May 18, 2010

APPROVED

you will see these tests everyday, so you need to be able to interpret them!
-some of this material will be covered in the cpc due to short time during the lecture

DUKE
UNIVERSITY
HEALTH SYSTEM

Abnormalities of Blood Count :
Pathophysiology and Laboratory Diagnosis of Anemias and other Blood Disorders

Anand Shreeram Lagoo, MD, PhD
Associate Professor of Pathology
Director, Clinical Flow Cytometry Laboratory

Phone: 668-0921, Pager 970-2903

Learning Objectives

numeric values

- Recognize common quantitative abnormalities in complete blood count (CBC) and qualitative abnormalities on a peripheral blood smear (PBS)

morphology

- Interpret hematologic laboratory values to diagnose various types of anemias
- Define the terms used to identify hematologic abnormalities
- Understand the morphological and etiologic classification of anemias and the pathophysiological basis of anemias
- Perform a differential diagnosis in a case of anemia and select additional laboratory tests to define cause of anemia
- Recognize the common white cell and platelet abnormalities

parallel but not mutually exclusive

Lecture Outline

we are going to use cases to demonstrate the pathophysiology to understand anemia

- Basic mechanisms of hematological abnormalities
- Automated blood count (ABC) – Method and parts of a typical ABC
- Red blood cells
  - Classification of anemias (Note: Hereditary causes of anemia will be covered in CPC on May 23)
  - Case 1 – Iron deficiency anemia. Pathophysiology of iron metabolism. Additional tests.
  - Case 2 – Pernicious anemia. Pathophysiology of megaloblastic anemias. Metabolism of folate, B12.
  - Case 3 – Anemia of chronic inflammation. Hepcidin and related molecules controlling iron.
  - Case 4 – Autoimmune hemolytic anemia. Causes and mechanisms.
- White blood cells
  - Case 5 – CML. Philadelphia chromosome.
  - Case 6 – Polycythemia vera. Jak2 mutations and myeloproliferative neoplasms.
  - Case 7 – Aplastic anemia.
- Platelets
  - Case 8 – ITP. Causes of thrombocytopenia.

role of b12 and folate important

common in hospital patients

BIZARRO


Would you mind putting that thing on vibrate?
BIZARROCOMIC.BLOGSPOT.COM
Dist. by King Features
DAN PIRARO
5-15-10
W/WAYNO
BABY
BIZARRO.COM

Peripheral Blood Cells : Basic Facts

	Number / cmm	Life Span in Days	Produced in	Destroyed in
Red Cells*	5×10^6	120	BM	Spleen
Platelets	5×10^5	5-7	BM	Spleen
White Cells	5×10^3	<1 (PMN)	BM, lymph nodes	Tissues

- *Reticulocytes: Without a nucleus, but contain RNA.
  Need 2 days in BM & 1 day in PB to mature to RBC. Normally 1% of RBC.

General Approach to Diagnosis of Hematological Abnormalities

- Is there an abnormality in the blood count?
- Which cell line(s) affected?

red cells, white cells, platelets

- Morphology of affected cells –
  - Normal?
  - Abnormal?

MCV, MCH

NOTE: Calculated “indices” provide similar information

- What is the likely cause of the abnormality?
  - Additional tests

rational treatment needs to be directed towards cause

Initial Division of Hematological Abnormalities

- Quantitative: one or more cell types may be involved
  - Reduced numbers of blood cells (=cytopenia)
  - Too many blood cells (=cytosis)
  - Complex: one cell type ↓, other ↑
- Qualitative
  - Presence of immature cells
  - Functionally abnormal cells
  - Presence of cells not belonging to blood
- Mixed

TOO LITTLE CELLS:
-white cells- leukocytopenia
-platelets- thrombocytopenia
-red cells- anemia

TOO MANY CELLS:
-white cells- leukocytosis
-platelets- thrombocytosis
-red cells- polycythemia

may have too little of one type of blood cell and too many of another- ex- leukocytosis with thrombocytopenia

based on morphology of cells

cells that do not belong in normal blood:
blasts - immature cells
tumor (leukemia) cells
-may be mixed

Quantitative Blood Cell Abnormalities -Basic mechanisms

- Causes of Cytopenias:
  - Decreased production
    - Lacks building blocks (nutritional, other)
    - Problems with production site (marrow pathology)
  - Excessive destruction
    - Intrinsic vs extrinsic abnormalities
  - Abnormal compartmentalization
- Causes of increased cell number:
  - Excessive production (reactive vs neoplastic)
  - Increased life-span (neoplastic)
  - Delayed exit from blood (steroids)

particularly in red cells- Hb about 97% of red cell mass so problem with Hb has great effect on red cell size and number

problem with bone marrow

enlarged spleen sequesters a lot of blood and lowers circulating red cell count

production increases
-reaction to insult-infection causes leukocyte count to increase
-malignancy - CML causes increase in platelets and white cells

CLL- cells live very long and don't die

white cell count may go up because neutrophils are not exiting the blood

Basic Laboratory Tests in Hematology

blood tests you will see for many patients
-automated blood count = cbc
-peripheral blood smear

- Automated blood count, with or without automated differential count

-CBC

- Peripheral blood smear

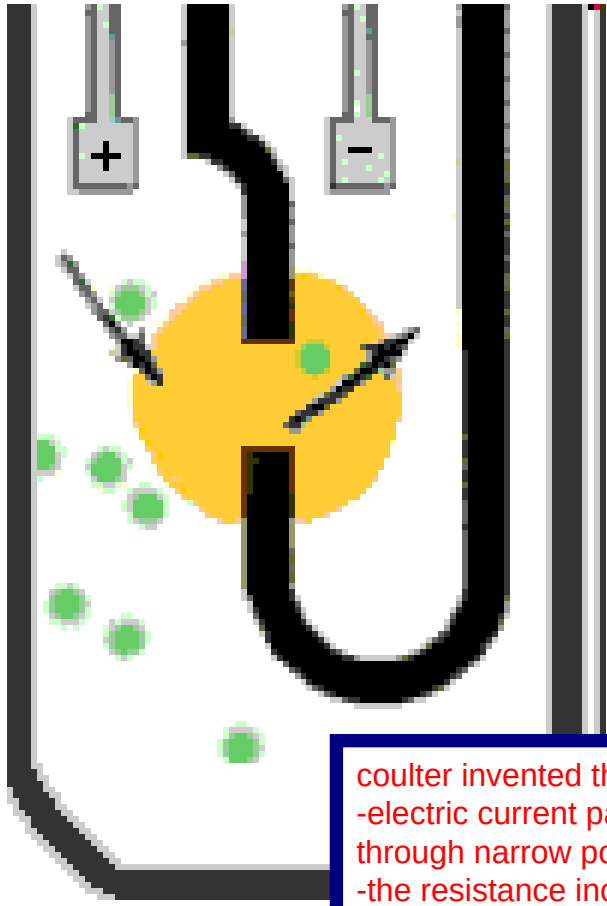
Automated Blood Analyzer

instrument for cbc
a couple hundred tests per hour

Can analyze 110 – 150 samples / hour

XE-2100

Automated Blood Analyzer: The Coulter Principle



When particles are pulled through an orifice, through which an electric current is flowing, there is a change in impedance that is proportional to the size of the particle.

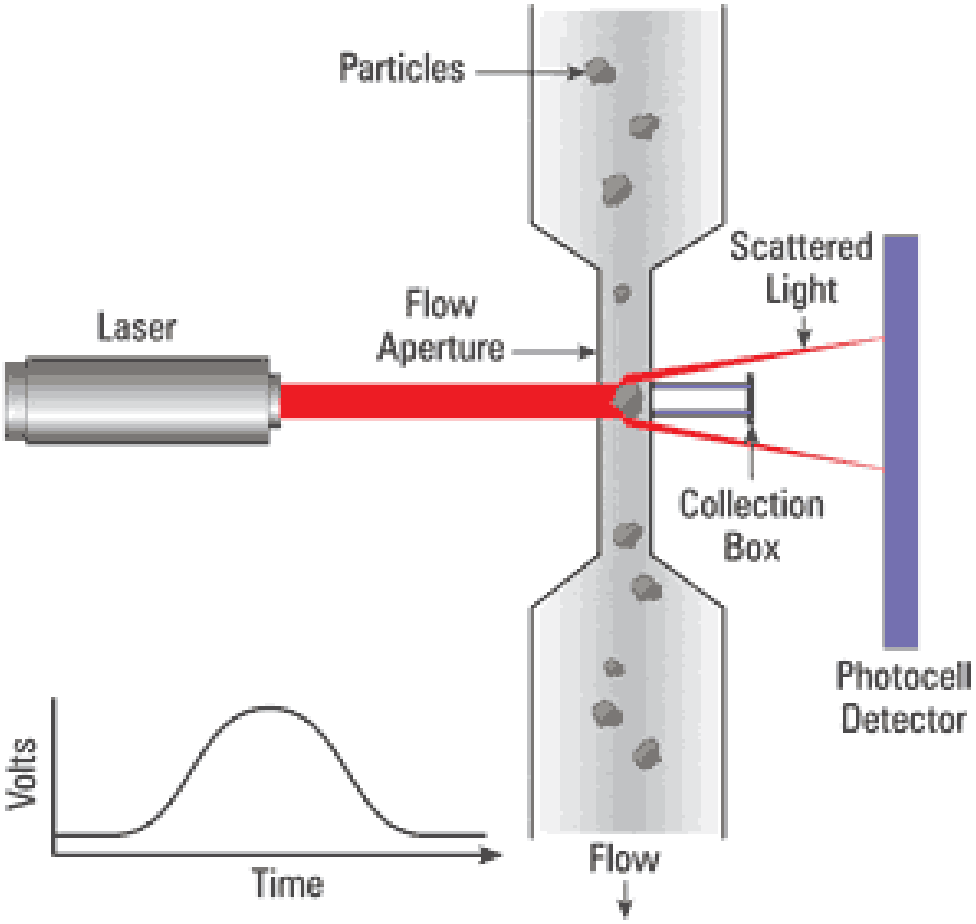
The Coulter principle was named for its inventor, Wallace H. Coulter

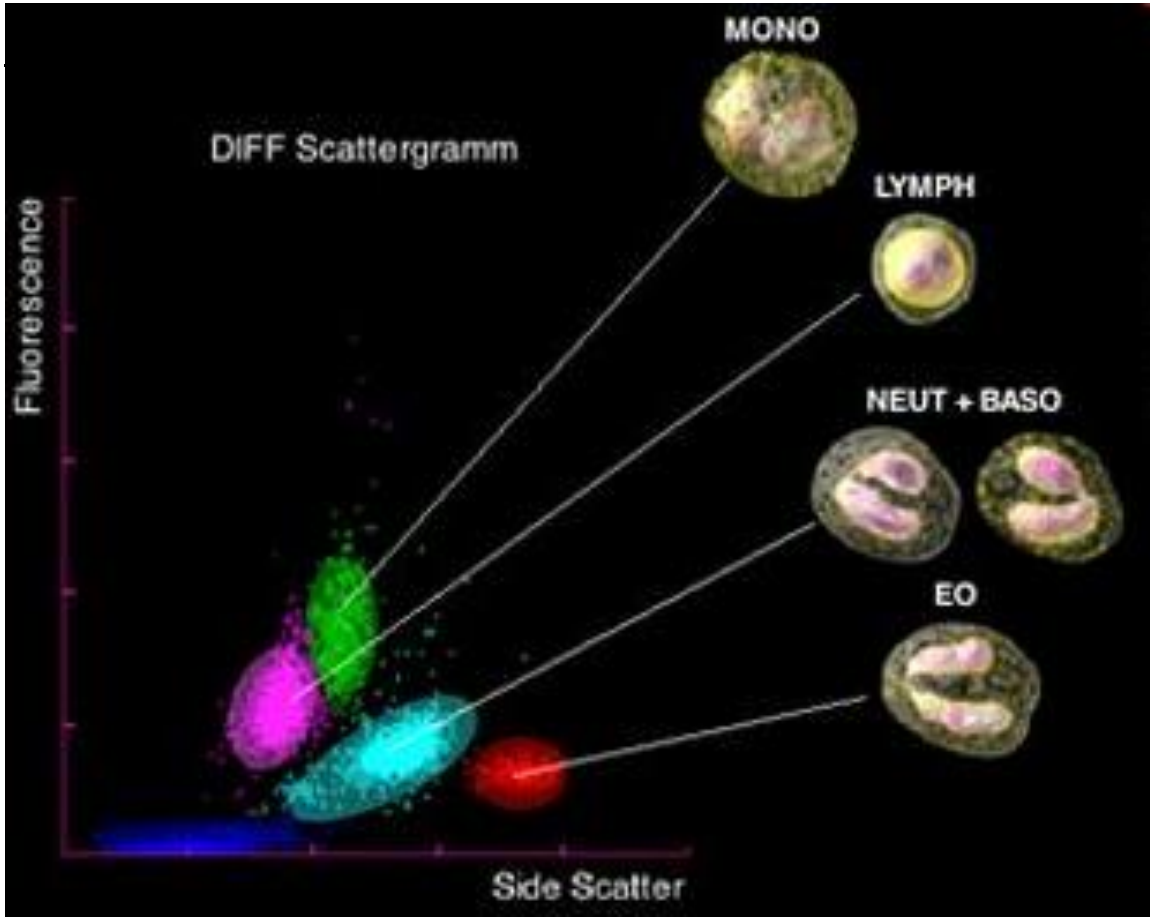
coulter invented the machine
-electric current passing through conducting fluid through narrow pore, there will be a certain resistance
-the resistance increases proportionally to cell size
-tells you how many cells passing through and size of different cells

1913 - 1998

Automated Blood Analyzer: Light Scatter

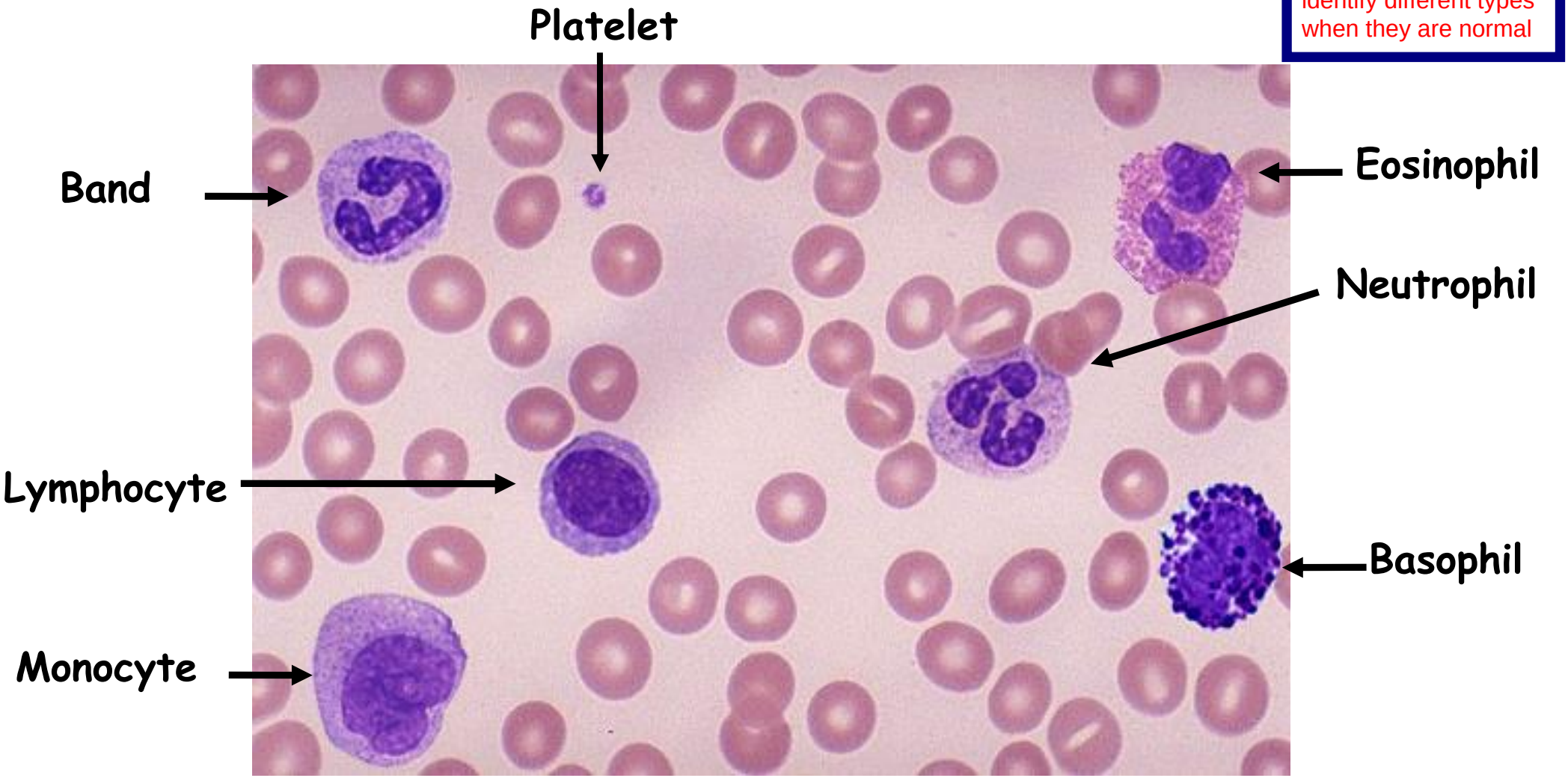
light scatter is also used to analyze blood
-laser points at single cells and angle of scatter tells you type of blood cell





Peripheral Blood Smear

stained to show different types of white cells
-should be able to identify different types when they are normal



Automated Blood Count

duke version of cbc
Boxed the values that he refers to later in the lecture

AUTO BLOOD CT WITH AUTO DIFF

			Reference
HEMOGLOBIN	15.5	g/dL	[13.7-17.3]
HEMATOCRIT	0.46	L/L	[0.39-0.49]
RED BLOOD CELL COUNT	4.95	X10^12	[4.37-5.74]
MCH	31.3	pg	[26.5-34.0]
MCHC	33.4	%	[31.5-36.3]
RDW-CV	13.4	%	[11.5-14.5]
MCV	94	fL	[80-98]
NUCLEATED RBC %	0.0	/100WC	
NUCLEATED RBC COUNT	0.00	X10^9	[0.00-0.00]
PLATELET COUNT /L	171	X10^9	[150-450]
WHITE BLOOD CELL COUNT	4.5	X10^9	[3.2-9.8]
NEUTROPHIL %	60.2	%	[37.0-80.0]
LYMPHOCYTE %	26.8	%	[10.0-50.0]
MONOCYTE %	10.1	%	[0.0-12.0]
EOSINOPHIL %	2.7	%	[0.0-7.0]
BASOPHIL%	0.2	%	[0.0-2.0]
NEUTROPHIL COUNT	2.7	X10^9	[2.0-8.6]
LYMPHOCYTE COUNT	1.2	X10^9	[0.6-4.2]
MONOCYTE COUNT	0.5	X10^9	[0.0-0.9]
EOSINOPHIL COUNT	0.12	X10^9	[0.00-0.70]
BASOPHIL COUNT	0.01	X10^9	[0.00-0.20]

Automated Blood Count

red box- red cell values
blue box- white blood cell values

AUTO BLOOD CT WITH AUTO DIFF

				Re
HEMOGLOBIN		15.5	g/dL	[13.7-17.3]
HEMATOCRIT		0.46	L/L	[0.39-0.49]
RED BLOOD CELL COUNT		4.95	X10^12	[4.37-5.74]
MCH	Mean cell Hb	31.3	pg	[26.5-34.0]
MCHC	Mean cell Hb concentration	33.4	%	[31.5-36.3]
RDW-CV	Red cell distribution width	13.4	%	[11.5-14.5]
MCV	Mean cell volume	94	fL	[80-98]
NUCLEATED RBC %		0.0	/100WC	
NUCLEATED RBC COUNT		0.00	X10^9	[0.00-0.00]
PLATELET COUNT /L		171	X10^9	[150-450]
WHITE BLOOD CELL COUNT		4.5	X10^9	[3.2-9.8]
NEUTROPHIL %		60.2	%	[37.0-80.0]
LYMPHOCYTE %		26.8	%	[10.0-50.0]
MONOCYTE %		10.1	%	[0.0-12.0]
EOSINOPHIL %		2.7	%	[0.0-7.0]
BASOPHIL%		0.2	%	[0.0-2.0]
NEUTROPHIL COUNT		2.7	X10^9	[2.0-8.6]
LYMPHOCYTE COUNT		1.2	X10^9	[0.6-4.2]
MONOCYTE COUNT		0.5	X10^9	[0.0-0.9]
EOSINOPHIL COUNT		0.12	X10^9	[0.00-0.70]
BASOPHIL COUNT		0.01	X10^9	[0.00-0.20]

platelet count in the middle and white cell count and differential on the bottom

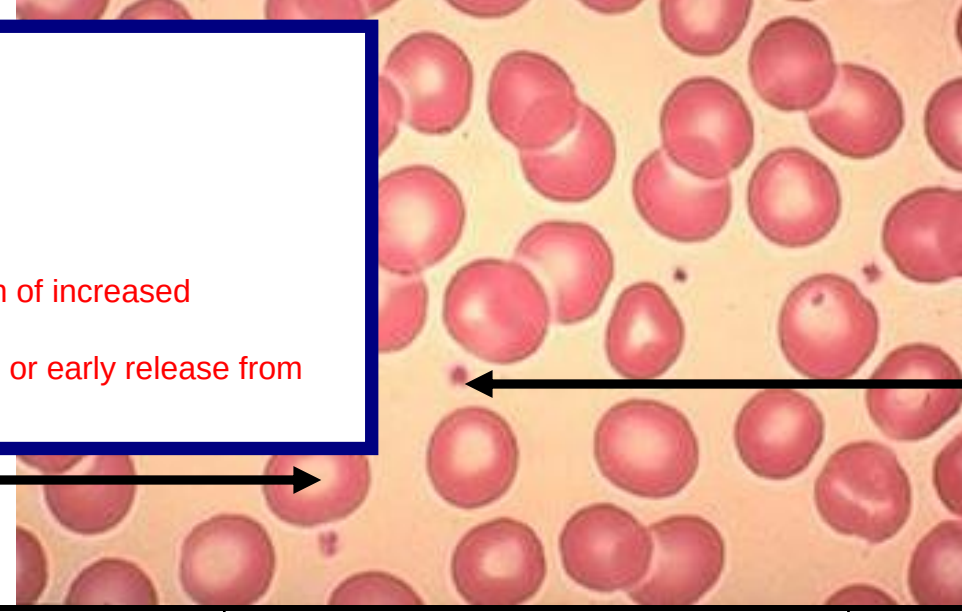
Automated Blood Count

when values are abnormal, they are marked in RED by the laboratory. Alert value is seriously abnormal and maybe life threatening. Lab must call a nurse or doctor with the result. In this case platelets are dangerously low. Dr. H

AUTO BLOOD CT WITH AUTO DIFF

			Reference
HEMOGLOBIN	*9.7	g/dL	[13.7-17.3]
HEMATOCRIT	*0.26	L/L	[0.39-0.49]
RED BLOOD CELL COUNT	*2.95	X10^12	[4.37-5.74]
MCH	32.9	pg	[26.5-34.0]
MCHC	*37.3	%	[31.5-36.3]
RDW-CV	*15.3	%	[11.5-14.5]
MCV	88	fL	[80-98]
NUCLEATED RBC %	0.0	/100WC	
NUCLEATED RBC COUNT	0.00	X10^9	[0.00-0.00]
PLATELET COUNT /L	*9	X10^9	[150-450]
This ALERT result has been called to BEAVEN by CYNTHIA KING on 13:39 and has been read back.			
WHITE BLOOD CELL COUNT	*19.0	X10^9	[3.2-9.8]
NEUTROPHIL %	*	%	[37.0-80.0]
LYMPHOCYTE %	**	%	[10.0-50.0]
MONOCYTE %	**	%	[0.0-12.0]
EOSINOPHIL %	**	%	[0.0-7.0]
BASOPHIL%	**	%	[0.0-2.0]
NEUTROPHIL COUNT	**	X10^9	[2.0-8.6]
LYMPHOCYTE COUNT	**	X10^9	[0.6-4.2]
MONOCYTE COUNT	**	X10^9	[0.0-0.9]
EOSINOPHIL COUNT	**	X10^9	[0.00-0.70]
BASOPHIL COUNT	**	X10^9	[0.00-0.20]
BLOOD FILM REVIEWED			

-Hb low- anemia
-Hb high - polycythemia
-MCV low - microcytic anemia
-MCV high - macrocytic anemia
-MCH low - hypochromic anemia
-MCH high - hyperchromic anemia
-reticulocytes low - decreased production of increased destruction in bone marrow
-reticulocytes high- increased production or early release from bone marrow



note that hemoglobin levels are different between the sexes!

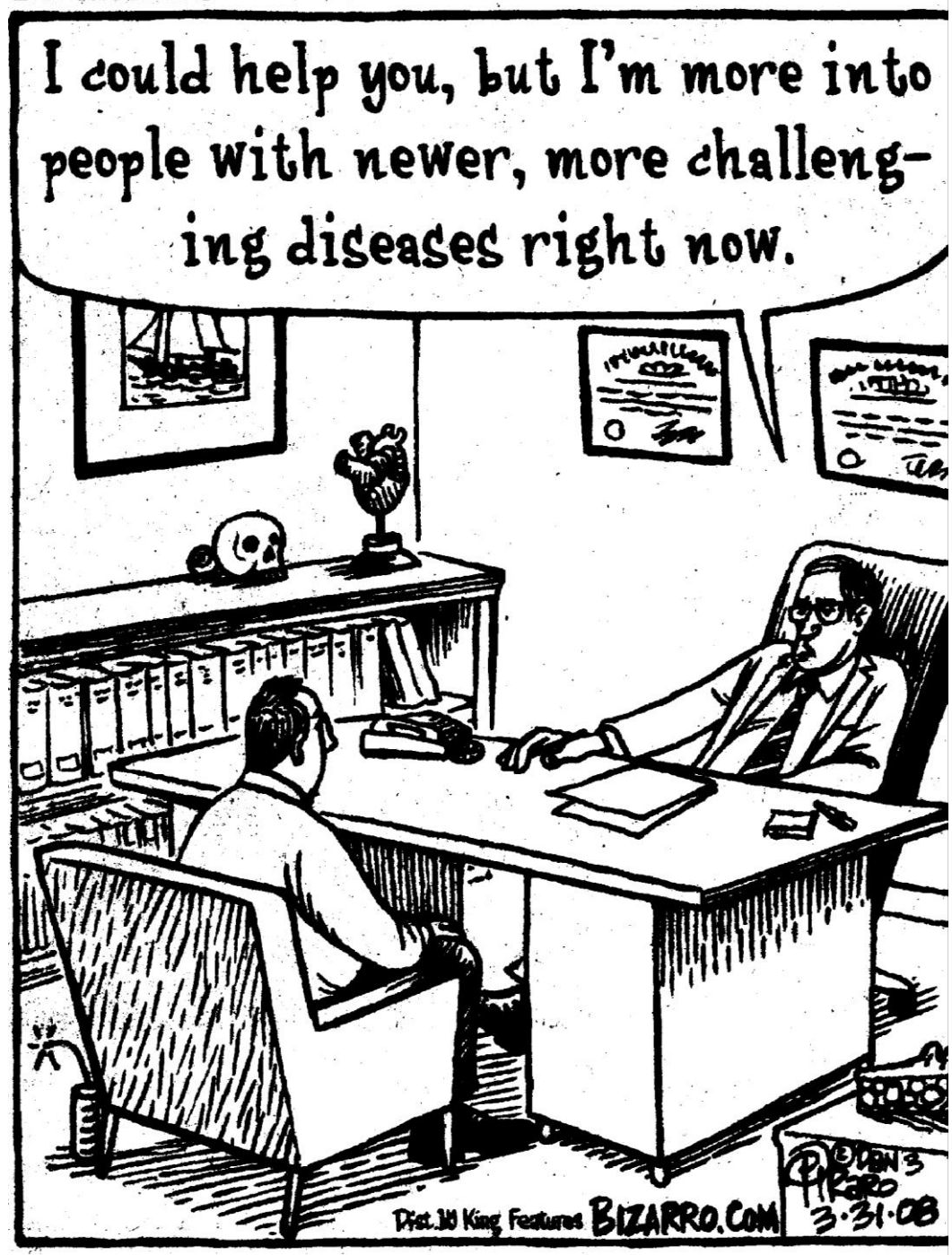
	Normal Range	Decreased below lower limit =	Increased above upper limit =
Hgb g/dL M F	14 - 18 12 - 16	Anemia	Polycythemia
MCV in fL	80 - 98	Microcytic	Macrocytic
MCH in pg	27 - 34	Hypochromic	Hyperchromic
Reticulocyte: % Abs /c mm	0.5 – 1.5 20k –100k	Decreased production or desctruction in BM	Increased production or early release from BM

Anemia : A Major Health Problem Worldwide

anemia is a huge problem worldwide and in the US

- Worldwide:
  - Anemia affects 42% children <5 years old and 53% children 5–14 years old
  - Anemia is 3rd leading cause of lost productivity in adult females
  - Over 1 billion people have iron deficiency (Am J Trop Med Hyg. 2007 Jul;77(1):44-51)
- In the US
  - 3.5% of all persons enrolled in one health insurance plan in 2000 were found to be anemic
  - Average annual cost for anemic patients was $14,535 compared to $9,451 in non-anemic patients J Manag Care Pharm. 2005 Sep;11(7):565-74.

BIZARRO


I could help you, but I'm more into people with newer, more challenging diseases right now.
Dist. by King Features BIZARRO.COM
3·31·08

Classifications of Anemias

definition of anemia = Hb low for the age of sex and patient
-severity based on how low Hb is

- Morphological classification- Based on size of RBC and their hemoglobin content
  - Normocytic vs Microcytic vs Macrocytic
  - Normochromic vs Hypochromic

based on cbc
-MCV- size - microcytic vs macrocytic
-MCH - chromic - hypochrombic vs hyperchromic

NOTE: The morphological classification suggests an etiologic differential which is confirmed by additional tests

- Etiological Classification
  - Decreased Hgb and/or RBC production
    - Deficiency of essential ingredients– Iron, Folate, B12, etc
    - Thalassemias
    - Decreased or defective progenitor cells
  - Defects of red cell survival
    - Hemoglobinopathies
    - Red cell membrane abnormalities
    - Red cell enzyme abnormalites
    - Immune destruction of RBC
    - Vascular and other extrinsic causes
    - Infections - Malaria

Case 1

The normal values you need to know for this case
-Hct - 0.39- 0.49
-Hb for a man - 14-18
-MCV- 80-98
-MCH- 27-34
-platelets - 150, 0000 - 450,000
-wbc count - 3200 - 9800

- 59 yo caucasian man
- Presents with fatigue and headache for 4 months
- He has noted some upper abdominal distress
- Physical examination is normal
- Lab data:
  - Hct: 27 %
  - Hgb: 8.9 gm/dL
  - MCV: 67 fL
  - MCH: 22.6 pg
  - Platelets: 600,000
  - WBC 4,900/cu mm

low - about 45% is normal

severe anemia - should be 14 or above for a man
-12-14 - mild anemia
-10-12 - moderate anemia
-below 10- severe anemia
-everything lowered by 2 in women

Anemia

fL - femtoliters - 10 -15 liters
-low

Microcytic

low

Hypochromic

slightly increased

Thrombocytosis

normal

Case 1: Microcytic, hypochromic anemia (continued)

- 59 yo caucasian man with Microcytic anemia and thrombocytosis
  - Hct: 27 %
  - Hgb: 8.9 gm/dL
  - MCV: 67 fL
  - MCH: 22.6 pG
  - Platelets: 600,000
  - WBC 4,900/cu mm

The normal values you need to know for this case
-reticulocyte count - 20-100 k/cumm

- Reticulocyte: 30,000/mm3 → **Inappropriately low**

low reticulocyte count

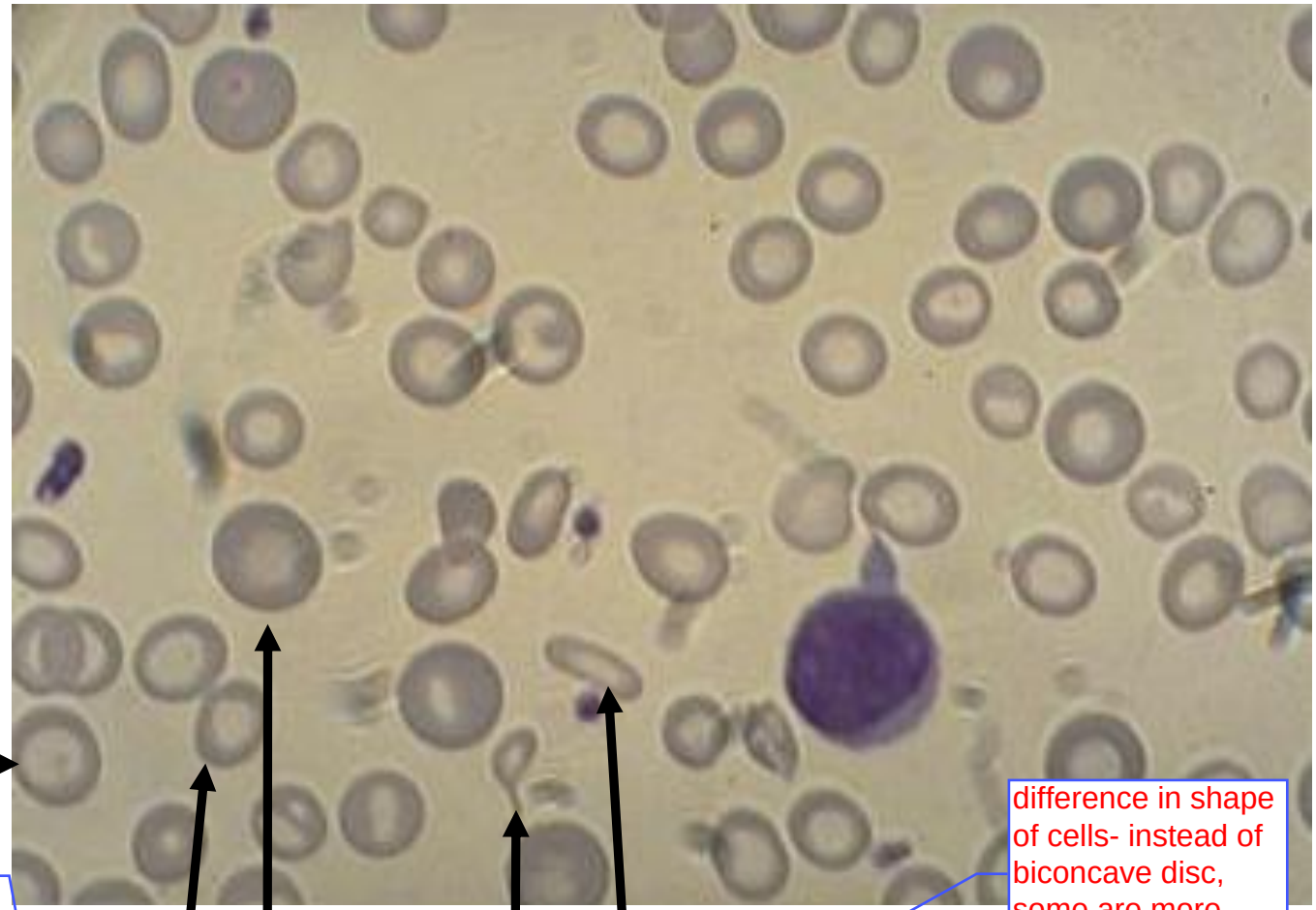
- Peripheral Blood Film-
  - WBC differential:
    - Neutrophils 65%
    - lymphocytes 33%
    - monocytes 2%

normal differential white blood cell counts

  - Abnormal RBC morphology

abnormal red cell morphology

Case 1- Peripheral Blood Film
Microcytic hypochromic anemia



central pallor in center of red cells is increased

Hypochromia

large variation in diameter of red cells

Anisocytosis
RDW=19.6

Poikilocytosis

difference in shape of cells- instead of biconcave disc, some are more pencil shaped

Microcytic hypochromic anemia: Etiological differential diagnosis

- **Iron deficiency anemia**
- Anemia of chronic inflammation
- Thalassemias

inherited cause, will see on monday

- Sideroblastic anemia
- Lead poisoning

Understanding iron metabolism:

- The body has no mechanism to excrete excess iron
- Absorption of dietary iron is strictly controlled to maintain total iron in the body
- Free iron is toxic, therefore it is bound to proteins –
  - Specific binding to transferrin and apoferritin
  - Non-specific binding to albumin

-body does not have a good mechanism for excreting iron so the intake of iron must be tightly regulated
-free iron is toxic so it must be bound to proteins- transferrin is primary transport protein

Understanding iron metabolism:

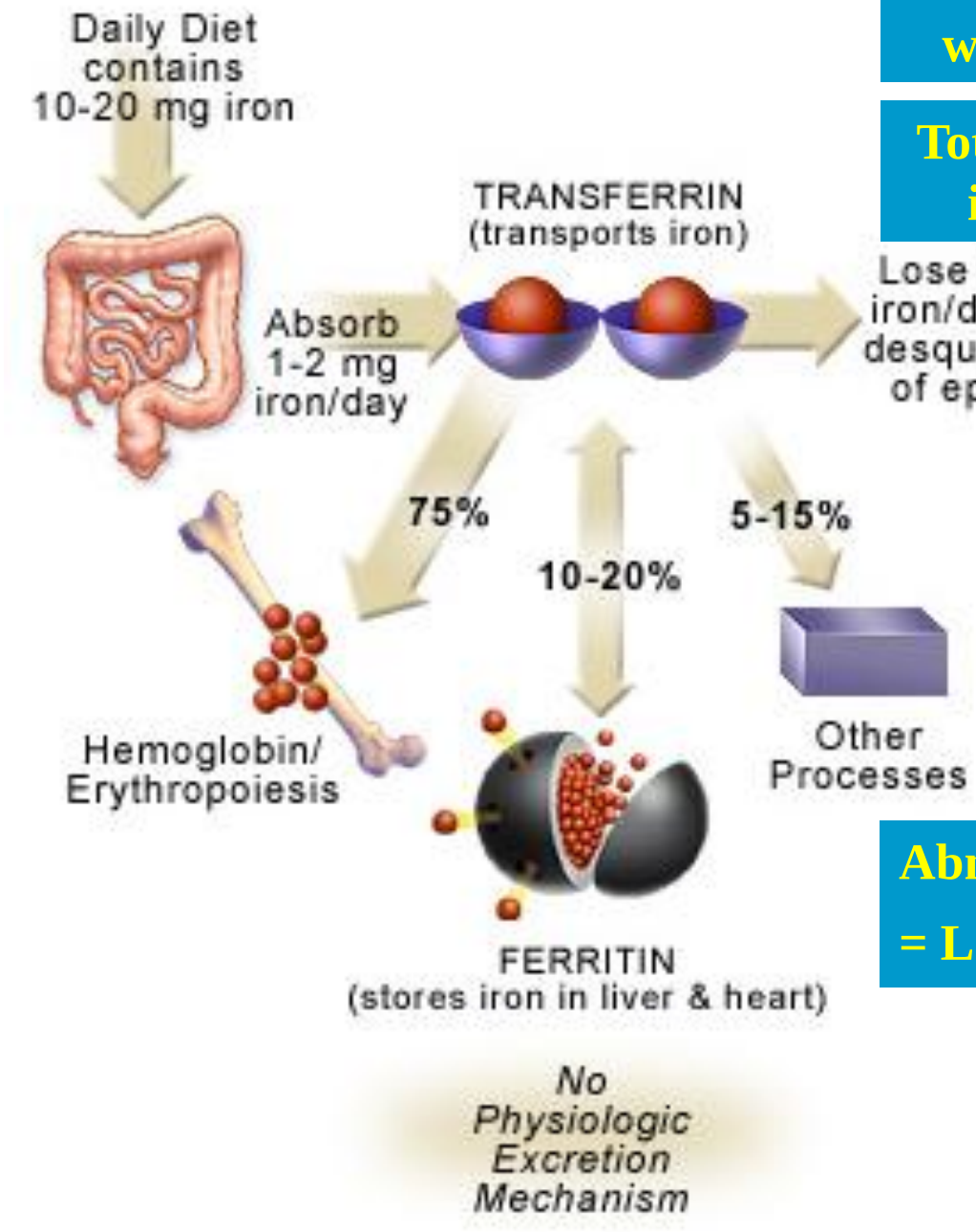
- Transferrin is the primary transport molecule for iron.
  - Blood transferrin level is referred to as "Total Iron Binding Capacity"
  - Proportion of transferrin molecules bound to iron = % saturation of iron binding capacity (about 1/3)
  - This iron is most readily available for Hgb synthesis
- Some iron binds to another protein called apoferritin (produced by liver) to form a water soluble molecule called ferritin (blood and tissues)
  - Ferritin is present in blood and ferritin iron can be easily delivered for Hgb synthesis.
- Excess iron is stored in bone marrow as water insoluble Hemosiderin

-transferrin bound iron is cash in your pocket that is the most readily available
-ferritin is like your atm card where you can go get cash if you need it
-hemosiderin is like your certificate of deposit that may be harder to access but may contain a lot of money

Additional Laboratory Tests In Microcytic, Hypochromic Anemia:

- Serum Iron level:
- Iron binding capacity = Transferrin level
- Transferrin saturation = % transferrin bound to iron
- Serum ferritin

any time you suspect problem with iron metabolism, do these 4 tests



Trasferrin levels increase when iron stores decline

Total Iron Binding Capacity (TIBC) increases but it is less saturated

-normally, iron is absorbed and binds to transferrin and most is used to make hemoglobin
-about 10% binds to ferritin and goes into the circulation
-when iron decreases, transferrin levels increase to try to absorb as much iron as possible so as soon as iron comes through intestine, it is bound up by high levels of transferrin
-when iron decreases, percent saturation of iron is low because there is little iron to bind to the transferrin
-ferritin is either high or low - inflammation can increase ferritin levels - low ferritin level suggests iron deficiency but normal ferritin level does not rule out iron deficiency

Abnormally Low blood Ferritin = Low/ Absent storage iron*

***Ferritin levels increase due to inflammation, even when iron stores are low. Therefore, normal or high Ferritin does NOT guarantee normal storage iron.**

Case 1 continued

- Additional laboratory tests:
- Serum Iron: 10 (low)
- Iron binding capacity: 450 (high)
- Transferrin saturation: 2% (low)
- Serum ferritin: 10 ng/mL (low)
- Diagnosis: Iron deficiency anemia

Must investigate causes of chronic blood loss in iron deficiency anemia in older adults. Dietary iron deficiency more common in children and reproductive age females.

- Stool samples positive for occult blood

do not miss the CAUSE of iron deficiency - in this case it was a GI malignancy
dietary causes are more common in younger patients whereas malignancies increase in likelihood in older patients

what is the cause of iron deficiency?
-adult male or post menopausal females --> GI tract malignancy?

need to do colonoscopy

The normal values you need to know for this case
-Hct - 0.39- 0.49
-Hb for a man - 14-18
-MCV- 80-98
-MCH- 27-34
-reticulocytes - 20-100 K/ cumm
-platelets - 150, 0000 - 450,000
-wbc count - 3200 - 9800

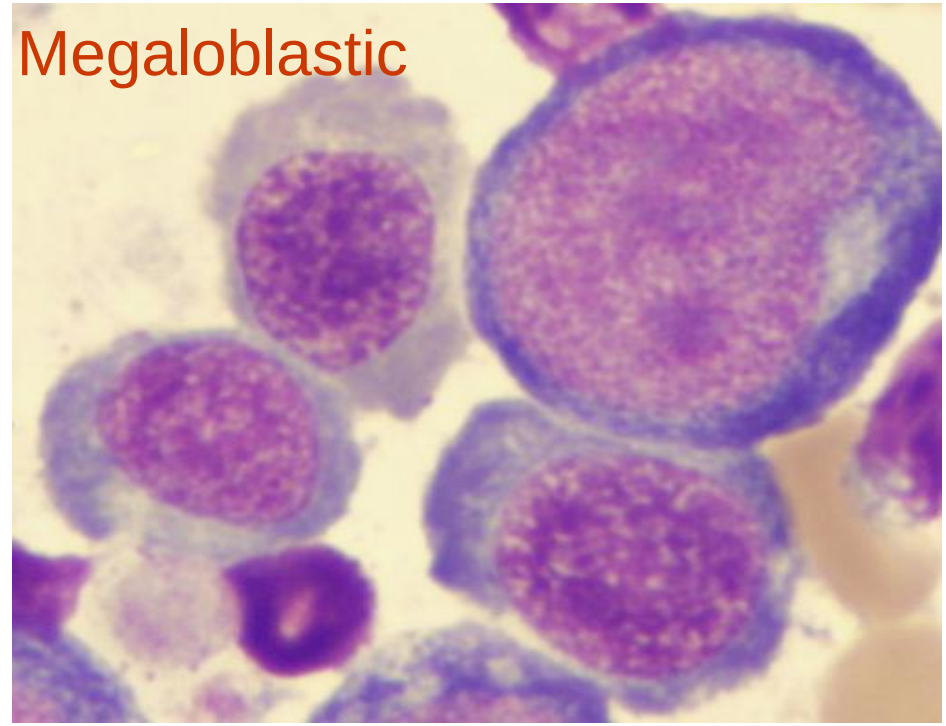
Case 2

- 54 yo man
  - Presents with nausea, poor appetite, mild diarrhea
  - PE: Normal
- CBC:
  - Hct: 35 % (low hematocrit)
  - Hgb: 12 gm/dl (Anemia) (moderate anemia)
  - MCV: 115 fl (Macrocytosis) (large red cells)
  - Retic: 65,000/ cu mm (not elevated, relatively low)
  - Platelets: 200,000
  - WBC: 4,000
- Blood film: Macrocytosis, WBC differential is normal
- Normal upper and lower GI studies

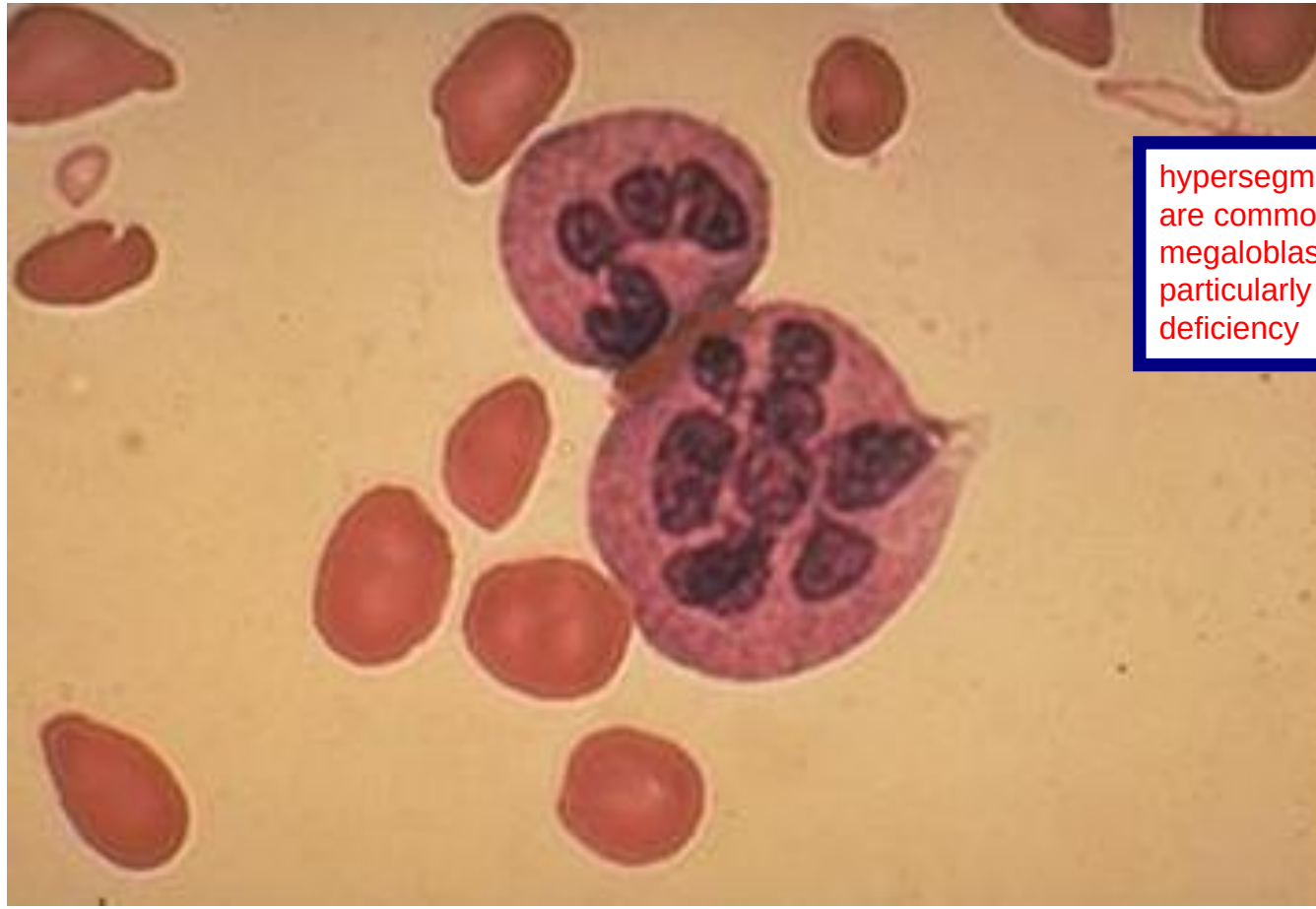
Macrocytic Anemias with low Retics: Megaloblastic or Normoblastic?

macrocytic anemias can be either:
-megaloblastic - abnormal erythopoiesis in bone marrow
-normoblastic - normal erythropoiesis

- Megaloblastic (specific morphological change in red cell precursors in bone marrow)
  - Vit B12 deficiency
  - Folate deficiency
  - Myelodysplastic syndromes
  - Drug-induced
- Normoblastic
  - Hypothyroidism
  - Liver disease
  - Alcohol



Case 2- Peripheral Blood Film



hypersegmented neutrophils are commonly seen with megaloblastic anemias- particularly vit b12 and folate deficiency

Hypersegmented neutrophil

Case 2 continued

difficulties with extremities

- Several months later -
  - Paresthesias of hands and feet
  - Difficulty using the clutch and gas pedals while driving
- PE:
  - Mild scleral icterus
  - Absent position and vibratory sensation
  - Diminished two-point discrimination

jaundice and neurological deficits

Case 2 continued

macrocytic anemia + neurological symptoms --> typical for vit b12 deficiency

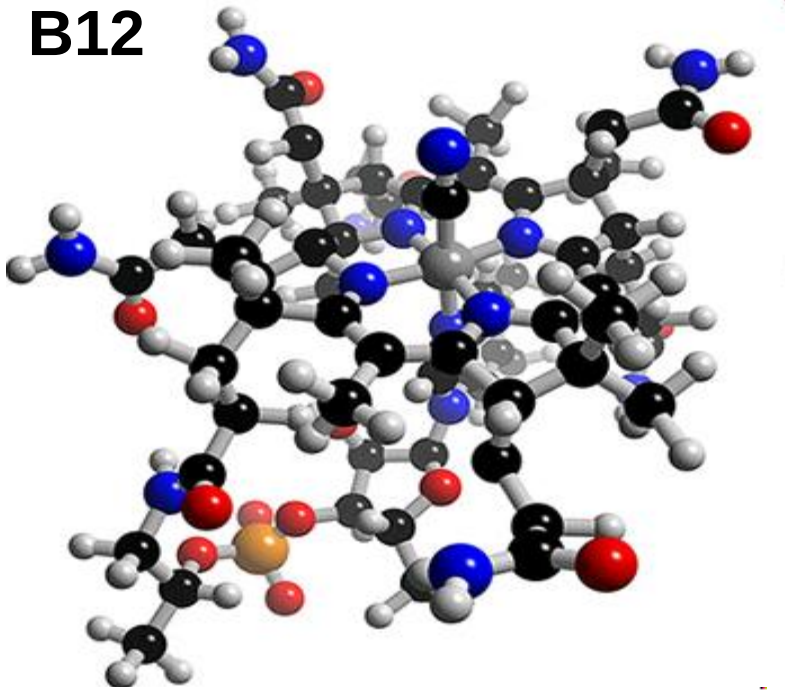
- Diagnostic laboratory evaluation-
  - Serum B12 level- 30 (normal > 180)
  - Anti-intrinsic factor antibodies positive

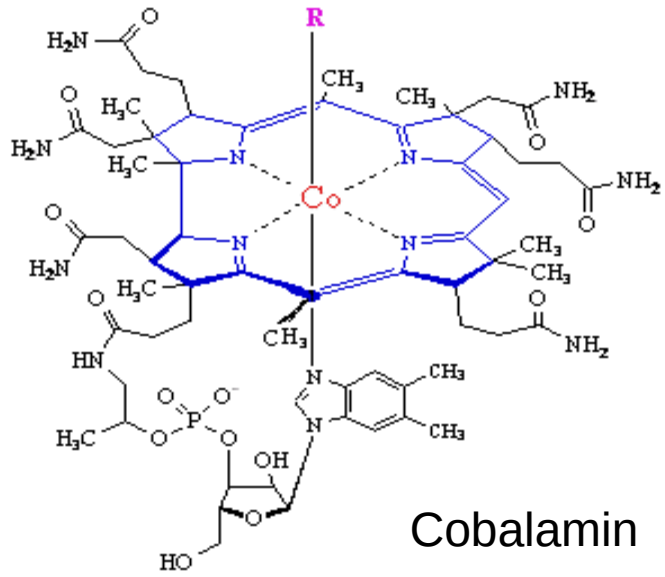
- Diagnosis- B12 deficiency
  Pernicious anemia

most common cause of b12 deficiency in adults
-autoimmune process in which absorption of vit b12 is impaired

Back to the Basics…

B12

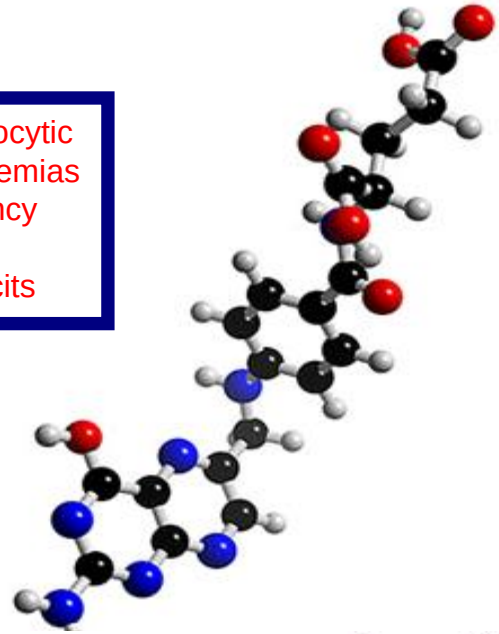


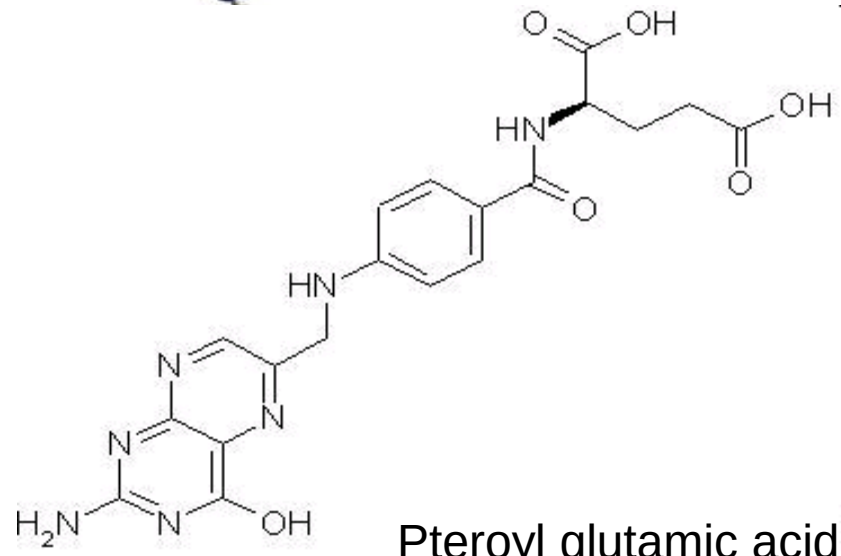


Cobalamin

Folate

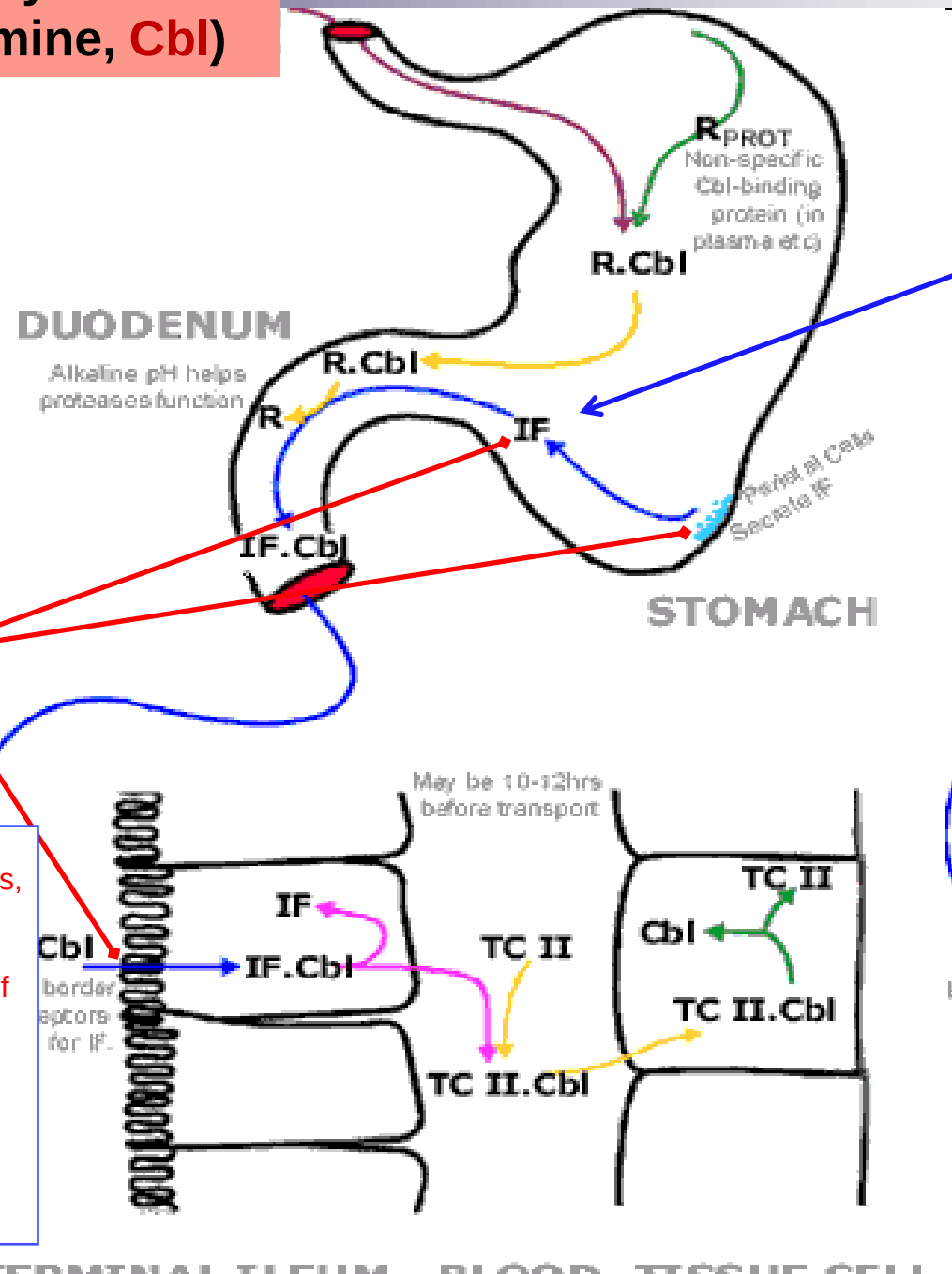
both cause macrocytic megaloblastic anemias but folate deficiency doesnt cause neurological deficits





Pteroyl glutamic acid

Dietary B12 (cobolamine, Cbl)



Intrinsic Factor (IF)
-Secreted by gastric parietal cells
-Required for absorption of B12

-in the stomach, dietary vit b12 binds to intrinsic factor
-intrinsic factor + vit b12 bind to a specific receptor in the small intestine and is absorbed

Autoantibodies disrupt B12 absorption in pernicious anemia

autoantibodies against IF, against parietal cells, or against receptor in small intestine all can cause malabsorption of vit b12
-takes a long time to develop bc vit b12 is stored in the body

Actions of B12 and Folate:

folate independent vit b12 actions are responsible for the neurological symptoms in vit b12 deficiency that not seen in folate deficiency

- Folate is directly required for Purine (DNA) synthesis, B12 is indirectly involved through folate metabolism
  - Only tetra-hydro folate (THF) can participate in purine synthesis
  - Dietary folate is converted to THF and then to methyl-THF
  - Methyl-THF can be converted back to THF if B12 is present
    - Only B12 can transfer the methyl group from Methyl-THF to homocysteine
  - In the absence of B12, most folate is “trapped” as methyl-THF , levels of THF decline, and DNA synthesis suffers
- Treatment with large doses of folate will form “new” THF, bypassing requirement for B12
- Treatment with folate will correct anemia due to folate deficiency or B-12 deficiency

but treating will worsen neurological symptoms

Anemia due to B12 or Folate Deficiency

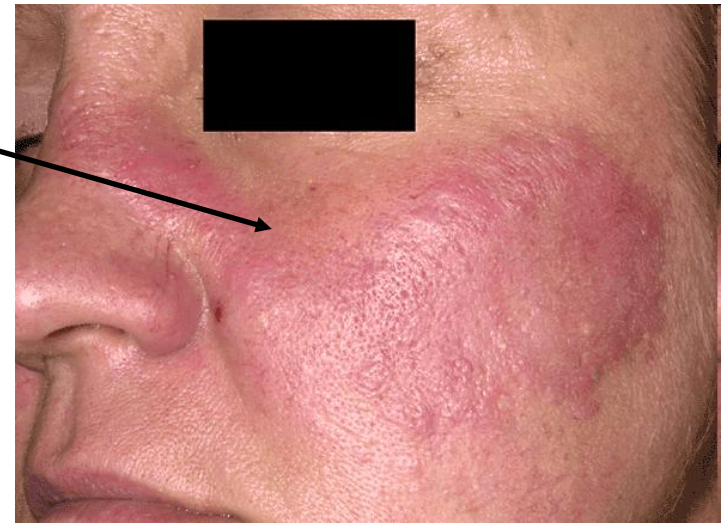
DO NOT treat vit b12 deficiency anemia with folate even though it corrects the anemia because it worsens the neurological symptoms
-can treat folate deficiency anemia with folate to correct the anemia

- Treatment with folate will correct anemia due to folate deficiency or B-12 deficiency
- Mitochondrial action of B12: (Folate independent)
  - Adenosyl-Cbl acts as coenzyme for conversion of methylmalonyl-CoA to succinyl-CoA
  - ? Associated with myelin formation and etiology of neuropathy observed in B12 deficiency
- **Neuropathy of B12 deficiency may be aggravated by folate administration**
- B12 administration will not correct anemia due to folate deficiency

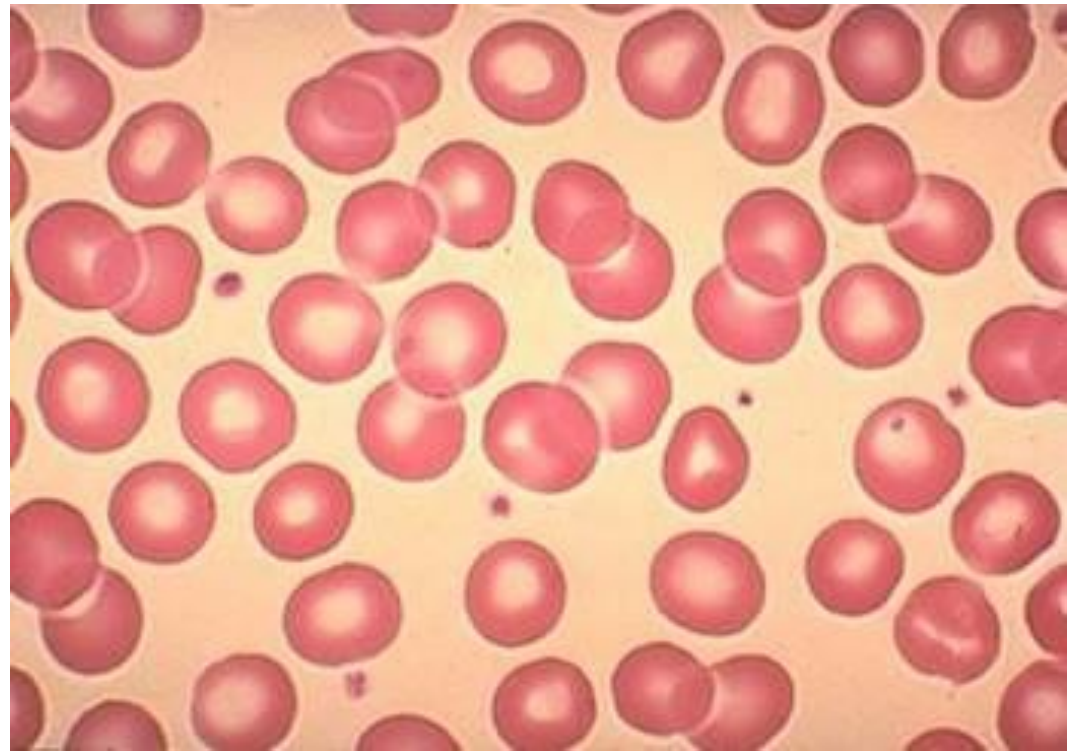
The normal values you need to know for this case
-Hct - 0.39- 0.49
-Hb for a woman - 12-14
-MCV- 80-98
-MCH- 27-34
-platelets - 150, 0000 - 450,000
-wbc count - 3200 - 9800
-reticulocytes - 20-100 K / cumm

Case 3

- 23 yo woman
  - Fatigue, arthralgias, skin rash for several months
  - PE: Malar rash
- Lab data:
  - Hct 29 %
  - Hgb 9.2 gm/dl ← anemia
  - MCV 82 fl ← borderline microcytic
  - Platelets: 150,000
  - WBC: 4,900
- Blood film:
  - Normochromic, normocytic RBCs
  - WBC diff: Normal ← white blood cell count normal
- Retic: 60,000/cu mm (inappropriately low) → reticulocyte count low



Case 3- Peripheral Blood Film



normal morphology of rbcs

Normochromic, normocytic RBCs

Normocytic - Normochromic Anemia and Low Retic Count: differential diagnosis

- Primary BM (stem cell) disorders
  - Aplastic anemia
  - Pure Red Cell aplasia
  - Infiltrative disorders
- Secondary to systemic illness
  - Anemia of chronic inflammation
  - Renal insufficiency
  - Endocrine disorders

primary causes of normal red blood cells + anemia + low reticulocyte count
-remember that when there is anemia, the reticulocyte count should be higher to compensate, so something is wrong if the reticulocyte count is also low

systemic inflammatory causes

usually microcytic anemia

Case 3: Additional Tests

- ESR: 80 mm/hr
- BUN: 42
- Creatinine 2.0
- Anti Nuclear Antibody 1:1256
- Complement C3/C4 Low
- Anti-ds DNA Positive

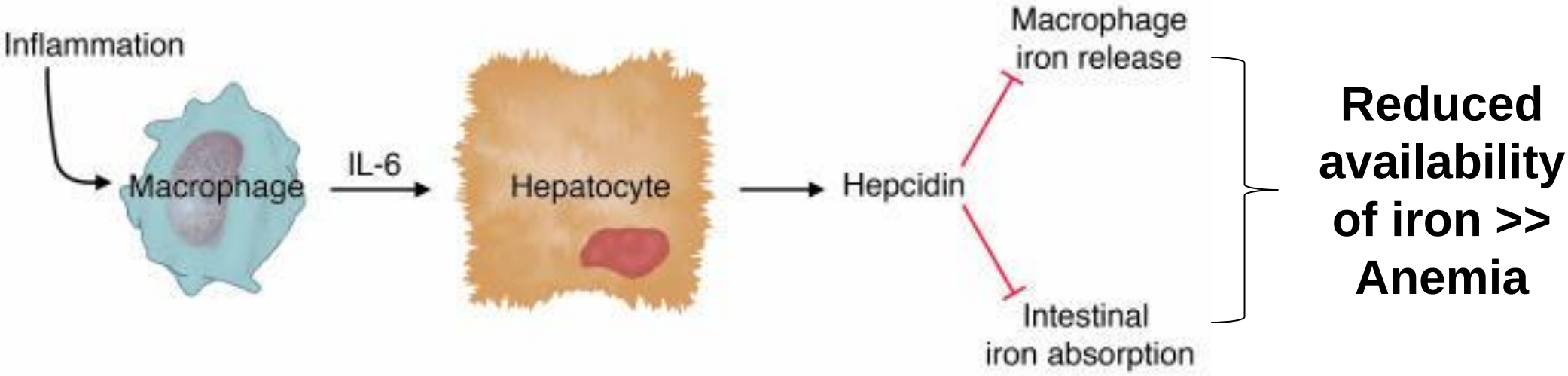
- Diagnosis
  - Systemic Lupus Erythematosus (SLE)
  - Renal insufficiency
  - Anemia of Chronic Disease (= anemia of inflammation)
    - Possibly worsened by low erythropoietin

chronic autoimmune disease- anemia of chronic inflammation

inadequate erythropoiten production may worsen anemia

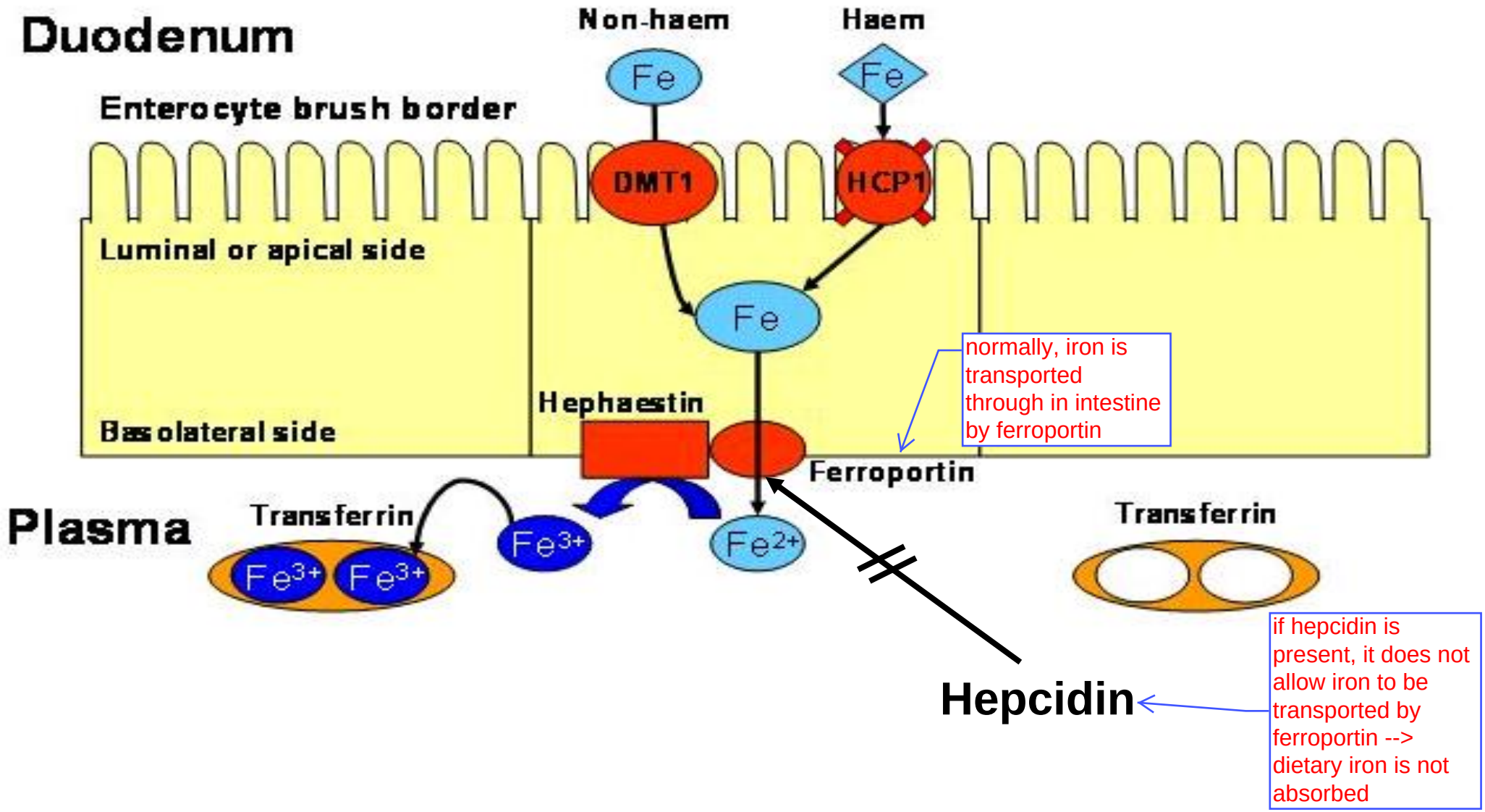
Hepcidin: The inflammation-anemia connection

-macrophages make IL6 in response to inflammation
-IL6 causes hepatocytes to make hepcidin
-hepcidin reduces intestinal absorption of iron and inhibits macrophages from releasing iron that they are storing



Reduced availability of iron >> Anemia

Anemia of inflammation: the cytokine-hepcidin link
Nancy C. Andrews
J. Clin. Invest. 2004, 113:1251


Duodenum
Non-haem
Haem
Fe
Fe
Enterocyte brush border
DMT1
HCP1
Luminal or apical side
Fe
Hephaestin
Basolateral side
normally, iron is transported through in intestine by ferroportin
Ferroportin
Plasma
Transferrin
Fe3+
Fe3+
Fe3+
Fe2+
Transferrin
Hepcidin
if hepcidin is present, it does not allow iron to be transported by ferroportin --> dietary iron is not absorbed

Other Molecules Involved In Iron Absorption

these are not related to anemia but are related to hepcidin

- These molecules are required for appropriate synthesis of Hepcidin
  - Mutations lead to reduced hepcidin and excess iron absorption = **HEMOCHROMATOSIS**

excess iron absorbed due to reduced hepcidin production

- Hemochromatosis (HFE) gene
  - Mutations cause adult hemochromatosis
- Hemojuvelin
  - Mutations cause a severe hemochromatosis in children
- Transferrin receptor 2

Case 4

The normal values you need to know for this case
-Hct - 0.39- 0.49
-Hb for a man - 14-18
-MCV- 80-98
-MCH- 27-34
-platelets - 150, 0000 - 450,000
-wbc count - 3200 - 9800
-reticulocytes - 20-100K / cumm

-if anemia develops slowly, cardiovascular/ respiratory adaptation is benefecial
-if anemia develops acutely, cardiovascular/ respiratory adaptation is deliterious

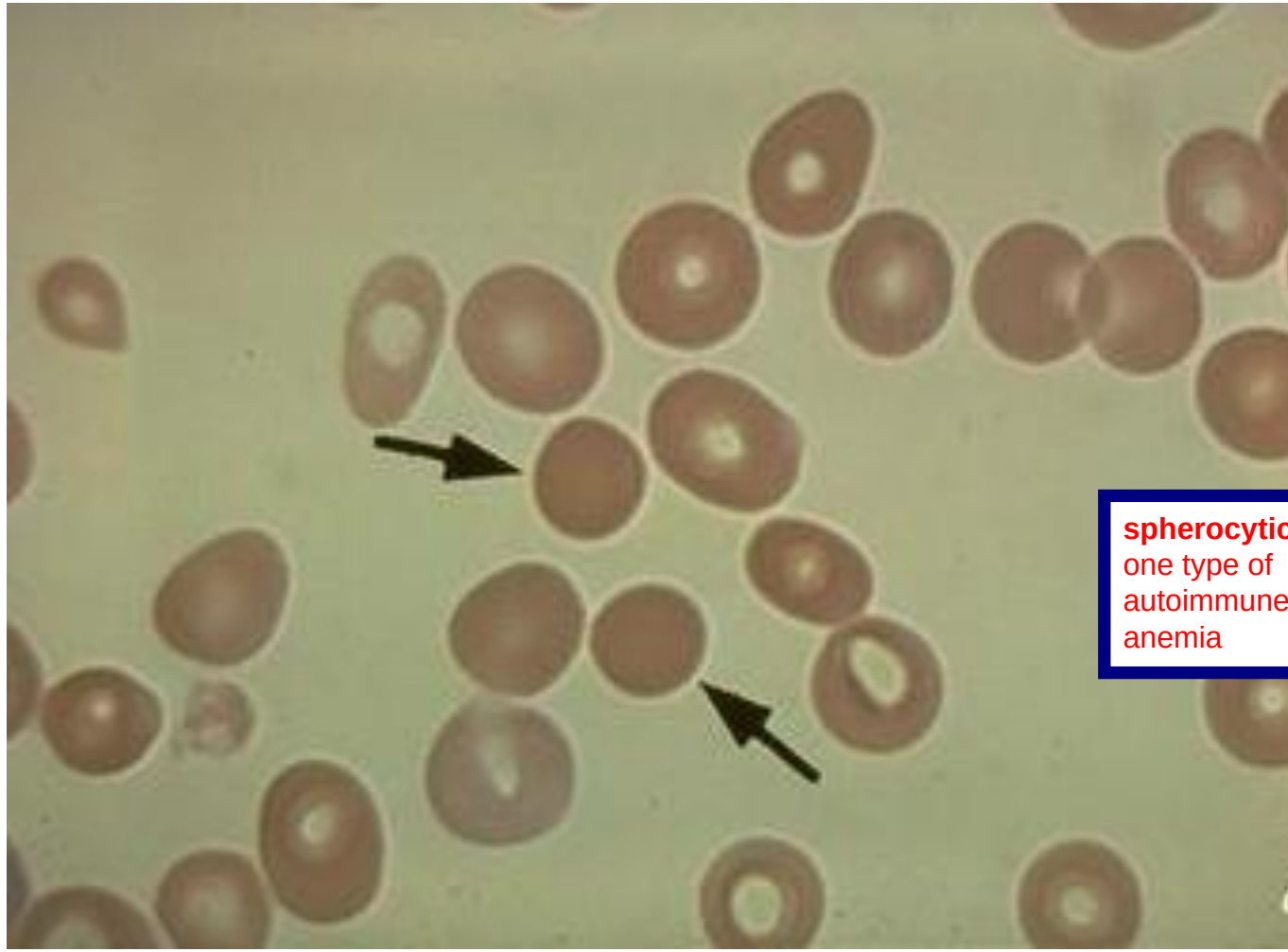
- 55 yo man (acute)
  One month history of fatigue and palpitations
- PE: Pallor
  Palpable spleen tip (splenomegaly)
- Lab data:
  - Hct: 20 %
  - Hgb: 6.9 gm/dl (severe anemia)
  - MCV: 100 fl (slightly elevated)
  - Platelets: Normal
  - WBC: Normal
  - Retic: 154,000/ cu mm → HIGH (high reticulocytes)

Anemia with Reticulocytosis: Differential Diagnosis

anemia with high reticulocyte count
- bleeding- think about bleeding with body trying to make more blood
-hemolytic anemias- mostly congenital. one that is acquired is malaria which isn't a problem in us but huge cause of anemia world wide - others autoimmune, prosthetic valves, MAHA
-hypersplenism- large spleen sequestering blood

- Bleeding Rule out first
- Hemolytic Anemias
  - Immune- Autoimmune, alloimmune, drug induced
  - Inherited- Hemoglobinopathies
    RBC membrane/enzyme disorders
  - Mechanical Prosthetic valves,
    Microangiopathic (MAHA)
  - Infections- Malaria, babesia
- Hypersplenism

Case 4- Peripheral Blood Film



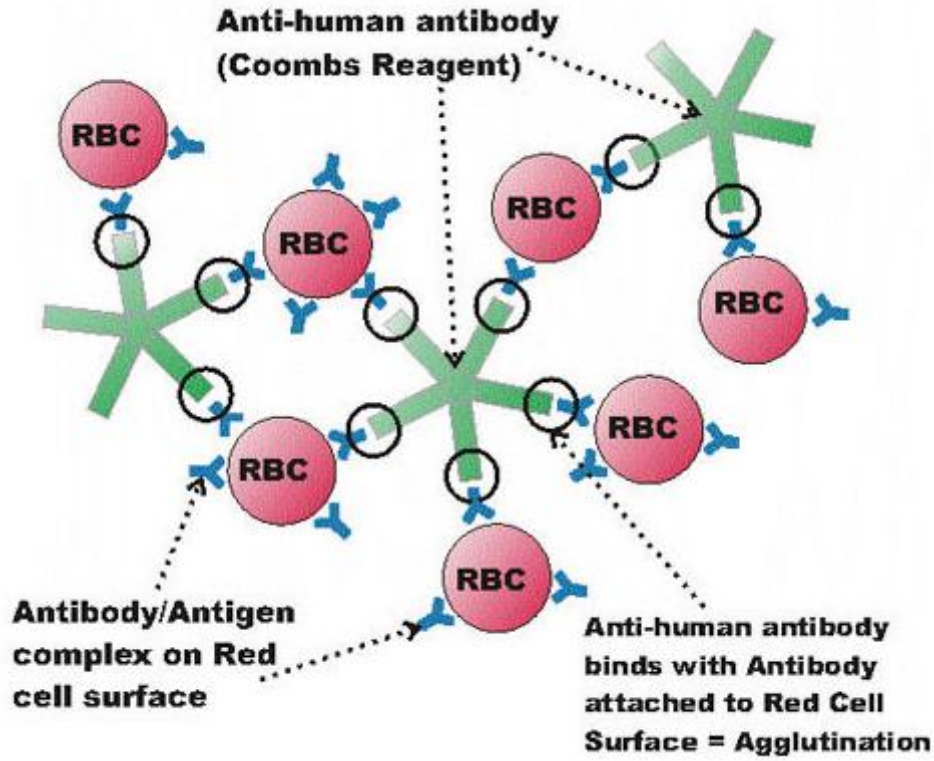
spherocytic cells in one type of autoimmune hemolytic anemia

Microspherocytes

Case 4 continued

Coombs test- looking for presence of antibodies bound to red cells
-take another antibody to recognize human antibodies (Coombs reagent), and these antibodies bind to that autoantibodies on the red cells and cause agglutination that can be seen directly

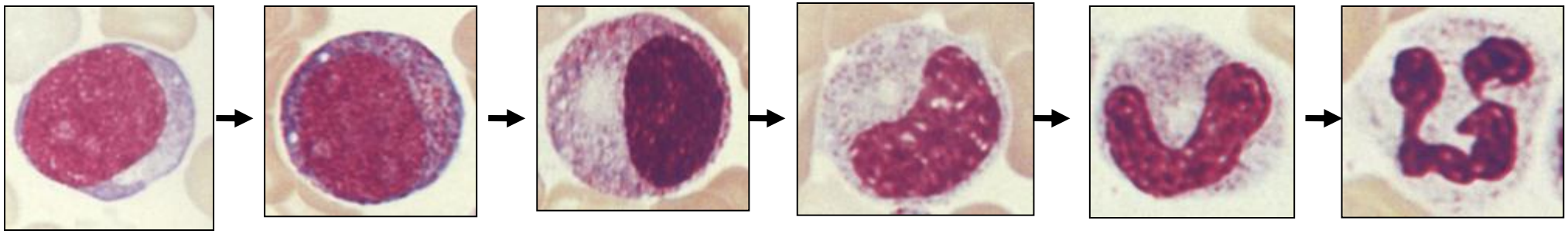
- Diagnostic laboratory evaluation
  - Direct Coombs test: Positive, 4+, IgG
  - Warm autoantibody eluted from RBCs



- Diagnosis: Autoimmune hemolytic anemia

Myeloid Differentiation

Bone marrow | **Peripheral blood**



Blast → Promyelocyte → Myelocyte → Metamyelocyte → Band → PMN

Case 5

The normal values you need to know for this case
-Hb for a man - 14-18
-platelets - 150, 0000 - 450,000
-wbc count - 3200 - 9800
- Neutrophils - 37-80%
- lymphocytes - 10-50%
-monocytes - 0-12%
-basophils - 0-7%
-eosinophils - 0-2%

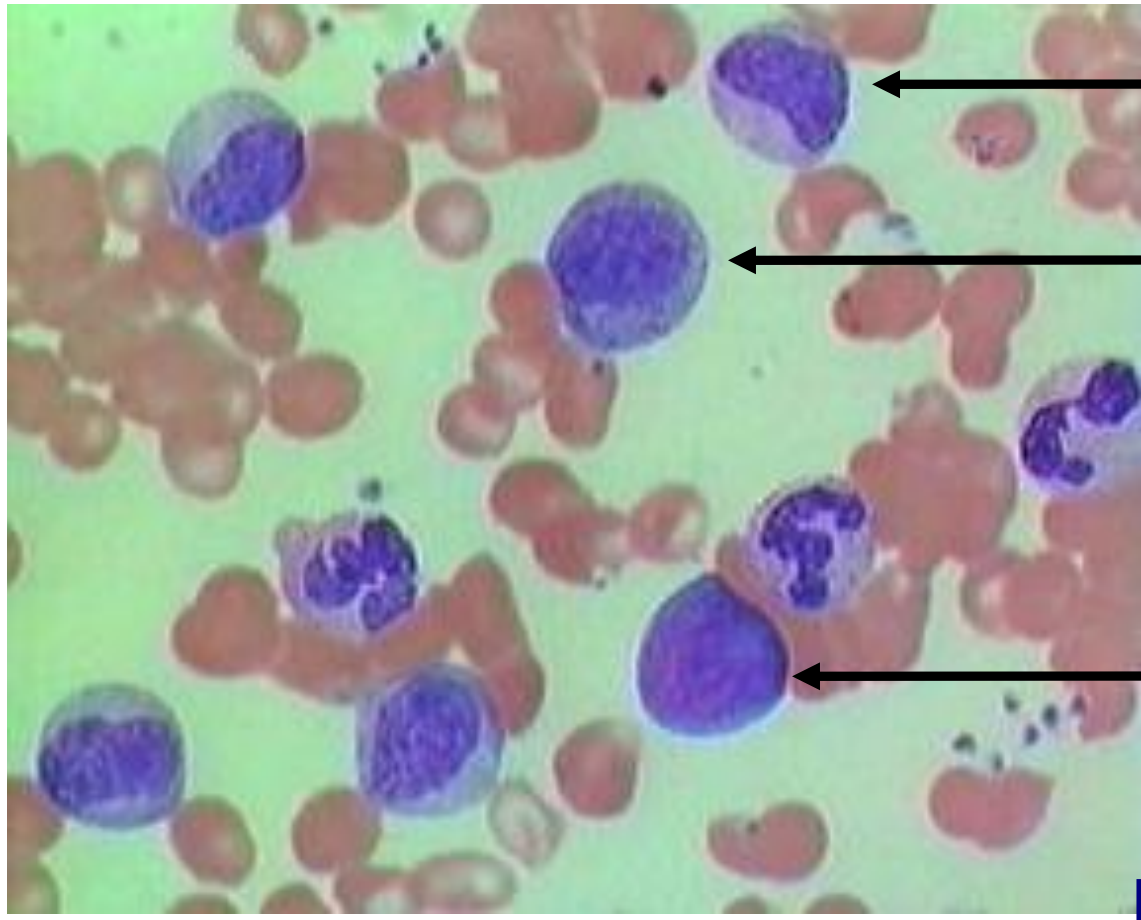
- 42 yo dentist
  - Turned down as a blood donor because of Hgb of 11.5
- PE Splenomegaly 4cm below left costal margin
- Further testing revealed:
  - WBC: 47,000/ cu mm
  - WBC diff: Neutrophils 40%
  - Bands: 20%
  - Metamyelocytes:16%
  - Myelocytes: 8%
  - Promyelocytes:6%
  - Blasts: 2%
  - Eos: 2%
  - Basos: 4%
  - Monos: 2%
  - Platelets: 680,000/ cu mm

moderate anemia

high white cell count with immature myeloid cells
-basophils were increased
-platelets were increased
-basically diagnostic for **CML** but need a blood smear and test for philadelphia chromosome

Immature myeloid cells

Case 5- Peripheral Blood Film



lots of immature myeloid cells
elevated basophils and platelets

Leukocytosis with left shift

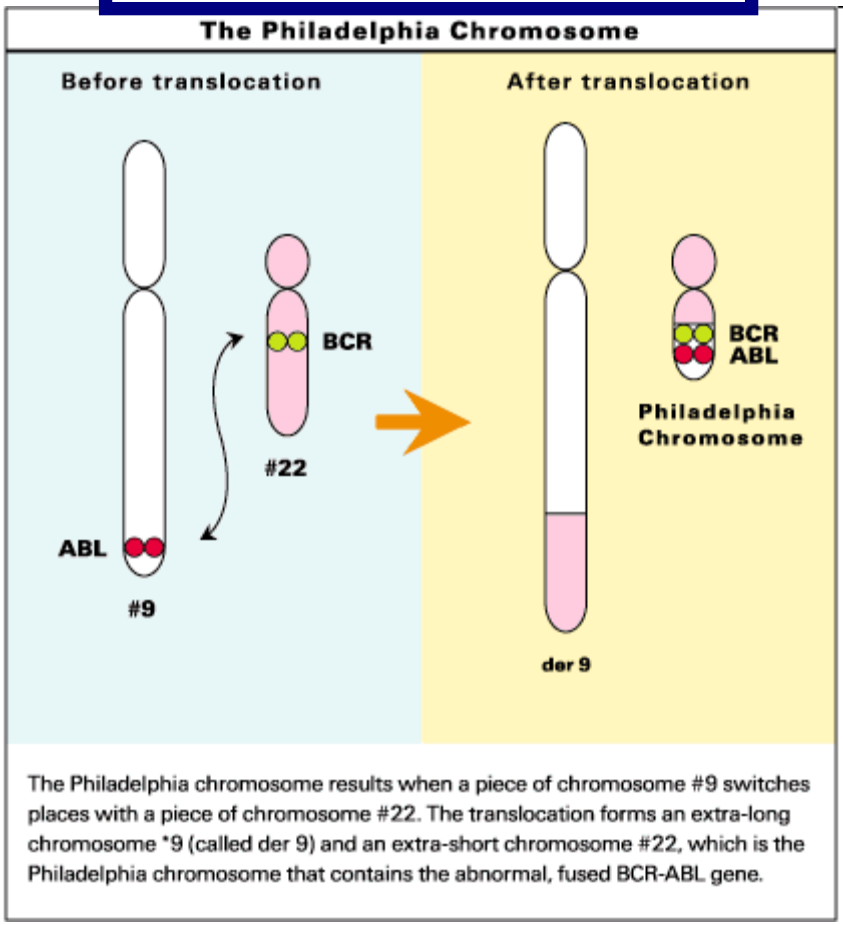
Case 5 continued

- Diagnostic evaluation

  Cytogenetics- Philadelphia chromosome +
  (due to translocation between chromosomes 9 and 22, producing an abnormal product by splicing ABL and BCR genes)

- Diagnosis: Chronic myelogenous leukemia (CML)
  - A type of chronic myeloproliferative neoplasm

philadelphia chromosome - presence is diagnostic of CML
-translocation of chromosomes 9 and 22 producing a fusion product BCR ABL
-gleevec specifically binds to tyrosine kinase site on BCRABL and is revolutionary drug for treating CML



The Philadelphia chromosome results when a piece of chromosome #9 switches places with a piece of chromosome #22. The translocation forms an extra-long chromosome *9 (called der 9) and an extra-short chromosome #22, which is the Philadelphia chromosome that contains the abnormal, fused BCR-ABL gene.

Chronic Myeloproliferative Neoplasms (MPN)

- Chronic myelogenous leukemia (CML) – ↑Neutrophils, basophils
- Polycythemia vera (PV) - ↑RBC
- Essential thrombocythemia (ET) - ↑Plt
- Idiopathic myelofibrosis (MF) - ↑Fibrosis

primarily neutrophils proliferating in CML

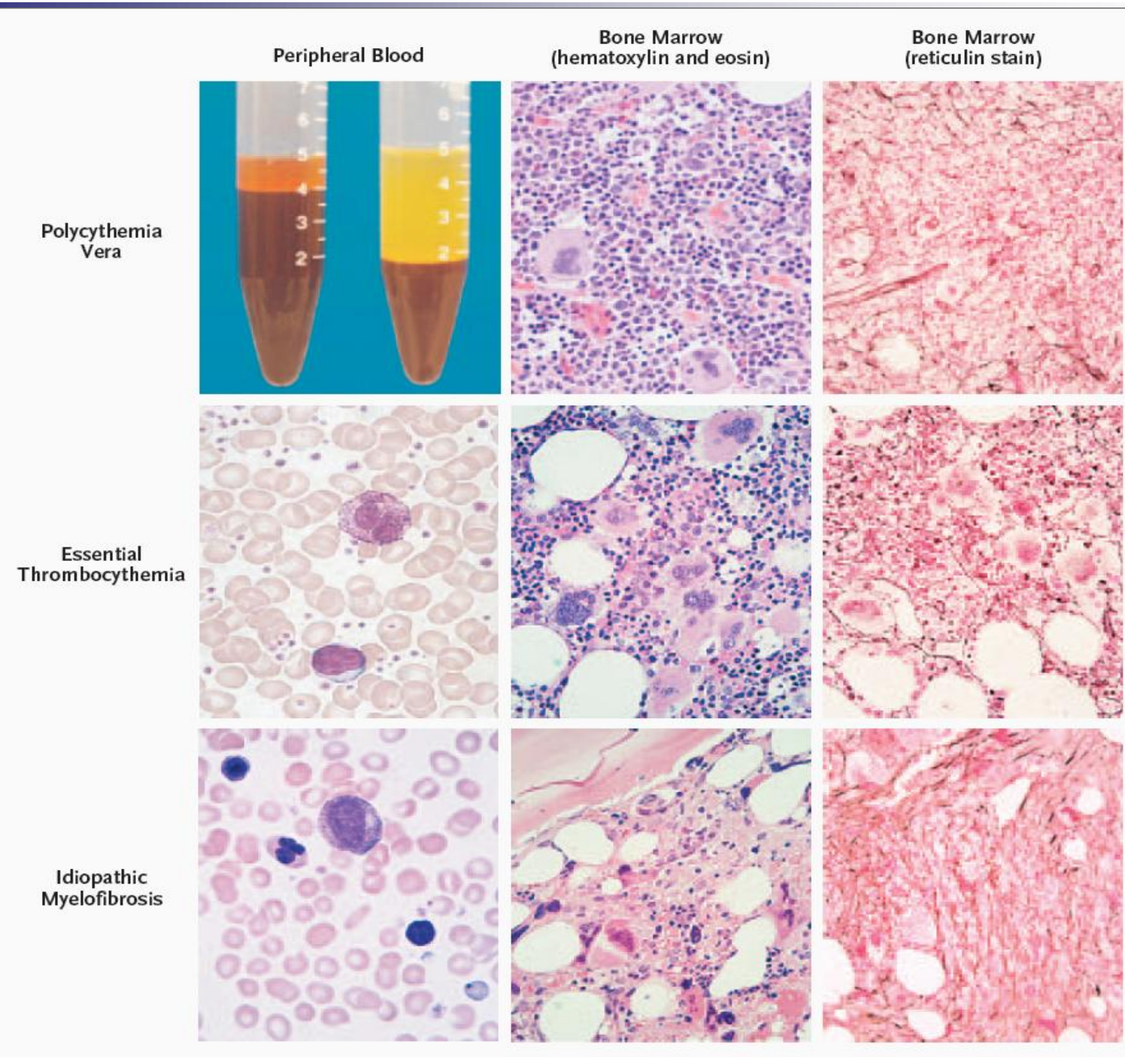
primarily red cells proliferating

primarily platelets proliferating

lots of fibrosis

Chronic Myeloproliferative Neoplasms: Clinical Features

- Enlarged spleen (except in Essential Thrombocythemia)
- Present with abnormal WBC, RBC, or platelet count
- Thrombosis and bleeding → ? Platelet dysfunction
- Must be distinguished from a reactive state, i.e.,
  - ↑ RBC → due to: Hypoxic stimulation Excess Erythropoietin
  - ↑ Plts → due to: infection, inflammation
  - ↑ WBC
- Natural history evolve over years. ie. not acute
- Usually NOT associated with fever, night sweats etc


Peripheral Blood
Bone Marrow (hematoxylin and eosin)
Bone Marrow (reticulin stain)
Polycythemia Vera
Essential Thrombocythemia
Idiopathic Myelofibrosis

Case 6

The normal values you need to know for this case
-Hb for a woman - 12-14
-MCV- 80-98
-platelets - 150, 0000 - 450,000
-wbc count - 3200 - 9800

- 60 yo woman
  Presents with pruritus, headache and early satiety
- PE Splenomegaly 5cm below left costal margin
- CBC
  - Hgb: 20 gm/dL (elevated - polycythemia)
  - MCV: 88 fl
  - Platelets: 580,000 / cu mm
  - WBC: 18,500
  - WBC diff: Normal

} Polycythemia

- Smear: No immature cells. Neutrophilia. Thrombocytosis

Case 6 continued

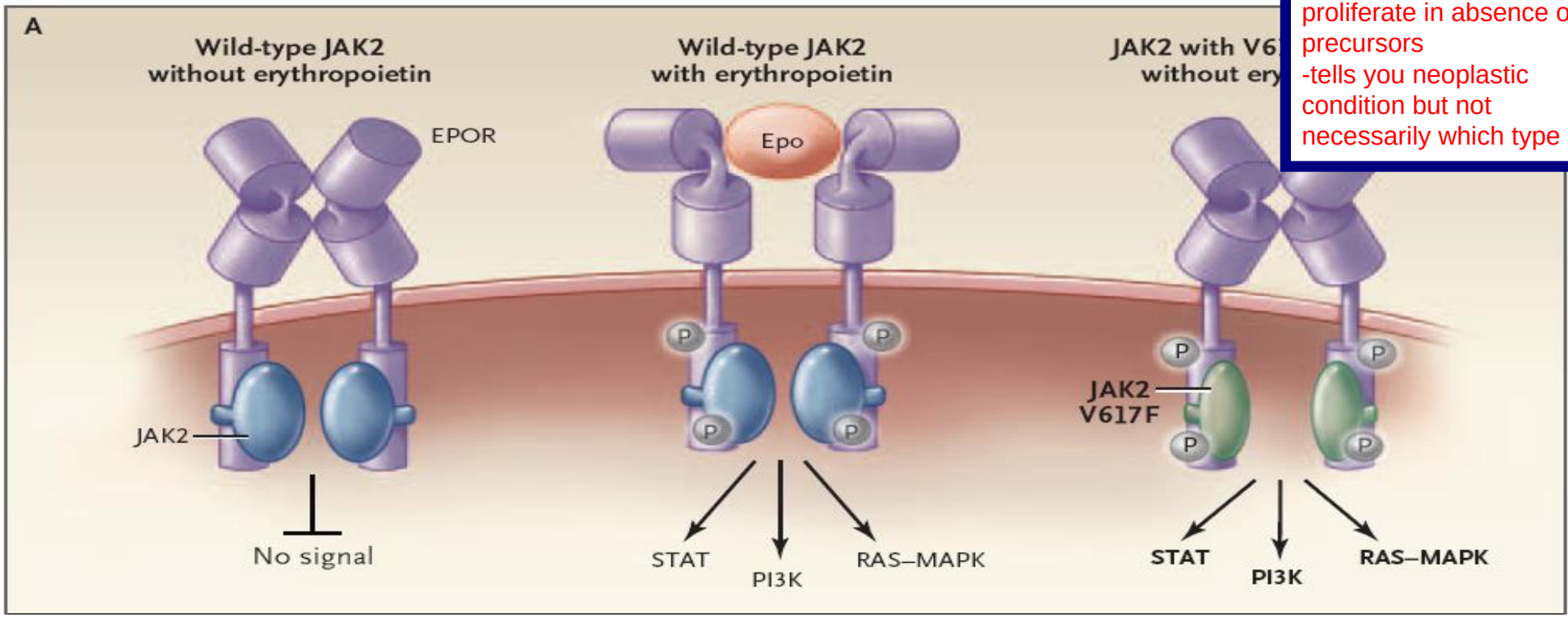
polycythemia may be caused by a reactive process or a neoplasm, so you want to rule out that polycythemia is not a reactive process before jumping to a neoplasm diagosis

- Differential Diagnosis of Polycythemia
  - Secondary
    - Smoking
    - Excessive erythropoietin
  - Primary = Polycythemia vera
- Diagnostic test:
  - Mutation analysis of JAK2 gene - POSITIVE
  - DIAGNOSIS - Polycythemia Vera

test used to test for neoplasm polycythemia

JAK-2 mutation results in activation of JAK-STAT pathway in absence of ligand – “cytokine independent constitutive activation”

JAK2- membrane bound kinase activated by binding of erythropoieten causing proliferation of erythroid precursors- with this mutation, red cells will proliferate in absence of precursors
-tells you neoplastic condition but not necessarily which type



Case 7

The normal values you need to know for this cas
-Hb for a man - 14-18
-MCV- 80-98
-reticulocytes - 20-100K/cumm
-platelets - 150, 0000 - 450,000
-wbc count - 3200 - 9800
-neutrophils - 37-80%
-lymphocytes - 10-50%
-monocytes - 0-12%

- 22 yo mechanic
  Admitted with fever, sore throat and numerous bruises

sudden

- PE - Purulent tonsillitis, petechiae and ecchymoses
- CBC:
  - Hgb: 6.1 gm/dl
  - MCV: 106 fl
  - Retic: 5,000/ cu mm
  - Platelets: 5,000 / cu mm
  - WBC: 1,900
  - WBC diff: Neutrophils 10%
  - Lymphs: 88% (relative lymphocytosis)
  - Monos: 2%

anemia

Pancytopenia

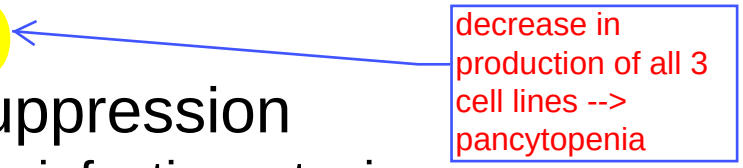
decrease in platelets

white cell counts low

- Blood Smear: No immature cells. Severe neutropenia and thrombocytopenia confirmed. RBCs normal

Differential Diagnosis of Pancytopenia

- Reduced Production:
  - Hematologic malignancy – Acute leukemia
    Myelodysplasia
    Myelofibrosis
  - Aplastic anemia
  - Bone marrow suppression
    - Drugs, radiation, infections, toxins
  - Metastatic tumor in marrow
  - B12/folate/copper deficiency
- Increased destruction:
  - Paroxysmal nocturnal hemoglobinuria
  - Hemophagocytic syndrome
  - Hypersplenism

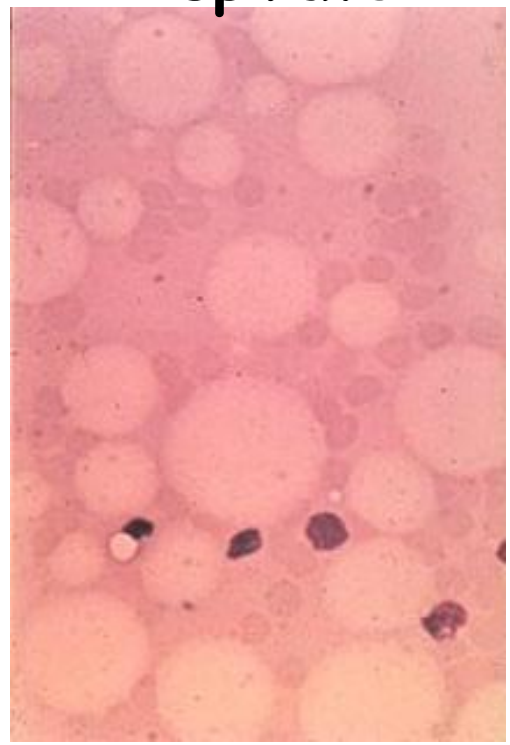


Case 7- Bone marrow

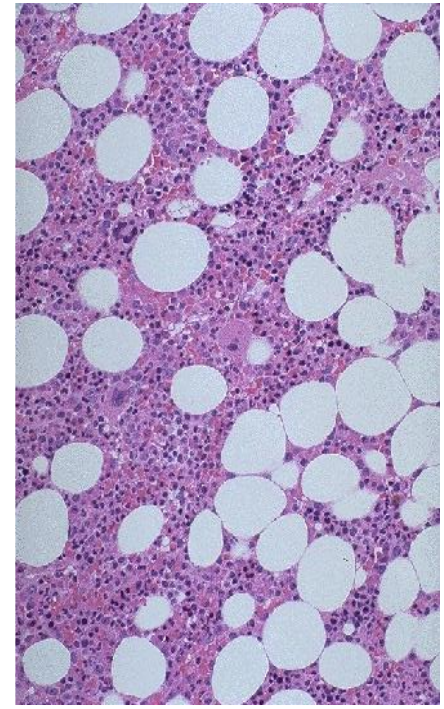
aplastic anemia show hypocellular bone marrow

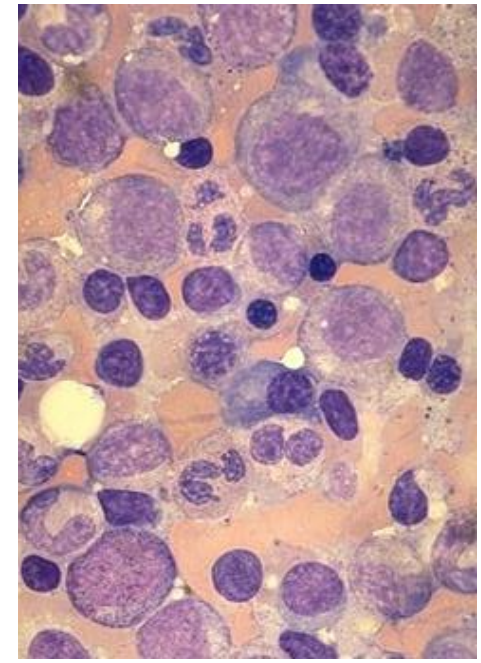
Biopsy

Aspirate



Normal BM





Diagnosis: Aplastic Anemia

The normal values you need to know for this case
-Hb for a woman - 12-14
-MCV- 80-98
-platelets - 150, 0000 - 450,000
-wbc count - 3200 - 9800

Case 8

- 29 yo woman, previously healthy

  Presents with heavy menstrual bleeding, numerous bruises

  PE: Petechiae and ecchymoses. No splenomegaly

- Lab data:
  - Hgb: 13.4 gm/dL
  - MCV: 85 fl
  - Platelets: 5,000 / cu mm ← thrombocytopenia
  - WBC: 10,500
  - WBC diff: Normal
  - Smear: No immature cells. Thrombocytopenia. No schistocytes
- DIAGNOSIS: Immune thrombocytopenic purpura (ITP)

Differential Diagnosis of Thrombocytopenia

- Impaired production
- Accelerated destruction
- Disorder of distribution (hypersplenism)
- Multifactorial

in this case it was autoimmune destruction of platelets- antibodies against platelets- underlying cause largely unknown - may also have alloantibodies from multiple blood transfusions
-is production low? is there destruction?

Differential Diagnosis of Thrombocytopenia

- Impaired production
  - Drugs
  - Infections
  - Aplastic anemia
  - Hematologic malignancy
  - Myelophthisis
  - Myelodysplasia
  - B12/folate deficiency

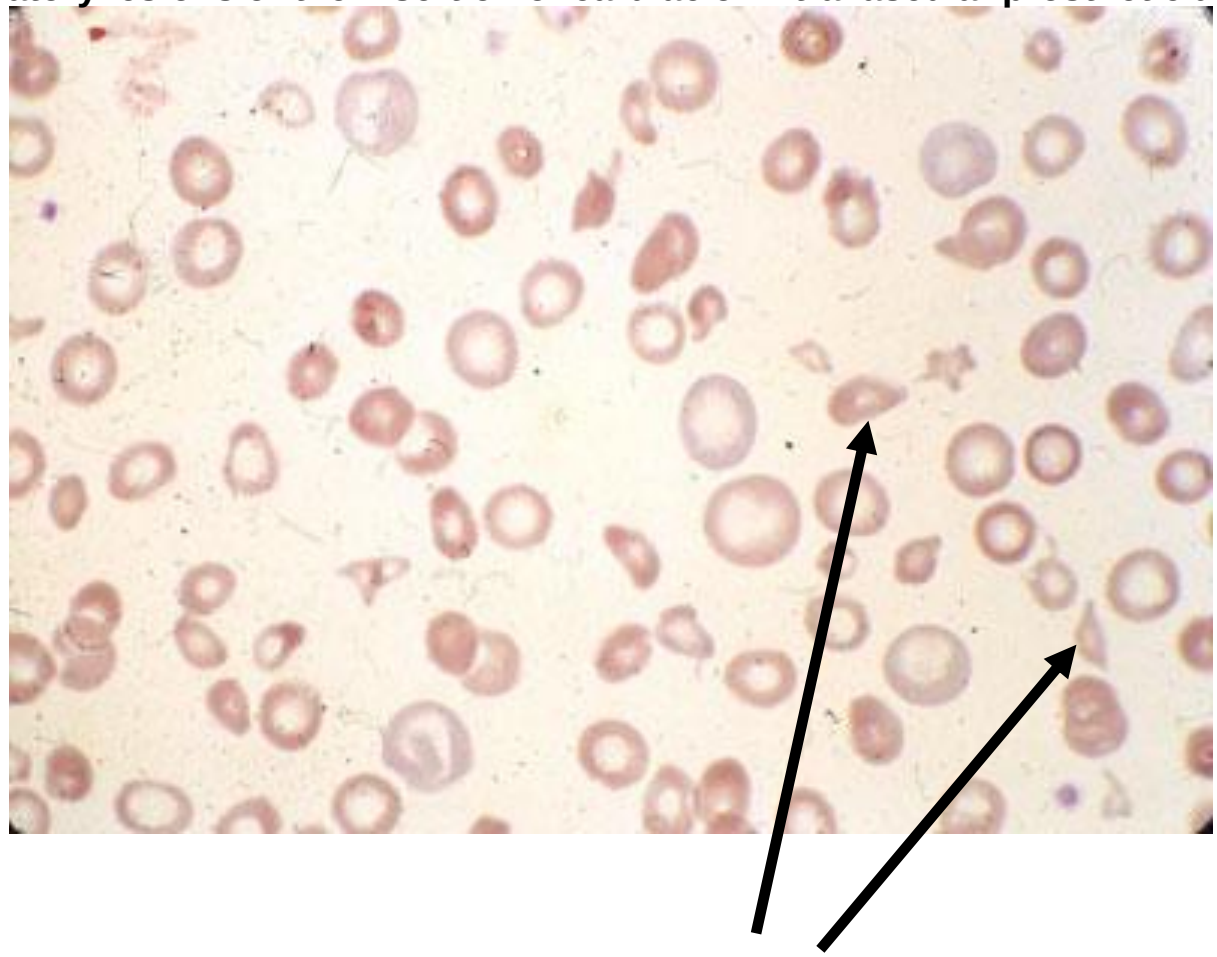
Differential Diagnosis of Thrombocytopenia

- Impaired production
- Accelerated destruction
  - ITP
  - Drugs, including Heparin
  - Collagen vascular diseases
  - Infections including HIV
  - Disseminated intravascular coagulation (DIC)
  - TTP/HUS
  - Alcohol
  - Inherited platelet disorders
  - Post-transfusion purpura
  - Non-Hodgkin lymphomas
- Disorder of distribution (hypersplenism)
- Multifactorial

clinically most important (life threatening) are red

Microangiopathic hemolytic anemia

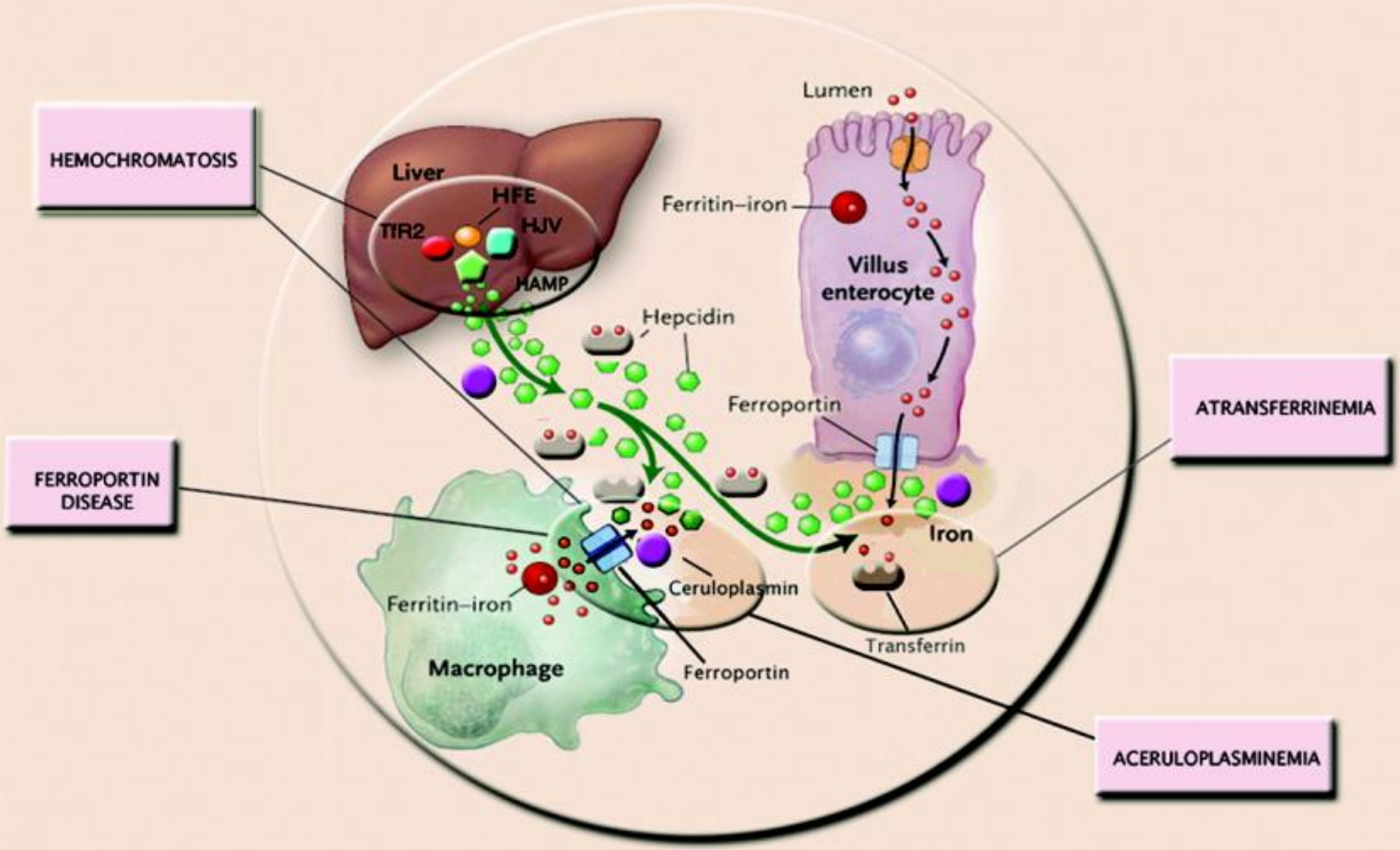
Hemolysis due to intravascular fragmentation of red blood cells; may be due to microcirculatory lesions or the insertion of cardiac or intravascular prosthetic devices.



-thoroughly familiarize yourself with cbc
-make diagnosis on which cell line is defective
-look at morphology to classify type of anemia
-think about likely etiologies

Summary

- CBC and peripheral blood smear are the mainstays of diagnosing disorders of blood cells
- Anemia is very common worldwide and has many causes
- Anemias are classified based on red cell morphology followed by an etiological classification using special tests
- Leukocytosis is often reactive but various leukemias must be considered
- Immune destruction of platelets is a common cause of thrombocytopenia but decreased production due to bone marrow abnormalities must also be considered.



Hepatology, Volume 46, Issue 4 , Pages 1291 - 1301

I added these tables that I mostly adapted from BRS pathology- i thought it was a good summary of the anemias- just FYI!

EXAMPLES OF ANEMIA RESULTING FROM DECREASED RED CELL PRODUCTION

Type	Mechanism	Diagnostic Features	Major Etiologic Factors
Iron Deficiency Anemia	Impaired heme synthesis	Hypochromia and microcytosis; decreased serum iron and increased total iron binding capacity; decreased serum ferritin	Dietary deficiency in infants and preadolescents; excess menstrual bleeding; chronic blood loss from the GI tract such as malignancy
Pernicious Anemia	Autoimmune gastritis leading to lack of gastric intrinsic factor and failure of vit B12 absorption; vit b12 deficiency delays DNA replication because it a cofactor in synthesis of THF	Pancytopenia, oval macrocytes, and hypersegmented neutrophils; megaloblastic hyperplasia; achlohydria; anti-intrinsic factor antibodies; hyperreflexia; absent position and vibration sensations; impaired vit b12 absoprtion corrected by intrinsic factor	Autoimmunity
Folate Deficiency	Delayed DNA replication	Pancytopenia, oval macrocytes, and hypersegmented neutrophils; megaloblastic hyperplasia	Dietary deficiency; malabsorption syndromes
Aplastic Anemia	Greatly diminished hematopoiesis	Pancytopenia, reticulocytopenia, marked hypocellularity of the bone marrow	Toxic drugs and chemicals; often idiopathic
Anemia of chronic disease	Diverse mechanisms; macrophages produce IL6, which causes hepatocytes to produce hepcidin and reduce iron absorption	Anemia most often normochromatic and normocytic or macrocytic; may be hypochromic and microcytic with decreased serum iron-binding capacity	Various chronic diseases, especially rheumatoid arthritis or SLE, renal disease and chronic infection
Myelophthisic	Bone marrow replacement; usually by a malignant tumor	Severe anemia; small numbers of nucleated red cells and immature granulocytes in the peripheral blood; tumor cells in the bone marrow	Malignancy

we covered all of these in this lecture except this last one

we only covered this one in this lecture.
Sickle Cell is most common type seen at Duke.
Dr. H

OF ANEMIAS RESULTING FROM INCREASED RED CELL PRODUCTION

Type	Mechanism	Diagnostic Features	Comments
Warm antibody autoimmune hemolytic anemia (primary and secondary forms)	IgG autoantibodies combine with red cell surface antigens; Fc combining site of IgG antibody further reacts with Fc receptor of phagocytic cells	Anemia, spherocytosis, and reticulocytosis; unconjugated hyperbilirubinemia and acholuric jaundice; positive direct Coombs test	Often secondary to lymphocytic neoplasms , Hodgkins disease, or autoimmune disease; sometimes associated with methyldopa or penicillin therapy
Hemolytic disease of the newborn (erythoblastosis fetalis)	Maternal alloimmunization of fetal red cell antigens; classically of Rh system; can also be caused by alloimmunization to ABO blood groups	Rising titer of maternal anti-Rh antibodies during the later part of pregnancy; cord blood at delivery contains immature red cell precursors; direct Coombs test positive on cord blood; progressive increase in postnatal unconjugated bilrubin	Prevented by administration of anti-Rh antibody to mother at time of delivery of first and subsequent children
Hereditary spherocytosis	Red cell membrane skeletal protein abnormality	Autosomal dominant; anemia, spherocytosis, and reticulocytosis; increased mean corpuscular hemoglobin concentration; unconjugated hyperbilirubinemia and acholuric jaundice; increase erythrocyte osmotic fragility in hypertonic saline; splenomegaly	Quantitative deficiency of spectrin due to diverse mechanisms
Glucose 6 phosphate dehydrogenase deficiency	Failure of erythrocyte hexose monophosphate shunt under oxidative stress	Self limited hemolytic anemia; reduced activity of erythrocyte G6PD	X linked inheritance
Sickle cell anemia	B globin hemoglobinopathy	Anemia and reticulocytosis; sickle shaped erythrocytes demonstrable on peripheral blood smear; homozygosity for hemoglobin S demonstrated with electrophoresis	Severe anemia, recurrent painful and asplastic crisises, and nonhealing leg ulcers; recurrent splenic infarcts with progressive fibrosis result in autosplenectomy
B thalassemia major	Diverse mutations in B globin gene causing decreased synthesis of B globin chains, aggregation of alpha chains causes hemolytic anemia and ineffective erythrocytosis	Severe anemeia; thalassemic red cell morphology; increase hemoglobin F	Occurs frequently in Mediterranean populations
Alpha thalessemia	Deletion of one or more of the four alpha globin genes	Differ according to the number of deletions	No clinical abnormalities with one gene deletion' mild to moderate thalessemic state with 2 or 3 deletions; intrauterine death with 4 deletions- hemoglobin barts in fetal life and hemoglobin H in adult life