# Hematology Essentials: A Foundation for Accurate Smear Reviews

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### Differential Training Program

\* Current Challenges

\* System Wide Approach

\* Standardization

# Training Area



### How does the program work?

#### Training Material

- Trainer and Trainee
   Checklists
- \* Reference guides
- \* Actual patient slides
- Case Study Power Point
- CompetencyChecklist



### Trainee Feedback



"I have to tell you I was dreading the diff training BUT it was AWESOME! Where I worked before I didn't have any formal diff training, you knew the basics and that was it. I love all the hand outs you provided. Things made more sense after the class."

### Manager Feedback



- \* "I was worried about the time commitment, but every employee came back saying how valuable the training was..."
- \* "I was impressed to hear how excited my employees were about hematology after the training"
- \* "Employees feel they really have the tools now to provide great patient care"

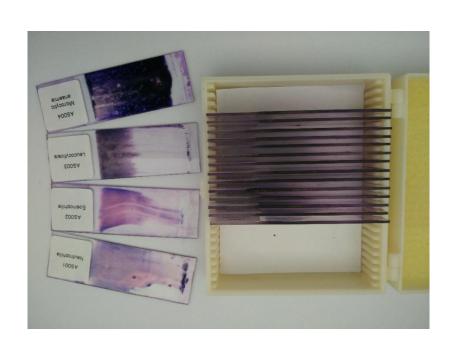
### Post Training

- \* Follow up post training with Cellavision images for competency
- \* Ongoing competency assessment
- \* Adapted for smaller sites and/or affiliates

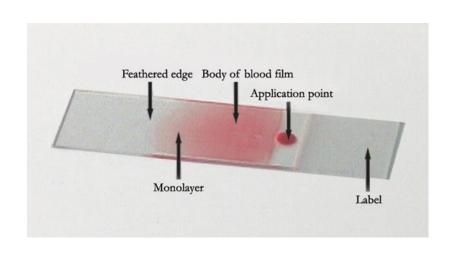
### Proper Slide Preparation

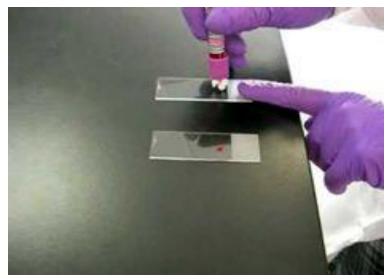
- \* smooth, homogenous film
- \* 1/2 to 3/4 the slide length
- \* straight feather edge
- \* at least 1/4 inch examination area
- \* pink RBCs and appropriate WBC blues under gross examination (Rainbow feather edge)

### Bad slide prep



## Good slide prep





### The Good and Bad



### Starting your slide examination

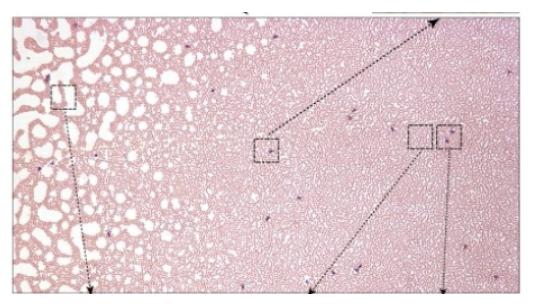
- \* Examine on 10X: Check for good cell distribution, free of precipitate
- \* Examine extreme feather edge:
  - Platelet clumps
  - Look for abnormal cells: More dense and larger cells will be pushed to the feather edge

### Starting your slide examination

- \* Area between extreme feather edge and "Zone of Morphology" is the cobblestone area. DON'T do the morph or diff in this area.
- \* "Zone of Morphology"-area where cells evenly distributed, RBC's close but not touching. Diff and morphology should be performed here

# Zone of morphology

feathured edge



too thick

### **WBC** Estimate

- \* Make sure slide has been made correctly
- \* If the slide has been pushed too hard when making the slide, WBC's will be concentrated at extreme feather edge and estimate will not match instrument result.

### **WBC** Estimate

- \* Estimate the white count under 10x or 40X/50x.
- \* Under low power 10X: 5 WBC's = 1,000/cumm
- \* Under 40X/50X: 1 WBC = 2,500/cumm
- \* The white count estimate may not be reported, but every manual differential white count is checked in this manner

### Performing a manual differential

#### In "Zone of Morphology":

- Switch to 40x/50X or 100X to count 100 WBC cells.
   Note: Perception at 100x can be distorted
- \* Manual differential vs analyzer differential
- \* Must drop to 100X for RBC morphology and Platelet estimate.
- \* Platelet Estimate = (Total # of PLTs Counted in 10 Fields Using 100X) X 15,000

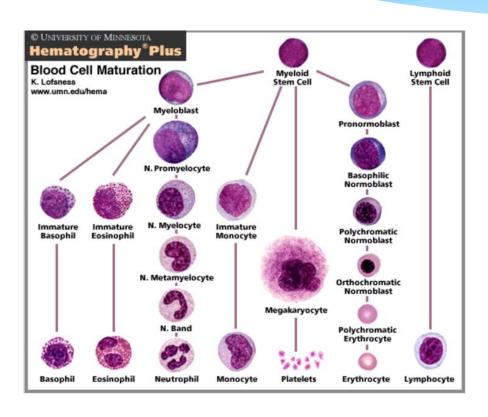
# Know appropriate morphology reporting

- \* Morphology not reported: Anisocytosis, Macrocytosis, Microcytosis, Poikilocytosis, Stomatocytes
- \* Morphology reported as present: Toxic Granulation, Dohle Bodies, Auer Rods, Hypersegmented Neutrophils, Hyposegmented Neutrophils, Vacuolated Neutrophils, Reactive Lymphocytes, Smudge Cells, Large Platelets, Agranular Platelets, Dwarf Megakaryocytes, Atypical Platelets, Basophillic Stippling, Pappenheimer Bodies, Howell Jolly Bodies, Sickle Cells, Rouleaux

# Know appropriate morphology reporting

- \* Slight, Moderate, Marked: Hypochromasia, Polychromasia
- \* Few, Moderate, Many: Target, Acanthocytes, Echinocytes, Schistocytes, Spherocytes
- \* Platelet estimate choices: Decreased, Adequate, Increased, Clumped

### Review Blood Maturation Chart

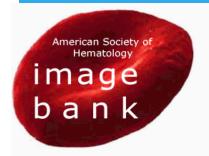


# Myeloid Series-5 characteristics to look for

- \* N/C Ratio
- \* Chromatin pattern-clumped or fine
- \* Nucleoli
- \* Cytoplasm-Color of granules, inclusions
- \* Size of cell

### Normal Slide

- \* PMN- coarse chromatin
- \* <u>Lymph</u>-N/C ratio 5:1 to 2:1 chromatin pattern clumped. Sky blue cytoplasm
- \* <u>Large Lymph</u> nucleus off center/clear cytoplasm (size determined by type of lymph, B,T,Killer)
- \* Basophil-large purple granules-see increase in reactive conditions such as MPD(myloproliferative disease.)
- \* Monocyte
- Eosinophil-contain bright orange-red granules evenly distributed in the cytoplasm-rarely overlie the nucleus.
- \* Band-narrowing of nucleus by 50%

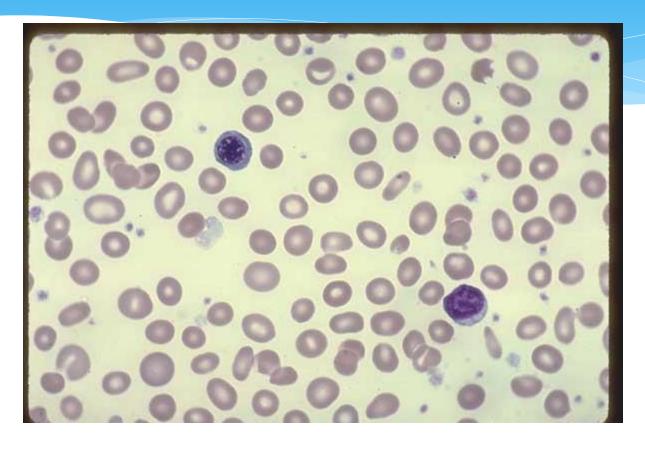


### Neutrophil



Maslak, P. ASH Image Bank 2002;2002:100360

### Lymphocyte



7-16 µm, nucleus is the size of a normal RBC, condensed chromatin, granules may be present

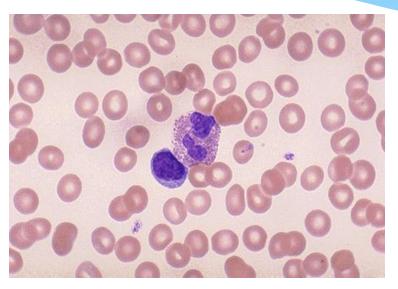
### Monocyte

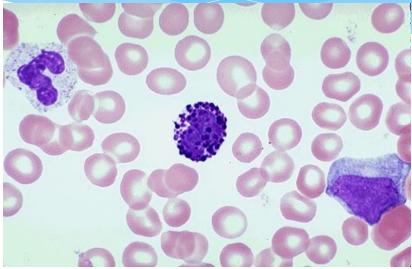


12-20 µm, folded nucleus, lacy chromatin, blue-gray cytoplasm, fine granules

http://library.med.utah.edu/WebPath/HEMEHTML/HEME003.html

### **Eosinophil and Basophil**



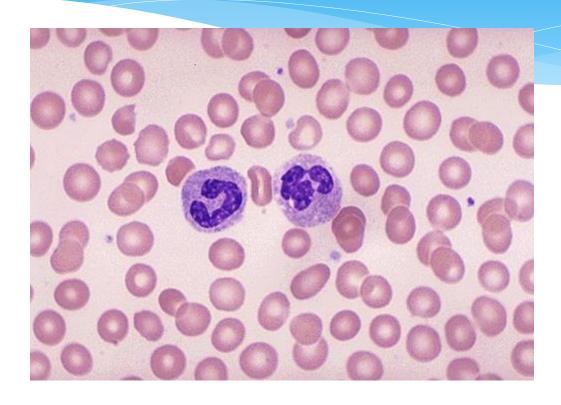


12-15 µm, 2-3 lobed nucleus, prominent reddish-orange granules

10-15 µm, segmented nucleus, prominent blue granules

Slides courtesy of http://library.med.utah.edu/WebPath/HEMEHTML/H EME003.html

### **Band Neutrophil**

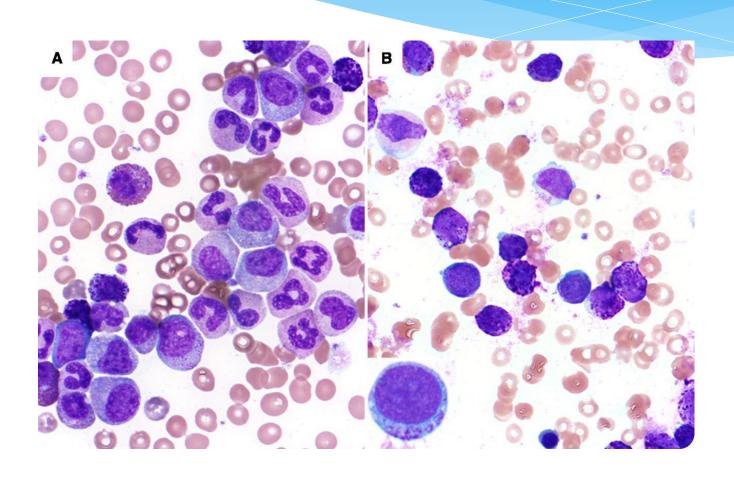


9-15 µm, horseshoe shaped nucleus, chromatin present in any filaments

### Chronic Myeloid Leukemia

- \* Leukemia is the uncontrollable growth of cells.
- \* Demonstrates a variety of immature cells, including blasts
- \* Basophilia and a left shift can be some of the first signs of CML
- \* Cells to be identified on slide:
  - \* Myelocyte
  - Metamylocyte-Nucleus kidney bean shaped
  - \* Promyelocyte-(granules can overlap nucleus) Basophilic cytoplasm-Chromatin pattern is fine 1-2 nucleoli
  - \* NRBC
  - \* Myeloblast-Most immature cell in the myeloid series, N/C ratio high-fine chromatin pattern, basophilic cytoplasm

### CML



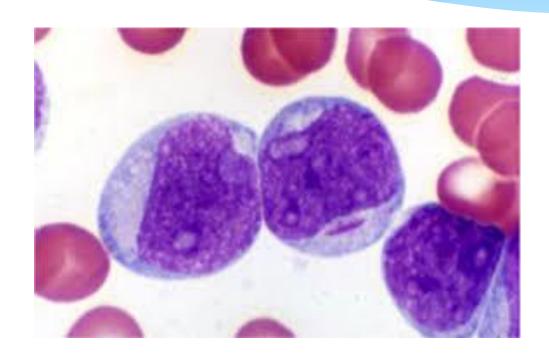
### Acute Myeloid Leukemia

- \* Mononuclear cells seen on slide
- Not seeing RBC's overlapping on slide
- Not seeing many platelets
- \* Pancytopenia-All three cell lines are affected
- \* Don't see many neutrophils (neutropenia)
- Large lymphs (clear cytoplasm/offset Nucleus)
- \* Blasts: Note-If you see Auer Rods this indicates cell is in the myeloid lineage

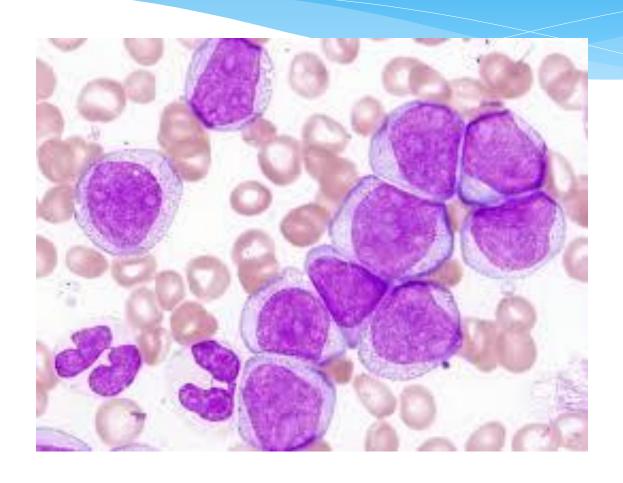
### Acute Myeloid Leukemia

- \* RBC morphology sometimes seen on slide:
  - Basophilic stippling
  - \* Polychromatic
  - \* Elliptocytes (Ovalocytes)
  - \* Teardrops
  - \* NRBCs

### **AML**



### **AML**



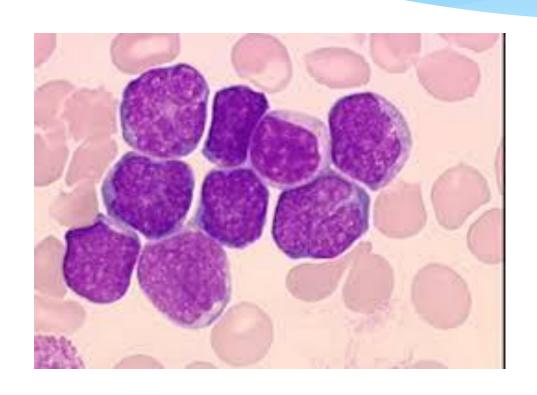
## Blast vs Lymph



#### ALL

- \* 4yr old, cough, fatigue
- \* High WBC count, low Hgb-3.8g/dl, low Plt-20,000
- Mononuclear cells with high N/C ratio, fine very fine, smooth chromatin pattern
- \* Slide full of Blasts

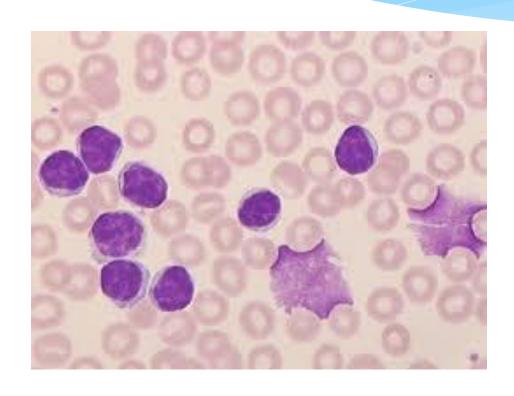
### ALL



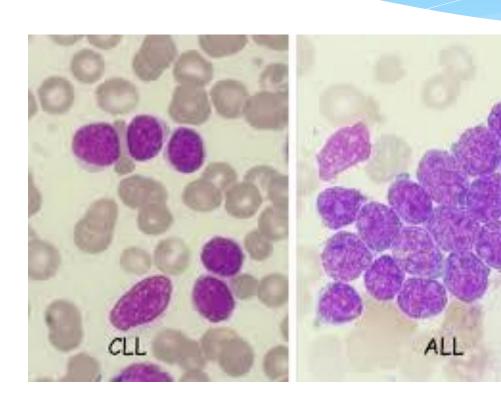
#### **CLL**

- Affects B-cell lymphocytes
- \* Typical Lymphocytosis >5.0 absolute
- Characteristic nucleus that looks like "cracked earth" or a soccer ball
- Cells are fragile, resulting in smudge cells present on smear
- \* Albumin slides made to reduce smudge cells, diff should be performed on albumin slide, RBC/WBC morphology should be performed on the original slide

#### CLL



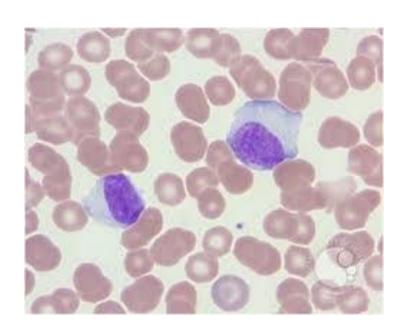
#### CLL vs ALL



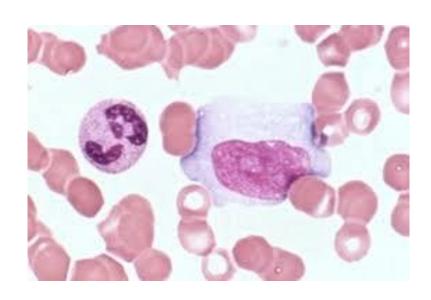
#### Reactive Lymphs

- \* Variability of cellular size and shape as well as nuclear size, shape and chromatin pattern
- \* Seen in many viral illnesses-infectious mononucleosis
- \* Nucleus attached to cell wall
- Cytoplasm surrounding RBC's
- \* Reactive lymph vs Monocyte

# Reactive Lymphs



# Reactive Lymph



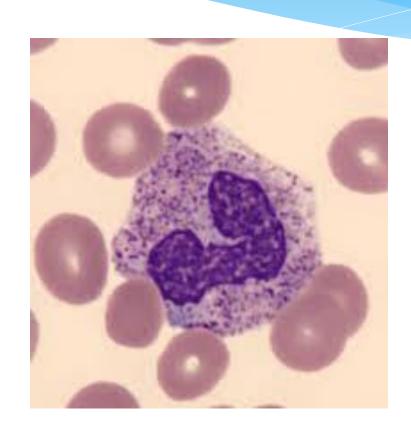
# GCSF: Neulasta, Neupogen

- Used to boost WBC following chemo
- \* Toxic granulation
- \* Dohle Bodies-sometimes
- \* Immature cells

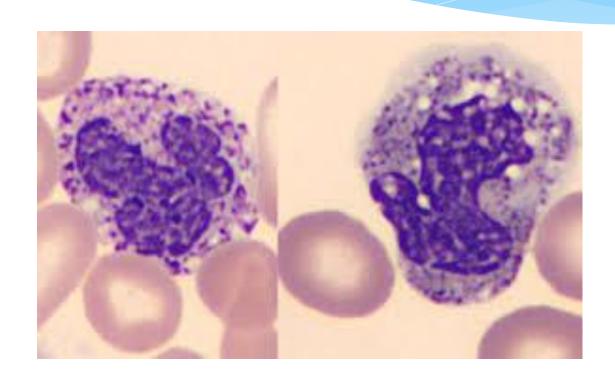
# Toxic gran, Dohle Bodies, Vacuolated Neutrophils

- \* Toxic Granulation-Large, purple or dark blue azurophilic granules, resembling the primary granules of promyelocytes, in the cytoplasm of neutrophils, bands and metamylocytes. Seen in severe infection, chemical poisoning, and other toxic states
- \* <u>Dohle Bodies</u>-Appear as single or multiple light blue or gray staining area in the cytoplasm of neutrophil. RNA and represent failure of cytoplasm to mature. Seen in infections, poisoning, burns and following chemotheraphy
- \* <u>Vacuolated Neutrophils</u>-seen in cytoplasm of neutrophils and bands and represent the sites of phagocytosed material. Seen in association with toxic granulation

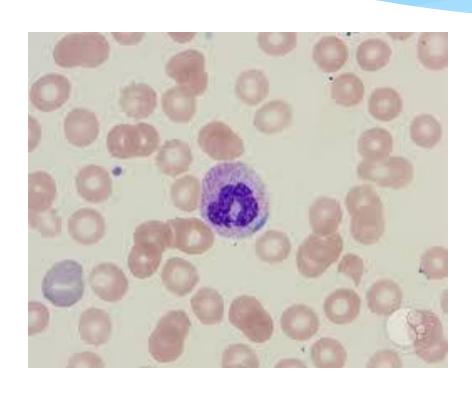
#### **Toxic Granulation**



#### Toxic Granules with Vacuoles



#### Toxic Granules + Dohle Bodies



# Hypersegmented Neutrophils

- Neutrophil with 5 or more lobes
- \* Need to see a # of them to call
- \* Seen in megaloblastic anemia, B12/Folate deficiency
- Seeing macrocytosis-MCV is 130 on this patient

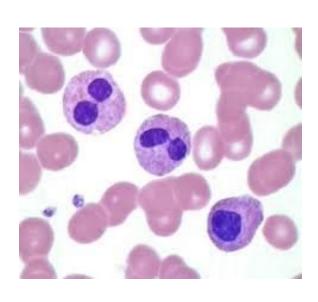
# Hypersegmented Neutrophil

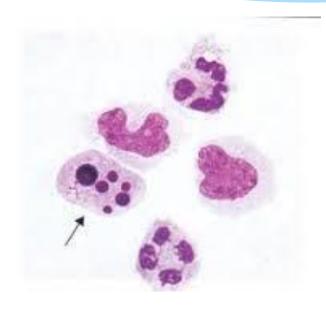


# Pelger Huet

- \* Unilobed neutrophil
- Genetic Disorder (benign)
- \* Cells will function fine
- \* Pelger vs pseudo Pelger vs pyknotic

# Pelger Huet vs Pyknotic





# True hypogranular, hypolobulated neutrophils



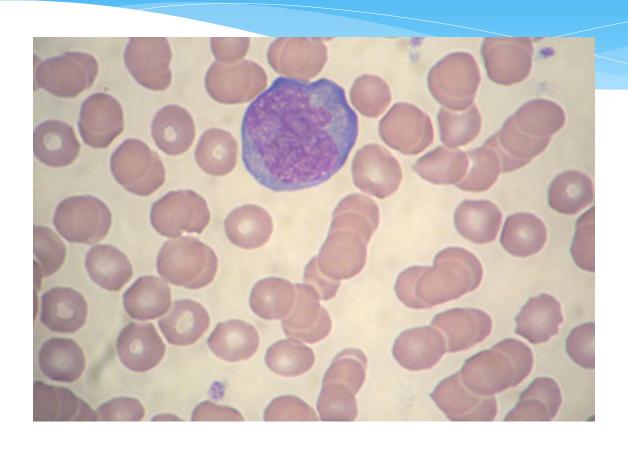
# Case Study Time!

- \* 22 yr old female presents at college health services
- \* Patient complains of sore throat, fever, and swollen glands

#### **CBC** results:

#### Differential results:

WBC RBC HGB HCT MCV	16.0 4.22 12.8 37.5 89	thou/cu mm mil/cu mm g/dL % fL	Neutrophils Lymphocytes Monocytes Eos	26 63 10 1
MCHC MCHC RDW PLT	30.4 34.2 12.6 213	pg % % thou/cu mm		





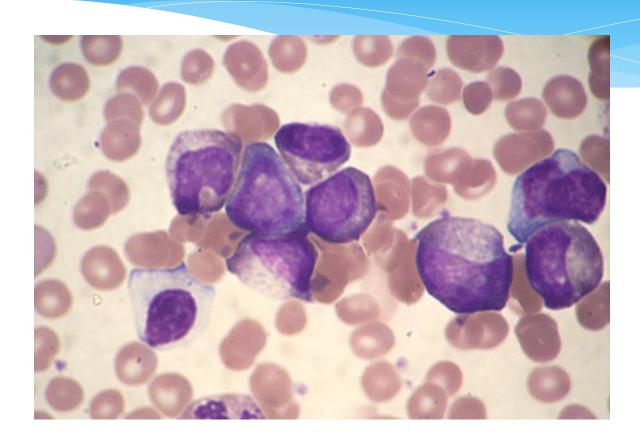
- \* Manual Differential reveals 3+ reactive lymphs
- \* Heterophile Antibody Test confirms infectious mononucleosis diagnosis

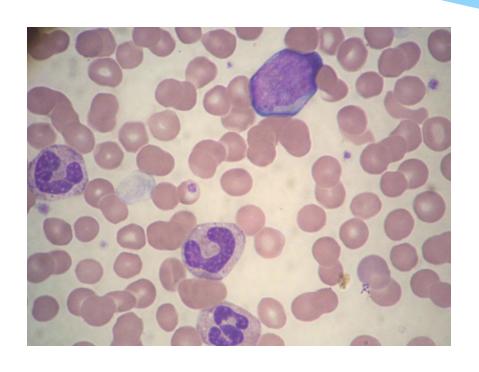
- \* 63 yr old female presents in ED
- \* Left lower quadrant pain, fever, chills
- \* History of diverticulitis, breast cancer
- Patient is quadriplegic due to the effects of polio as a child

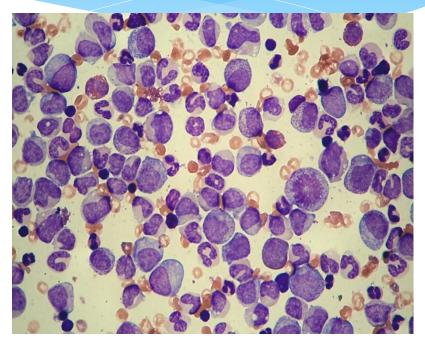
#### CBC results:

#### Differential results:

WBC	124.3	thou/cu mm	Neutrophils	48
RBC	4.31	mil/cu mm	Lymphocytes	10
HGB	13.3	g/dL	Monocytes	5
HCT	39.9	%	Eos	2
MCV	93	fL	Baso	3
MCH	30.9	pg	Bands	14
MCHC	33.3	%	Meta	7
RDW	17.1	%	Myelo	11
PLT	189	thou/cu mm		

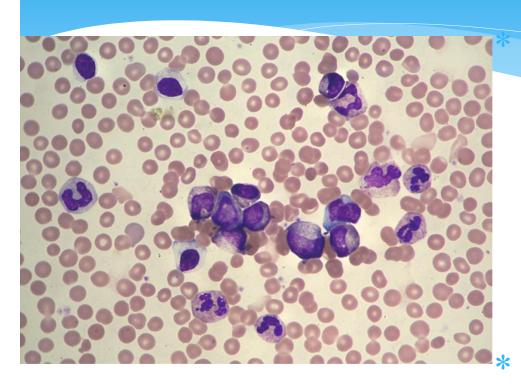






Blast-peripheral blood

Bone marrow-ME slide



Initial

Hematology/Oncology consult determined increase in WBC was due to infection since Hgb and Plts were normal

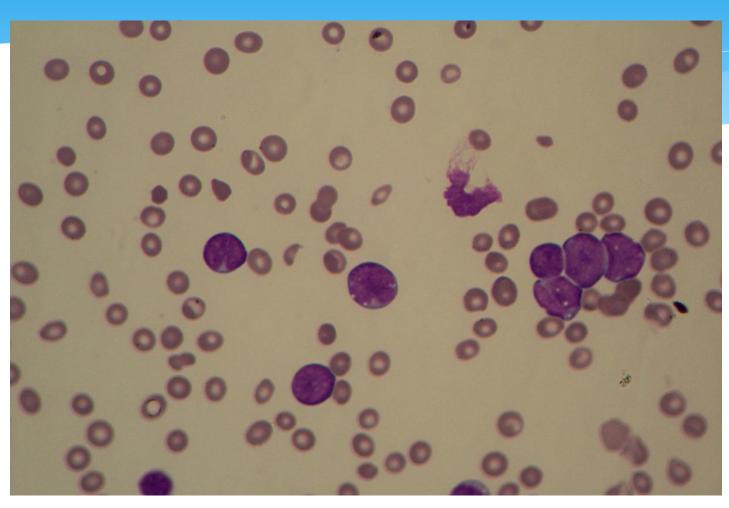
Next step?

- Smear was referred to pathologist
- Pathologist sent blood for BCR/ABL gene
  - Specific for Chronic Myelogenous Leukemia (CML)
  - Results are positive
  - Second Oncology consult results in bone marrow biopsy
  - \* Bone marrow confirms CML diagnosis

- \* Child
- \* Presented to clinic with cough and fatigue
- \* Pediatrician ordered CBC/Differential
- \* CBC results revealed the following:

```
WBC 32,000
Hgb 3.8 g/dl
Plt 19,000
```

- \* Peripheral smear review:
  - \* High % mononuclear WBC's
  - Irregular, clefted nuclei
  - Vacuoles present
- Pediatrician informed of possible abnormal cells;
   requires confirmation by Pathologist
- Slide sent STAT to hospital
- Blasts confirmed by Pathology





- \* Pediatrician notified by Pathologist
- \* Flow Cytometry: Lymphoid
- \* B Cell ALL
  - \* Cytogenetics t(12;21)
  - \* Prognosis: favorable
  - 5-year overall survival rate for childhood ALL 89%
- \* Treatment: Induction/Consolidation

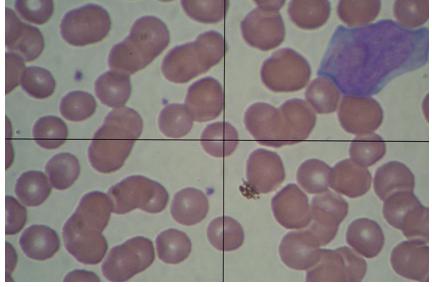
- \* Pre-op for total knee replacement
- \* Routine labs included urinalysis, BMP, and CBC
- \* CBC revealed low platelet count =86
- \* Slide reviewed
- \* No abnormalities revealed
- \* Next day platelet count low
- \* Slide reviewed (rule, Blast flag)

# Case Study #4 Images



Blast w/ prominent nucleolus

Blast w/Auer Rod



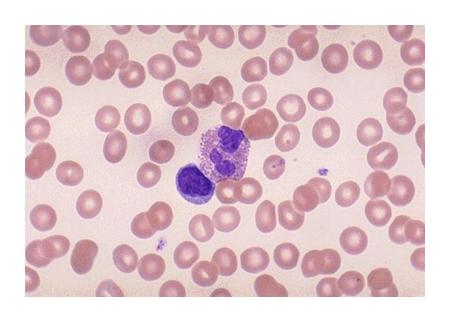
- \* Slide review revealed 2-3 blast type cells with possible auer rods
- \* Pathologist reviewed, contacted physician for further workup
- \* Initial slide reviewed to see if we missed anything
- Surgery delayed
- \* Patient had bone marrow biopsy

- \* Morphology
  - Large blast cells
  - Basophillic cytoplasm/granules
  - \* Auer rods

- \* AML with t(8;21)
- \* Prevalence ~25% adult AMLs
- \* Prognosis: Good, 70% 5 year survival rate
- \* Treatment: Patient starts induction chemo followed by consolidation therapy

# Break!

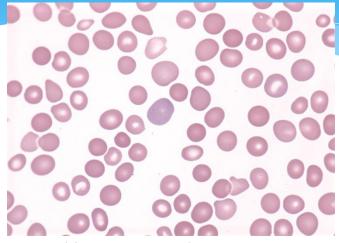
#### Normal Red Blood Cells



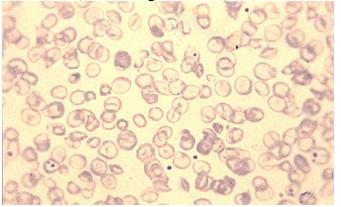
- \* Function
- \* Size
- \* Color
- \* Central Palor

- \* Note area of review
- \* Stain quality

### Polychromasia



Maslak, P. ASH Image Bank 2004;2004:101122



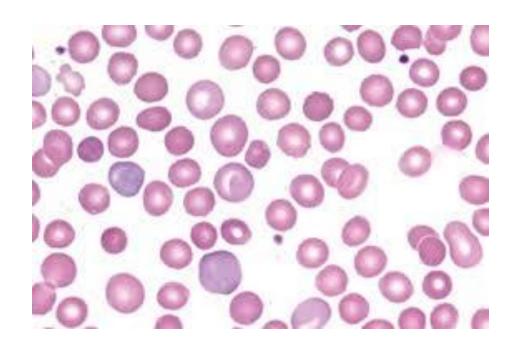
Schrier, S. ASH Image Bank 2001;2001:100208

- \* Acute/chronic bleed
- \* Hemolysis
- \* Newborns
- Hypochromasia
- \* IDA
- \* Thalassemias

### Hereditary Spherocytosis

- Spherocytes and many times, polychromasia
- Inherited hemolytic anemia
- \* Defect in the protein that forms the outer membrane of RBC
- \* RBC's become spherical and lose central palor
- \* Cells break down more quickly and are destroyed in spleen
- Bone marrow will start producing more RBC

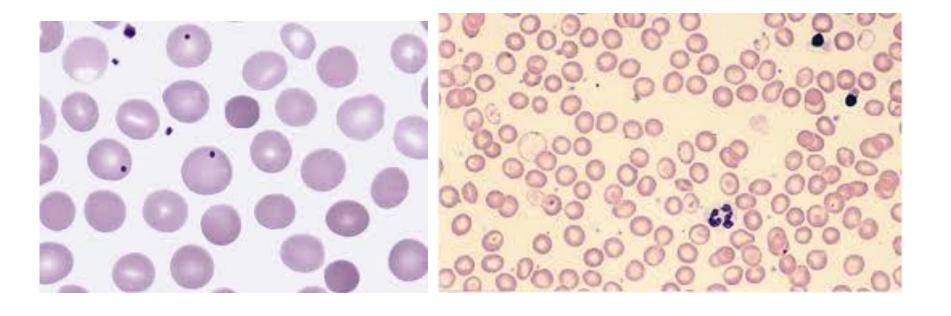
# Spherocytes



# Hereditary Spherocytosis

- \* Same patient as previous slide after spleen removed
- \* Seeing Howell-Jolly bodies in RBC's
- \* Round, purple nuclear fragments composed of DNA
- Seen following splenectomy
- Notice not seeing polychromasia because bone marrow doesn't have to work as hard

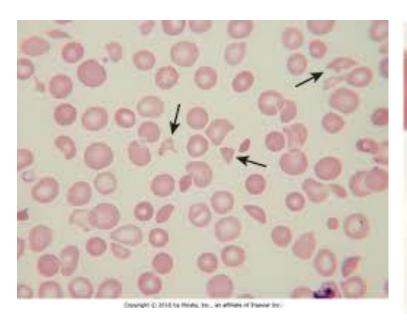
# Post splenectomy

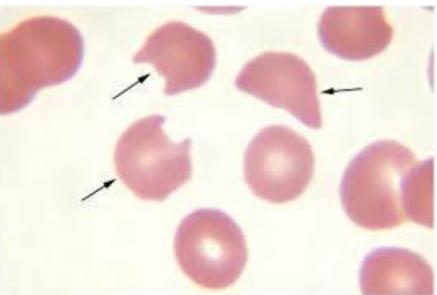


# Fragmented RBCs

- Marked increase in fragmented RBC (schistocytes)
- \* May be of any size or shape including helmet cells, keratocyte and other irregular, unusual shapes
- \* Look sheared or cut

# Fragmented RBCs





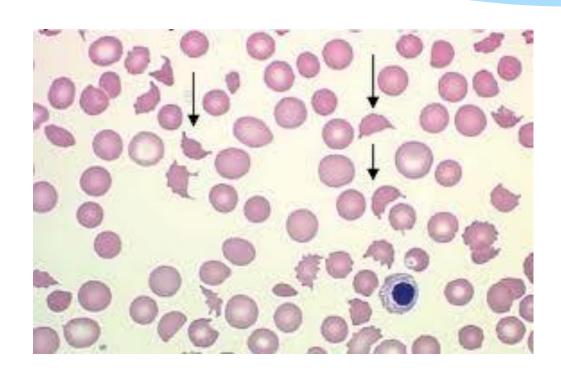
# Fragmented RBCs

- \* Clinically significant and often seen in 3 conditions
  - Mechanical heart valve shearing RBCs
  - \* Burn victims
  - \* Microangiopathic anemias that includes disseminated intravascular coagulation (DIC), Hemolytic Uremic Syndrome (HUS), or Thrombotic thrombocytopenic Purpura (TTP)-these are heme emergencies. A physician and/or pathologist should be notified immediately.

#### TTP

- \* Extensive microscopic clots are formed in small blood vessels
- \* Caused primarily by autoimmune inhibition of the ADAMTS13 enzyme that cleaves Von Willebrand factor. The increase in vWF increases platelet adhesion
- \* Treatment is plasma exchange to reduce circulating antibodies and increase the ADAMTS13 enzyme

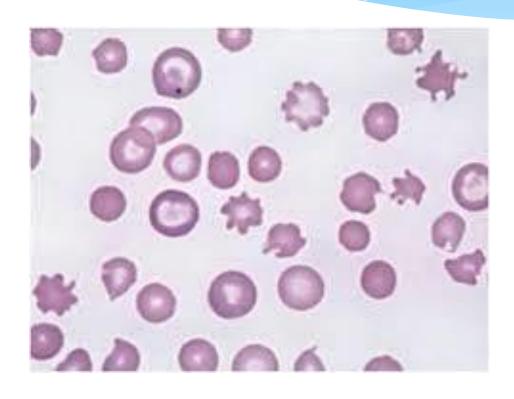
### TTP



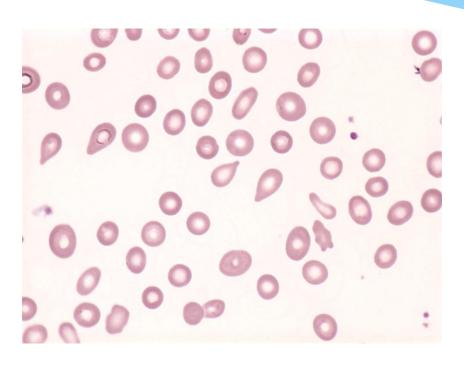
# Acanthocytes (Spur Cells)

- \* RBC's that lack central pallor with multiple oblong projections (rounded ends)
- \* Form due to alteration in the lipid content of the RBC membrane
- \* Seen in abetalipoproteinemia (genetic and rare disease)
- \* Also seen in severe liver disease

# Acanthocytes



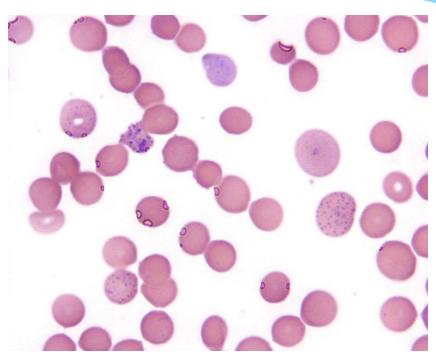
# Tear Drops



- \* Myelofibrosis
- \* Thalassemias

Maslak, P. ASH Image Bank 2002;2002:100453

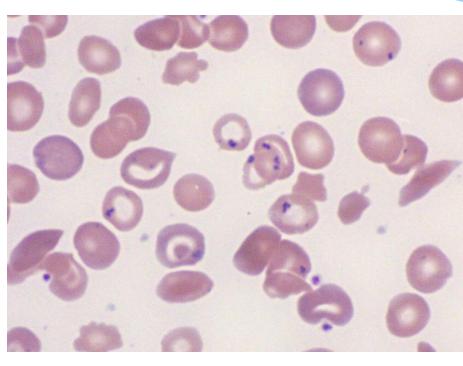
# Basophillic Stippling



Lazarchick, J. ASH Image Bank 2007;2007:7-00025

- \* Numerous fine or coarse granules
- \* Evenly distributed
- \* Composed of RNA
- \* Lead Poisoning
- \* Thalassemias

### Pappenheimer Bodies



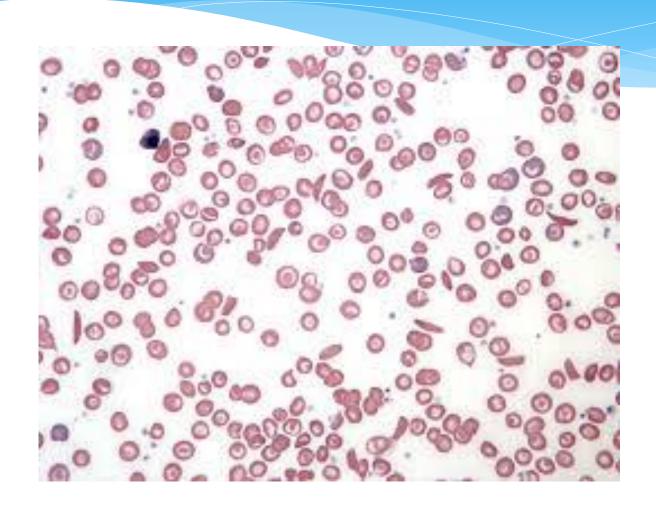
Lazarchick, J. ASH Image Bank 2007;2007:7-00013

- \* Fine, irregular granules
- \* Usually in clusters
- \* Composed of Iron
- \* Splenectomy
- \* Hemoglobinopathies
- \* Hemolytic anemia
- \* Sideroblastic anemia

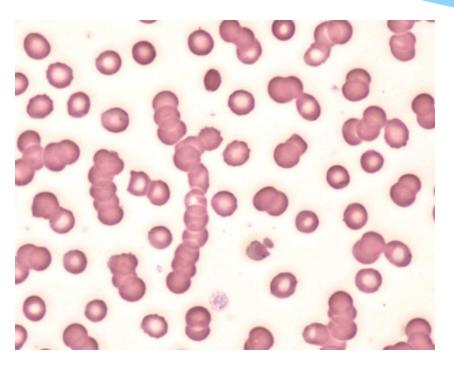
#### Sickle Cell

- \* RBC's appearing in the shape of a sickle with two pointed ends
- Can also appear as crescent-shaped, boat shaped and lack central pallor
- \* Also see many target cells on this slide

# Sickle cell



### Rouleaux



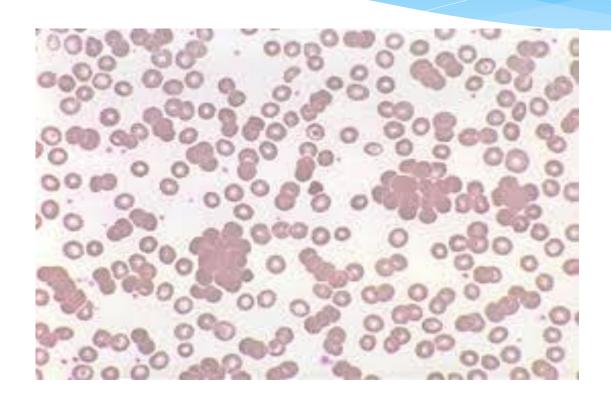
Maslak, P. ASH Image Bank 2004;2004:101153

- \* RBCs stack like coins
- \* Due to increased protein concentration
- \* Multiple Myeloma
- \* Blue slide

# RBC Agglutination

- \* See clumps of RBC's
- Caused by cold agglutinins

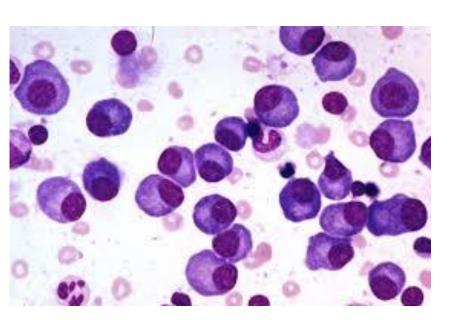
# RBC Agglutination

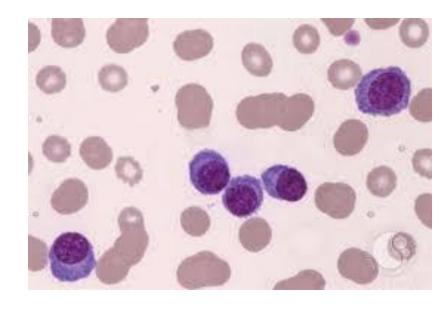


#### Plasma Cell Leukemia

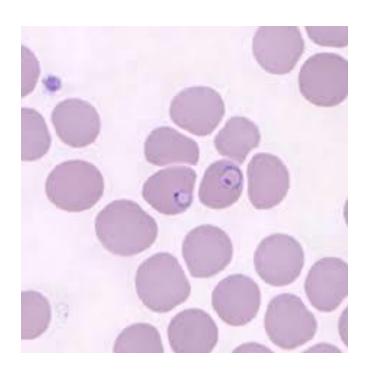
- Note Rouleaux (as compared to agglutination)
- \* Plasma cells have eccentric nucleus, "clockface" nuclei
- \* Plasma vs reactive lymphs

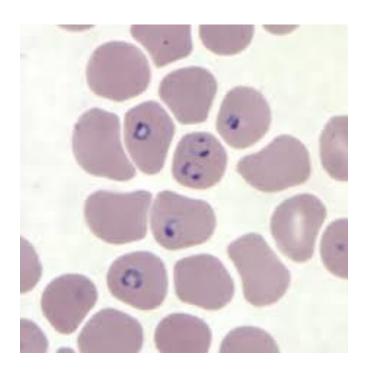
### Plasma cells





# Malaria

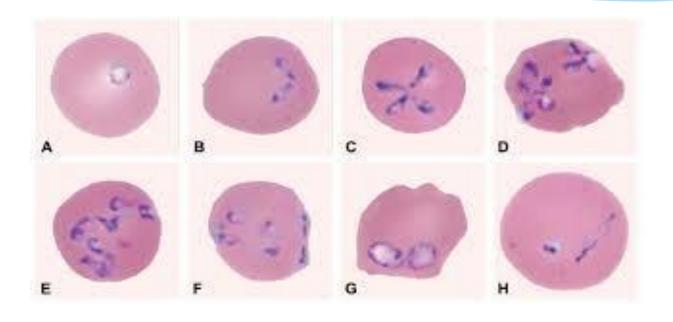




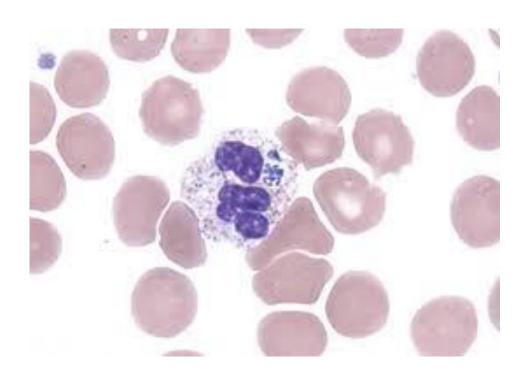
### Babesia

- \* Look for maltase cross, often in rings
- \* Unlike malaria, can have extracellular ring forms

# Babesia



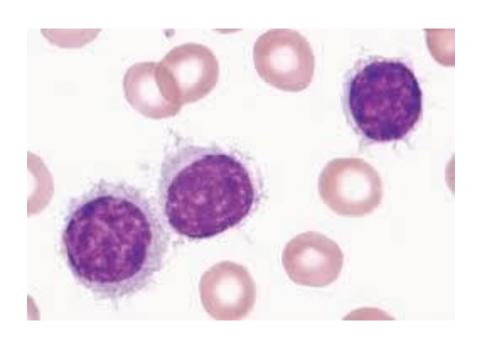
# Anaplasma



# Hairy Cell

- \* Abnormal B Lymphocytes
- \* Hair-like cytoplasmic projections
- \* TRAP stain can identify hairy cells

# Hairy Cells



# Case Study Time!

- \* 47 yr old female presents at clinic
- \* History of gastric bypass surgery
- \* 3 week history of fever with unknown origin
  - \* 101.8 F
- \* Experiencing sweats and chills 1-2 times/day
- Recently was treated with amoxicillin for strep throat
- Peripheral smear referred

#### **CBC** results:

#### Differential results:

WBC RBC HGB HCT MCV MCH MCHC RDW	4·3 3·84 9·7 31.8 83 25·3 30·5 16.7	thou/cu mm mil/cu mm g/dL % fL pg %	Neutrophils Lymphocytes Monocytes Eosinophils Myelocytes NRBC	33 57 7 2 1 2
RDW PLT		% thou/cu mm		

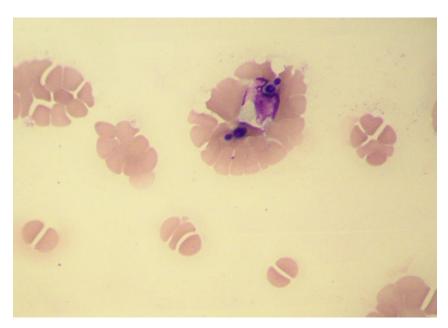
#### Additional history reveals

- \* Patient underwent gastric bypass surgery 17 yrs ago that was unsuccessful
- \* Patient had corrective surgery but developed short bowel syndrome and subsequent chronic malnutrition
- Patient had a Hickman catheter placed to receive nutrition (TPN) at night

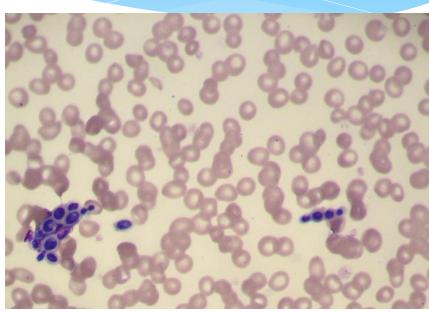


Peripheral smear: Neutrophil- What's in it?

### Case Study #1-Examine the entire slide



Don't forget the feathered edge!



Peripheral smear

#### Yeast!

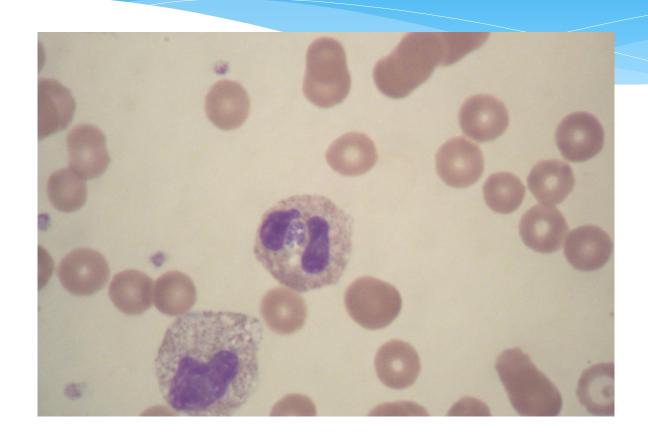
- Pathologist notified and primary physician called immediately
- \* Patient admitted
- \* Catheter removed
- Blood and catheter cultures revealed Rhodotorula Species

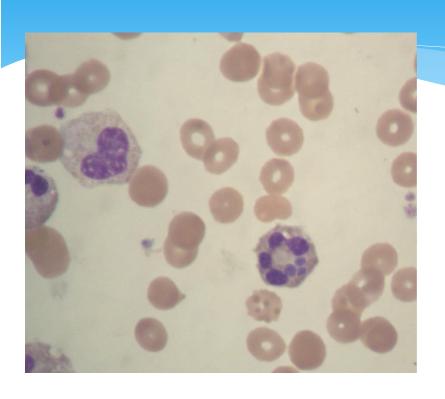
- \* 68 yr old male presents in ER
- \* 2 week history of nausea, diarrhea, chills, weight loss, and mild confusion
- \* Right upper quadrant pain

#### CBC results:

### Differential results:

WBC	5.37	thou/cu mm	Neutrophils	27
RBC	3.64	mil/cu mm	Lymphocytes	3
HGB	11.1	g/dL	Monocytes	4
HCT	31.1	%	Bands	62
MCV	85.4	fL	Metas	4
MCH	30.5	pg		
MCHC	35.7	%		
RDW	13.5	%		
PLT	18	thou/cu mm		





Additional history reveals

\* One week prior to this episode he spent time at his cabin in Western Wisconsin with his wife

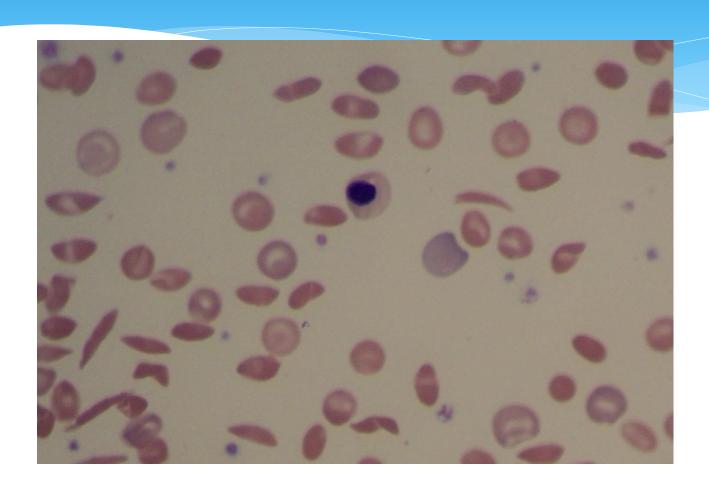
- \* Human Anaplasmosis revealed on buffy coat smear
- \* Present in neutrophils
- \* Physician alerted immediately
- Patient started on IV doxycycline
- \* DNA by PCR was positive

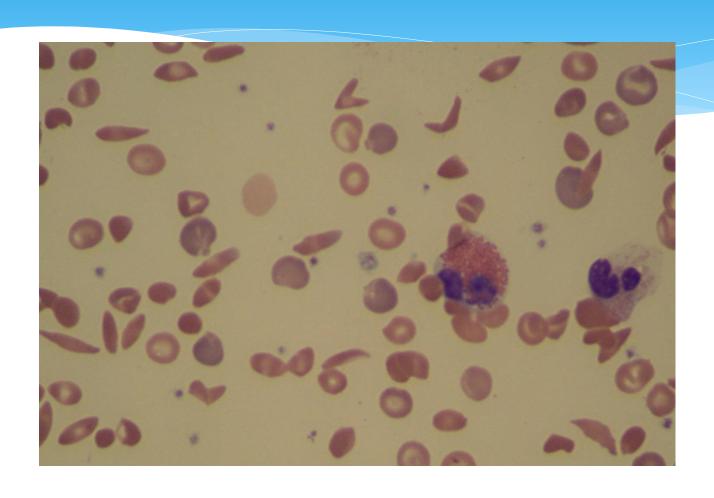


34 yr old male presents to the ED with the following:

- \* Sternal chest pain
- \* Back Pain
- \* Groin Pain
- \* Mild Shortness of Breath

```
CBC:
WBC 13.8
HGB 8.1
PLT 370
Other labs:
Total Bilirubin: 15.3
ALT 52
AST 58
```





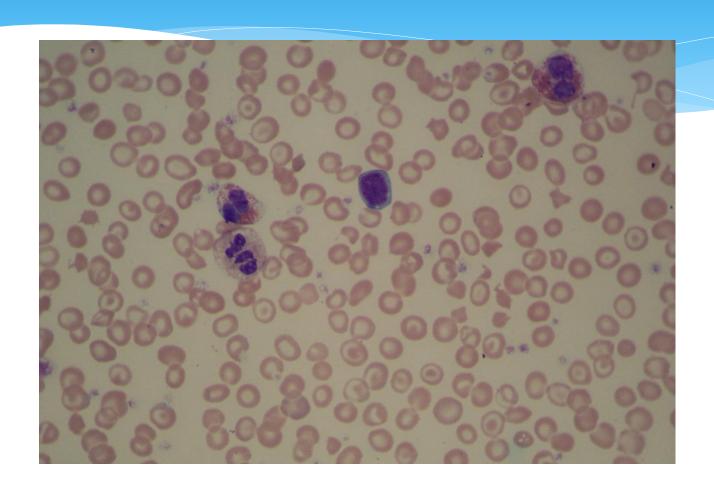
### Sickle Cell Crisis

- Homozygous Hemoglobin S Disease
- \* Present in 0.3-1.3 % of African Americans
- \* Sickle Cell Trait: 8-10%
- \* Deoxygenated state produces sickled cells
- \* Sickled cells jam in capillaries causing pain
- \* Anemia caused by hemolysis
- \* Lifespan of a sickle cell: 14 days
- \* Treatment: Hydroxyurea

84 yr old female presents with the following:

- Left hip fracture after a fall
- \* Moderate fatigue
- \* History of CAD

```
CBC:
HGB 10.5
MCV 73
MCH 18.5
MCHC 28.3
RDW 18.2
PLT 320
Other labs: Ferritin 31(normal 25-400)
Soluable Transferrin Receptor 14.5 (normal 1.9-4.4)
```



### Advanced Stage Iron Deficiency Anemia

- \* HGB decreased
- \* MCV < 75
- \* Ferritin 31 <15 is diagnostic
- \* Increased STfR
- \* Microcytic, hypochromic (MCH, MCHC)
- \* Target cells
- \* Therapy: Iron replacement

67 yr old male presents with:

- \* Fatigue
- \* Shortness of Breath
- \* Post aortic valve replacement

CBC:

WBC 8.9

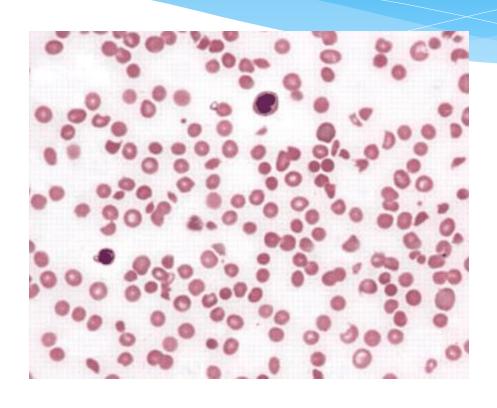
HGB 7.8

Retics 6.3%

Other labs:

LDH increased

Haptoglobin decreased

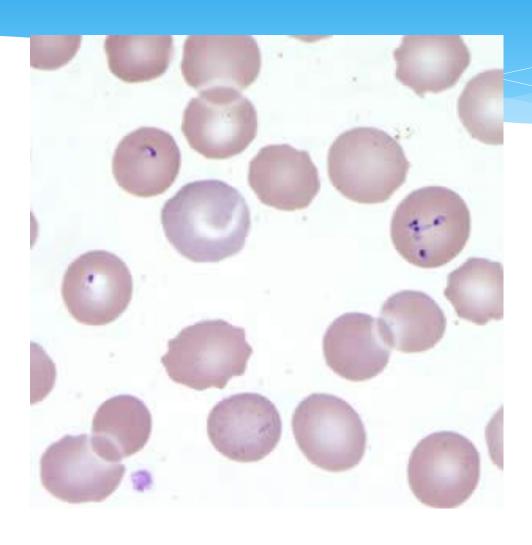


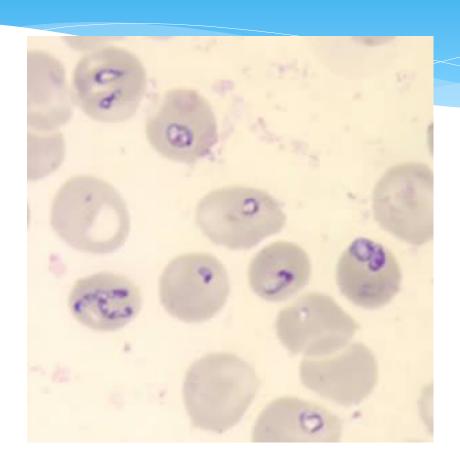
- Microangiopathic Hemolytic Anemia (MAHA)
- \* Secondary to a poorly functioning heart valve
- \* Schistocytes present
- \* Will probably have to have valve replaced

### Case #6

### 34 yr old male presents with:

- \* Fever
- \* Fatigue
- \* Chills
- \* Sweats



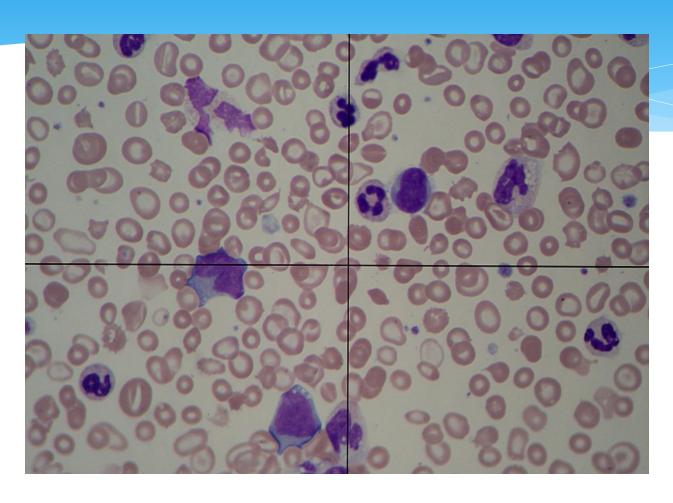


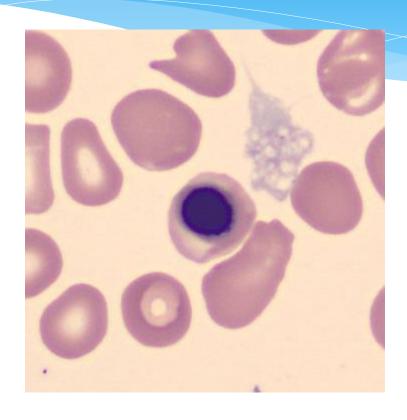
- \* Babesia microti
- \* Transmitted by the tick Ixodus scapularis (deer tick) present in the Minnesota
- \* Important to distinguish babesia from other RBC inclusions or malaria
- \* Often found in tetrads, vary in size,
- \* Treatment: Clindamycin and quinine

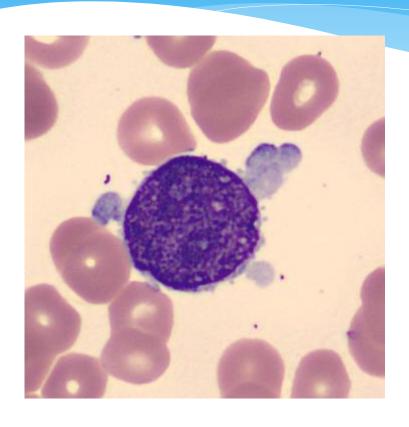
- Middle aged patient
- \* Symptoms-fatigue, general "ill" feelings
- \* CBC results: WBC 25.6, RBC 5.90, HCT 58, RDW 26, PLT >750,000

- \* Polycythemia vera
- \* WHO: Chronic Myeloproliferative Disease
- \* Molecular on PB-JAK2
- \* Treatment-hydroxyurea

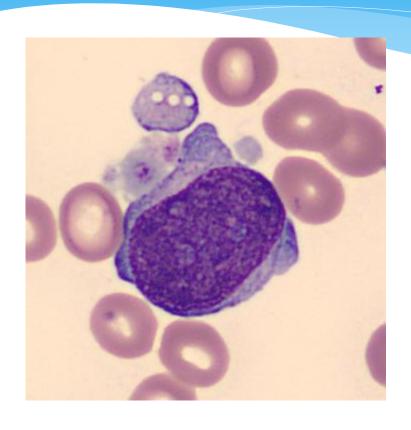
- \* Same patient, 3 years later, presents with bone pain
- \* CBC revealed pancytopenia, bizarre platelet morphology
- Bone marrow biopsy-reticulin stain
- \* Addition of chromosome 9
- \* CMPD-Myelofibrosis
- \* Treatment
  - \* Splenectomy
  - Continued hydroxyurea

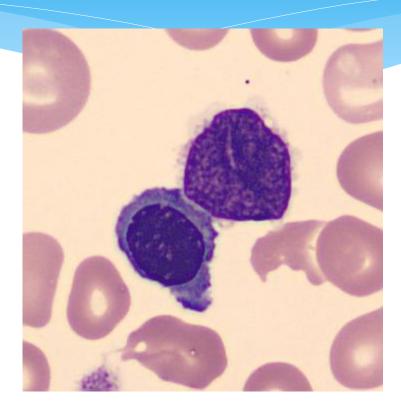












- \* Same patient, 13 years later presents with continued bone pain, poor quality of life
- \* CBC and Differential-PB, WBC >100,000, increased blasts ~20%
- \* No bone marrow biopsy, confirmed by flow for CD34+ cells
- \* Transformation to Acute Leukemia
- \* WHO: AML w/multilineage displasia (w/prior MDS)

# Thank you