MYELOPROLIFERATIVE NEOPLASMS

CLPC Fall Seminar – 2016
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Myeloproliferative Disorders

First Described by Dameshek in 1951

A Heterogenous Group of Hematological Conditions Characterized by Cellular Proliferation of One or More Cell Lines --- Distinct from Acute Leukemia

MPDs

- Chronic disorders which lie in the "gray area" between reactive disorders and clearly malignant disorders, such as acute leukemia.
- Now known as **MPNs** (Myeloproliferative Neoplasms)

Who Classification Scheme for MPNs

- Chronic Myeloid Leukemia
- Chronic Neutrophilic Leukemia
- Polycythemia Vera
- Primary Myelofibrosis
- Essential Thrombocythemia
- Myeloproliferative Neoplasm, Unclassifiable
- Chronic Eosinophilic Leukemia

Classification of Myeloproliferative Neoplasms (MPNs) by Predominance of Cell Types

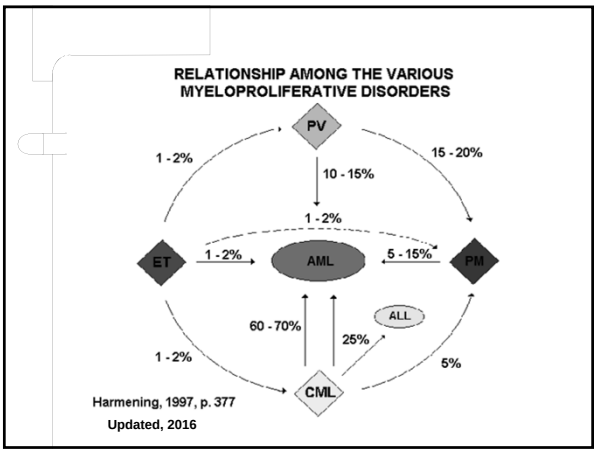
Involved Cell Line	MPN
Myeloid	Chronic myelogenous leukemia (CML)
Erythroid	Polycythemia vera (PV)
Megakaryocytic	Essential thrombocythemia (ET)
Fibroblast	Primary Myelofibrosis* (PMF)

*The fibroblast in PMF is not a part of the neoplastic process but is increased because of a reactive process

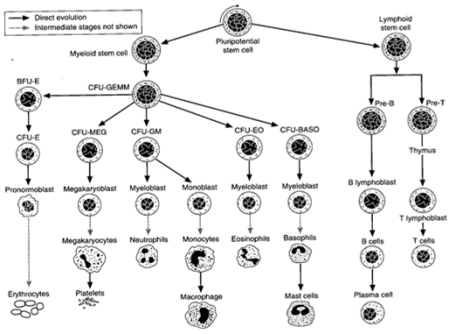
Modified from McKenzie, 2015, p. 448

COMMON FEATURES

- Pluripotent, clonal, stem cell disorder
- Usually more than one cell line involved
- Transitions may occur
  - One disorder to another
  - Some other malignant disorder



Stem Cell Diagram



GENERAL FEATURES

- Middle aged or elderly
- Gradual onset, chronic course
- Clinical: hemorrhage, thrombosis, infection, pallor, weakness, splenomegaly
- Anemia or polycythemia
- Leukoerythroblastic blood picture with
  - Bizarre platelets
  - Increased basophils

and...

GENERAL FEATURES

- Hypercellular bone marrow
  - may progress to fibrotic
- Extramedullary hematopoiesis
- Abnormal leukocyte alkaline phosphatase
- Cytogenetic abnormalities common
- May terminate in acute leukemia

CHRONIC MYELOCYTIC LEUKEMIA (CML)

Chronic Granulocytic Leukemia (CGL)
Chronic Myelogenous Leukemia

CML - GENERAL FEATURES

- Neoplastic Growth - Primarily Myeloid Cells
- Three phases
  - Chronic Phase
    - 3-5 yrs untreated
    - Responds well to therapy
  - Accelerated (transitional) Phase
  - "Blast Crisis"
    - Resembles Acute Leukemia (25-30% Blasts)
    - Responds poorly to therapy
    - Death Within 3-6 Months
    - 50% Myeloid, 25% Lymphoid, 25 % Undifferentiated

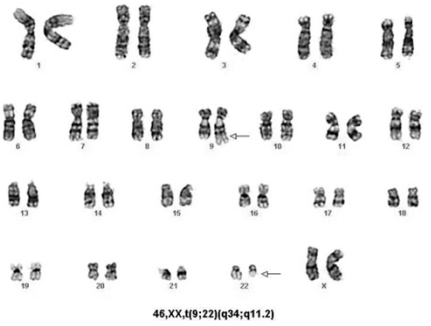
CML-Cytogenetics

- First chromosomal abnormality linked to disease pathogenesis (1960)
- PHILADELPHIA (Ph^1) CHROMOSOME
  - Acquired somatic mutation
  - Balanced translocation t(9q+;22q-)
  - Present in ***all*** neoplastic granulocytic, erythrocytic, monocytic, and megakaryocytic precursor cells

CML - Cytogenetics

- Ph^1 is absent in 5–10% of patients with CML phenotype
  - Usually detected at the molecular level
    - BCR/ABL gene
  - However if Ph- and BCR/ABL-, then usually CNL, aCML or CMML
- Malignant cell is pluripotential stem cell (CFU-GEMM)

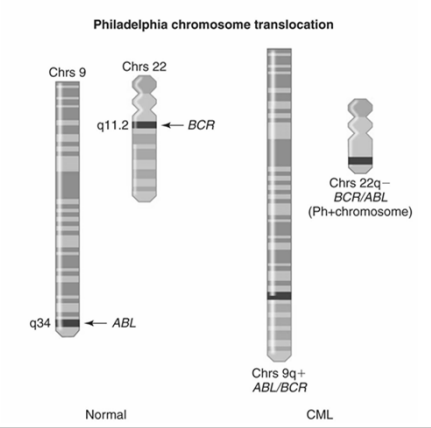
Philadelphia Chromosome

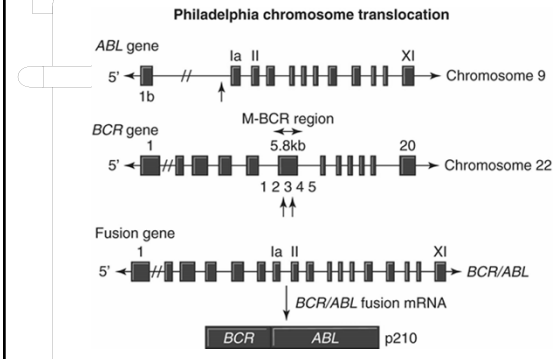


46,XX,t(9;22)(q34;q11.2)

CML - Molecular

- BCR/ABL is the molecular equivalent of Ph^1
  - Hybrid oncogene BCR/ABL formed
  - New protein (p210) produced
    - Abnormal fusion protein with molecular mass of 210kD
    - Increased Tyrosine Kinase Activity
      - regulator of metabolic pathways
      - serves as receptor for growth factors
      - suppresses apoptosis





CML - Molecular

- One-third of Ph[1] negative CML patients are BCR/ABL positive
  - Similar disease course to Ph[1] pos.
- Ph[1] neg and BCR/ABL neg patients
  - Thought to have another MPN or one of the myelodysplastic syndromes

CML - CLINICAL FEATURES

- Peak age 40-59 years
- Males / females equally affected
- Slow onset; chronic course
- Median survival
  - 3 years before Gleevec
  - Now the 5 year survival rate is >90%
- **Ph[1] Neg – 8 months**
  - **aCML or possibly CMML**
    - **Now considered MDS/MPD in WHO**

CML - CLINICAL FEATURES

- Ph[1] Neg – 8 months average survival
  - aCML (atypical CML) *or*
  - possibly CMML (Chronic MyeloMonocytic Leukemia
  - Now considered in the MDS/MPN WHO category

CML - CLINICAL FEATURES

- Presenting Symptoms
  - Weakness
  - Fever, Sweats
  - Weight Loss
  - Abdominal Fullness
  - GI Bleeding
  - Retinal Hemorrhage

CML - CLINICAL FEATURES

- Physical Exam
  - Pallor
  - Sternal tenderness
  - Splenomegaly
  - Occ'l hepatomegaly
  - Rarely "chloroma"
    - Greenish tumor – due to myeloperoxidase
    - Extramyeloid mass

CML-LAB FEATURES

- Leukocytosis (>30,000; Frequently >100,000)
- Normocytic, normochromic anemia
- Normal to increased platelets
  - Some abnormal

CML - LAB FEATURES

- All stages of granulocytes
  - 20% or fewer blasts and promyelocytes
  - Increased basophils and eosinophils
- Nucleated red blood cells
- Pseudo pelger-huet cells
- Decreased LAP
- Increased serum uric acid, LD and B_{12}

CML - LAB FEATURES

- Bone Marrow
  - Hypercellular
  - M:E 10:1 to 40-50:1 (granulocytic hyperplasia)
  - Myelocytes predominant
  - May become fibrotic late in disease
    - may resemble myelofibrosis

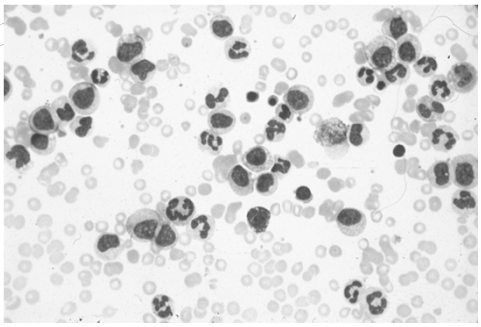
CML vs LEUKEMOID REACTION

CML	LEUKEMOID REACT.
• MKD. INCREASED WBC	• MOD. INCREASED WBC
• NC/NC ANEMIA	• NO ANEMIA
• RBC ABNORMALITIES	• RBCS NORMAL
• NUCLEATED RBCS	• NO NRBCS
• FEW BLASTS AND PROMYELOCYTES	• NO BLASTS AND PROMYELOCYTES

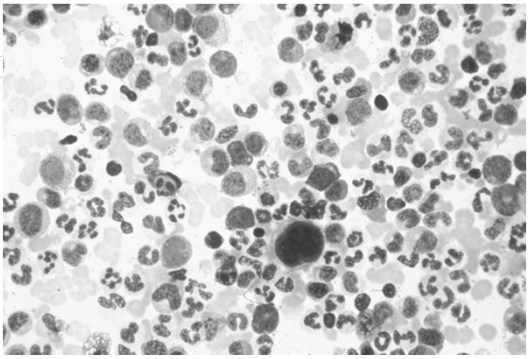
CML vs LEUKEMOID REACTION

CML	LEUKEMOID REACTION
• INCREASED EOS AND BASOS	• NORMAL EOS AND BASOS
• DECREASED LAP	• LAP NORMAL TO INCREASED
• PHILADELPHIA CHROMOSOME	• NO PHILADELPHIA CHROMOSOME

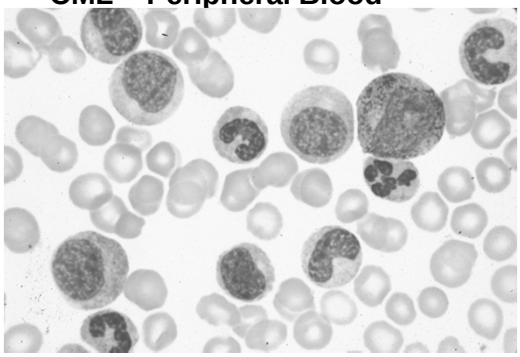
CML – Peripheral Blood



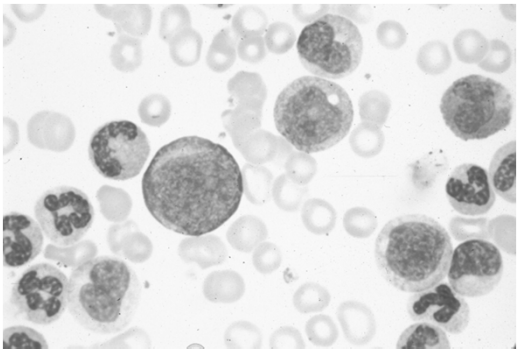
CML – Bone Marrow



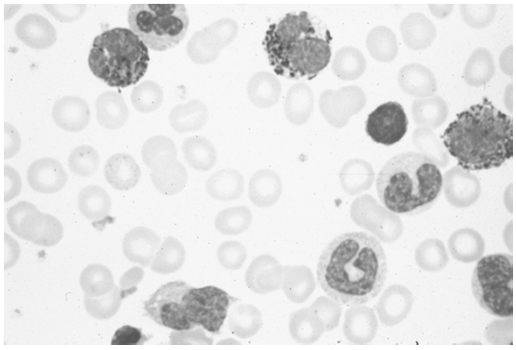
CML – Peripheral Blood



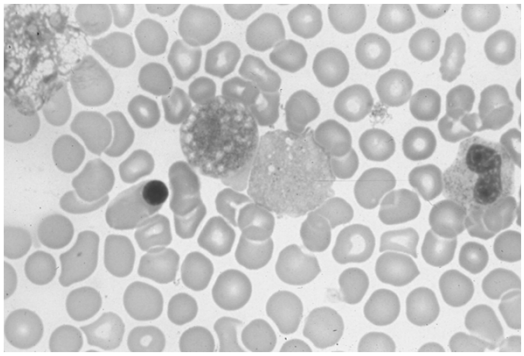
CML – Peripheral Blood



CML – Peripheral Blood



CML – NRBC; Giant Plts



CML - TREATMENT

- CHEMOTHERAPY
  - Remission may last 2-3 years
  - Blast crisis responds poorly; median survival about 10 weeks
- New Drug – Gleevec (imatinib mesylate)
  - Approved by FDA in 2002
  - Inhibits abnormal Tyrosine kinase produced by BCR/ABL oncogene
  - Became the most effective therapy for initial treatment of CML

http://www.dnalc.org/view/15082-Shutting-down-cancer-with-Gleevec-Brian-Druker.html
http://www.dnalc.org/view/15525-How-Gleevec-works-to-alleviate-symptoms-of-myeloid-leukemia-3D-animation-with-basic-narration.html

CML – TREATMENT (cont)

- 83% show complete hematologic response to Gleevec at 12 months
- 96% show complete response at 60 months
- Some patients develop resistance to Gleevec

Newer TK inhibitors

- Dasatinib and Nilotinib
  - Second generation drugs with improved response
  - Approved in 2007 for
    - Resistance or intolerance to prior imatinib therapy
    - Accelerated phase of CML
  - Both FDA approved in 2010 for first line treatment of CML

CML – TREATMENT (cont)

- BONE MARROW TRANSPLANT
  - Must be <65
  - Syngeneic (identical twin) – best option
  - Allogeneic (HLA compatible donor)
    - Overall survival for HLA-Matched Sibling Donor – 86% cure rates exceeding 3 years of leukemic free survival
    - Newer research – overall survival rates for Unrelated Donors (with 8/10 to 9/10) HLA loci has improved

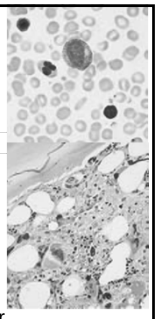
CML - SUMMARY

- WBC – Markedly increased
- PLT – Normal to increased
- NC/NC anemia; NRBCs
- Differential
  - All stages of granulocytes
  - Few promyelocytes and blasts
- Increased eos and basos
- Philadelphia Chromosome
- LAP – Decreased
- Often ends in “Blast Crisis”

Primary Myelofibrosis (PMF)

Agnogenic Myeloid Metaplasia (AMM)
Mylofibrosis with Myeloid Metaplasia(MMM)
Idiopathic Myelofibrosis (IMF)

PMF - GENERAL FEATURES



- Splenomegaly
- Leukoerythroblastosis
- Progressive bone marrow fibrosis
  - Reactive fibroblast proliferation secondary to underlying clonal disorder
  - Fibrosis inhibits normal hematopoiesis
- Extramedullary hematopoiesis - (myeloid metaplasia) in spleen and liver, etc.

PMF - GENERAL FEATURES

- Fibroblasts stimulated by growth factors (cytokines) from malignant megakaryocytes, platelets, monocytes
  - Transforming Growth Factor (TGF-B)
  - Platelet Derived Growth Factor (PDGF)
- 50% have JAK2 somatic mutation
- 5% have *MPL* gene (*M*yelo*P*roliferative *L*eukemia protein)

PMF - CLINICAL FEATURES

- Most frequent:
  - Anemia
  - Splenomegaly (90%)
- 1/3 Asymptomatic at diagnosis
  - May remain asymptomatic 3-5 Years
- May have bleeding problems
- Median survival 5 years; may terminate in acute leukemia

PMF - LAB FEATURES

- Normocytic, normochromic anemia
- Platelets increased early; decreased later in disease
- Giant, bizarre platelets
- WBC
  - 15,000 to 30,000/uL range
  - Left shift

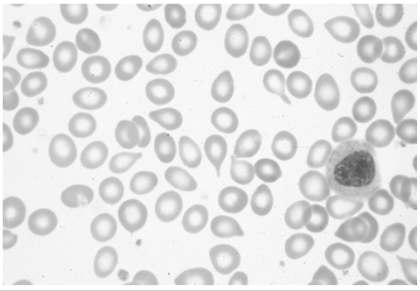
PMF - LAB FEATURES

- Hallmarks
  - Leukoerythroblastic blood picture
  - Dacryocytes (teardrop RBCs)
- LAP normal to increased
- JAK2 mutation is found in ~50% of patients
  - Associated with longer survival

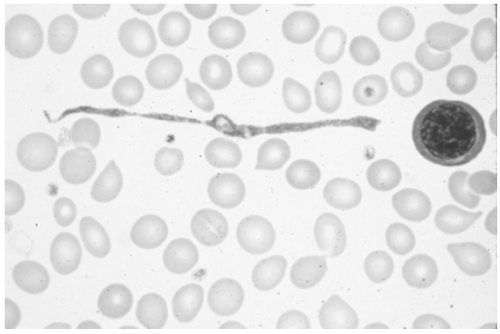
PMF - LAB FEATURES

- Marrow aspiration - “Dry Tap”
- Marrow biopsy
  - Hypercellular (early); Hypocelluar (late)
  - Varying degree of fibrosis
  - Aggregates of megakaryocytes

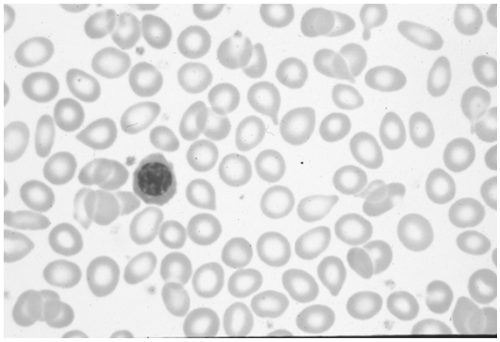
PMF – Peripheral Blood

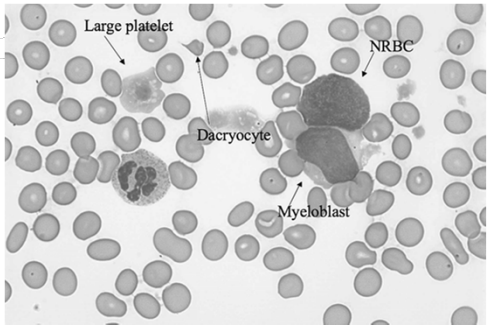


PMF – Peripheral Blood

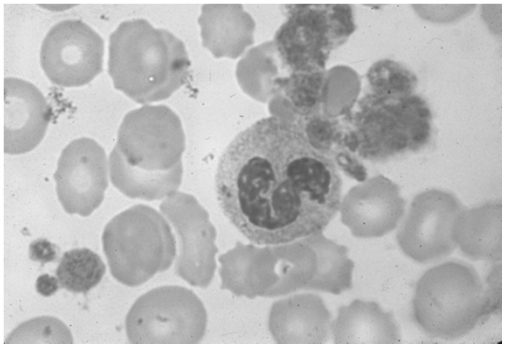


PMF – NRBC; Teardrop Cells

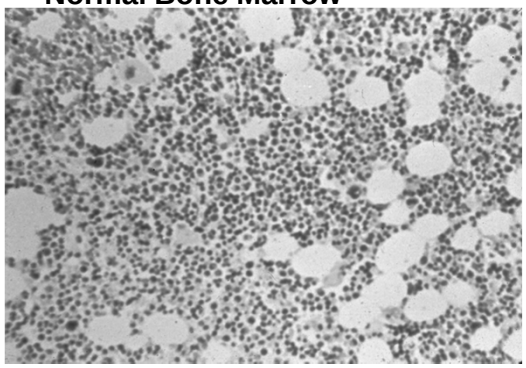




Megakaryocyte Fragments

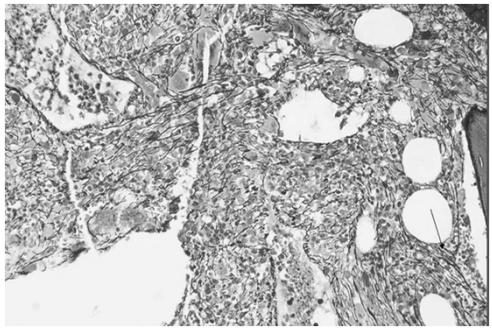


Normal Bone Marrow



Bone Marrow – Fibrotic

Reticulum Stain showing increased collagen



PMF - PROGNOSIS

- Least favorable of all MPNs
  - Median Survival approximately 5 years
  - < 20% alive at 10 years
  - About 10 – 15% transform Into acute leukemia (either AML or ALL)
  - Causes of death: hemorrhage, infection, cardiovascular disease

PMF - TREATMENT

- Alleviation of symptoms and improvement of quality of life
  - Androgens and corticosteriods for anemia and thrombocytopenia
  - Hydroxyurea/irradiation and other chemotheraputic agents for organomegaly
  - Therapeutic splenectomy
- Jakafi (ruloxitinib) – JAK2 inhibitor
  - First FDA approved drug for PMF
- Allogeneic stem cell transplantation is only curative therapy

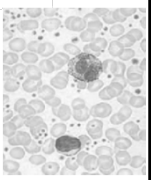
PMF - SUMMARY

- Leukoerythroblastic blood picture
- Dacryocytes (Teardrop RBCs)
- WBC – Usually elevated
- PLT – Variable
  - Giant platelets
  - Megakaryocyte Fragments
- NC/NC Anemia
- Bone Marrow – Fibrotic/"Dry Tap"
- LAP – Normal to Increased

ESSENTIAL THROMBOCYTHEMIA (ET)

ET - GENERAL FEATURES

- Neoplastic growth
- Usually affects all three cell lines
- Primarily megakaryocytes affected
  - Extremely Elevated Platelet Counts
- Median age at diagnosis - 60
- Annual incidence 1.5 – 2.4/100,000
  - Least common MPN



ET - GENERAL FEATURES

- One-third of patients have thrombotic/hemorrhagic complications
  - Major cause of death
  - Pathophysiology not clearly understood
  - May be a qualitative platelet defect
  - Abnormal platelet function
    - Platelet hyperaggregability

ET - CLINICAL FEATURES

- May be asymptomatic
- One-third present with vasomotor symptoms (headache, dizziness, visual disturbances)
- 10-25% present with thrombosis
- Splenomegaly in about 50%

ET - LAB FEATURES

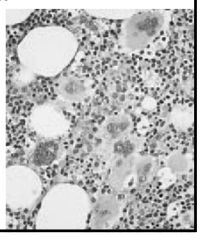
- Extreme persistent thrombocytosis (>600,000; may be >1,000,000/uL)
- Must rule out reactive thrombocytosis
  - Splenectomy, chronic infection, etc.)
- Platelets often clumped, giant or aytpical
- Megakaryocyte fragments

ET - LAB FEATURES

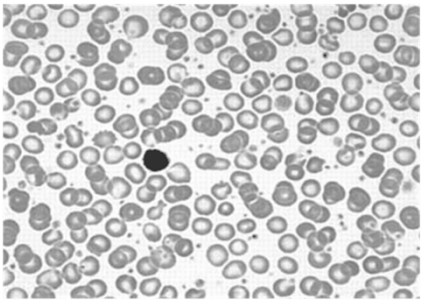
- Slight to moderate anemia
- WBC often elevated
- Repeated low levels of IL-6 or C-reactive protein
  - rules out reactive thrombocytosis

ET – LAB FEATURES

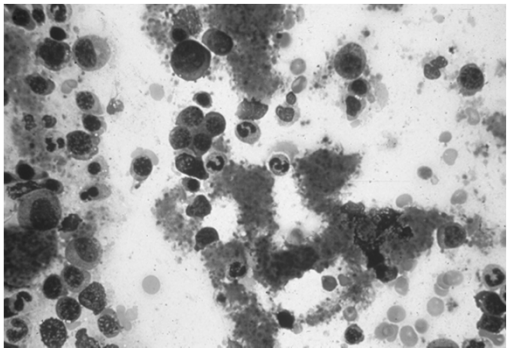
- Bone Marrow
  - Megakaryocytic hyperplasia
  - Abnormal megakaryocyte morphology
  - Fibrosis absent or < one-third area of biopsy



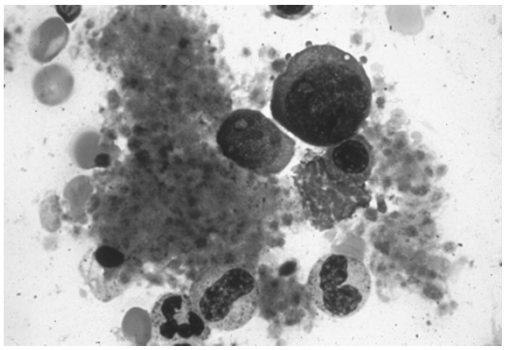
ET – Peripheral Blood



ET – Bone Marrow



ET – Bone Marrow



ET – TREATMENT / PROGNOSIS

- Chemotherapy to lower platelet count
  - in patients > 60, or those with history of thrombosis, or cardiovascular risk factors
- Plateletpheresis to quickly decrease plt count below 1 million and prevent CVA
- Chronic course
  - life expectancy 1-5 years
  - Usually die from thrombosis or bleeding
- May transform into PV, Myelofibrosis or Acute Leukemia

ET – SUMMARY

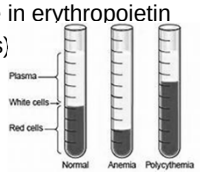
- PLT – Markedly increased
- PLTs clumped, giant, atypical, abnormal function
- WBC – Often elevated
- Slight to Moderate anemia

POLYCYTHEMIA VERA (PV)

(Polycythemia Rubra Vera)

POLYCYTHEMIA VERA

- Only type of polycythemia classified as a Myeloproliferative Neoplasm
- Absolute increase in RBC production with NO corresponding increase in erythropoietin
- Pancytosis (or Panmyelosis)



PV - GENERAL FEATURES

- Neoplastic Growth - primarily erythroid cells
- Pathophysiology
  - Two populations of RBC precursors
  - Malignant clone very sensitive to erythropoietin
- Annual Incidence 10 million cases in U.S. (0.6 – 1.6 / million)
- Typically affects 40-60 age range
- Slightly more common in males and whites

PV - CLINICAL FEATURES

- Common Symptoms
  - Plethora, headache, dizziness,
  - Weakness, blurred vision, night sweats
  - Pruritis, tinnitis
  - Splenomegaly, hepatomegaly
- Thrombohemorrhagic complications
  - Usually correlate with Hct
  - Frequent and severe

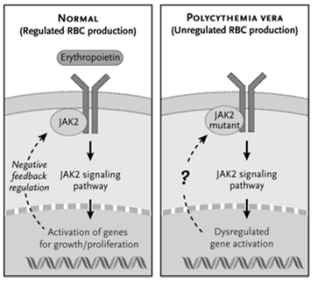
PV - LAB FEATURES

- Hallmark – Pancytosis
  - Elevated RBCs, WBCs and Platelets
- May see increased basophils, eosinophils, and/or immature granulocytes
- RBC mass (Volume) elevated
- Platelets morphologically and functionally abnormal

PV – LAB FEATURES

- *JAK2 (V617F) mutation (Janus Kinase2)*
  - Nonspecific molecular mutation found in >95% of PV patients
  - JAK2 gene codes for a tyrosine kinase involved in cell signaling
    - Mutated JAK2 gene gives rise to a turned-on cytokine receptor which leads to increased production of all cell lines
  - Has also been found in some cases of ET and IMF

JAK2 Mutation



PV – Lab Features

- Elevated Leukocyte Alkaline Phosphatase
- Arterial oxygen saturation **normal**

PV – Lab Features

- If HCT >55%, amount of anticoagulant in coagulation sample tube may need to be adjusted for the decreased amount of plasma
  - Too much Ca++ will be removed by the excess Na Citrate
  - May cause false prolongation of clotting tests
- Must redraw sample
  - Formula for calculating amount of anticoagulant

PV – Lab Features

- Bone Marrow
  - Increased cellularity
  - Trilinear hyperplasia
  - Increased megakaryocytes
  - Markedly decreased or absent iron stores

PV - TREATMENT

- Intermittent phlebotomy
  - Hct <45%
  - May lead to iron deficiency
  - Does not control thrombocytosis
  - Elevated hct only predictive factor for increased risk of thrombosis
- Radioactive phosphorus (^{32}P)
- Myelosuppressive therapy with chemotherapy (Hydroxyurea)
  - Carries less risk of causing secondary leukemia

PV - TREATMENT

- Ruxolitinib
  - JAK inhibitor
  - Showing promising results in clinical trials*
  - Currently being used only for hydroxyurea resistant or intolerant patients

*Haematologica. 2016 Jul;101(7):821-9. doi: 10.3324/haematol.2016.143644. Epub 2016 Apr 21.

PV - PROGNOSIS

- Median survival 10-20 years with current disease management
  - Untreated 18 months
- About 15 - 30% develop marrow fibrosis
  - Resembles PMF
- About 5 - 10% terminate in Acute Myeloid Leukemia
  - Seems to develop at higher rate in patients treated with myelosuppressive drugs as opposed to phlebotomy alone

PV - SUMMARY

- RBC, WBC, PLT – elevated
- May see increased Eos, Basos, immature Granulocytes
- Increased RBC Mass/Volume
- Erythropoietin – Normal to decreased
- LAP – Usually elevated
- JAK2 mutation usually present

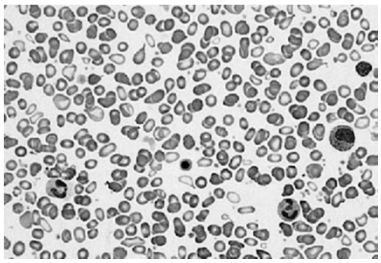
Case Study 1

- 52 year old male with hyperuricemia
- In clinic for follow-up evaluation for splenomegaly discovered 18 months ago
- Originally denied fatigue, fever, discomfort
- Patient now complaining of fatigue, weakness, dyspnea, bone pain and abdominal discomfort
- PE revealed a slightly enlarged liver and palpable spleen
- CBC ordered

CBC

- Hgb 11.6g/dL MCV 97fL
- Hct 35% MCHC 33g/dL
- RBC 3.6 x 10^6/uL
- WBC 26.2 x 10^3/uL
- Platelets 853 x 10^3/uL
- Differential and smear evaluation
  - Marked anisocytosis with 3+ teardrops and many nRBCs
  - Immature myeloid cells with basophilia and large platelets

Peripheral Blood Picture



Which of the following terms best describes this blood picture?

A. Blast crisis
B. Pancytosis
C. Leukoerythroblastosis

Bone Marrow Ordered

- Aspiration unsuccessful
- Biopsy
  - moderate hyperplasia, platelet clusters, abnormal megakaryocyte morphology and fibrotic marrow spaces
- Cytogenetic studies
  - negative for Ph chromosome, JAK2, BCR/ABL1
  - positive for MPL

What diagnosis do these results suggest?

A. Chronic Myelocytic Leukemia (CML)
B. Polycythemia vera (P.V.)
C. Primary MyeloFibrosis (PMF)
D. Essential Thrombocythemia (ET)

Treatment

- Main goal is to control symptoms and improve quality of life
- Patient is young enough for hematopoietic stem cell transplantation if suitable HLA donor is found
- Will be watched for disease progression and possible transplant

Case Study 2

- 34 year old woman
- 2 month history of increasing weakness, persistant cough, fever and chills with night sweats and 13 lb. weight loss
- Treated with ciprofloxacin and cough improved

Follow-up

- Continued to grow weaker
- Returned to physician
- PE revealed tenderness and fullness in left upper quadrant
- Spleen was palpable
- No hepatomegaly or swollen glands noted
- CBC ordered

CBC Results

Hgb	9.5g/dL	Diff:	
Hct	26.3%	Neutrophils	44%
WBC	26.3 x 10^3/uL	Bands	4
Plt	449 x10^3/uL	Lymphocytes	10
		Eosinophils	3
		Basophils	7
		Myelocytes	30
		Promyelocytes	1
		Blasts	1
		nRBC	3

Additional Lab Tests

Uric Acid	8.1 mg/dL	(4 to 6mg/dL)
LDH	692 IU	(140 to 280 IU)

Additional Tests?

Results of Genetic Studies

- Cytogenetics
  - t(9;22) - positive
- FISH
  - BCR/ABL1 - positive

Therapy of Choice?

- Imatinib (Gleevec) to induce remission
- Since patient was only 34 and HLA-matched donor was available
  - Patient underwent stem cell transplantation
  - Curative and patient remains disease free 3 years post transplant

Case Study 3

- 42 year old male
- 2 year history of fatigue and pruritus of the legs
- Smoked 1 pack/day for 15 years
- 5 to 6 alcoholic drinks/day

Physical Exam

- Unremarkable with no rash or palpable spleen
- CBC ordered

CBC Results

HGB	21.9	(14.0 – 18.0g/dL)
RBC	6.96	(4.50 – 6.0x10^6/uL)
MCV	90	(80.0 – 99.0fL)
WBC	12.1	(4.5 – 10.8x10^3/uL)
PLT	154	(150 – 400x10^3/uL)

Diff: 71% neutrophils
18% lymphocytes
8% monocytes
2% eosinophils
1% basophils

RBC morphology – essentially normal

Additional Lab Results

Iron Studies:

Serum Ferritin	9 mg/mL	(26 – 388)
Serum Iron	55 ug/dL	(65 – 175)
TIBC	431 ug/dL	(250 – 450)
% Saturation	13%	(22 – 55)

Retic count – 1.1% (0.2 – 2.4)

Bone Marrow Results

- Trilineage hematopoiesis
- No overt dysplastic features
- M:E - 1.0
- 70% cellular
- Slightly increased number of megakaryocytes
- Some iron seen on aspirate
- No stainable iron on clot or biopsy

Flow Cytometry and cytogenetics on marrow

- No diagnostic abnormalities
- 46,XY

More lab results

- Erythropoietin level of 1mU/mL (4-24)
- PCR analysis
  - Positive for JAK2 V617F mutation

Most Likely Diagnosis?

A. Chronic Myelocytic Leukemia (CML)
B. Polycythemia vera (P.V.)
C. Primary MyeloFibrosis (PMF)
D. Essential Thrombocythemia (ET)

Iron Studies

MCV 90fL

Explanation?

Follow-up

- 2.5 years later
  - Patient is receiving therapeutic phlebotomy every other month
  - Has not needed chemotherapy

References

McKenzie, Shirlyn B., Williams, J. Lynn, Clinical Laboratory Hematology, Upper Saddle River, New Jersey: Prentice Hall, 3rd ed. 2015.

Rodak, B. Hematology, Clinical Principles and Applications, 4th edition, St. Louis, MO, Saunders, 2011

Haematologica. 2016 Jul;101(7):821-9. doi: 10.3324/haematol.2016.143644. Epub 2016 Apr 21.

http://www.bostonbiomedical.com/cancer-stem-cells/signaling-pathways/?utm_source=bing&utm_medium=cpc&utm_campaign=Pathways&utm_term=%2Bjak%20%2Bstat%20%2Bpathways&utm_content=JAK%2FSTAT

https://hms.harvard.edu/sites/default/files/assets/News/2008/April/gilliland_schematic.gif

https://upload.wikimedia.org/wikipedia/commons/thumb/a/a4/Erythromelalgia.jpg/220px-Erythromelalgia.jpg

http://diseasespictures.com/wp-content/uploads/2012/10/Polycythemia-Vera-2.jpg