

# Anemias

# Initial Laboratory Evaluation

- Complete blood count with indices:
  - MCV – indication of RBC size.
  - RDW – indication of RBC size variation.
- Examination of the peripheral blood smear.
- Reticulocyte count:
  - Measurement of newly produced young RBC's.
  - Considered a measure of bone marrow responsiveness.

# Additional Labs

- Vitamin levels: iron profile, ferritin, Vitamin B12, folate.
- LDH, bilirubin, haptoglobin.
- Coomb's test
- Hemoglobin electrophoresis
- SPEP
- Creatinine
- TSH
- Urinalysis
- Stool guaiac
- Consider bone marrow examination

# Definition of Anemia

- Reduction in the volume of RBC's (hematocrit) or concentration (hemoglobin) when compared to similar values from a reference population.
  - Hgb = expression of amount (g/dL).
  - Hct = expression of volume (% or decimal fraction).
  - RBC = expression of number ( $\#/mm^3$ ).

# Morphology Classification

- Microcytic (MCV  $<80 \mu^3$ ):
  - Iron deficiency, thalassemia, chronic disease/inflammation, sideroblastic anemia, lead poisoning.
- Normocytic (MCV  $80-100 \mu^3$ ):
  - Acute blood loss, chronic disease, hypersplenism, bone marrow failure, hemolysis.
- Macrocytic (MCV  $>100 \mu^3$ ):
  - B12 or folate deficiency, hemolysis with reticulocytosis, chemotherapy, hypothyroidism, MDS.

# Case 1

- A 35 year old woman has a history of pleurisy and has been told she may have SLE.
- Previous CBC's have been normal.
- She presents to your office with complaints of exertional dyspnea, fatigue, and yellowing of her eyes.
- Physical exam is normal except for mild scleral icterus and moderate splenomegaly.

# Case 1

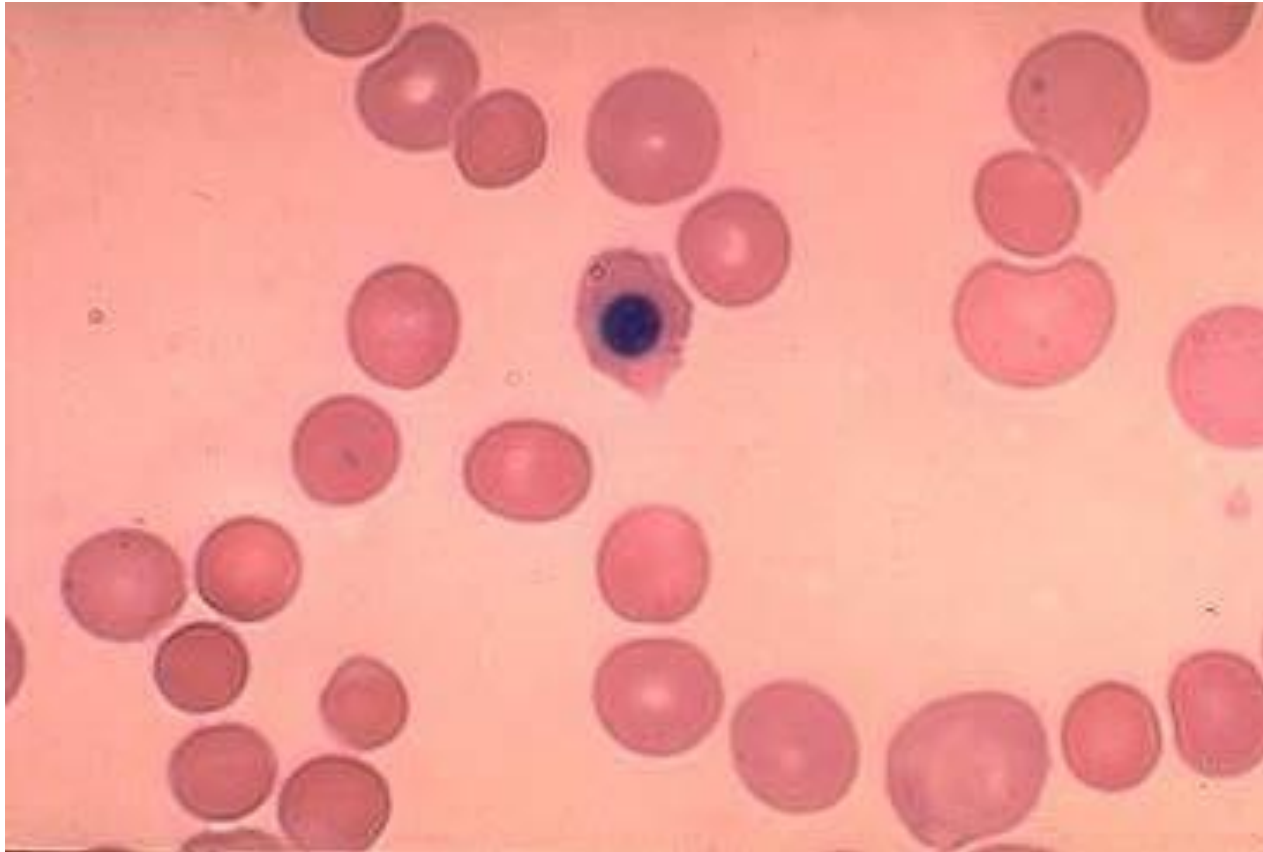
- Initial Labs:
  - Hemoglobin 7.9 gm/dL
  - HCT 23.9%
  - WBC 4000/mm<sup>3</sup> with a normal differential
  - Platelet 138,000/mm<sup>3</sup>
- What other labs would you like to see?

# Case 1

- MCV 114
- Uncorrected Retic count 14.2%
- LDH 2343 U/L
- Bilirubin 4.3mg/dL
- Direct bili .8mg/dL
- The peripheral blood smear reveals macrovalocytes, polychromasia, and an occasional nucleated red blood cell.



# Case 1



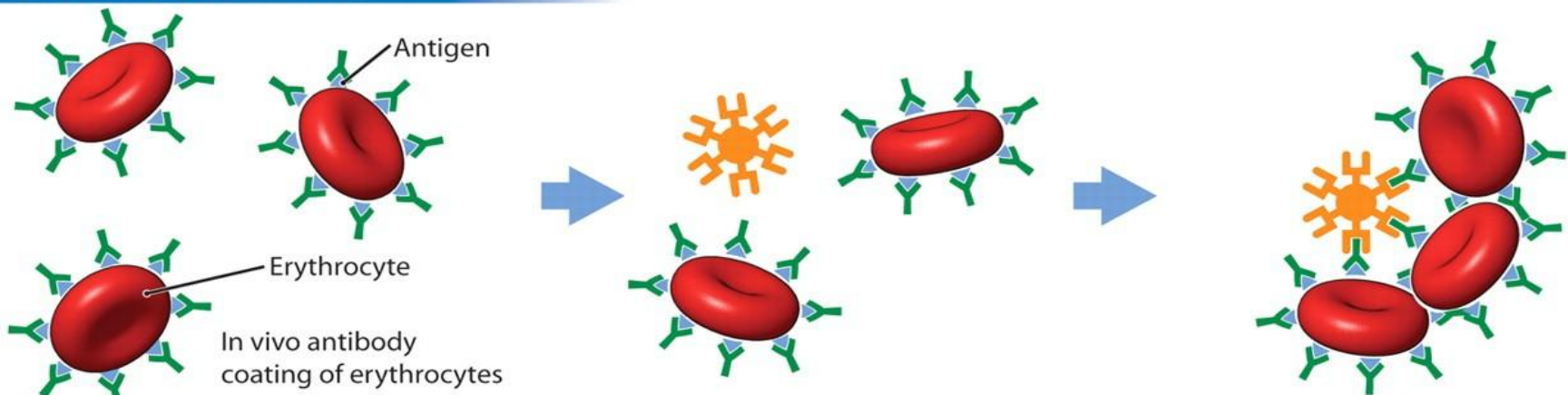
# Case 1

- You suspect an autoimmune hemolytic anemia based on her history, Physical Exam, labs, and smear.
- What further tests would you like to order?

# Case 1

- Direct Coombs Test (AKA direct antiglobulin test):
  - RBC's are washed, removing serum and then incubated with antihuman globulin (Coombs reagent).
  - This binds immunoglobulin or complement factors fixed on the RBC surface, causing agglutination and therefore a positive DAT.

## Direct Antiglobulin Test



Anti-IgG AHG reagent added  
after erythrocytes are washed

AHG reagent causes IgG-coated  
erythrocytes to agglutinate

# Case 1

- Direct Coombs test results are as follows:
- DAT: Positive 3+
  - IgG: Positive 3+
  - Complement: Negative
- What type of autoantibody is this?
- What conditions are typically associated with this type of antibody?

# Case 1

- You diagnose a warm-antibody hemolytic anemia and suspect an underlying autoimmune disorder.
- Etiology:
  - Idiopathic
  - Autoimmune
  - Lymphoproliferative Disorder
  - Viral
  - Immunodeficiency
  - Medications

# Case 1

- You decide to avoid blood transfusion because of:
  - The difficulties in obtaining cross match compatible blood, AND
  - The expected short half life of transfused blood.
- What treatment do you recommend?

# Case 1

- Treatment:
  - Prednisone
  - Splenectomy
  - Rituximab (monoclonal antibody)
  - Immunosuppressive agents
    - Cytoxan, imuran, cyclosporin
  - Danazol
  - Plasma exchange

# Case 1

- You begin her on 60 mg Prednisone and she has a good response. Upon several attempts at tapering the prednisone she has a relapse with worsening of the hemolytic anemia.
- You are concerned about the long term side effects of Prednisone, thus you send her for a procedure.



# Case 1

- She responds well to splenectomy, but still requires very low maintenance doses of Prednisone.
- Over the years she occasionally requires higher doses of Prednisone when she is ill or has a lupus flare.

## Case 2

- You are referred a pleasant 34 year old African American woman who has been known to have a mild microcytic anemia, which was picked up some years ago on routine blood work. She is entirely asymptomatic. She has been prescribed iron several times over the years without a response.
- What data would you like to review?

# Case 2

- Labs:
  - HCT 31-34%
  - Hgb 10.6-11.4gm/dL
  - PLT 232-312K/mm<sup>3</sup>
  - WBC 6500-8000/mm<sup>3</sup> with a normal differential
  - MCV 72-74 cubic microns
- What is in your differential diagnosis?

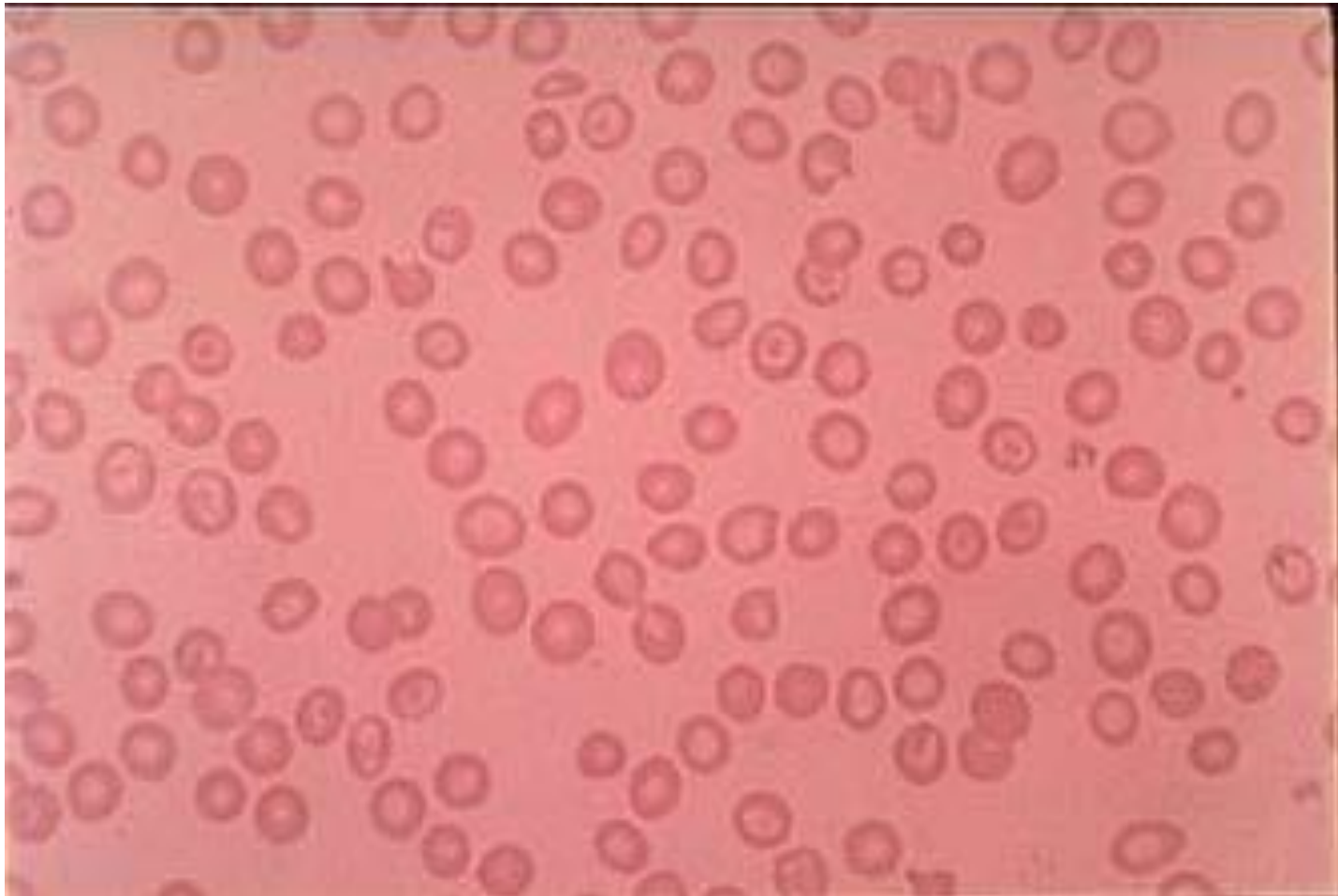
# Case 2

- Differential Diagnosis:
  - Iron deficiency anemia
    - Noncompliance, inadequate dosing, incorrect formulation.
  - Beta thalassemia
  - Alpha thalassemia
  - Anemia of chronic inflammation/disease
  - Sideroblastic anemia
  - Lead
- What additional lab tests would you like to order?

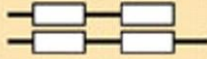





## Case 2

- Iron studies are normal.
- Chemistries, liver function tests, thyroid studies are normal.
- No history of lead exposure.
- You ask to see a peripheral blood smear and one other study. What is it?

# Case 2



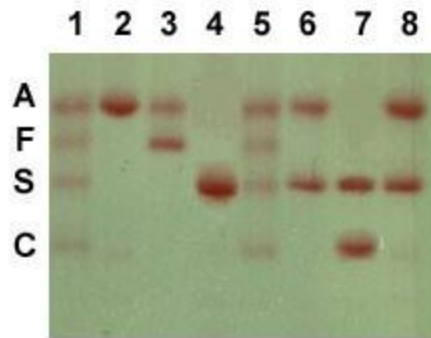
# Case 2

$\alpha$ -Thalassemia Syndromes		
$\alpha$ Gene Map	$\alpha$ Genotype	$\alpha$ Clinical Syndrome
	Normal	Normal
	Heterozygous $\alpha$ - Thal - 2 (also called $\alpha^+$ )	Silent Carrier of $\alpha$ Thalassemia
	Heterozygous $\alpha$ - Thal - 1 (also called $\alpha^0$ )	$\alpha$ - Thalassemia Trait
	Homozygous $\alpha$ - Thal - 2 (also called homozygous $\alpha^+$ )	$\alpha$ - Thalassemia Trait
	Compound Heterozygous $\alpha$ - Thal - 1 & 2 (also called $\alpha^+/\alpha^0$ )	Hb - H Disease
	Homozygous $\alpha$ - Thal - 1	Hydrops Fetalis

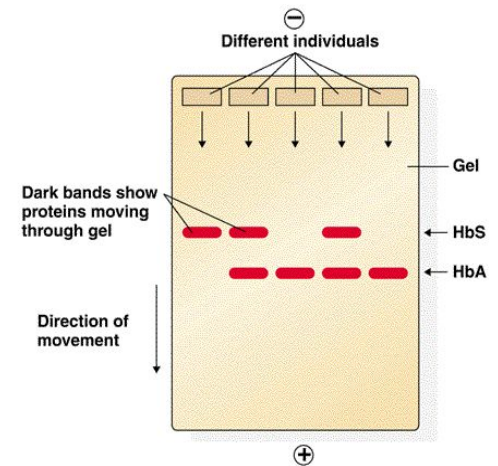
# Case 2

- You also request a hemoglobin electrophoresis.

- Hgb A = 97.5%
- Hgb A2 = 2.1%
- Hgb F = 0.6%



Tobin/Dusheck, Asking About Life, 2/e  
Figure 10.15



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- What is your diagnosis of exclusion?



## Case 2

- Alpha thalassemia with a double gene deletion.
  - No treatment is necessary.
  - Anemia is not progressive.
  - No other systemic problems.
  - Often mistaken for iron deficiency and treated with iron or for anemia of chronic disease.

## Case 3

- A 32 year old previously healthy woman with no prior medical history presents to your office stating that she has felt “unwell” for the past month. In the past 3 days she has experienced fevers, a “rash” on her legs, weakness, and shortness of breath. She has been too weak to get out of bed the last 24 hours and has experienced spontaneous nose bleeds.

# Case 3

- Physical exam confirms an ill appearing woman.
  - T = 38.6, pulse 122, BP 123/54
  - Dried blood in the nares, pale conjunctiva
  - Dried mucous membrane
  - Tachycardia and tachypnea
  - Petechiae over the lower extremities

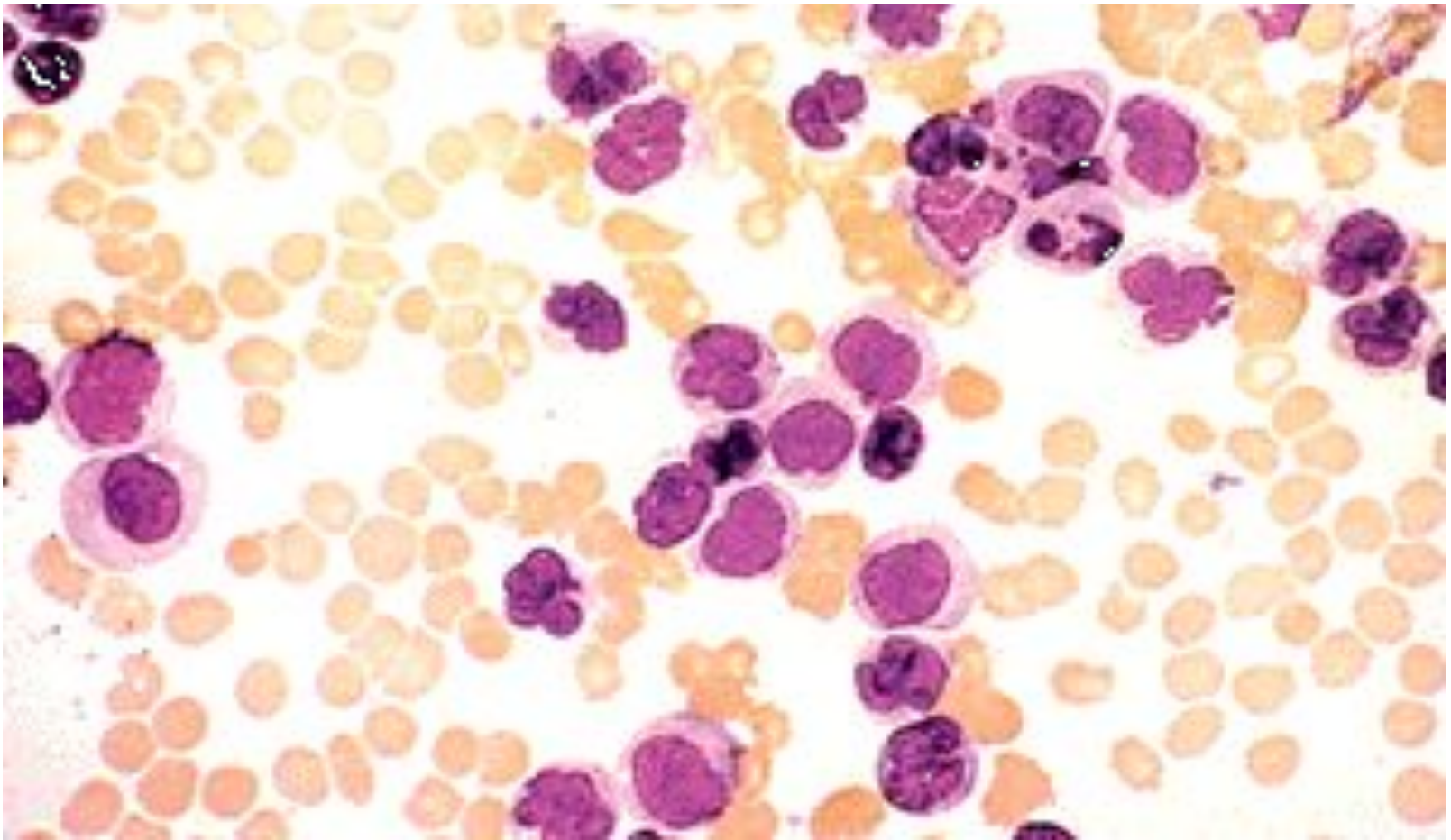
# Case 3

- Routine labs:
  - WBC = 1700/mm<sup>3</sup>
  - Hgb = 5.6 gm/dL
  - HCT 18.2%
  - Platelet – 12,000/mm<sup>3</sup>
- What important piece of laboratory information is missing from the WBC?

## Case 3

- Differential of the white blood cell count reveals at least 50% of young white cells, the lab thinks are blasts.
- Reticulocyte count = 0.4%
- Electrolytes are consistent with dehydration, potassium and creatinine are slightly elevated.

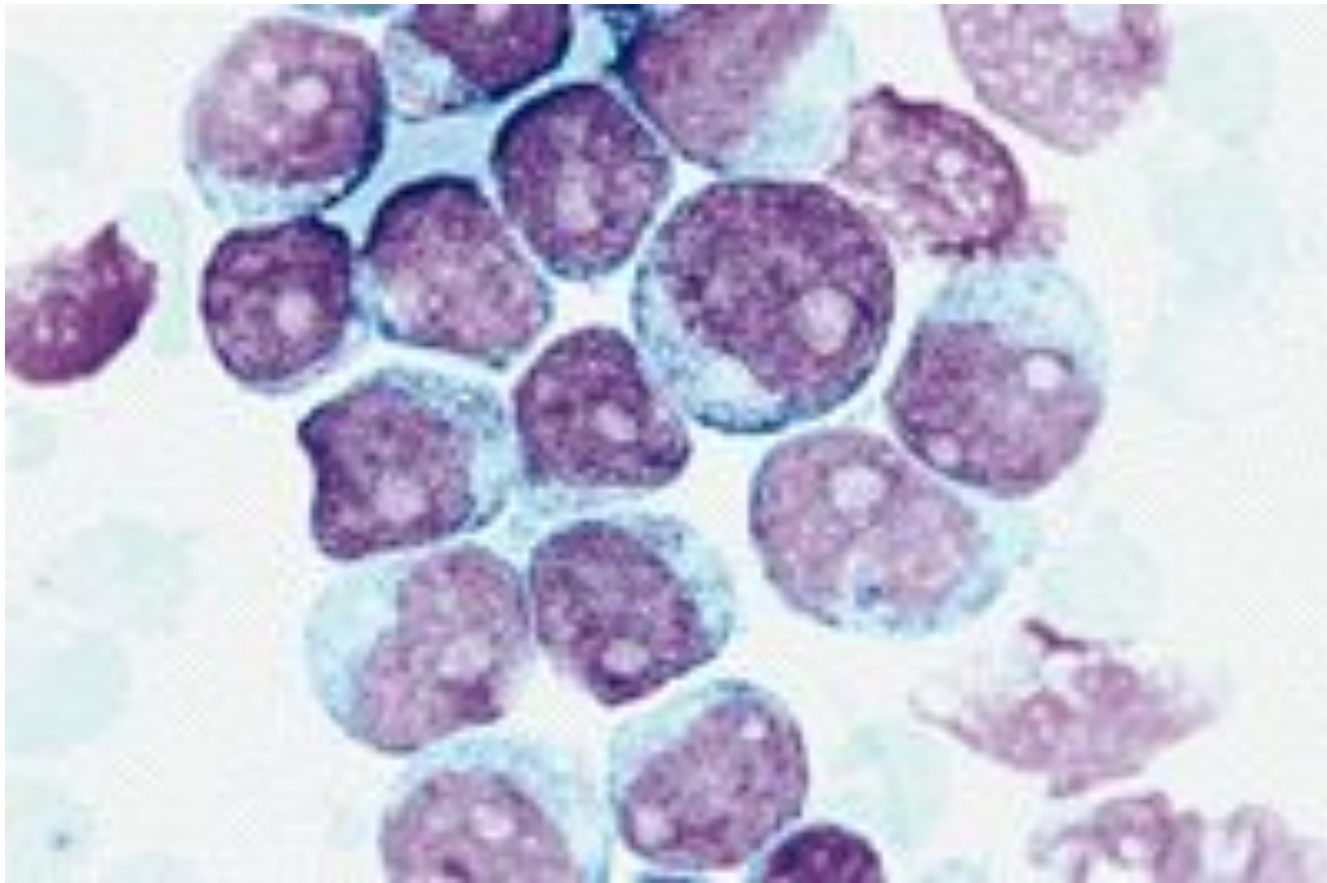
# Case 3



# Case 3



# Case 3





# Case 3

- Hematopathology evaluation revealed AML subtype M1.
- Chromosomal studies were normal.
- What should you do prior to beginning chemotherapy in this patient?

# Case 3

- The patient tolerated induction chemotherapy well and remains in remission 4 months after completing induction and consolidation therapy. Her blood counts have all now normalized.
- If she was to relapse what therapy would you recommend?

# Case 4

- You are asked to see a 43 year old previously healthy woman who presented to the ER complaining of fevers, weakness, and bleeding from her gums.
- Her only significant past history is a recently resolved viral syndrome. Her family notes she has been somewhat confused over the last 24 hours.

# Case 4

- The ER attending notes:
  - The patient to be ill appearing
  - T 38.3, HR 123, BP 126/76, RR 26
  - Dried blood in the nares and mouth
  - Petechiae on lower extremities
  - Patient slightly confused, but no focal neurologic findings.
- Before you arrive in the ER she has a seizure.

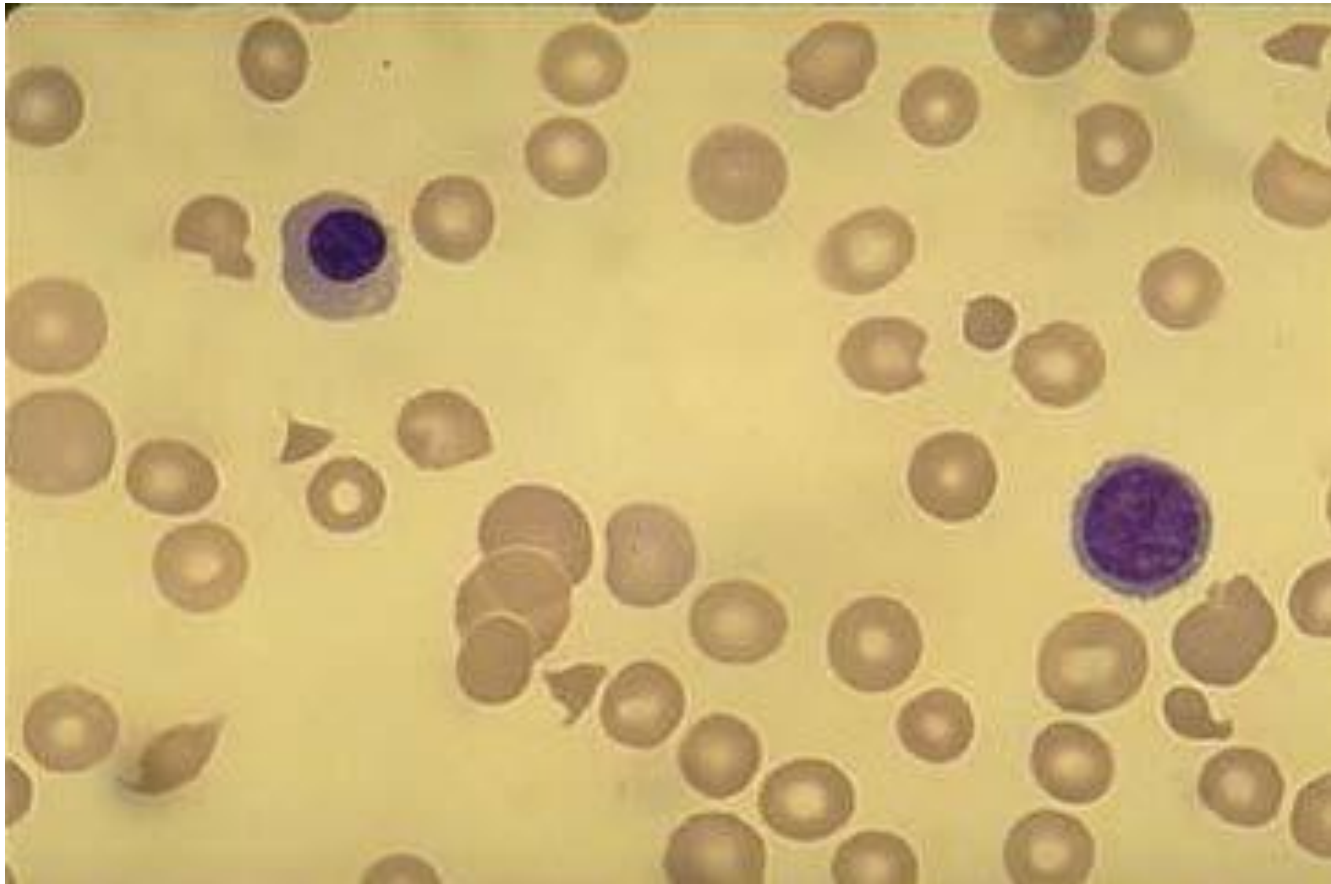
# Case 4

- Labs:
  - WBC 5600/mm<sup>3</sup>, normal differential
  - HCT 16.3%
  - Hgb 5.3 gm/dL
  - Platelets 21,000/mm<sup>3</sup>
  - PT 11 sec
  - PTT 29 sec

# Case 4

- Labs:
  - Creatinine 2.4 mg/dL
  - LDH 3000 U/L
  - Bili 3.2 mg/dL, mostly indirect
  - Reticulocyte count 6.2%
  - Haptoglobin <10mg/dL
  - Urine: 2+ hemoglobin, neg RBC

# Case 4



# Case 4

- What is your differential diagnosis based on the history, physical, labs and blood smear?
- What else could give you a similar blood smear?



# Case 4

- You diagnosis TTP based on the classic pentad and consistent blood smear:
  - Microangiopathic hemolytic anemia
  - Thrombocytopenia
  - Fever
  - Renal Failure
  - MS changes
- What is the first line treatment of TTP?

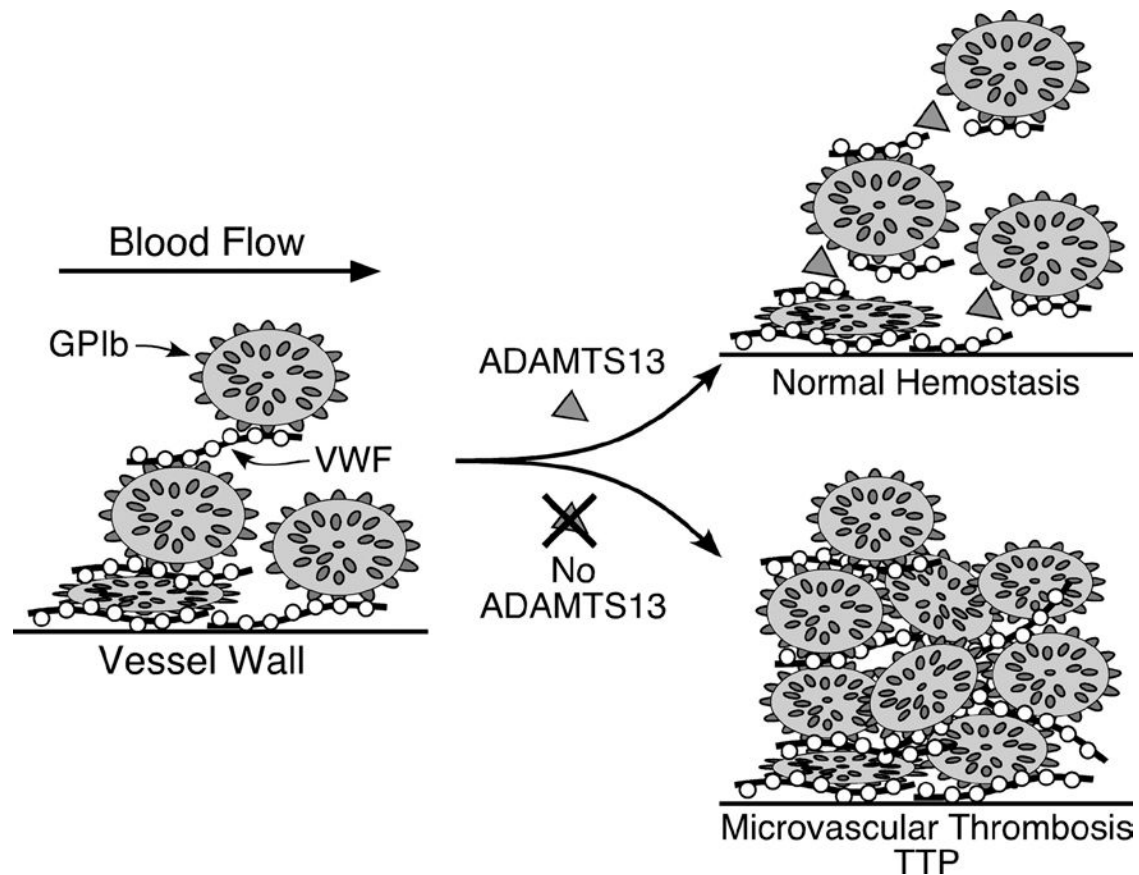
## Case 4

- You begin daily plasma exchange procedures using FFP as your replacement fluid. Her mental status improves by the next day. Her platelet count normalizes and LDH decreases over the next week. Her anemia slowly improves as does her renal failure. She is weaned off plasma exchange and has a single relapse, which responds to similar therapy.

# Case 4

- Pathophysiology:
  - Typically an inhibitor against ADAMTS- 13, a vWF cleaving to protease leading to accumulation of High Molecular Weight vWF multimers leading to small vessel thrombosis and microangiopathic hemolytic anemia.
  - Plasmapheresis:
    - Removes the offending antibody
    - Supplies the deficient ADAMTS-13

# Case 4



## Case 5

- A 36 year old Caucasian man with a history of progressive renal failure over the last 4 years comes to you for work up of weakness and progressive anemia. He has recently begun dialysis. His renal failure initially appeared after an acute febrile illness and the etiology was never determined.

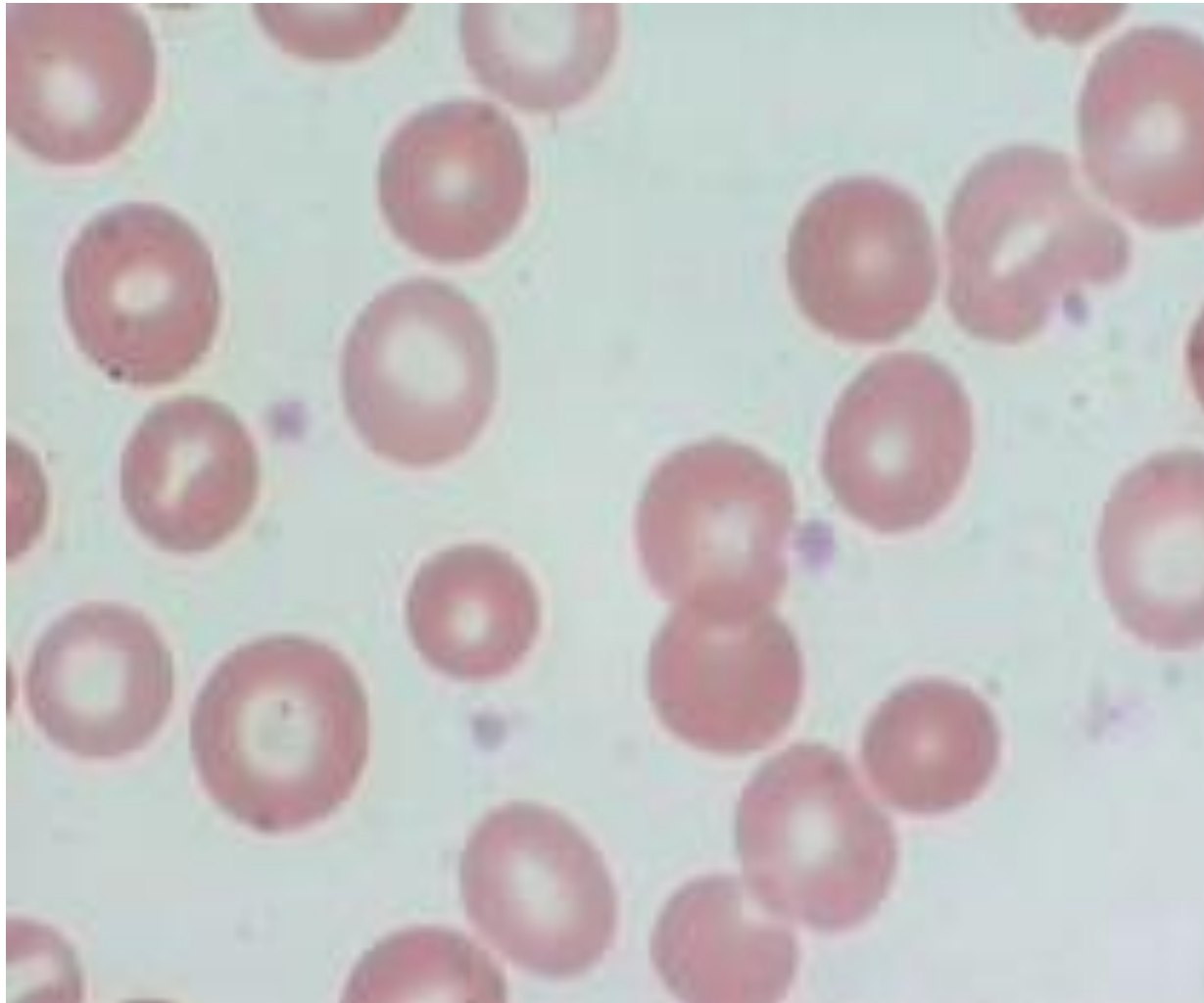
# Case 5

- Physical exam is significant for pale sclera and an AV shunt in his left arm.
- CBC:
  - WBC 6400/mm<sup>3</sup>
  - Hgb 7.6 gm/dL
  - HCT 23.8%
  - Platelet 242,000/mm<sup>3</sup>
  - MCV 78/mm<sup>3</sup>

# Case 5

- Prior labs reveal a hematocrit that was normal 4 years ago, but has steadily declined as his renal function worsened.
- Reticulocyte count corrected= 0.7%
- Fe 26 mcg/dL
- TIBC 224 mcg/dL
- Iron saturation 11%
- Ferritin 145 ng/mL
- What is in your differential diagnosis?

# Case 5





# Case 5

- Differential Diagnosis:
  - Anemia of renal failure
  - Iron deficiency anemia
  - Thalassemia
  - Lead poisoning
  - Myelodysplasia
  - Mixed microcytic and macrocytic anemia.
- You request one more study to support your suspicion?

# Case 5

- You order an erythropoietin level which returns at 12 IU ( 4.1-19.5 ) within the normal limits.
- What do you make of this value?
- Should you proceed to a bone marrow exam?

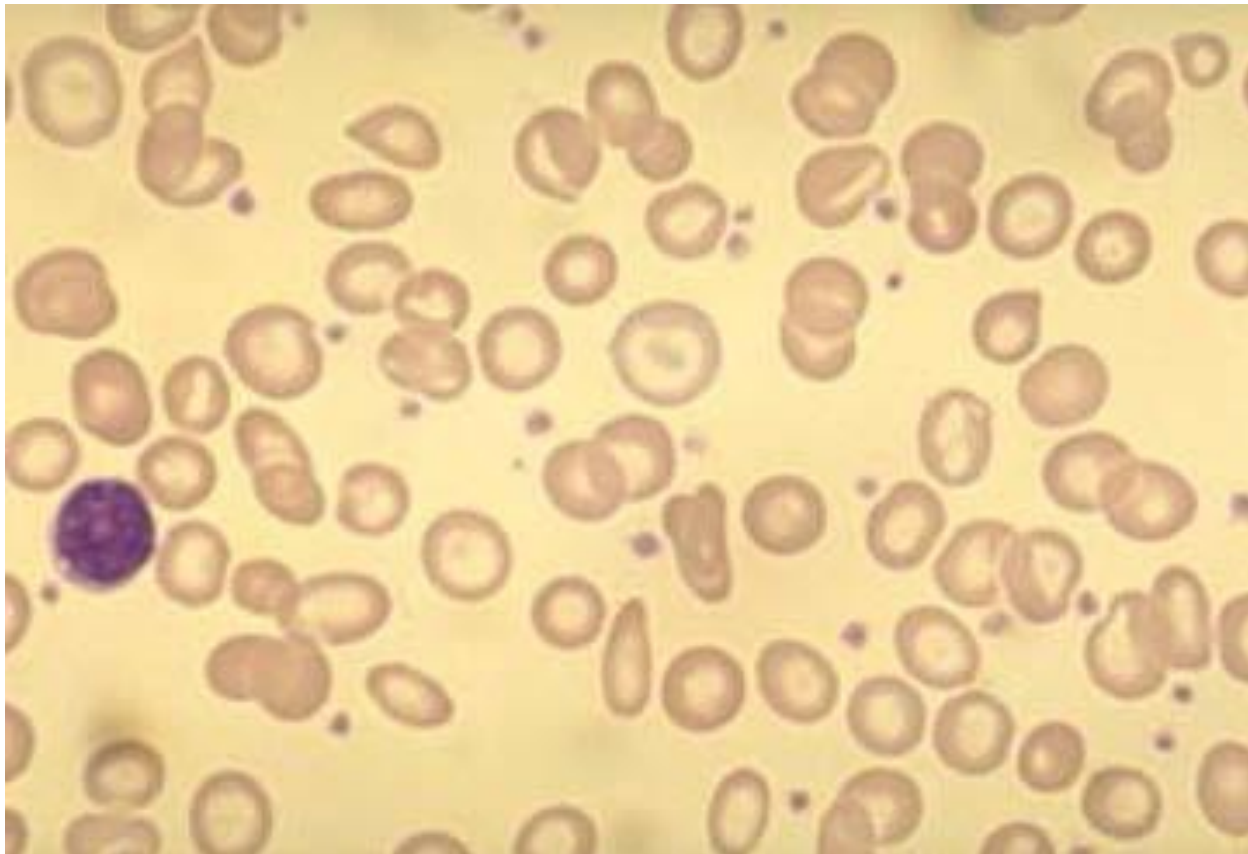
# Case 5

- You place the patient on 10,000 units of EPO SQ tiw with dialysis. He comes to see you in one month feeling better.
- Labs:
  - HCT 34%
  - Hgb 11.2 gm/dL
  - MCV 83/mm<sup>3</sup>
  - Retic 3.2%

# Case 5

- He continues on his EPO injections for four months. Soon after he again becomes fatigued, but otherwise doing well. Labs reveal:
  - WBC 6200/mm<sup>3</sup>
  - Hgb 8.9 gm/dL
  - Platelet 234,000/mm<sup>3</sup>
  - MCV 76 cubic microns
  - Retic .6%

# Case 5



# Case 5

- You suspect with his increased reticulocytosis and hgb/hct over the last few months he has become iron deficient. You confirm this with lab assay.
- You give him 1 gram of intravenous iron and ask him to come back in 2 weeks.

# Case 5

- He again feels much improved and is tolerating the iron fairly well. His hematocrit has increased to 33%, corrected reticulocyte count to 3.2%, and the MCV is now 80 cubic microns.
- He continues on Iron and EPO injections and is considering renal transplant.

# Case 5: Anemia of Chronic Inflammation

- Mild to moderate anemia that is persistent for greater than 1-2 months in patients with infectious, inflammatory, or neoplastic diseases.
  - Other causes have been excluded.
  - Hypoproliferative, low reticulocyte count.
  - Normocytic-MCV 80-100:
    - May be microcytic in later stages.
  - Iron studies show low serum iron, low percent saturation, and normal to elevated ferritin.
    - Adequate reticuloendothelial iron stores.



# Case 5: Pathophysiology

- Epo production is inhibited or decreased, so inappropriately low levels.
- Increased levels of inflammatory cytokines:
  - IL-1, TNF  $\alpha$ .
  - IL-6 causes induction of hepcidin synthesis (decreased iron absorption).
- Alterations in iron metabolism.
- Suppression of erythropoiesis.
- Moderate decrease in RBC survival.

# Case 5: Laboratory Abnormalities

	Fe deficiency	AOCD
<i>Standard</i>		
Serum Fe	↓ to NI	↓ to NI
TIBC	High NI to ↑	↓ to low NI
% iron saturation	↓↓	↓
Serum ferritin	↓↓*	NI to ↑↑
MCV	↓ to ↓↓	↓ to NI
RDW	↑	NI to ↑ <sup>†</sup>

# Case 5

- ACI does not usually require treatment.
- Iron replacement is typically not necessary.
- Erythrocyte-stimulating agent can be given in the setting of renal disease however caution regarding hypertension and thrombosis.
- Treat the underlying cause!

# Case 6

- A 53 year-old female complains of fatigue for 3 months. She falls and breaks her left femur.
- On reviewing her labs the creatinine is 1.3 mg/dl, calcium 10.1 mg/dL. Comprehensive metabolic panel is otherwise normal.
- Hematocrit is 25%. White blood count and platelet counts are mildly depressed at 3.9K/mm<sup>3</sup> and 147K/ mm<sup>3</sup>.

# Case 6

- What is the most likely diagnosis?
- How do you want to proceed with your evaluation?

# Case 6

- Serum protein electrophoresis (SPEP)
  - M- spike of 4.2 gm/dl
- Immunofixation (IFE)
  - IgG lambda noted in serum.
  - Free lambda light chains in urine.
- Quantitative immunoglobulins
  - Moderately suppressed levels of IgM (31 mg/dL) and IgA (32 mg/dL).
  - IgG elevated at 3875 mg/dL

# Case 6

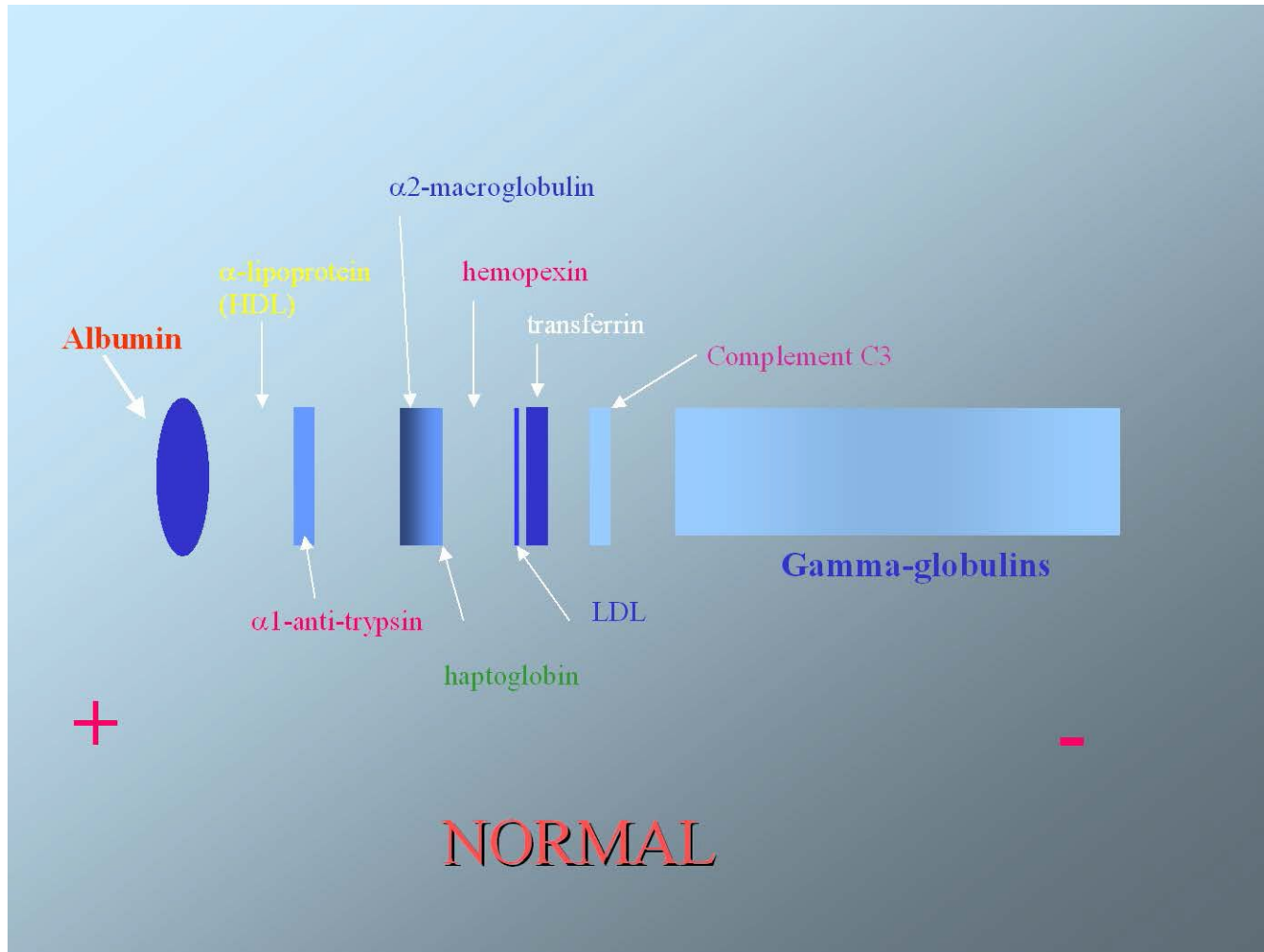
- Skeletal survey
  - Multiple lytic lesions throughout the skeleton
- Bone Marrow aspirate and biopsy
  - Sheets of malignant appearing plasma cells identified

# Case 6

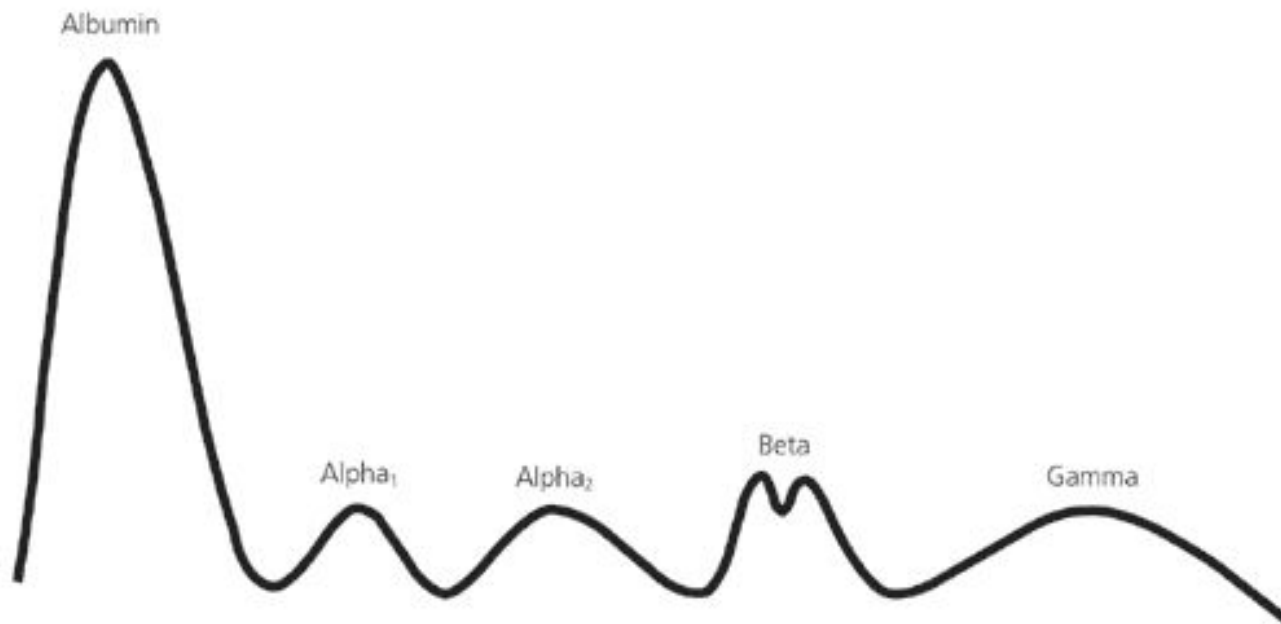
- An M-protein is usually seen as a discrete band on agarose gel electrophoresis in the  $\gamma$ ,  $\beta$ ,  $\alpha_2$  region of the densitometry tracing.
  - Immunoglobulins primarily in  $\gamma$  component, but also in  $\beta$  and  $\alpha_2$  region
- A polyclonal response produces a broad band or a broad-based peak limited to the  $\gamma$  region



# Case 6: SPEP

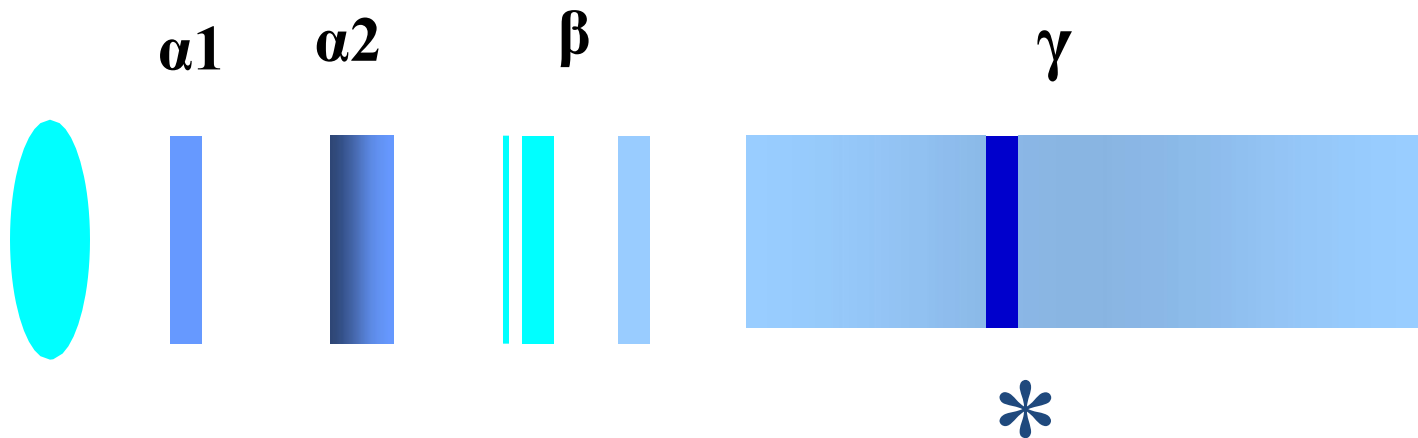


# Case 6: SPEP



NORMAL

# Case 6: Myeloma



+

-

# Case 6: Density Scan

+

-

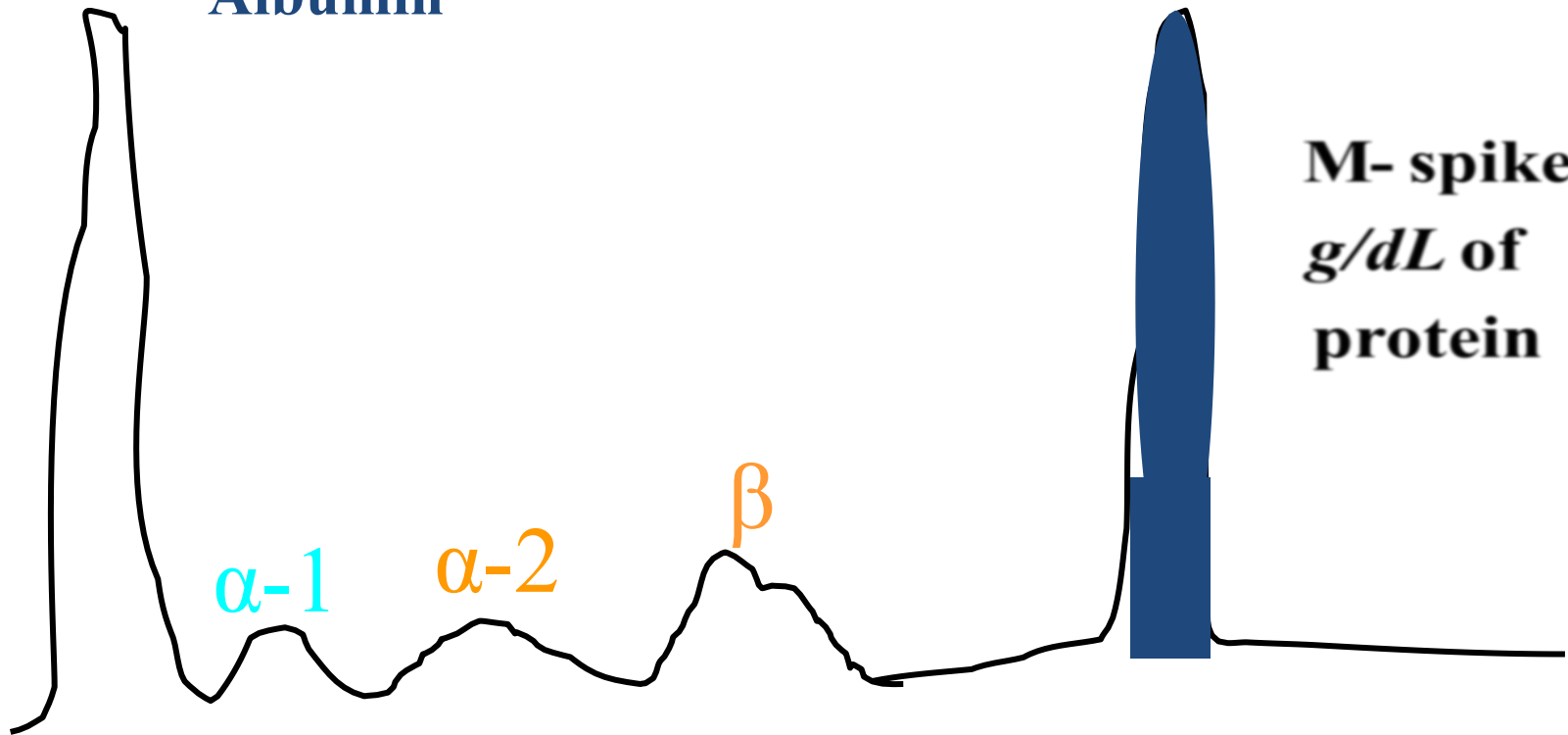
Albumin

$\alpha-1$

$\alpha-2$

$\beta$

**M- spike in  
*g/dL* of  
protein**



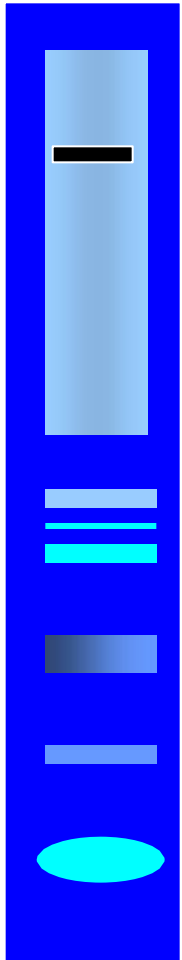
# Case 6

- When to order an SPEP:
  - When you suspect multiple myeloma, Waldenstrom's macroglobulinemia or amyloidosis.
- With unexplained:
  - Weakness, fatigue, anemia, back pain, fractures, hypercalcemia, renal insufficiency.
  - Recurrent infections.
  - Sensorimotor neuropathy, carpal tunnel syndrome, CHF, syncope.

# Case 6

- Immunofixation (IFE):
  - Proteins are fractionated on electrophoretic strips:
    - Each lane overlaid with monospecific antisera against IgG, IgA, IgM, and light chains
    - Immunoglobulins are precipitated by antisera
      - Wash away nonprecipitated proteins
    - Precipitated proteins are stained

# Case 6: Normal IFE



SPE



gamma



alpha



mu



kappa



lambda

# Case 6

- When to order an IFE:
  - To type the paraprotein (M spike) identified on SPEP
  - Further evaluate an equivocal SPEP
  - To search for a low level paraprotein with a negative SPEP:
    - clinical suspicion of a lymphoplasma-cytic disorder is high
    - unexplained symptoms such as neuropathy, renal failure, etc..
  - When searching for Bence-Jones proteinuria
  - In treated myeloma patients with a negative SPEP



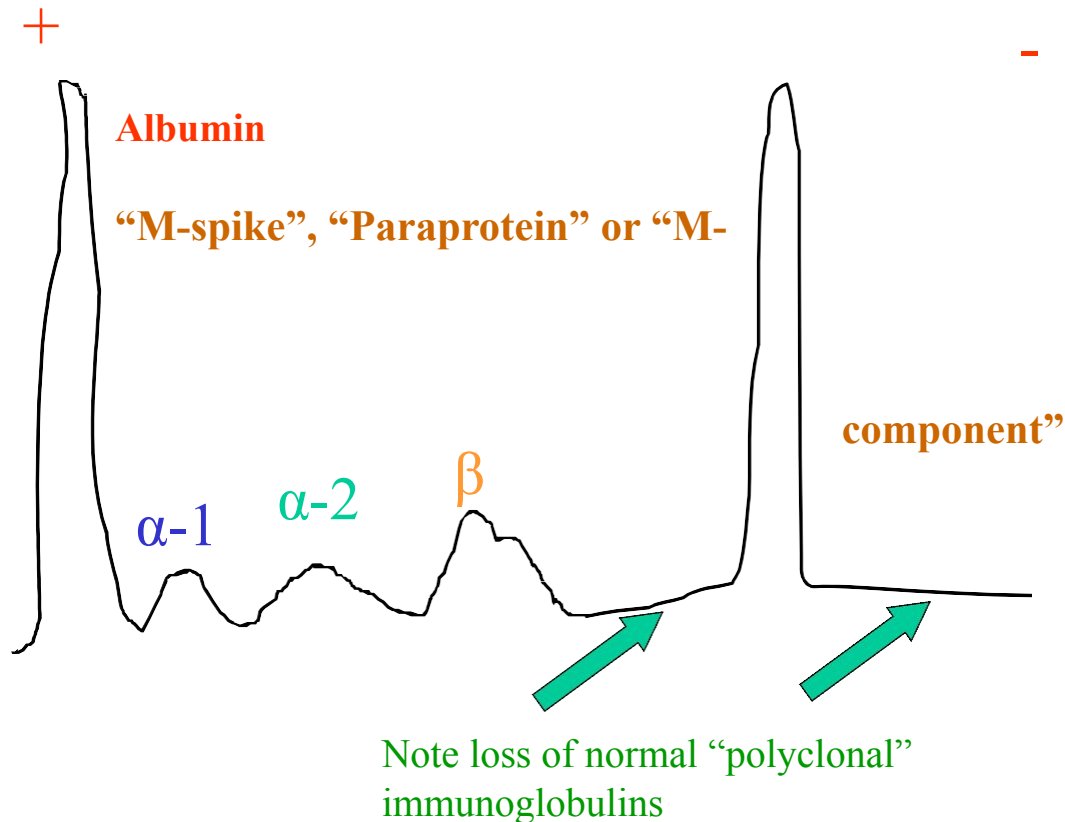
# Case 6: Quantitative Immunoglobulins

- Useful to quantitate:
  - The amount of monoclonal protein
  - Suppression of uninvolved immunoglobulins in a monoclonal disorder
  - Identify a congenital or acquired deficiency state of an individual immunoglobulin

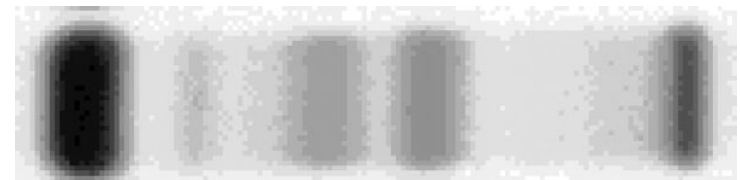
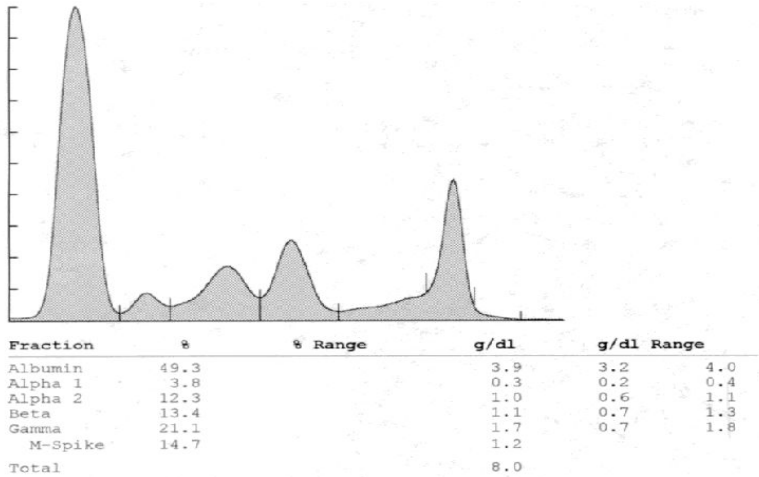
# Case 6

- Serum light chain assays are newer and a very sensitive technique for measuring serum light chains.
- Either an adjunct to or replacement of urine protein electrophoresis.

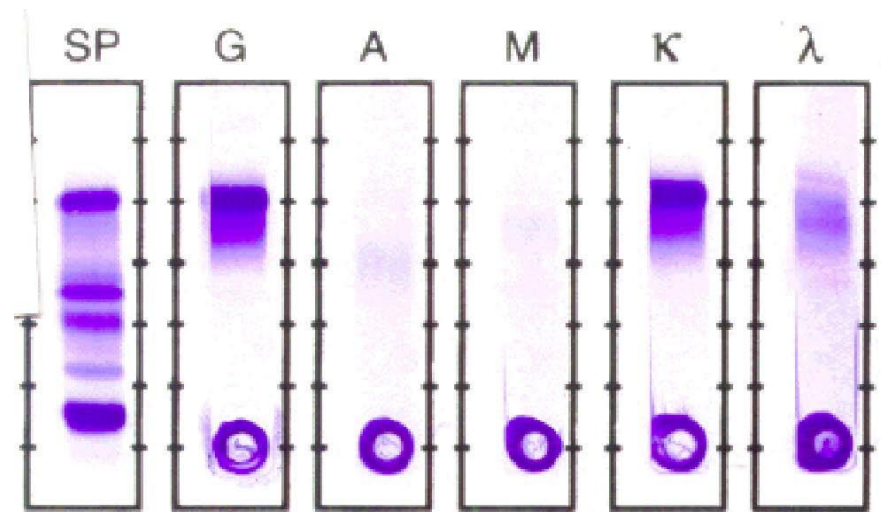
# Case 6: Quantitative Immunoglobulins



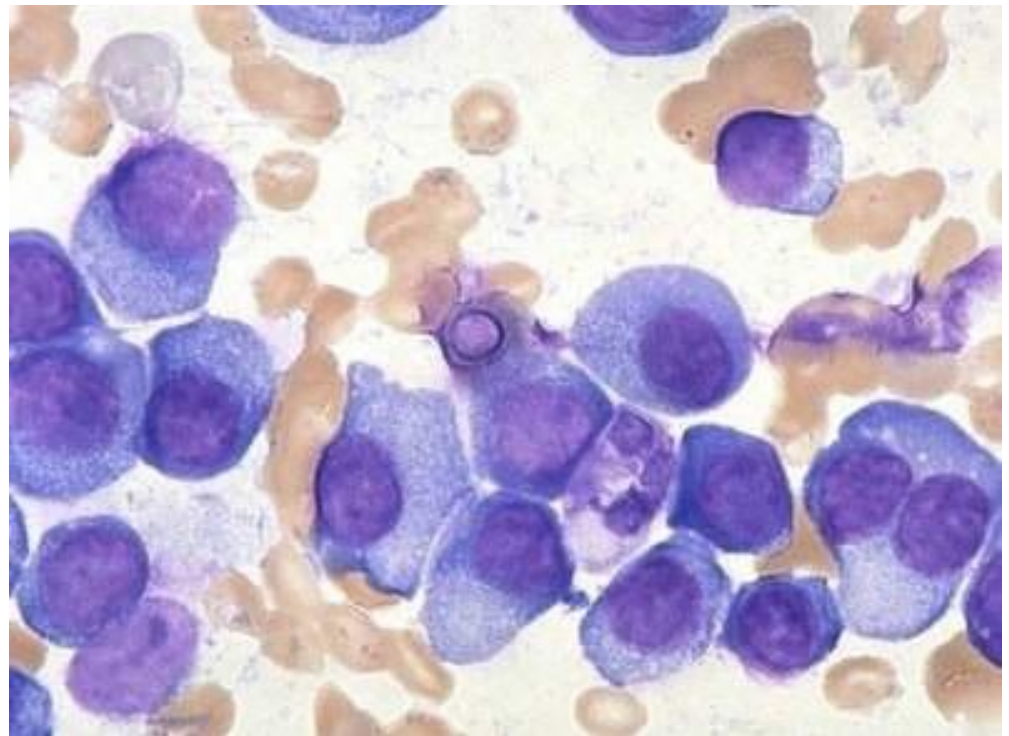
# Case 6



Our patient



# Case 6



# Case 6: Stage III Multiple Myeloma

- The patient is started on systemic chemotherapy and an autologous bone marrow transplant is planned.
- Multiple new and effective agents exist for MM:
  - Thalidomide or Revlimid and other anti-angiogenic agents.
  - Proteasome inhibitors (velcade)
  - Double autologous transplant
  - Mini allogeneic transplant
- Remains non-curative except possibly for allogeneic transplant

# Case A

- A 45 yo white male presents to his physician complaining of tiredness and fatigue over the last several months. He is otherwise healthy. His only other complaint is vague abdominal discomfort. The only medications he takes are daily NSAIDS for a knee injury he sustained while playing tennis.
- You are concerned he might be anemic. What questions should you ask in the H & P?

# Case A

- Progression of symptoms
- Blood in stool
- Other medications or toxins, including EToH
- Prior history of anemia, prior CBC's
- Other constitutional symptoms, recent illness
- Family history
- Diet
- Craving ice, starch, or dirt



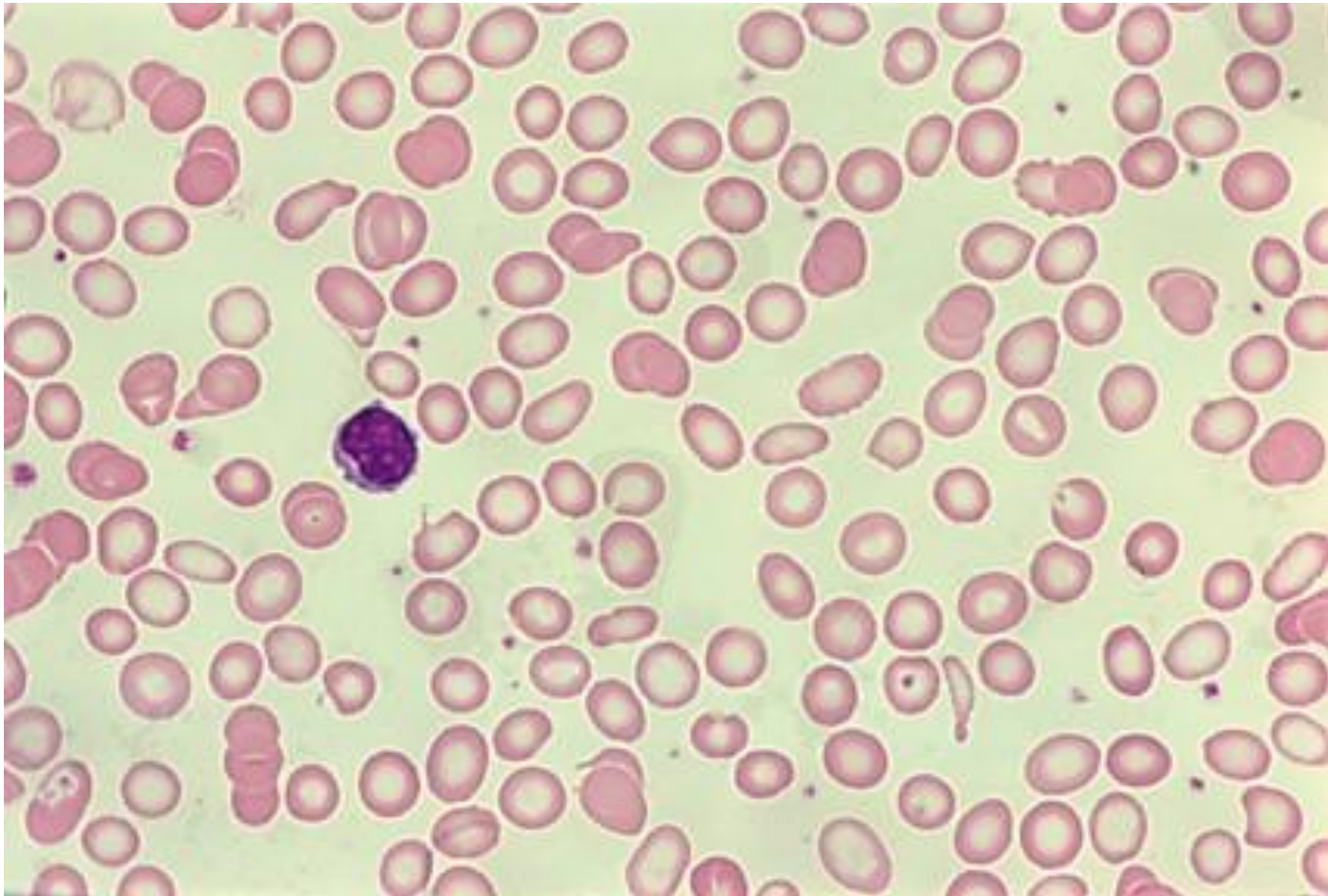
# Case A

- The patient does note he has had black tarry stools for a few weeks prior to his visit. You suspect GI blood loss induced by NSAID use.
- What should you key in on the physical exam?

# Case A

- You order a CBC which shows the following results:
  - Hbg 8.2 gm/dL
  - HCT 26%
  - RBC count 3.82 million/mm<sup>3</sup>
  - MCV 73 cubic microns
  - Platelet count 516,000/mm<sup>3</sup>
  - WBC 7,000/mm<sup>3</sup>
- What is your interpretation of these values?

# Case A



# Case A

- Iron studies:
  - Fe 14 ug/dL
  - TIBC 426 ug/dL
  - % sat 3%
  - Ferritin 10ng/mL
- Corrected reticulocyte count 1.1%
- Are any other studies (bone marrow) needed?

# Case A

- You confirm iron deficiency likely secondary to NSAID use. An endoscopy confirms a H.pylori negative ulcer with chronic bleeding. He is started on Omeprazole and his NSAID is stopped.
- You prescribe oral Fe Sulfate 325 mg titrated to TID which he tolerates fairly well.
- He comes back to see you in 4 weeks.

# Case A

- He feels much better and has experienced resolution of his fatigue and lethargy.
  - Hgb 12gm/dl
  - HCT 35%
  - MCV 82 cubic microns
  - Retic count 4.2%
- Why do you think his retic count is elevated?
- Can you stop his Fe sulfate when his hgb/hct become normal?

# Case A



# Case B

- A 67 year old woman with a history of IDDM and treated hypothyroidism is referred to you for evaluation of anemia. Her complaints leading to this diagnosis included weakness, fatigue, weight loss, and mild numbness in her feet bilaterally.
- Physical exam was essentially normal except for mild loss of proprioception in her feet bilaterally.



# Case B

## Current Labs:

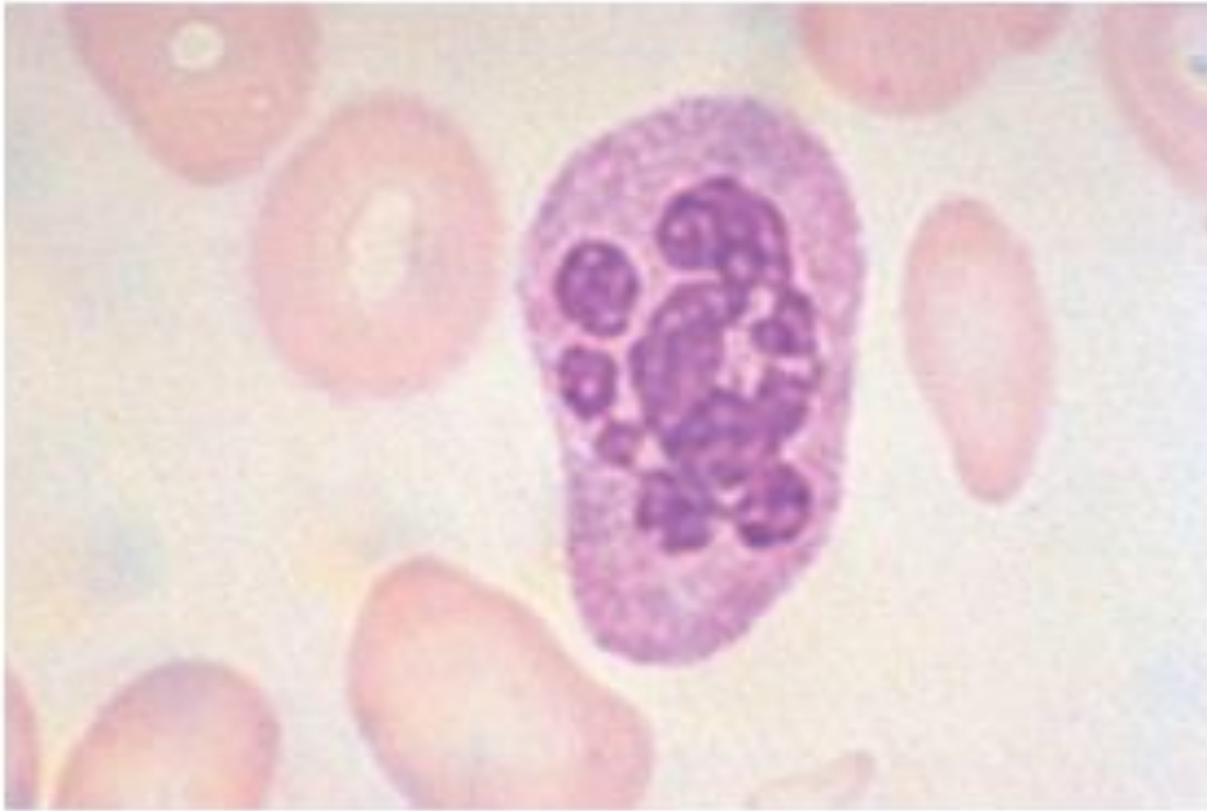
WBC	3,700/mm <sup>3</sup> (4-10 thou/mm <sup>3</sup> )
Hemoglobin	8.3 g/dL (12-16 g/dL)
Hematocrit	25% (35-45%)
Platelet	152,000/mm <sup>3</sup> (150-450 thou/mm <sup>3</sup> )
MCV	123 $\mu^3$ (80-100)
Retic count	.3% (.3-1.7%)

## 2 years ago:

WBC	4800/mm <sup>3</sup> (4-10 thou/mm <sup>3</sup> )
Hemoglobin	11.2 g/dL (12-16 g/dL)
Hematocrit	33% (35-45%)
Platelet	187,000/mm <sup>3</sup> (150-450 thou/mm <sup>3</sup> )
MCV	103 $\mu^3$ (80-100)
Retic count	1.1% (.3-1.7%)

How do you interpret these values?

# Case B



\*Hypersegmented neutrophil, macroovalocytes

# Case B

- What tests or procedures do you want to perform to further evaluate this patient?

Folate	8.3 ng/mL (>3)
Vitamin B12	73 pg/mL (180-914)
TSH	1.5 mIU/mL (.4-4.0)

# Case B

- You diagnose B12 deficiency and prescribe B12 injections 1000ug weekly x4 then 1000ug a month indefinitely.
- In 1 month the patient feels remarkably better and her blood counts have all improved.

# Case B: Vitamin B12 Deficiency

- Found primarily in animal meats.
- Large hepatic reservoir.
- Deficiency from decreased oral intake takes many years.
- In the duodenum cobalamin binds to intrinsic factor produced by parietal cells of the stomach.
- In the terminal ileum, cobalamin-IF complex is transported through the enterocyte into the blood.
- Almost always due to malabsorption:
  - Pernicious anemia, achlorhydria, IBD, celiac disease, pancreatic insufficiency, bacterial overgrowth, alcohol.

# Case B: Vitamin B12 Deficiency

- Clinical findings:
  - Insidious onset.
  - Glossitis, weight loss, pale yellow skin.
  - Neurologic manifestations:
    - Loss of position or vibratory sense.
    - Can progress to spastic ataxia.
    - May occur with mild anemia and may be irreversible.
  - Psychiatric disorders can occur without evidence of hematologic abnormalities.
    - Hallucinations, dementia, psychosis, “megaloblastic mania”.
    - May be irreversible.

# Case B: Vitamin B12 Deficiency

- Lab findings:
  - Cobalamin <200ng/L.
  - If low-normal (200-350ng/L), check homocysteine and MMA.
    - Both elevated in Vitamin B12 deficiency.
  - Methylmalonic acid level is more sensitive than low-normal cobalamin alone.
  - Pernicious anemia:
    - Autoantibodies to parietal cells or intrinsic factor (50-70% sensitive, 100% specific).

# Case B: Vitamin B12 Deficiency

- Treatment:
  - Parenteral:
    - 1000ug IM for 5 days followed by weekly for 4-5 weeks, then monthly.
  - Oral:
    - 1000-2000ug/day.
- Reticulocytosis in 3-5 days, blood counts normalize by 2-3 months.
- Folate may correct the anemia but not the neuropsychiatric manifestations, so you must check both prior to administration of folate.