	Extra-hematopoietic features	Other cancers	Implications for management
Fanconi anemia	21 genes to date	AR AD (FANCR) X-linked (FANCB), loss-of-function Missense, FS/NS, splicing, CNV	AYA (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, CNV	Adult > Ped (range 2-77)	AA, MDS, AML	Nail dystrophy, oral leukoplakia, reticular skin pigmentation, pulmonary fibrosis, liver disease, vascular abnormalities, stenosis, severe congenital	SCC of head, neck and anogenita	Attenuated regimen, radiosensitive

SCREENING FOR GERMLINE MUTATIONS IN HUMAN BONE MARROW FAILURE

Syndrome	Gene(s)	Inheritance, mechanism Mutation types	Predominant age of reported MDS/AML onset (range)	Heme features	Extra-hematopoietic features	Other cancers	Implications for management
Familial MDS/AML with GATA2 mutation	GATA2	AD, haploinsufficient Missense, NS/FS, duplication, splicing, regulatory, CNV	AYA (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, haploinsufficient or dominant negative, depending on mutation Missense, FS/NS, duplication, CNV	Adult > Ped (range 5-72)	MDS, AML, T-ALL, hairy cell leukemia, CMML Mild-to-moderate thrombocytopenia Normal plt size Abnormal function	Case report of co-occurring eczema	--	

SCREENING FOR GERMLINE MUTATIONS IN HUMAN BONE MARROW FAILURE

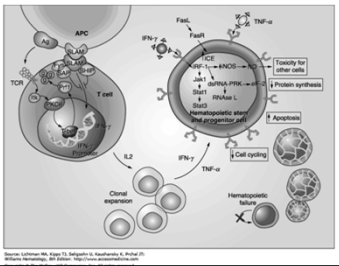
Syndrome	Gene(s)	Inheritance, mechanism Mutation types	Predominant age of reported MDS/AML onset (range)	Heme features	Extra-hematopoietic features	Other cancers	Implications for management
Germline SAMD9/SAMD9L	SAMD9 SAMD9L	AD, gain-of-function Missense	Ped, rare adult (range 1-56)	AA, MDS, AML, CMML[a] Increased prevalence of monosomy 7	MIRAGE syndrome (SAMD9) Ataxia-Pancytopenia (SAMD9L)	--	
Diamond-Blackfan anemia	22 genes to date	AD, X-linked (GATA1, TSR2) haploinsufficient, loss-of-function Missense, FS/NS, splicing, CNV, 3' UTR	Adult > Ped (range 2-57)	Macrocytic pure red cell aplasia, MDS, AML, ALL	Growth retardation, congenital malformations in <50% (head, limbs, GU, heart)	Osteosarcoma, colon, possibly others	
Shwachman-Diamond	SBDS DNAJC21 EFL1	AR, loss-of-function Missense, FS/NS, splice	AYA (range 2-53)	Neutropenia, MDS, AML	Exocrine pancreatic insufficiency, neurodevelopmental & skeletal	--	

SCREENING FOR GERMLINE MUTATIONS IN HUMAN BONE MARROW FAILURE

Syndrome	Gene(s)	Inheritance, mechanism Mutation types	Predominant age of reported MDS/AML onset (range)	Heme features	Extra-hematopoietic features	Other cancers	Implications for management
ANKRD26-related thrombocytopenia	ANKRD26	AD, suspected to be gain-of-function SNV or small deletion in 5' UTR, N-terminal truncating variants, missense[a]	Adult (range 26-70)	MDS, AML, CML CMML, CLL Thrombocytopenia Normal plt size Abnormal function	--	--	Mild bleeding tendency
ETV6-related thrombocytopenia	ETV6	AD, incompletely understand, possibly dominant negative	Ped + Adult (range 8-82)	AA, B-ALL, MDS, AML, CMML, DLBCL Variable thrombocytopenia Normal plt size	Not shared across pedigrees (developmental delay, dysmorphisms, autoimmunities)	Colon, breast, meningioma	Mild-to-moderate bleeding tendency

IMMUNE APLASTIC ANEMIA
(PATHOPHYSIOLOGY)

T cell immune attack at the CD34 progenitor cell



CLASSIFICATION OF AA: CAMITTA CRITERIA

Peripheral Blood Cytopenias	Non-severe (Moderate) aplastic anemia (not meeting criteria for severe disease)	Severe aplastic anemia (any 2 of 3)	Very-severe aplastic anemia (meets criteria for severe disease and absolute neutrophils < 200)
Bone marrow cellularity	< 25%	< 25%	< 25%
Absolute neutrophil count		< 500 / μl	< 200 / μl
Platelet count		< 20,000 / μl	
Reticulocyte count		< 1.0% corrected or < 60,000 / μl	

Camitta BM et al Blood. 1976;48:63–70

CLASSIFICATION OF AA: CAMITTA CRITERIA

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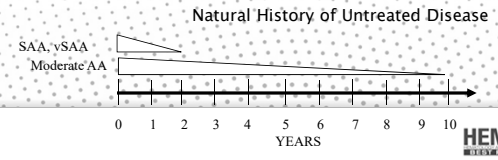
Camitta BM et al Blood. 1976;48:63–70

	Aplastic Anemia	MDS
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

Brodsky, RA Wintrobe's Clinical Hematology

DECISION TO TREAT

Based on disease severity
- Severe and Very Severe AA require prompt therapy
- Moderate AA does not necessarily



HEMONC BEST PRACTICES

SUPPORTIVE CARE

Central Venous Catheter
- considered for all patients with AA, given the frequency of phlebotomy, transfusions, and administration of therapeutic medications (PICC, Hickman, Mediport)

Blood transfusions
- *Irradiated* -- prevent transfusion associated GVHD
- *Leukofiltered* -- reduce viral infections and prevent alloimmunization

Growth factors
- May provide clinical benefit but do not induce disease remissions

Infections
- Granulocyte transfusions ~controversial
- Antibiotics = important

Marsh J, et al. BrJHaematol 2010.
Quillen K et al. Haematologica 2009
Marsh J et al. Semin Hematol 2007.

HEMONC BEST PRACTICES

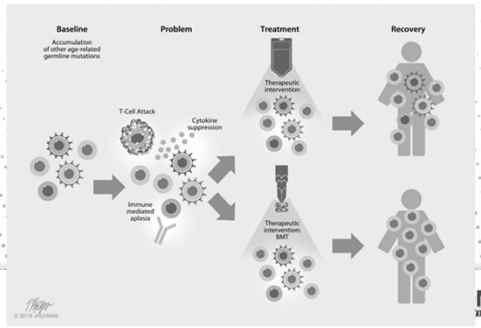
IDEAL THERAPY FOR SAA

Available to all patients
- Not limited by age and donor status

Low toxicity
- Rapid hematopoietic reconstitution
- Low risk for graft failure/GVHD/infections

Reduces or eliminates risk of secondary clonal disease
- MDS/Leukemia
- PNH

HEMONC BEST PRACTICES



IMMUNOSUPPRESSIVE THERAPY (IST)

STANDARD OF CARE:

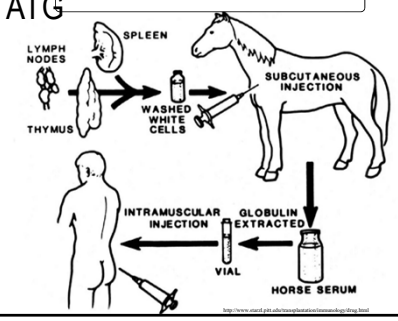
Anti-thymocyte globulin (ATG) (anti lymphocyte globulin ALG)

Polyclonal buffed serum from horse or rabbit that has been immunized with human T cells
- HORSE >RABBIT in USA

Often ATG is usually combined with pill immunosuppressants as well- usually CYCLOSPORINE (CsA)

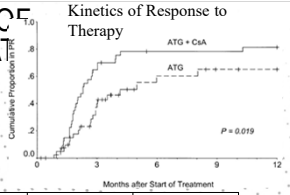
Metrics to evaluate treatment: response, relapse, survival, clonal evolution (getting MDS, AML, PNH)

PREPARATION AND USE OF ATG



2003 REPORT OF RESPONSE RAT

ATG vs ATG CsA



	ATG alone	ATG + CsA	P value
Response Rate	41%	70%	0.015
Relapse Rate	45%	30%	0.4
Overall Survival	54%	58%	0.6
Clonal Evolution	The actuarial probability of malignant diseases was 18% at 11.3 years. It was 8% for MDS or leukemia. The interval from treatment of AA to the diagnosis of MDS or leukemia was 6.6 to 9.5 years.		

Blood. 2003 Feb 15;101(4):1236-42.

HORSE ATG + CSA

Response

Cumulative incidence of relapse at 3 years= 28%

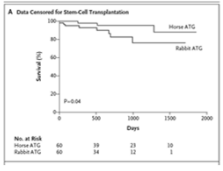
Incidence of clonal evolution= 21%
- Monosomy 7 (=MDS)
- Leukemia

Other studies
- 35% relapse at 5 years
- Clonal evolution to MDS or PNH as high as 10%

Kojima et al, *Blood* 2002
Rosenfeld et al, *JAMA* 2003

Overall Survival

96% at 3 years



Scheinberg et al *NEJM* 2011

IST

Antithymocyte globin (ATG) + Cyclosporine (CsA)
- →60-70% response rate!!
- Horse (71% response rate) versus Rabbit (43% response rate)
- 2005-2010 hematologic response at 6 mos (blood counts) 120 patients (60 in each group)

Table 2. **Hematologic Response at 3 and 6 Months to Horse ATG and Rabbit ATG.**

Response	Horse ATG (N=60)	95% CI	Rabbit ATG (N=60)	95% CI	P Value
	no. (%)		*no. (%)*		
At 3 mo	37 (62)	49–74	20 (33)	21–46	0.002
At 6 mo	41 (68)	56–80	22 (37)	24–49	<0.001

ELTROMBOPAG ADDED TO HATG/CSA TO ENHANCE CR RATES

hATG> rATG is US standard- 60-70% response rate (CR and PR)

- Immunosuppression alone has induced complete responses in approximately 10% of patients historically.
- In a phase 2 clinical trial, eltrombopag plus standard IST resulted in a 6-month complete-response rate of 58% among patients receiving eltrombopag for 6 months

Townsley et al NEJM 2017

HEMONC BEST PRACTICES 37

Eltrombopag Added to Standard Immunosuppression for Aplastic Anemia

Danielle M. Townsley, M.D., Phillip Scheinberg, M.D., Thomas Winkler, M.D., Ronan Desmond, M.D., Bogdan Dumitriu, M.D., Olga Rios, R.N., Barbara Weinstein, B.S.N., Janet Valdez, P.A., Jennifer Lotter, P.A., Xingmin Feng, Ph.D., Marie Desierto, B.S., Harshraj Leuva, M.B., B.S., Margaret Bevans, Ph.D., Colin Wu, Ph.D., Andre Larochelle, M.D., Ph.D., Katherine R. Calvo, M.D., Cynthia E. Dunbar, M.D., and Neal S. Young, M.D.

ATG + Cyclosporin + Eltrombopag

- Improved complete response (CR) rates at 6 months
- Improved overall response (OR) rates at 6 months

FDA APPROVED

Table 2. Hematologic Response in Patients Treated with Immunosuppression and Eltrombopag.*

Cohort and Response	Rate at 3 Mo	Rate at 6 Mo	P Value
Cohort 1			
No. of patients	30	30	
Response — no. (% [95% CI])			
Overall response	23 (77 [61–93])	24 (80 [65–95])	
Partial response	18 (60 [41–79])	14 (47 [28–66])	
Complete response	5 (17 [3–31])	10 (33 [15–51])	0.01
Cohort 2			
No. of patients	31	31	
Response — no. (% [95% CI])			
Overall response	24 (77 [62–93])	27 (87 [75–100])	
Partial response	16 (52 [33–70])	19 (61 [43–79])	
Complete response	8 (26 [9–42])	8 (26 [9–42])	0.06
Cohort 3			
No. of patients	31	31	
Response — no. (% [95% CI])			
Overall response	27 (87 [75–100])	29 (94 [84–103])	
Partial response	12 (39 [21–57])	11 (35 [18–53])	
Complete response	15 (48 [30–67])	18 (58 [40–76])	<0.001
All cohorts			
No. of patients	92	92	
Response — no. (% [95% CI])			
Overall response	74 (80 [72–89])	80 (87 [80–94])	<0.001†
Partial response	46 (50 [40–60])	44 (48 [37–58])	
Complete response	28 (30 [21–40])	36 (39 [29–49])	<0.001

ClinicalTrials.gov: NCT01623167

HEMONC BEST PRACTICES

RESPONSE TO EPAG AT 6 MONTHS BY AGE

	Adults		Pediatrics	
	IST n=286	EPAG n=131	IST n=87	EPAG n=40
Overall Response	57%	82%	72%	70%
	P<0.001		p=0.78	

Groarke et al ASH 2019

HEMONC BEST PRACTICES

RESPONSE TO IMMUNOSUPPRESSION

PNH clones and telomeres predict response to IST in Pediatric AA

Prospective study of 113 children (Ages 0-16)

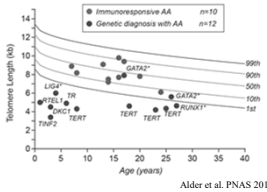
All had PNH clones and telomeres done in CLIA certified way before IST

Response assessed based on PNH clone presence and telomere length

- If PNH clone + and telomeres "long"- ~70% response
- If PNH clone neg and telomeres "short"- ~19% response
- **Suggestion for upfront HSCT if in this group**

Narita et al. Haematologica 2015

TELOMERES ARE "LONG" IN PATIENTS WITH IMMUNE RESPONSIVE AA COMPARED TO INHERITED AA

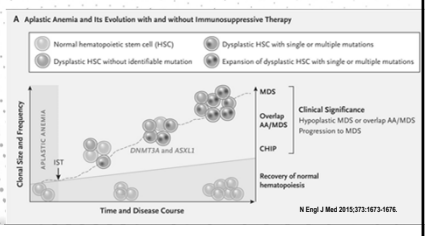


Alder et al. PNAS 2018

CLONALITY INCREASINGLY A PROBLEM

As survival is improving, more late development of MDS/AML in ≥15% of patients= clonal evolution

Extrapolation from MDS field→ somatic mutational testing



HEMONC BEST PRACTICES

TARGETED SEQUENCING OF MDS ASSOCIATED GENES IN AA INCREASED REPORTED

Paper	N	Dx Age of patients (yrs)	N genes/ Method	# mutations 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	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, NCI in 9	2 (5.3)	**SLIT1, 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 SNP6 platform	29 (19.3 %)	**ASXL1, DNMT3A, BCOR**	20 % (<10 % in 41 % patients)	17 (11.3%)
Huang 2015	138 (106SAA, 71 SAA, 57 NSAA) 20 pts with PNH clone	30.7 (5-76)	6; Sanger sequencing (focus on TET2 and ASLXL1)	24(17.4%)	**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 NGS: 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	16 (72 %); 2 (9 %) with an MDS-associated SM	**PIGA, LOH6p, STAT5B, CAMK2b**	>20 %	n/a
Negoro 2017	258 AA and 59 PNH PRE and POST IST	Need supplemen ta	WES and targeted deep sequencing # genes	69/133 AA (32/71 presentatio n vs. 42/74 after IS)	**ASXL1, DNMT3A, PIGA**	P	N/A

SIDE EFFECTS OF ATG

USUALLY TEMPORARY

Decrease in blood counts further
- Increased need for transfusions
- Increased risk of bleeding
- Increased risk of infection

Liver toxicity
- Rise in transaminases (AST/ ALT)

Kidney Toxicity
- Rise in creatinine

- SERUM SICKNESS

SIDE EFFECTS OF C

USUALLY DECREASE WHEN DRUG DECREASED/

GI toxicity (nausea, diarrhea)
High blood pressure
Kidney toxicity
Headaches
Tremor (can limit driving rarely)
Infections
Thickening of gums in mouth
Increased hirsutism
Peripheral neuropathy

HEMONC BEST PRACTICES

SERUM SICKNESS

Reaction to the horse (or rabbit) proteins
- Immune complex hypersensitivity reaction

Flu-ish feeling when getting ATG

High fevers, flushing, myalgias

Usually within 4-10 days of ATG

To reduce the incidence of this, methylprednisolone 1mg/kg should be administered with the ATG and then steroids continue and are tapered over the subsequent month

HEMONC BEST PRACTICES

SUMMARY OF IST

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

HEMONC BEST PRACTICES

BMT TRIALS FOR SAA: HISTORICAL PERSPECTIVE ON MATCHED SIBLING TRIALS

Institution	Years	N	Median Age (Range)	Engraftment (%)	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	1998-2007	239	42 (30-67)	86	61	4	20	25

CONTEMPORARY MATCHED DONOR TRANSPLANT FOR SAA

Retrospective report from CIBMTR for BMT 2008-2013 at 145 centers
- Received h- or r-ATG transplanted with bone marrow grafts from a HLA-matched sibling or unrelated donor- comparisons by ATG type
- 833 patients age 1-71 yo with acquired SAA
  - 546 MSD and 287 URD

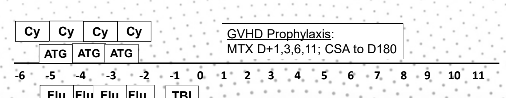
- Engraftment 86-90% with median time to ANC >500 17-19 days
- cGVHD 9-20% (less with r-ATG)- none received post transplant CY
- OS at 3yrs 92-87%

Late effects reasonable with RIC/ NMA conditioning
- fertility preserved with matched donor ~90%

Kekre et al Haematologica Jul 2017:1291-1298; Eapen et al. Br J Haem 2000 Dec;111(3):754-60; Loren et al. BBMT 2011 Feb;17(2):157-66.

HEMONC BEST PRACTICES

SOC Treatment Schemas For Matched BMT



GVHD Prophylaxis:
MTX D+1,3,6,11; CSA to D180

Cy = Cyclophosphamide 50mg/kg (or 35)
ATG = hATG range 60-150 mg/kg; r-ATG 6-15 mg/kg
Flu = Fludarabine 30 mg/m² IV daily
TBI = 200 cGy

Storb et al Blood 1994

HEMONC BEST PRACTICES

UPFRONT ALTERNATIVE DONORS INCREASING IN POPULARITY

MUD and mMUD in UK= Similar OS

29 SAA children had unrelated donor HSCT without prior IST in UK (24 10/10, 5 mismatched)

Compares each with 3 matched controls from the database of the SAAWP of the EBMT with MSD HSCT

No PTCy CSA alone or CSA + MTX

compared to 91% in MSD and 94% in IST

1 failure to engraft

@ 1 yr CI of cGVHD 19%

Dufour et al BJH 2015

Haplos Upfront in China

89 upfront Haplos

Retrospective registry study

No PTCy--- CSA, MMF, MTX

OS @2 yrs: 86 %

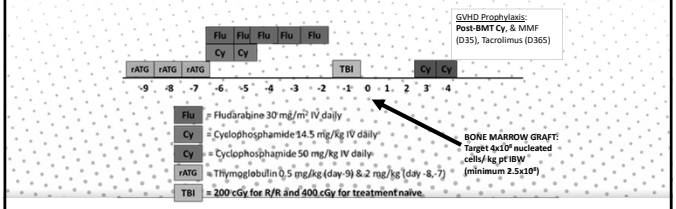
EFS @2yrs 85%

Increased CI grades II-IV aGVHD 30%

extensive cGVHD 3.4%

Xu et al Hematol Oncol 2017

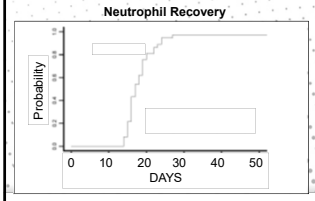
HAPLO BMT: THE HOPKINS EXPERIENCE



HEMONC BEST PRACTICES

DeZern et al BBMT 2016 and ASH 2019

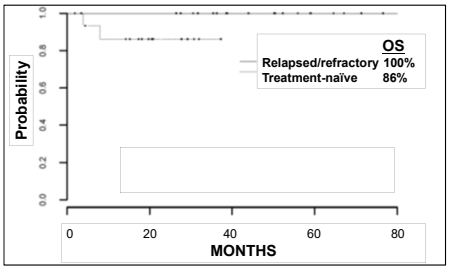
RAPID HEMATOPOIETIC RECOVERY



DAYS	Relapsed/ Refractory	Treatment – Naïve	Overall Median
Neutrophil engraftment	18 (14-39)	17 (14-88)	17
Red cell engraftment	20 (6-58)	19 (14-291)	19
Platelet engraftment	29.5 (15-108)	24 (18-25)	25

OVERALL SURVIVAL IS EXCELLENT

95% FOR ALL PATIENTS



HIGH CURE RATE WITH HAPLO BMT IN SAA

RELAPSE and REFRACTORY

Excellent Disease Free Survival

- All 20 alive, transfusion-independent, without clonality (KPS 100)
- One primary graft failure (engrafted with 2nd BMT from different donor)
- Acute GVHD grade II-IV 2/2 (9.5%)
- Extensive chronic GVHD 1/20 (5%)

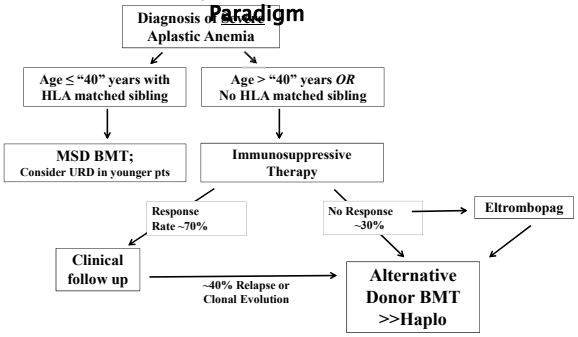
Multicenter BMT CTN trial ongoing

TREATMENT- NAÏVE

- **Excellent Disease Free Survival**
  - 15/17 (88%) alive, transfusion independent without clonality (KPS 100)
  - 400 cGy appears superior to 200 cGy in upfront setting
    - 5/7 engrafted with 200 TBI
    - 10/10 engrafted with 400 TBI
  - Longer follow-up and more experience necessary

HEMONC BEST PRACTICES

2020 Severe Aplastic Anemia Treatment Paradigm



XYSMAL NOCTURNAL HEMOGLOBINURIA

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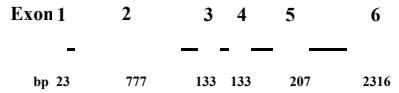
HEMATOLOGY AND MEDICAL ONCOLOGY
BEST PRACTICES

PNH PATHOPHYSIOLOGY

- Acquired Clonal Multipotent Hematopoietic Stem Cell Disease
- PIG-A mutation
  - X(p22.1)
- PIG-A gene product necessary for 1st step in the biosynthesis of GPI anchors
- PNH cells have deficiency or absence of all GPI anchored proteins

HEMONC BEST PRACTICES

PIG-A CODING REGION



PIGA Mutations

- **Frameshift – small insertions/deletions → Stop codon**
- **Nonsense → Stop codon**
- **Splice defect → Deleted exon**
- **Missense (substitution) → May have residual activity**

PNH

Pathogenesis of hemolytic anemia

CD59
- **Membrane inhibitor of reactive lysis**
- **Prevents incorporation of C9 into C5b-8; thus, MAC does not form**

CD55
- **Decay accelerating factor**
- **Block C3 convertase**

Protect cells from complement-mediated destruction

HEMONC BEST PRACTICES

CLASSIFICATION OF PNH

IPIG CRITERIA

Classical PNH
- **hemolytic and thrombotic patients who have evidence of PNH in the absence of another bone marrow failure disorder**

PNH in the context of other primary bone marrow disorders
- aplastic anemia or myelodysplastic syndrome

Subclinical PNH
- small PNH clones but no clinical or laboratory evidence of hemolysis or thrombosis

Parker et al. Blood 2005

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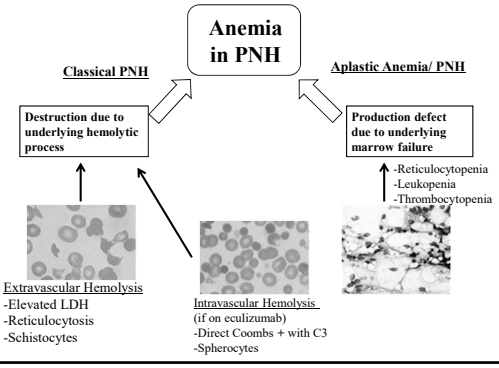
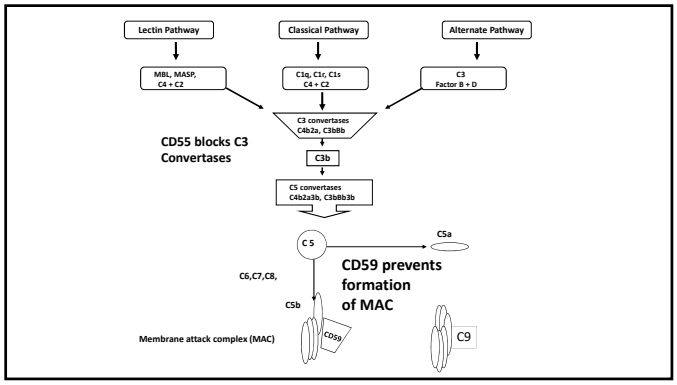
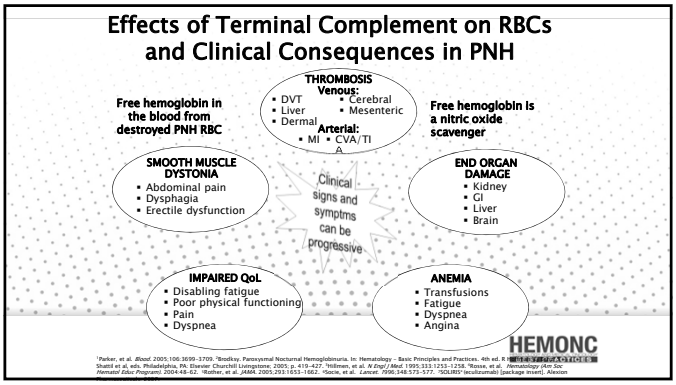




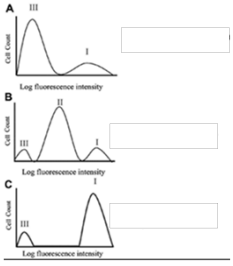


WHICH PATIENTS SHOULD BE SCREENED FOR PNH?

- Hemoglobinuria
- Hemolytic anemia
- Bone marrow dysfunction
  - Aplastic anemia (AA) or MDS screened periodically
- Coombs-negative intravascular hemolysis
  - Elevated serum LDH
- Unusual or unexplained venous thrombosis
  - Budd-Chiari syndrome
  - Mesenteric, portal, cerebral, or dermal veins
- Unexplained arterial thrombosis
- Episodic dysphagia or abdominal pain with evidence of chronic hemolysis

HEMONC BEST PRACTICES

TYPES OF PNH CELLS



Type III cells: no GPI-AP

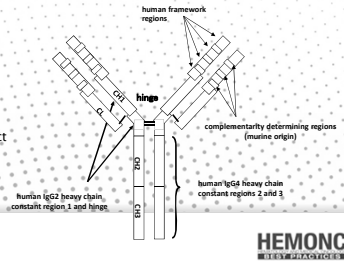
Type II cells: partial GPI-AP Expression

Type I cells: Normal GPI-AP

COMPLEMENT INHIBITION IS A HIGHLY EFFECTIVE THERAPY FOR CLASSICAL PNH

Eculizumab

- Humanized monoclonal
- Antibody binds to C5
- Terminal complement activity is blocked
- Proximal functions of complement remain intact
- Weak anaphylatoxin
- Immune complex clearance
- Microbial opsonization



HEMONC BEST PRACTICES

ECULIZUMAB STUDIES IN PNH

Pilot Study – *NEJM* 2004
N = 11

TRIUMPH – *NEJM* 2006
Pivotal Phase III, Double-Blind, Placebo-Controlled Trial, N = 87

Long-Term Extension Trial
Evaluated long-term safety, efficacy and effect on thrombosis; Placebo patients switched to eculizumab
N = 187

SHEPHERD – *Blood* 2008
Broader patient population, including those receiving minimal transfusions or with thrombocytopenia, N = 97

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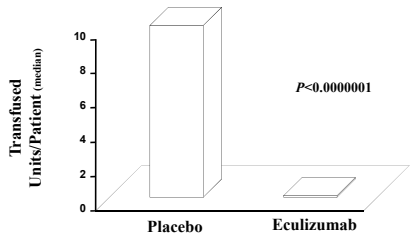
DOSING SCHEDULE
USED FOR ECULIZUMAB

Pretreatment		Induction Phase				Maintenance Phase				
≥ 2 weeks before induction	Week →	1	2	3	4	5	6	7	8	9 and every 2 weeks thereafter
Neisseria meningitidis vaccination	SOLIRIS® dose, mg →	600	600	600	600	900	X	900	X	900

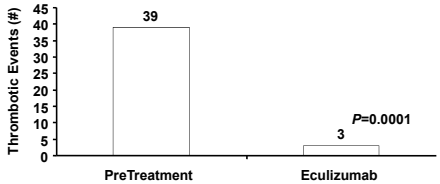
- Administered via IV infusion over 35 minutes every 7 days during induction and every 14 days during maintenance
- Eculizumab dose adjustment to every 12 days may be necessary for some patients to maintain LDH reduction

SOLIRIS® (eculizumab) [package insert]. Alexion Pharmaceuticals; 2007.

EFFECT OF ECULIZUMAB ON TRANSFUSION



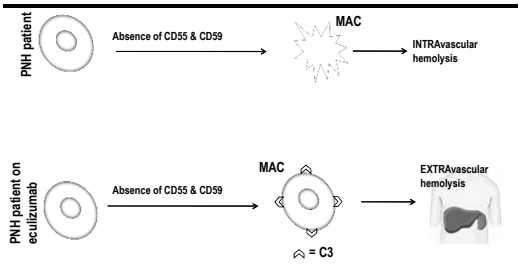
CLOTS IN PATIENTS WITH AND WITHOUT ECULIZUMAB



92% Fewer thrombotic events with SOLIRIS treatment
7.37 clots/100 pt yrs vs 1.07 clots/100 pt yrs
Most patients (63%) received concomitant anticoagulants
The effect of anticoagulant withdrawal was not studied

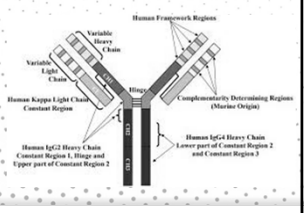
Hillmen P, et al. *Blood*. 2007;110: 4123-4128

INTRAVASCULAR AND EXTRAVASCULAR HEMOLYSIS IN PNH



RAVULIZUMAB: FDA APPROVAL DEC 2018
FOR THE TREATMENT OF ADULT PATIENTS WITH PNH

- Long acting C5 blockade- 4 fold longer dosing interval compared to eculizumab (q8 wks)
- Randomized phase 3: 246 adults with classical PNH randomized 1:1 to either ravulizumab (n=125) or eculizumab (n=119)
- By 26-week follow-up, ravulizumab was noninferior to eculizumab
- Administed at a loading dose of 2,400 mg, 2,700 mg, or 3,000 mg (depending on weight) D1
- Maintenance doses of 3,000 mg, 3,300 mg, or 3,600 mg on day 15 and every eight weeks thereafter



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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

RE RED CELL APLASIA

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BEST PRACTICES

PURE RED CELL APLASIA
CLINICAL CHARACTERISTICS

rare
females>males
clinical associations:
- thymoma
- CLL
- neoplasia, collagen vascular disease
- medical drugs (anti-epileptics)
- viruses

immune mechanisms
- antibodies to erythroid precursors
- **autoimmune cytotoxic T cells**
- antibodies to Epo (iatrogenic; historical)

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DIFFERENTIAL DIAGNOSIS OF PRCA

Self-limited
- transient erythroblastopenia of childhood
- transient aplastic crisis of hemolytic disease (B19)

Pregnancy

Fetal
- nonimmune hydrops fetalis (B19)

Constitutional
- Diamond-Blackfan anemia

Acquired
- persistent B19 parvovirus infection
- immune-mediated (including thymoma-associated)
- MDS (5q-)

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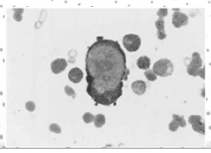
PARVOVIRUS B-19 INDUCED RED CELL APLASIA

Globoside (p-antigen)
- Viral target on erythroid progenitors

Susceptible hosts
- Immunosuppressed pts
- Chronic hemolytic anemia

Diagnosis
- DNA based assays
- Antibodies
- Giant pronormoblast



Treatment - IVIG

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PARVOVIRUS B19 AND ANEMIA

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)
Immuno-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)

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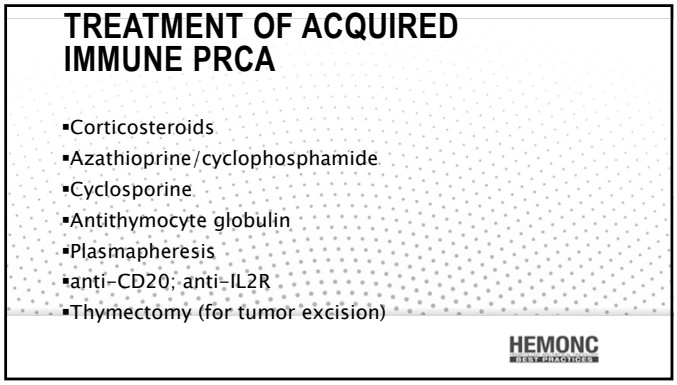
DeZern et al. *Exp Hem* 2013.



Consumptive Thrombohemorrhagic Disorders (DIC, TTP, HUS)

Michele Lambert, MD

August 15, 2020

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

23 – Consumptive Thrombohemorrhagic Disorders (DIC, TTP, HUS)

Michele Lambert, MD

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DISCLOSURES

- **Consultant:** Novartis, Dynamed(EBESCO), Octapharma, Sysmex, CSL Behring, Dova, Shionogi, Bayer, Rigel, Principia, Argenx, DOJ;
- **Research Funding:** OctaPharma, Novartis, Astra Zeneca, Quansys, Sysmex;
- **Medical Advisor:** PDSA, 22q11.2 Society, CdLS Foundation;
- **Medical-legal firms:** Consulting, Testimony

Off Label Usage

- Rituximab for TTP
- Eculizumab for TMA (not aHUS)

2

THROMBOTIC MICROANGIOPATHIES…

Hemolytic Anemia	Thrombocytopenia	Organ Injury

Infection-induced HUS	Atypical HUS	Acquired TTP	Congenital TTP

TA-TMA	Drug induced-TMA	Other congenital TMA

HIT	CAR-T	HELLP	CAPS	ECMO
Cancer	Cardiac Devices	Metabolic	SLE	TAMOF

3

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

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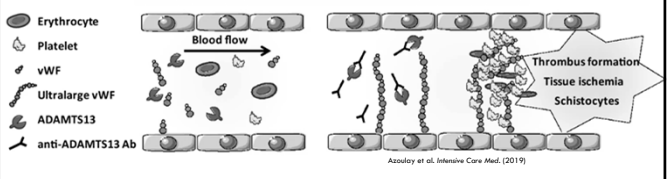
THROMBOTIC MICROANGIOPATHIES



Azoulay et al. *Intensive Care Med.* (2019)

5

TREATMENT OF TTP

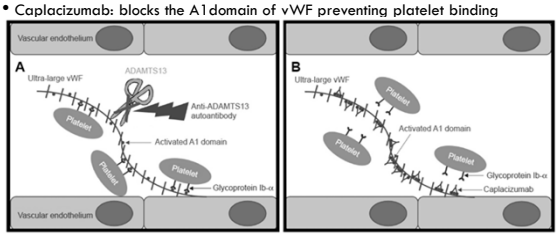
- Replenish ADAMTS13 activity (FFP, plasma exchange)
- Remove inhibitors (if acquired) (steroids and rituximab, plasma exchange)
- Remove ULVWF (plasma exchange)

- If complement activation is playing a role, strategies to block complement may be necessary as adjunctive therapies

6

TREATMENT OF TTP

- Caplacizumab: blocks the A1 domain of vWF preventing platelet binding



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TREATMENT OF TTP

- ISTH recommendations:
  - 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 recommended

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CLASSICAL DIFFERENTIAL DIAGNOSIS

HUS	TTP
Renal dysfunction **(Can have neurological dysfunction)** **Hemolytic anemia** **Thrombocytopenia** ***Shiga-toxin / neuraminidase*** ***(E coli, Strep)*** ***Complement-mediated (atypical)***	**Neurologic symptoms** **(Can have renal failure)** **Hemolytic anemia** **Thrombocytopenia** ***ADAMTS13 deficiency (congenital or acquired)***

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HEMOLYTIC UREMIC SYNDROME

- infection associated
  - Shiga-toxin producing *E.Coli* - STEC
  - *s. pneumonia*
  - Others (Influenza, H1N1, HIV) COVID19?
- atypical HUS
  - 50-60% alternative complement pathway
  - 5% thrombomodulin mutations
  - Some mediated by anti-complement factor H antibodies

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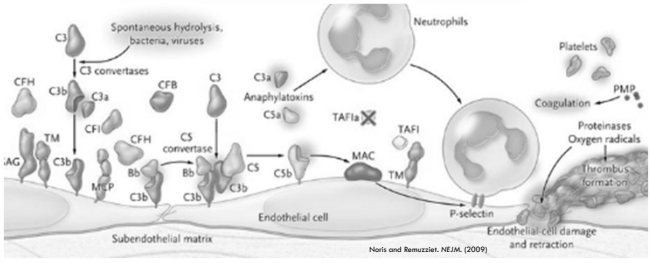
HEMOLYTIC UREMIC SYNDROME

- Metabolic Disease related
  - Cobalamine C defects
  - DGKE mutations
- Underlying conditions
  - Transplant associated
  - Malignancy
  - Autoimmune disorders (SLE, CAPS)
  - Medication induced (calcineurin inhibitors)
  - Malignant HTN

11

HUS – COMPLEMENT DYSREGULATION



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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)

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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

14

SO WHAT ABOUT DIC…

How does that fit in with the TMAs and how do we tell the difference?

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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

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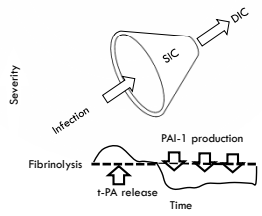
SEPSIS INDUCED COAGULOPATHY(SIC) DIC

☐ Thrombotic phenotype
☐ Fibrinolytic phenotype



Iba, T.; Levy, J.H.; Raj, A.; Warkentin, T.E. Advance in the Management of Sepsis-Induced Coagulopathy and Disseminated Intravascular Coagulation. *J. Clin. Med.* **2019**, *8*, 728.

17

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
- Based on the trigger, DIC may look different
  - Sepsis mediated DIC more organ dysfunction and thrombosis, higher fibrinogen
  - Fibrinolytic (non-sepsis) DIC have more bleeding (malignancy, placental abruption)

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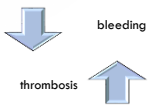
CLINICALLY RELEVANT DIC

- 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



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SEPSIS INDUCED COAGULOPATHY SCORE

- Platelet count
- INR
- SOFA score (sequential organ failure assessment) (used to confirm presence of sepsis and is limited to 2 points);

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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 10^9/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 was only 28%	
≥100 mg/dL	0
<100 mg/dL	1

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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
- Important to make the right diagnosis

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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

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Review on DIC, TTP, HUS and HIT and Limb Gangrene

Theodore (Ted) E. Warkentin, MD

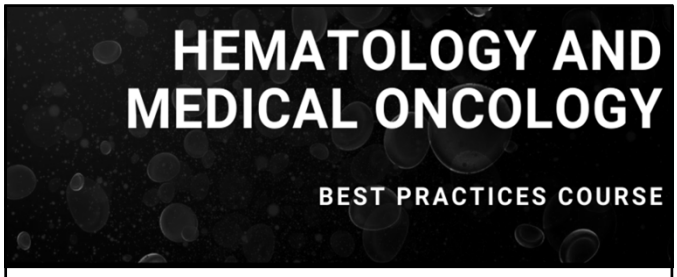
August 15, 2020

25

Hematologic Complications of Pregnancy

Robert S. Siegel, MD

August 15, 2020



25 - Hematologic Complications of Pregnancy

Robert S. Siegel, MD

1

Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

- Consultant: CVS/Caremark (Formulary Review)
- Research Funding: Janssen Pharmaceuticals, Medivation, Pfizer, Suncoast Community Clinical Oncology

2

Topics

1. Anemia
2. Thrombocytopenia and Other Bleeding Disorders
3. Venous Thromboembolism

3

Anemia

4

Anemia in Pregnancy

- Common complication
- Often dilutional:
  - Plasma volume increases by 40%-50%
  - Red cell mass increases by 20%-30%
- Prevalence of iron deficiency anemia in pregnant women in the United States estimated to be 19%

1. 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.

5

Anemia in Pregnancy

Definition of anemia in pregnancy by ACOG and WHO:

- First trimester – Hemoglobin <11 g/dL
- Second trimester – Hemoglobin <10.5 g/dL
- Third trimester - Hemoglobin <10.5 to 11 g/dL
- Postpartum – Hemoglobin <10 g/dL

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Anemia in Pregnancy

- Association between severe anemia and poor pregnancy outcomes in multiple observational studies
  - 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 ACOG[2]

1. Daru J, Zamora J, Fernández-Félix BM, et al. Risk of maternal mortality in women with severe anaemia during pregnancy and post partum: a multilevel analysis. *Lancet Glob Health*. 2018;6(5):e548-e554.
2. Pena-Rosas JP, Viteri FE. Effects of routine oral iron supplementation with or without folic acid for women during pregnancy. *Cochrane database of systematic reviews*. 2006;(3):CD004736-.

7

Iron Requirements

- Pregnant women have increased iron requirements, 27 mg per day
- In the United States, daily supplementation with 30 mg is recommended if patient is not anemic[1]
- Women with IDA and hemoglobin <9 g/dL or hematocrit <27% should be treated with 60-120 mg/day of iron[2]

1. Institute of Medicine (US) Committee on the Prevention, Detection, and Management of Iron Deficiency Anemia Among U.S. Children and Women of Childbearing Age; Earl R, Woteki CE, editors. Iron Deficiency Anemia: Recommended Guidelines for the Prevention, Detection, and Management Among U.S. Children and Women of Childbearing Age. Washington (DC): National Academies Press (US); 1993. Recommended Guidelines For Preventing And Treating Iron Deficiency Anemia In Pregnant Women.
2. McDonagh M, Cantor A, Bougatsos C, et al. Routine Iron Supplementation and Screening for Iron Deficiency Anemia in Pregnant Women: A Systematic Review to Update the U.S. Preventive Services Task Force Recommendation [Internet]. Rockville (MD): Agency for Healthcare Research and Quality (US); 2015 Mar. (Evidence Syntheses, No. 123.)

8

Folate Requirements

- Macrocytosis due to vitamin B12/folate deficiency can be masked by simultaneous iron deficiency[1]
- 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.

9

Folate Requirements

Higher folate doses needed:

- Hemoglobinopathy
- Patients on anti-convulsants
- Multiple gestation
- Neural tube defect in prior pregnancy

10

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)

11

Sickle Cell Disease

Infants of SCD mothers have increased risk of:

- Prematurity
- Small for gestational age

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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
- Meta-analysis: prophylactic transfusion associated with a reduction in:
  1. Maternal mortality (7 studies, 955 participants; odds ratio [OR] = 0.23)
  2. Vaso-occlusive pain episodes (11 studies, 1219 participants; OR = 0.26)
  3. Pulmonary complications (9 studies, 1019 participants; OR = 0.25)
  4. Pulmonary embolism (3 studies, 237 participants; OR = 0.07)
  5. Neonatal death (5 studies, 374 participants; OR = 0.26)
  6. Preterm birth (9 studies, 1123 participants; OR = 0.59)

Malinowski AK, Shehata N, D'Souza R, et al. Prophylactic transfusion for pregnant women with sickle cell disease: a systematic review and meta-analysis. *Blood*. 2015;126(21):2424-2437.

14

Thrombocytopenia

15

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
- Platelet count is 100-150,000/dl[1]
- Mothers can be delivered normally
- No association with fetal thrombocytopenia
- Occurs in 5-7% of all pregnancies
- Patients should receive routine OB care

1. Reese JA, Peck JD, Deschamps DR, et al. Platelet Counts during Pregnancy. *N Engl J Med*. 2018;379(1):32-43.

17

Immune Thrombocytopenia

- Occurs in 1 to 10 in 10000 women[1]
- Incidence of severe ITP (platelet count <50,000 or thrombocytopenia requiring treatment) occurs in 0.83 per 10000 women[2]
- A diagnosis of exclusion
- Platelet count lower than in gestational thrombocytopenia
- Diagnosis is suggested when a mother with pre-existing ITP develops profound thrombocytopenia

1. 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.
2. Care A, Pavord S, Knight M, Alfirevic Z. Severe primary autoimmune thrombocytopenia in pregnancy: a national cohort study. *BJOG*. 2018;125(5):604-612.

18

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%

Webert KE, Mittal R, Sigouin C, Heddle NM, Kelton JG. A retrospective 11-year analysis of obstetric patients with idiopathic thrombocytopenic purpura. *Blood*. 2003;102(13):4306-4311.

19

Immune Thrombocytopenia

- Concern for fetal thrombocytopenia[1]
  - 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]
- Scalp vein & percutaneous umbilical cord sampling should not be done[3]
  - Hemorrhagic risk to fetus
  - Underpredicts platelet count

1. Burrows RF, Kelton JG. Fetal thrombocytopenia and its relation to maternal thrombocytopenia. *N Engl J Med* 1993;**329**:1463–6.
2. Belkin A, Levy A, Sheiner E. Perinatal outcomes and complications of pregnancy in women with immune thrombocytopenic purpura. *J Matern Fetal Neonatal Med*. 2009;22(11):1081-1085.
3. Burrows RF, Kelton JG. Pregnancy in patients with idiopathic thrombocytopenic purpura: assessing the risks for the infant at delivery. *Obstet Gynecol Surv*. 1993;48(12):781-788.

20

Immune Thrombocytopenia

- Prednisone/IVIg used for bleeding or platelets <20,000[1]
- Splenectomy can be considered second-line for thrombocytopenia refractory to steroids and IVIg[2]
  - 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 indications[1]
- Platelets rise after delivery

1. Mahey R, Kaur SD, Chumber S, Kriplani A, Bhatla N. Splenectomy during pregnancy: treatment of refractory immune thrombocytopenic purpura. *BMJ Case Rep*. 2013;2013:bcr2013201778. Published 2013 Dec 20. doi:10.1136/bcr-2013-201778
2. Neunert C, Lim W, Crowther M, et al. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. *Blood*. 2011;117(16):4190-4207.

21

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%

22

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, Prabulos AM, Vandvik PO. VTE, thrombophilia, 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 Suppl):e691S-e736S.

23

HELLP Syndrome

- Hemolysis (microangiopathic)
- Elevated (Bili>1.2, SGOT>70)
- Liver enzymes (LDH >600)
- Low Platelets (<100,000/dl)

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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

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Thrombotic Thrombocytopenic Purpura

- Seen with pregnancy or post partum
- 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

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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**

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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**

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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**

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Other Bleeding Disorders

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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**

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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**

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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

- **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.

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Venous Thromboembolism

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Essential Thrombocytosis, JAK2, and Pregnancy

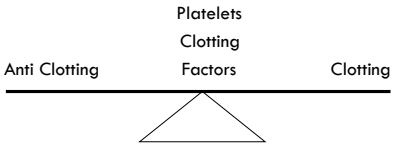
Retrospective single-institution analysis of 63 pregnancies in 36 women with ET
- **61% live births, 39% fetal loss**
- **10/20 patients studies had JAK2 mutation**

Predictors of outcome:
- **No correlation with maternal age, JAK2, WBC, HGB, platelet count**
- **Only factors that affected results: use of ASA**
- **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.

39

Balancing Act



Based on packets of proteins in blood in:
- Platelets
- Vessel walls
- Tissues (brain, heart)

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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**

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Hypercoagulability

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**

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Hypercoagulability

Virchow's Triad: Hypercoagulability, Stasis, Endothelial Damage

Stasis

- **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**

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Normal Pregnancy & Postpartum Period as an Acquired Hypercoagulable State

- ↑ concentration of coagulation factors II, V, VII, VIII,IX, X, XII, fibrinogen and vWF
- ↓ levels of free protein S, and ↑ 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)**

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Pregnancy-Associated Thrombosis

- **PE account for 15% of maternal deaths in developed countries**
- **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 Haemost*. 1997;78(4):1183-1188.

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Pregnancy-Associated Thrombosis

MEGA Study

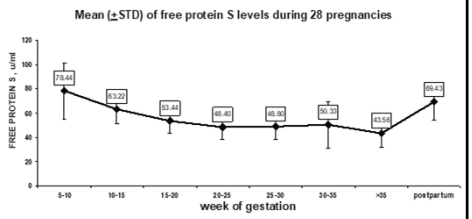
- 285 pregnant women vs. 857 controls
- 4 to 5 x increased risk of VTE during pregnancy
- 8.8x increased risk in 3rd trimester
- 52 x increased if patient V Leiden carrier
- 31 x increased if patient II G20210A carrier
- 84 x increased in the first 6 weeks post partum

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.

47

Pregnancy-Associated Thrombosis

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 Haemost*. 2002;88(1):32-36.

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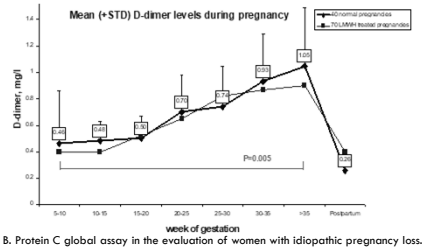
Pregnancy-Associated Thrombosis

The same results were obtained with PT 1+2 levels during pregnancies.



Sarig G, Lanir N, Hoffman R, Brenner B. Protein C global assay in the evaluation of women with idiopathic pregnancy loss. *Thromb Haemost*. 2002;88(1):32-36.

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Thrombosis & Pregnancy

Inherited
- **Protein C and S Deficiency**
- **APC Resistance**
- **Antithrombin Deficiency**
- **Factor V Leiden**
- **Prothrombin G20210A**

Acquired
- **Antiphospholipid Antibodies**
- **Ovarian Hyperstimulation**
- **Obesity**
- **Immobilization**
- **Age > 35**

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Prevalence of Congenital Thrombophilia (European Populations)

Thrombophilic defect	**Prevalence (%)**
Antithrombin deficiency	0.25 – 0.55
Protein C deficiency	0.20 – 0.33
Factor V Leiden heterozygotes	2 - 7
Prothrombin G20210A heterozygotes	2
MTHFR C677T homozygotes	10

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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)

1. McColl MD, Ellison J, Reid F, Tait RC, Walker ID, Greer IA. Prothrombin 20210 G-->A, MTHFR C677T mutations in women with venous thromboembolism associated with pregnancy. *BJOG*. 2000;107(4):565-569.
2. Gerhardt A, Scharf RE, Beckmann MW, et al. Prothrombin and factor V mutations in women with a history of thrombosis during pregnancy and the puerperium. *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 extremity)
- 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

Stewart JA, Hamilton PJ, Murdoch AP. Thromboembolic disease associated with ovarian stimulation and assisted conception techniques. *Hum Reprod*. 1997;12(10):2167-2173.

53

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 women
- Prophylactic anticoagulation is not recommended for average risk women

Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO. VTE, thrombophilia, 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 Suppl):e691S-e736S.
Arya R, Shehata HA, Patel RK, et al. Internal jugular vein thrombosis after assisted conception therapy. *Br J Haematol*. 2001;115(1):153-155.

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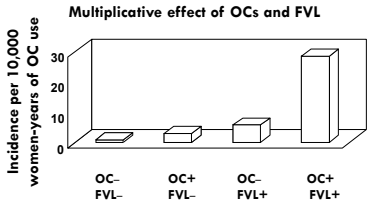
Managing Patient at Risk for VTE

- **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**

55

Risk of a First VTE



56

Vitamin K Antagonists

- 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, Vandvik PO. VTE, thrombophilia, 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 Suppl):e691S-e736S.

57

Use of Anticoagulants in Lactation

- The following can continue during breastfeeding:
  - Warfarin
  - UFH
  - LMWH
  - Danaparoid
  - 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, thrombophilia, 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 Suppl):e691S-e736S.

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

Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO. VTE, thrombophilia, 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 Suppl):e691S-e736S.

59

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.

60

Safety of Withholding Heparin

Results:
- 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.

61

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, thrombophilia, 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 Suppl):e691S-e736S.

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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, Vandvik PO. VTE, thrombophilia, 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 Suppl):e691S-e736S.

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VTE Prophylaxis: ≥ 2 VTE

Not associated with thrombophilia
- Antenatal: LMWH
  - Prophylactic or INT or ADJ dose LMWH
- Postpartum
  - 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, thrombophilia, 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 Suppl):e691S-e736S.

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, thrombophilia, 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 Suppl):e691S-e736S.

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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
- LMWH
- Warfarin (INR 2-3)

Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO. VTE, thrombophilia, 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 Suppl):e691S-e736S.

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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

67

Thrombophilia and Pregnancy Complications/Loss

- **A woman should be screened for antiphospholipid antibodies when she has:**
  - **Recurrent pregnancy loss (≥3) before 10 weeks**

Bates SM, Greer IA, Middeldorp S, Veenstra DL, Prabulos AM, Vandvik PO. VTE, thrombophilia, 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 Suppl):e691S-e736S.

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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, thrombophilia, 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 Suppl):e691S-e736S.

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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, Prabulos AM, Vandvik PO. VTE, thrombophilia, 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 Suppl):e691S-e736S.

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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.

71

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
  - Limitation: subgroups were small
- Conclusion: Neither ASA nor ASA + Heparin improved live birth rate

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.

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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.

73

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%)
- 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-molecular-weight heparin and low-dose aspirin in women with recurrent miscarriage. *Blood*. 2010;115(21):4162-4167.

74

Prevention of Pregnancy Complications in Women with Thrombophilia

Randomized trials and meta-analysis with similar findings:

1) 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.
2) 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.
3) 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.

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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

HEMATOLOGY AND MEDICAL ONCOLOGY

BEST PRACTICES COURSE

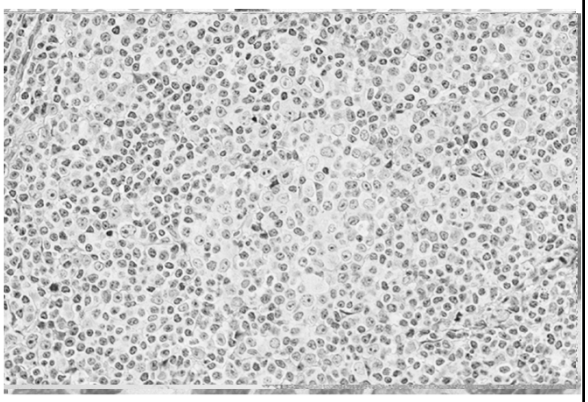
27 - Review of Cellular Morphology

Donald Karcher, MD

Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

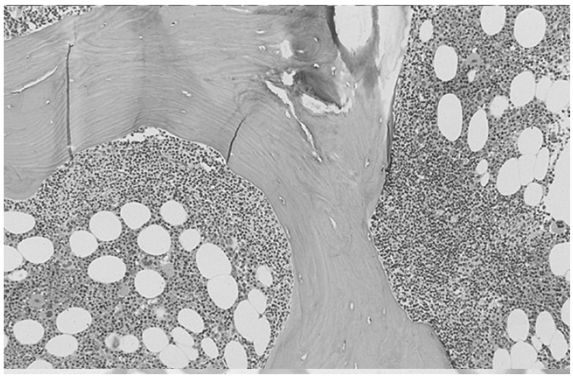
- None



Answer:

Chronic lymphocytic leukemia

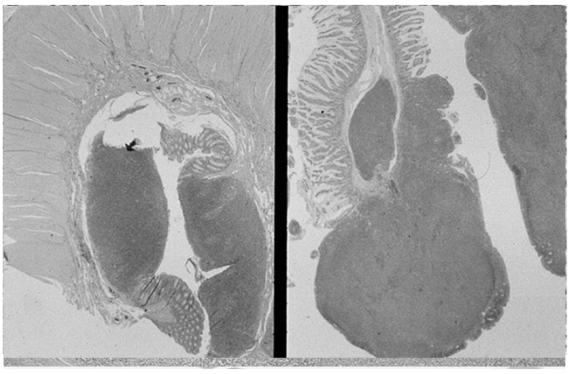
Special features –
- Small to medium size lymphocytes with densely clumped chromatin
- "Smudge" cells on peripheral smear
- May progress with an increase in prolymphocytes
- "Proliferation centers" in lymph nodes



Answer:

Follicular lymphoma, low-grade (grade 1-2 of 3)

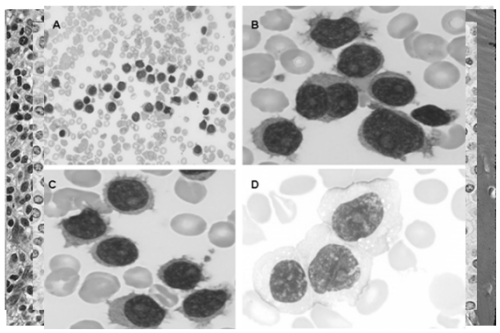
Special features –
- "Buttock" cells in peripheral blood
- Multiple uniform follicles in lymph node
- Predominance of centrocytes; less than 15 centroblasts per HPF
- Paratrabecular bone marrow infiltrate



Answer:

Mantle cell lymphoma

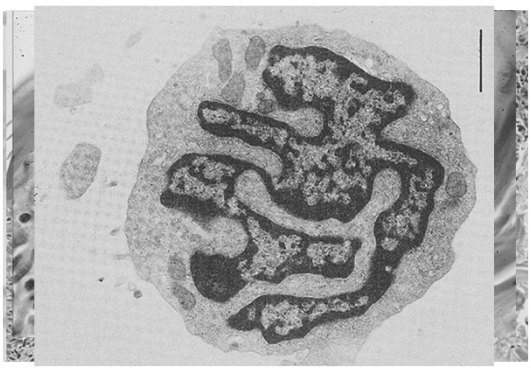
Special features –
- Circulating lymphoma cells
- Vaguely nodular growth pattern in lymph node
- "Naked" germinal centers in some cases
- Small lymphocytes with moderate mitotic activity
- Admixed histiocytes in some cases
- Blastoid variant → Fine chromatin, nucleoli, ↑ mitoses
- Cyclin D1 positivity
- In gut, "lymphomatous polyposis"



Answer:

Hairy cell leukemia

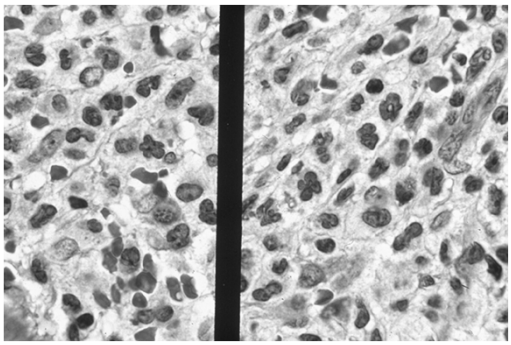
Special features –
- Indistinct cytoplasmic margins (rarely see "hairs")
- Round, bean-shaped, to irregular nuclei
- Cytoplasmic "ruffles" and ribosome lamellar complexes on EM
- Tartrate-resistant acid phosphatase (TRAP) positive
- "Fried egg" appearance on tissue sections
- Variant hairy cell leukemia → Higher WBC count, larger nucleoli

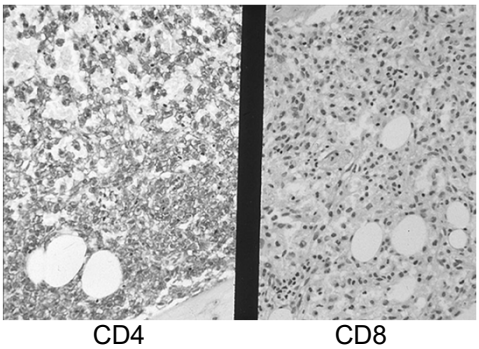


Answer:

Cutaneous T-cell lymphoma; mycosis fungoides

Special features –
- Circulating Sezary cells with "cerebriform" nuclei
- High dermal lymphocytic infiltrate with intra-epidermal Pautrier microabscesses
- Complex nuclear shape on EM





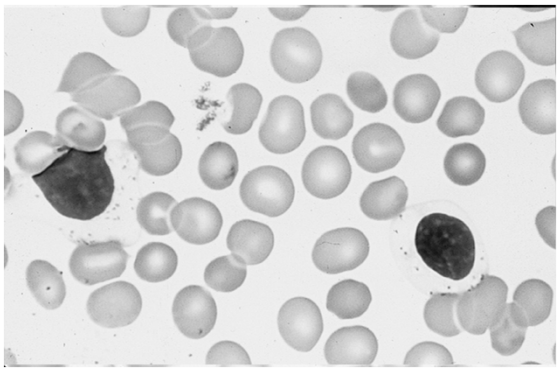
CD4 CD8

Answer:

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

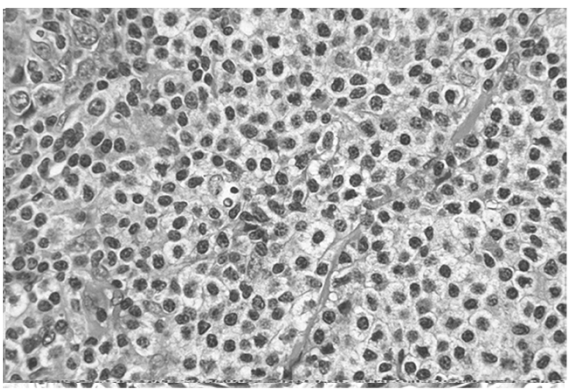


Answer:

T-cell large granular lymphocytic leukemia (formerly “large granular lymphocytosis”)

Special features –

- Typical large granular lymphocytes
- Immunophenotype: CD3+, CD8+, CD16+, CD57+

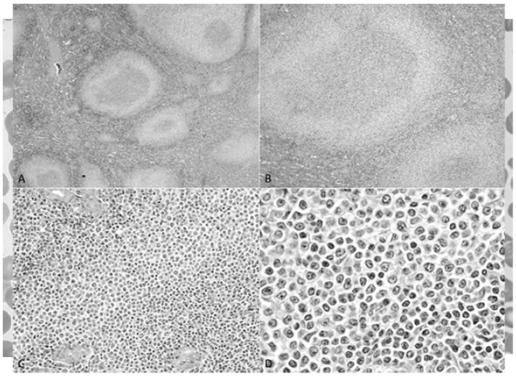


Answer:

Marginal zone B-cell lymphoma

Special features -

- Extranodal marginal zone B-cell lymphoma (MALT lymphoma) – 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

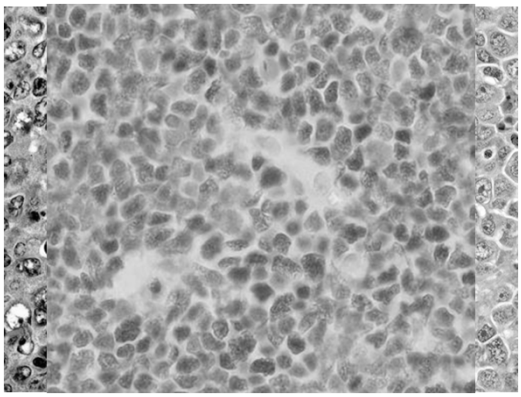


Answer:

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

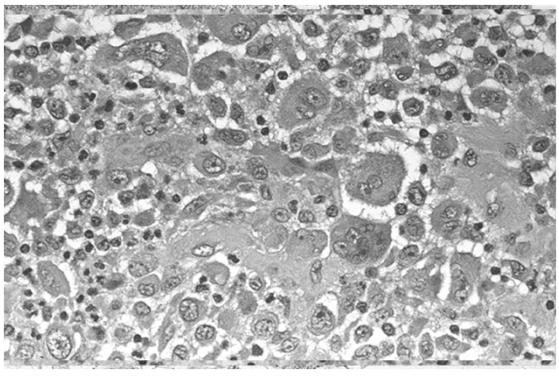


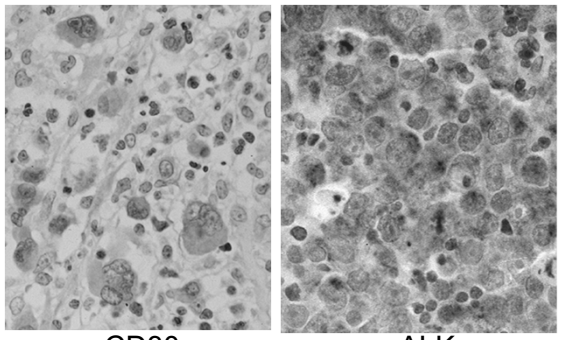
Answer:

Diffuse large B-cell lymphoma

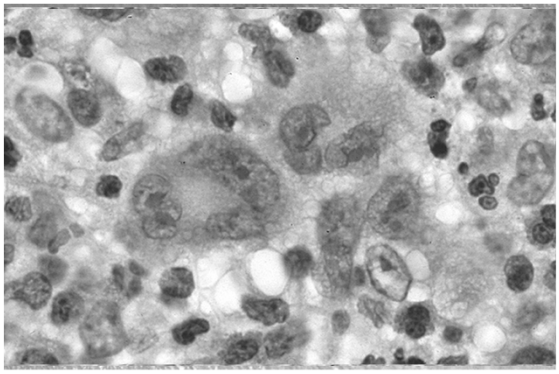
Special features –

- 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





CD30 ALK

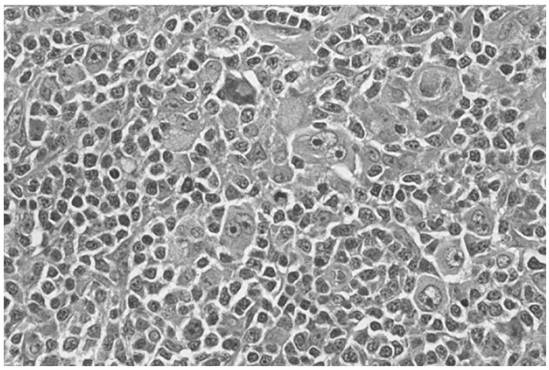


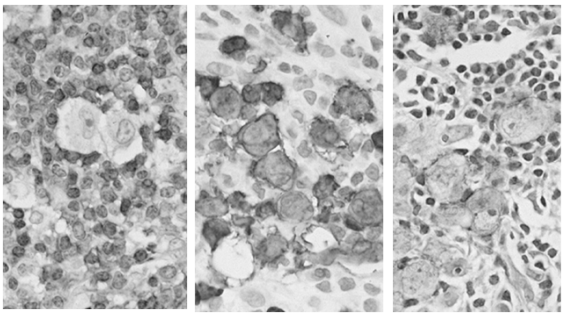
Answer:

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





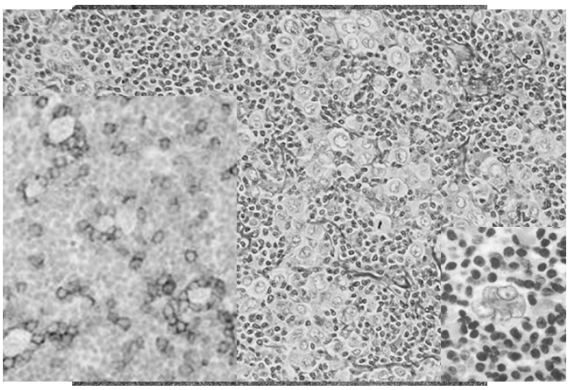
CD45 (LCA) CD15 CD30

Answer:

Classical Hodgkin's lymphoma, nodular sclerosis type

Special features –

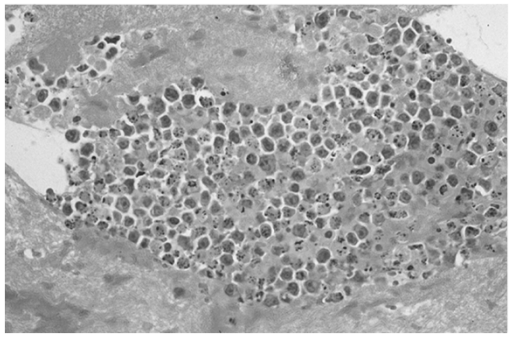
- Nodules surrounded by fibrotic bands
- Lacunar ± classical Reed-Sternberg cells, often loosely clustered
- Reed-Sternberg cells: CD45-, CD20-/+, CD15+, CD30+, PAX5 dim+, Oct2-, BOB.1-
- Background: Mostly CD4+ T cells



Answer:

Hodgkin's lymphoma, nodular lymphocyte predominant type

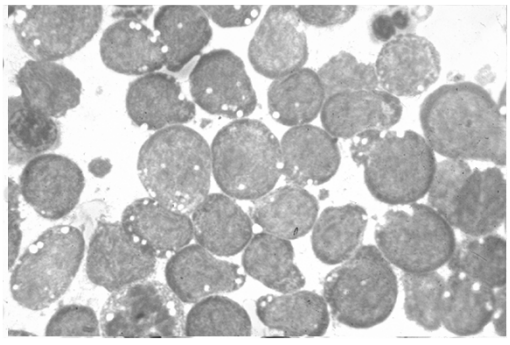
Special features –
- Vaguely nodular growth pattern
- "LP" or "popcorn" cells: CD45+, CD20+, CD15-, CD30-, PAX5 bright+, Oct2+, BOB.1+
- Background: B cells ± T cells
- Rosettes of CD57+, PD1+ T cells around LP cells



Answer:

Primary effusion lymphoma

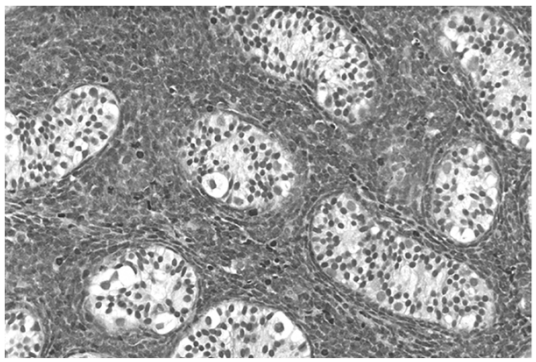
Special features –
- Frequently confined to body cavities
- Large immunoblastoid cells
- Negative for most B-cell antigens and surface immunoglobulin
- Strong association with HHV-8



Answer:

Burkitt's lymphoma

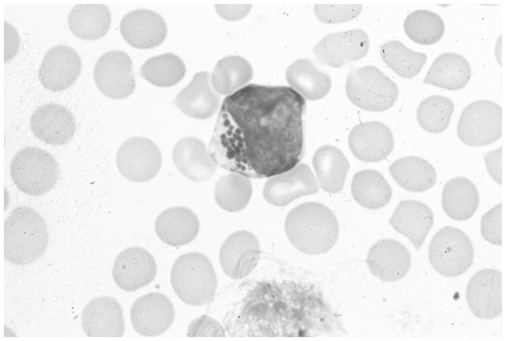
Special features –
- Frequently extranodal
- Uniform, medium size, blast-like cells with basophilic cytoplasm and clear vacuoles
- "Starry sky" cells (tingle-body macrophages)
- Very high mitotic activity
- Lymphoma cells often in peripheral blood and/or bone marrow ("Burkitt cell ALL", "ALL, L3")



Answer:

Acute lymphoblastic leukemia

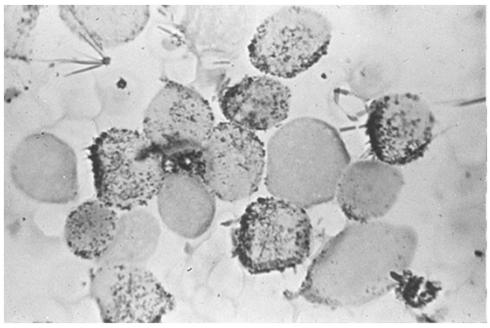
Special features –
- Variable blast morphology, may include mature-appearing cells
- PAS positivity in blasts
- "Hand-mirror" cells in some cases
- Testis as "sanctuary site"



Answer:

"Granular" precursor B-cell ALL

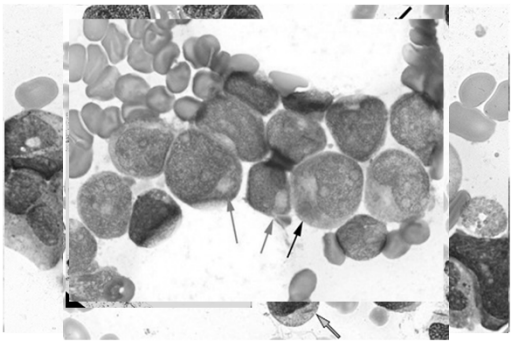
Special features -
- 5-15% of childhood ALL; <5% of adult ALL
- Large cytoplasmic granules (3 or more)
  - Myeloperoxidase (MPO) negative
  - Sudan black positive (light gray)
- Slightly worse prognosis than conventional precursor B-cell ALL



Answer:

Acute myeloid leukemia

Special features –
- "Classical" myeloblast morphology
  - Fine, "reticular" chromatin
  - Prominent "cookie-cutter" nucleoli
  - Auer rods in some cases
  - Myeloperoxidase (MPO) positive [cytochemical technique shown]

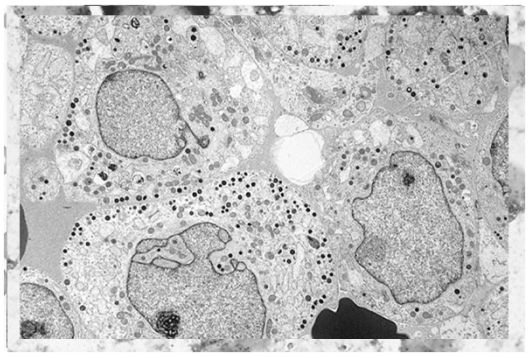


Answer:

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

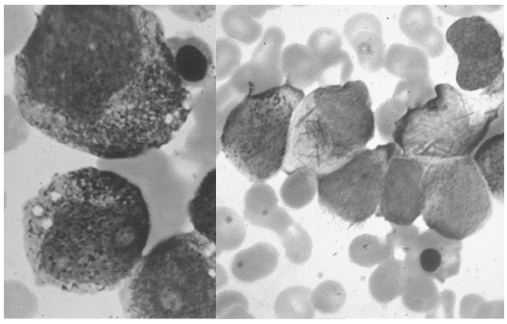


Answer:

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

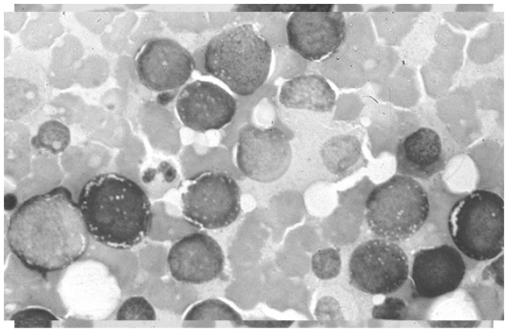


Answer:

Acute promyelocytic leukemia with *PML-RARA*, typical “hypergranular” subtype

Special features –

- Promyelocytes with very large primary granules
- Cells with multiple Auer rods (“faggot” cells)

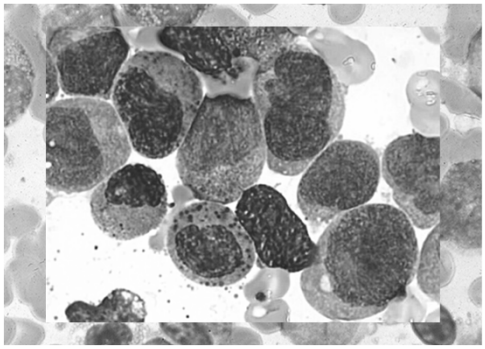


Answer:

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]

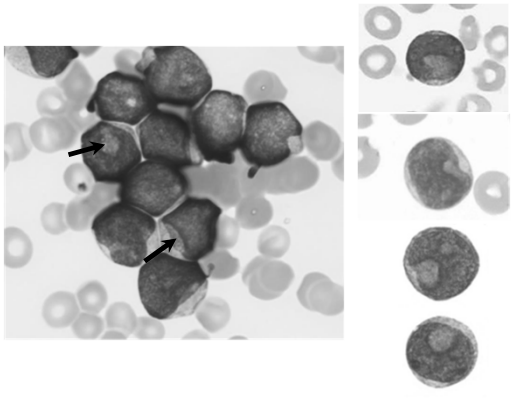


Answer:

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

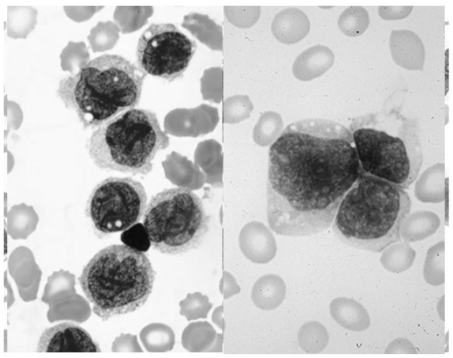


Answer:

Acute myeloid leukemia with *NPM1* and/or *FLT3* mutations

Special features –

- Myeloblasts with nuclear invaginations, so-called "cup-like" or "fish mouth" nuclei

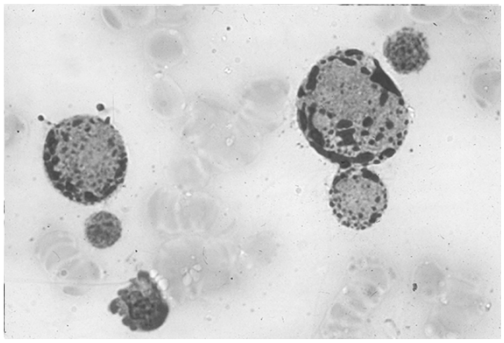


Answer:

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]

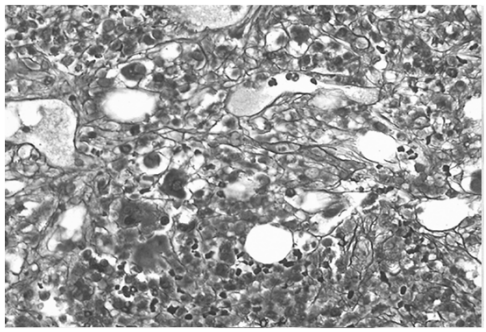


Answer:

Acute erythroid leukemia

Special features –

- Immature erythroblasts with:
  - Round nuclei
  - Basophilic cytoplasm
  - Large cytoplasmic vacuoles, PAS+
- Little or no myeloblastic component

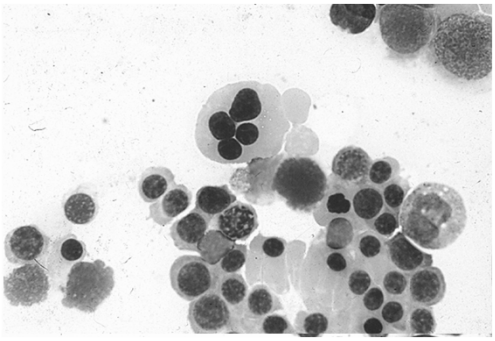


Answer:

Acute megakaryoblastic leukemia

Special features –

- Small to large megakaryoblasts, some exhibiting cytoplasmic blebs ("platelet budding")
- Reticulin fibrosis in marrow

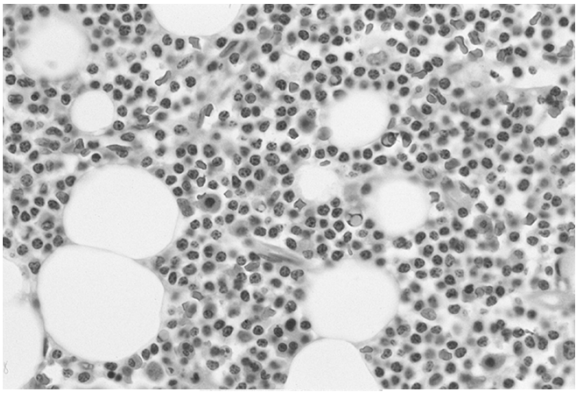


Answer:

Myelodysplasia

Examples shown –
- Dwarf and multinucleated megakaryocytes
- Multinucleated and multilobulated erythrocytic precursors

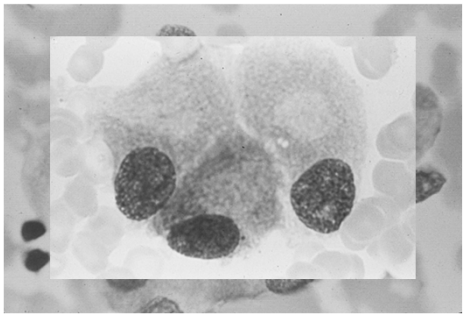
Associated with MDS, post-chemo, HIV, etc.



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

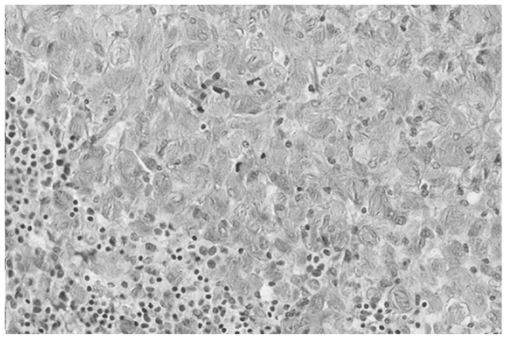
Lymphoplasmacytic lymphoma
- Lymphoplasmacytic infiltrate with Dutcher intranuclear pseudoinclusions



Answer:

Osteoblasts

Special features –
- May mimic plasma cells, but Golgi area distinct from nucleus (in plasma cells, adjacent to nucleus)
- Seen especially on bone marrow touch preps

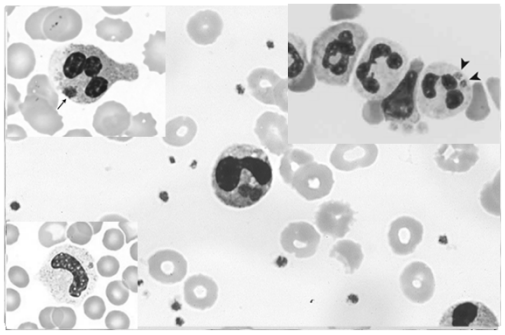


Answer:

Gaucher's disease

Special features –

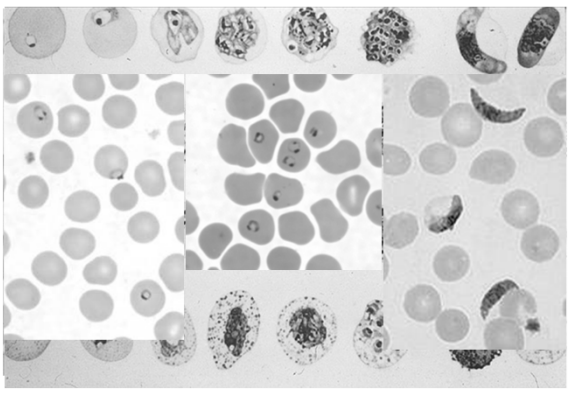
- On marrow aspirate smear: Histiocytes with "wrinkled tissue paper" appearance in cytoplasm
- On core biopsy: Histiocytes with PAS+ spindle shaped inclusions in cytoplasm



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:

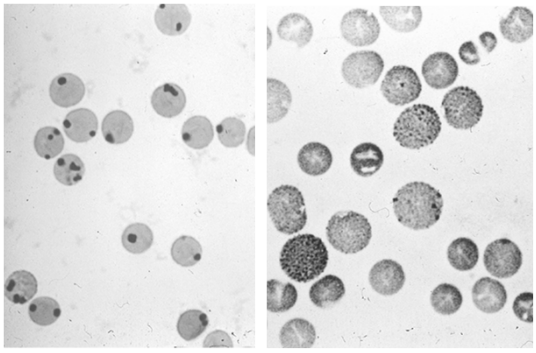
Intraerythrocytic microorganisms

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.



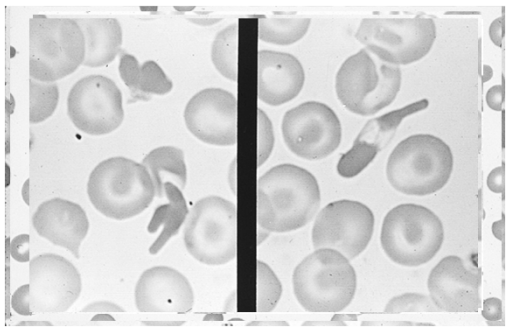
crystal violet brilliant cresyl blue

Answer:

Red cell inclusions associated with hemoglobin denaturation

With crystal violet: Heinz bodies (associated with G-6-PD deficiency, other enzyme deficiencies, alpha thalassemia)

With brilliant cresyl blue: "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

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Time's up . . . thanks!

GW