

HENATOLYMPHOID SYSTEM
THIRD YEAR MEDICAL STUDENTS-UNIVERSITY OF JORDAN

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MYELOID NEOPLASMS

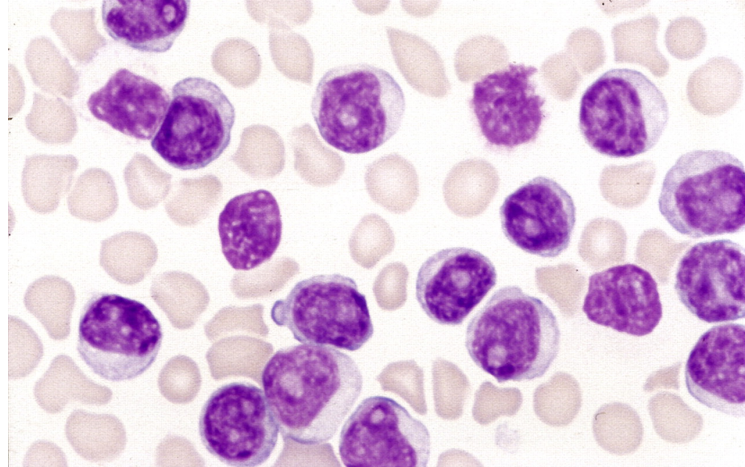
Introduction:

- Myeloid neoplasms are divided into three major categories:
 - Acute myeloid leukemia
 - Myeloproliferative neoplasms
 - Myelodysplastic syndrome.
- As discussed in the previous lectures, all hematolymphoid cells arise from the bone marrow, and all start as a pluripotent stem cell that gives rise to all the blood cells.
- The two major lines are lymphoid and myeloid.
- The stem cells undergo a series of maturation steps to give rise to the more mature progeny. Additionally, it undergoes proliferation to replace the stem cells that were utilized for production of more mature cells. In other words, stem cells undergo proliferation and maturation.
- These two roles (proliferation and maturation) are tightly regulated by at the molecular levels with different mediators, cytokines and negative feedback loops.
- Any derangement of these mechanisms results in myeloid neoplasia.
- The following terms are important:
 - Proliferation: the ability of the cell to undergo division to replace its own pool
 - Maturation: the ability of the cell to transform into more mature and functional cells.
- A defect in maturation (arrested maturation) coupled with increased proliferation gives rise to acute myeloid leukemia (AML).
- A defect in proliferation with sustained maturation gives rise to myeloproliferative neoplasms (MPNs).
- The third category of myeloid neoplasms is myelodysplastic syndrome, characterized by abnormal maturation and inability of the mature cells to be released into peripheral blood (ineffective hematopoiesis). There is defective proliferation but there is no arrest in maturation, in other words, the maturation is abnormal but, unlike AML, not arrested.

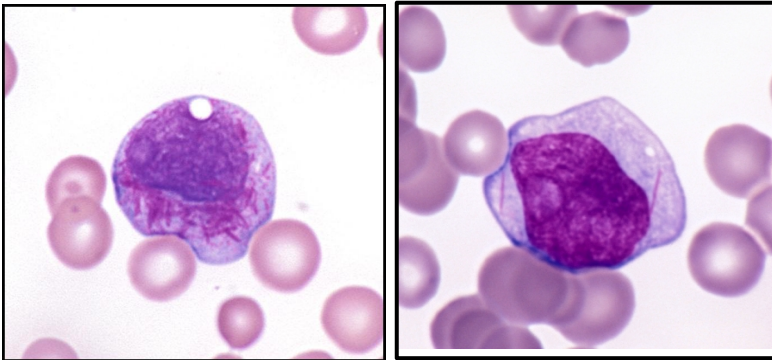
Acute myeloid leukemia (AML).

- Definition: a clonal myeloid neoplasm, characterized by uncontrolled proliferation of immature precursor cells (blasts) that constitute 20% or more of the bone marrow cells.
- AML is classified into four major categories:
 - AML with recurrent cytogenetic abnormalities
 - Therapy related AML
 - Myelodysplasia-associated AML
 - AML-not otherwise specified (NOS).
- AML with recurrent cytogenetic abnormalities: are AML (20% or more blasts in the bone marrow) with reproducible cytogenetic translocations, these leukemias have the best prognosis of all AML subtypes, examples:
 - AML with t(15;17), *PML-RARA*. Also called acute promyelocytic leukemia.
 - AML with t(8;21), this translocation involved the *RUNX* gene which plays an important role in myeloid maturation.
 - AML with inversion of chromosome 16 (inv.16), this genetic change involves the gene *CBFB*, again plays a vital role for myeloid maturation.
 - Please remember that none of these genetic changes is sufficient, by itself, to induce AML, in fact it is believed that the role of these changes is maturation arrest, and other genetic events must occur to promote the proliferation of these arrested cells.
- Therapy related AML: this is an acute myeloid leukemia that develops in patients who had received chemotherapy or radiation therapy in the past (as far as 8 years earlier); they are characterized by having poor prognosis. This AML typically occurs 1-8 years after receiving chemotherapy or radiation.
- Myelodysplasia related AML: this can be encountered in three situations:
 - AML developing in a patient who has a prior diagnosis of MDS
 - A de novo AML with morphologic changes of myelodysplasia affecting at least 50% of the cells (MDS morphologic changes will be discussed in the following sections.).
 - AML with genetic changes characteristic of MDS.
 - This AML is also characterized by poor prognosis.
- AML-NOS is an entity that describes AML that lacks the criteria of the previous three categories, in other words, this is an AML with no recurrent cytogenetic abnormalities, no history of chemotherapy or radiation therapy, no history of MDS and no morphologic changes associated with MDS.
- Clinical features: AML can occur at any age, however, in children it is less common than ALL.

- Patients typically present with the stigmata of bone marrow failure (the neoplastic cells occupy the bone marrow and suppress hematopoiesis). These include fatigue and pallor secondary to anemia, infections due to neutropenia and bleeding due to thrombocytopenia. Sometimes the blasts invade tissue other than bone marrow resulting in masses in the skin or gingiva.
 - When AML presents only with masses without bone marrow involvement, this is called myeloid sarcoma or chloroma, however, the treatment is exactly the same as AML as these tumors inevitably progress to AML.
 - CBC: half of the patients present with pancytopenia, and the other half present with high WBC count that may reach more than 100,000/microliter.
 - Peripheral blood is involved in the majority of cases; however, rare cases of AML have no blasts in the peripheral blood (called *aleukemic leukemia*), that's why a bone marrow biopsy is a must for diagnosis.
 - A particularly serious complication of acute promyelocytic leukemia (AML with t (15;17) is disseminated intravascular coagulation (DIC), this results from the production of procoagulant and fibrinolytic mediators from the neoplastic cells.
- Diagnosis
 - Morphology: the bone marrow or peripheral blood show the presence of 20% or more blasts (intermediate to large cells with scant to moderate cytoplasm, oval to round nuclei with fine chromatin and prominent nucleoli). Note that the morphology is not different from blasts seen in ALL, therefore, immunophenotype is critical in arriving at the correct diagnosis.
 - Auer rods: are specific for myeloid leukemia and not seen in ALL: these are eosinophilic, needle-like structures in the cytoplasm. Their presence is diagnostic of AML but their absence does not rule out AML.



Note the abundant blasts, to diagnose AML you need to count 500 cells in the bone marrow and calculate the percentage of blasts; if they are 20% or more, this is AML.



Auer rods: needle like structures in the cytoplasm; these are characteristic of myeloid blasts and are not seen in lymphoid blasts.

- Immunophenotype: the myeloid blasts are positive for CD34 (universal blast marker), negative for TdT and B-cell markers (CD19, CD20, CD22, CD79 and pax5), negative for T-cell markers (CD3) and positive for myeloid markers (MPO is the most specific, in addition to CD117, CD15, CD13 and CD33). AML with monocytic differentiation are also positive for monocytic markers such as CD14 and CD64. AML with erythroid differentiation are positive for erythroid markers such as CD71 and glycophorin A (CD235). And finally AML with megakaryocytic differentiation are positive for CD61, CD41 and CD42.
- Prognosis: Depends on the subtype (see above), overall 5-year survival is 15-30%. Treatment with chemotherapy and bone marrow transplantation

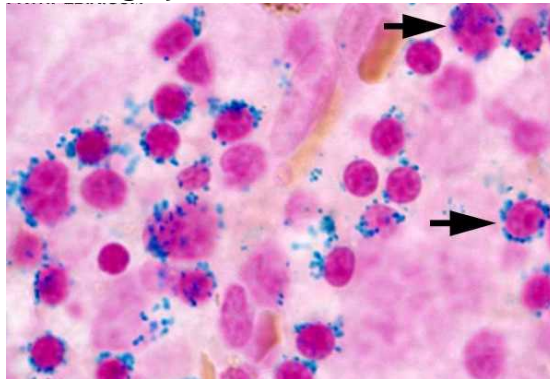
Myelodysplastic syndrome (MDS)

- Definition: MDS refers to a group of clonal stem cell disorders characterized by maturation defects that are associated with ineffective hematopoiesis with cytopenias and a high risk of transformation to AML.

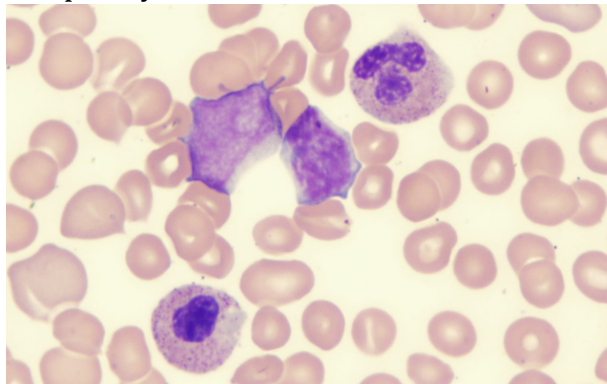
- By definition there is NO cytolysis in MDS. The cell counts are low or normal.
- Epidemiology: this is a disease of the elderly; mean age of presentation is 70.
- Clinical features: up to half of the patients are asymptomatic with their cytopenia discovered incidentally on CBCs done for other purposes.
 - When symptomatic, these patients present with fatigue, fever and infection, and bleeding; all secondary to pancytopenia.
 - On CBC: one or more cell lines show cytopenia, the patients may present with unicytopenia (anemia only, leukopenia only, or thrombocytopenia only), bi- or pancytopenia. Also the MCV is usually slightly above reference range (not as high as the MCV seen in cases of B12 or folate deficiency).
- Diagnosis: bone marrow examination is a must for diagnosis
 - Morphologic features of dysplasia
 - Granulocytes: decreased cytoplasmic granulation, hyposegmented nuclei (nuclei having one or two lobes only as opposed to the 3-5 lobes seen in normal neutrophils)
 - Erythroid cells: nuclear irregularity, nuclear bridging, nuclear budding, megaloblastoid changes (immature nucleus with fine chromatin and mature grey cytoplasm, similar to what's seen in B12 and folate deficiency). Ring sideroblasts (erythroid cells with perinuclear iron deposits seen by iron stains) are also dysplastic features.
 - Megakaryocytes: small megakaryocytes with small, monolobated nuclei showing hyperchromasia.
 - Blasts: in a subset of MDS cases, there will be an increase in the percentage of blasts; by definition they should be up to 19% (if 20% or more, it is AML!)
 - Keep in mind the following numbers: you need the dysplastic features to affect at least 10% of the cells to be considered significant and to qualify as dysplasia, one exception is ring sideroblasts which has to affect at least 15% of erythroid cells to be significant.
 - In cases of AML with myelodysplasia related changes you need dysplasia to be in at least 50% of the cells.



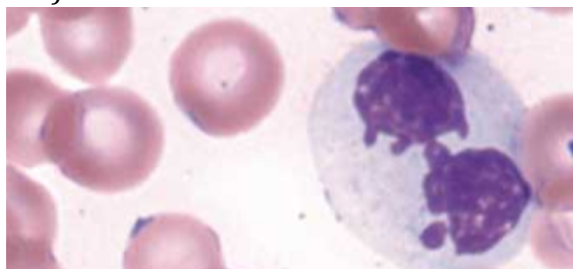
- Note the erythroid dysplasia in the form of irregular nucleus (cell on the left) and nuclear budding (two cells on the right).



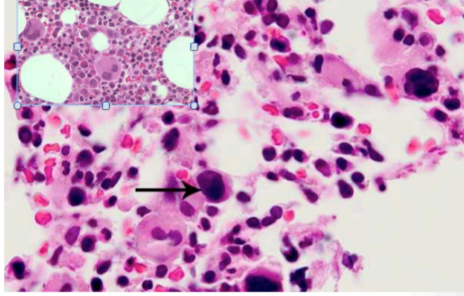
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- Note the ring sideroblasts: these are erythroid cells with perinuclear iron deposits covering, partially or completely, the nucleus.



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- Note the presence of a neutrophil with only one lobe (lower cell), also note the presence of a blast (middle left).



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- Note another neutrophils with only two lobes and severely hypogranulated cytoplasm (normally the cytoplasm should appear pink with numerous fine granules, they are completely absent in this neutrophil and therefore, the cytoplasm appears grey).

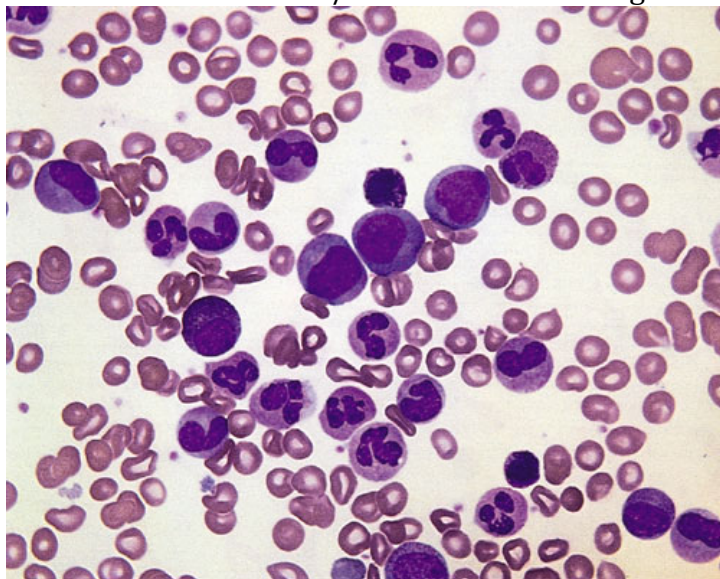


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- Note the dysplastic megakaryocytes with their small, hyperchromatic nuclei (compare them to the normal megakaryocytes in the upper left inset).
- Pathogenesis: genetic changes are key to pathogenesis, four functional categories are recognized:
 - Epigenetic changes: the term “epigenetic” refers to changes affecting the expression of the gene without affecting its sequence.
 - RNA splicing factors: mutations affecting the 3’ end of the RNA are seen in a subset of cases.
 - Transcription factors: mutations of some transcription factors that are important in myelopoiesis and maturation are also seen in some cases.
 - Around 10% of the cases have genetic mutations in the *TP53* gene; these cases are associated with poor prognosis.
- Prognosis: variable according to the subtype, chromosomal changes, severity of cytopenia and the number of blasts. Overall median survival is 9-29 months, but some patients might survive more than 5 years. Risk of transformation to AML is around 10-40%.

Myeloproliferative neoplasms

- Definition: a heterogeneous group of clonal myeloid proliferations characterized by uncontrolled proliferations that do not affect the maturation of the myeloid cells, dysplasia is absent.
 - A common feature to all these tumors is the presence of mutated, continually active tyrosine kinases that promote cell proliferation without the need of growth factor signaling.
- Four major diseases will be discussed in this manuscript:
 - Chronic myeloid leukemia (CML)
 - Polycythemia vera (PV).
 - Primary myelofibrosis (PMF)
 - Essential thrombocythemia (ET).
- Common among these neoplasms is the following:
 - Increased proliferative drive in the bone marrow

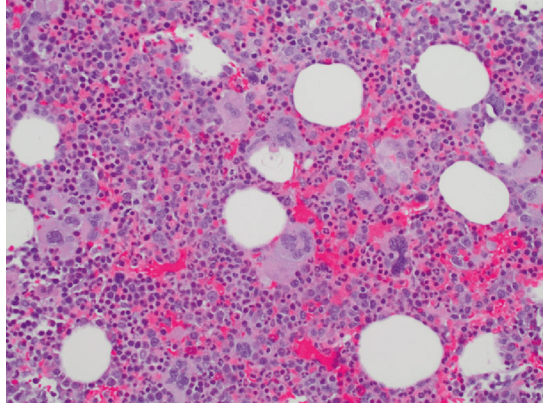
- Homing of the neoplastic stem cells to secondary hematopoietic organs, producing extramedullary hematopoiesis. Splenomegaly is marked in these tumors.
- Variable transformation to a spent phase characterized by marrow fibrosis and peripheral blood cytopenias.
- Variable transformation to acute leukemia.
- **CML:** this is a myeloproliferative neoplasm characterized by uncontrolled proliferation of granulocytic cells in various degrees of maturation and the presence of the BCR/ABL fusion gene that results in constitutively active tyrosine kinase, this is the only hematolymphoid malignancy in which molecular changes are required for diagnosis (you cannot diagnose CML without detecting the BCR/ABL fusion by karyotyping or polymerase chain reaction (PCR)).
- Clinical presentation: typically affects people above the age of 50, any age can be affected however.
 - Incidental finding of leukocytosis (may reach more than 100,000/microliter) on a CBC done for other purposes. Basophilia and thrombocytosis are also common.
 - Fatigue, anorexia and weight loss due to anemia and hypermetabolism caused by high tumor cell burden.
 - Abdominal heaviness due to splenomegaly, or abdominal pain due to splenic infarction.
- Morphology: the bone marrow and peripheral blood are hypercellular due to massive proliferation of maturing myeloid cells including neutrophils, bands, metamyelocytes and myelocytes. Blasts can be present and usually do not exceed 10%. Increases in basophils are also noted. Dysplasia is not significant. (Remember, even if dysplasia is noted, MDS is ruled out since the patient presents with leukocytosis, there is no Cytosis in MDS!).
 - Detection of BCR/ABL is a must for diagnosis.



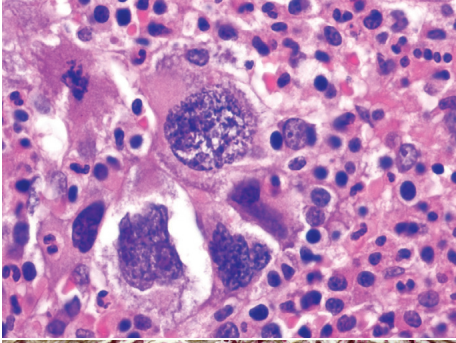
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Note in the image the presence of granulocytic cells in different stages of maturation (neutrophils, bands, metamyelocytes, myelocytes and promyelocytes) note the presence of basophils as well.

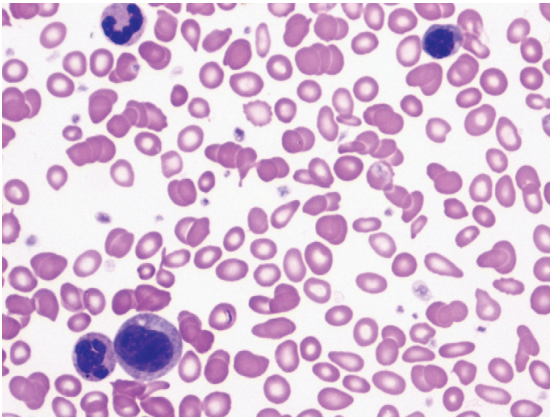
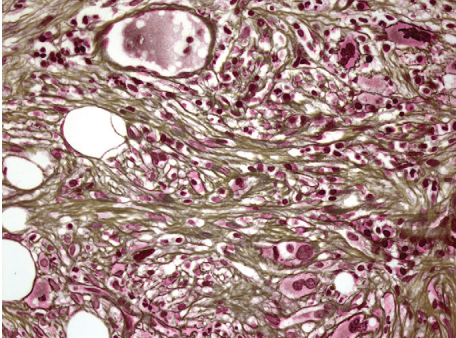
- Prognosis: indolent disease, even without treatment the median survival is 3 years.
 - May progress to accelerated phase characterized by cytopenia, increased basophilia, and massive splenomegaly.
 - May progress to acute leukemia if the blast count exceeds 19%, surprisingly the leukemia can be myeloid (AML) in two thirds of the cases or lymphoid (ALL) in one third of the cases.
- Treatment with targeted drugs that inhibit the activity of the BCR/ABL fusion protein results in markedly decreasing the cell counts and the risk of AML transformation.
 - Bone marrow transplantation might be considered in young patients.
- **Polycythemia vera**: A myeloproliferative neoplasm characterized by increased hemoglobin concentration. JAK2 mutation is present in > 95% of the cases.
- Criteria for diagnosis: there are three major criteria and one minor criterion:
 - Major criteria:
 - Increased hemoglobin (more than 16.5gm/dL in men and 16gm/dL in women)
 - Bone marrow hypercellularity due to increased proliferation of erythroid, myeloid and megakaryocytic cells (panmyelosis)
 - The presence of JAK2 mutation detected by PCR.
 - Minor criterion: low serum erythropoietin.
 - To diagnose PV, you need the three major criteria, or the first two major criteria with the minor criterion.
- Clinical features: this is a rare disease that typically affects middle to late age people. The symptomatology of PV largely stems from increased RBC counts and increase RBC mass with resulting hyperviscosity. Symptoms include headache, hypertension, visual disturbance, and parasthesia. Pruritus is also common and likely represents a manifestation of histamine release from basophils. In nearly 20% of cases, an episode of venous or arterial thrombosis (such as deep vein thrombosis, myocardial ischemia, or stroke) is documented in the medical history, and may be the first manifestation of PV.
- Morphology: the bone marrow is hypercellular with increased cell proliferation of all major cells (erythroid, myeloid and megakaryocytic) not only erythroid! There is no significant dysplasia and, by definition, the blast count is less than 20%.



- Note the hypercellular bone marrow (normally the amount of fat in the bone marrow should equal the patient's age, for example a 60-year-old woman should have 60% fat in the bone marrow), note also the increase in all cell lines (myeloid, erythroid and megakaryocytic, in other words: panmyelosis)
- Prognosis: About 25% of the patients develop major thrombotic events such as myocardial infarction, mesenteric thrombosis, and stroke; without treatment these complications can be fatal. Approximately 10% of the patients have bleeding. Ten to 20% of the patients progress into a "spent phase" where the bone marrow is severely fibrotic. Approximately 2% progress to acute leukemia, most of which is AML. Unlike CML, progression to ALL is exceptionally rare.
- Treatment with simple phlebotomy to decrease the hemoglobin concentration and hence the viscosity results in marked improvement of the patient's outcome. In fact, phlebotomy alone can extend the survival to 10 years. JAK2 inhibitors are promising form of targeted therapy.
- **Primary myelofibrosis:** A myeloproliferative neoplasm characterized by extensive bone marrow fibrosis, extramedullary hematopoiesis and blood cytopenia. JAK2 mutations are present in ~50% of the cases. Mutations in the *MPL* and *CALR* genes are also commonly seen.
- Clinical presentation: the typical patient is above the age of 60. The presentation is with severe anemia and abdominal fullness secondary to massive splenomegaly. Nonspecific symptoms of weight loss, fever and loss of appetite can occur. Hyperuricemia and gout can develop secondary to high cell turnover. Lab findings show normochromic normocytic anemia with mild thrombocytosis and normal to mildly low WBC count.
- Morphology: The bone marrow is replaced by strands of thick fibrotic tissue. The megakaryocytes are increased dramatically with bizarre morphology including hyperchromasia and markedly irregular nuclei. The peripheral blood shows leukoerythroblastic reaction, which means the presence of immature myeloid and erythroid cells in the blood. Additionally, tear drop RBCs are commonly seen.



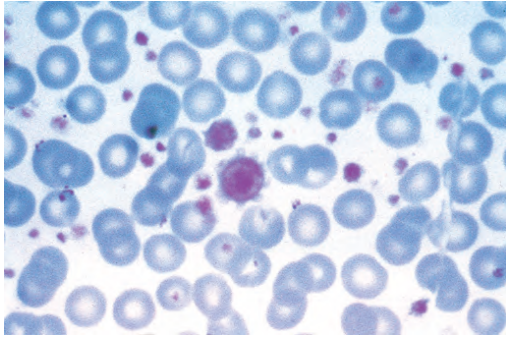
The first image shows a significant proliferation of megakaryocytes with markedly bizarre shapes. The second image shows severe fibrosis (the brown to black strands represent increase reticulin fibrosis).



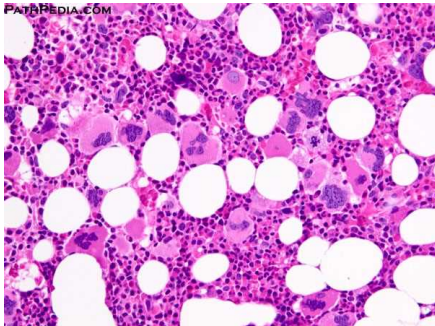
Note the leukoerythroblastic reaction in this image: the upper right corner has an erythroid cells (you should not see these in the peripheral blood normally). The lower left corner shows a neutrophil right next to an immature myeloid cell (A myelocyte). Note the presence of frequent teardrop cells in the image.

- Prognosis: Much more difficult to treat than PV. Patients may die secondary to infections, thrombosis or bleeding. Median survival is 3-5 years. Progression to AML occurs in 5-20% of the patients.
- Treatment with JAK2 inhibitors or bone marrow transplantation may extend survival.
- **Essential thrombocythemia:** A myeloproliferative neoplasm characterized by thrombocytosis. JAK2 mutations are present in ~50% of the cases. Mutations in the MPL and CALR genes are also commonly seen. Before rendering this disease, all secondary causes of thrombocytosis (such as inflammation and iron deficiency) should be ruled out. Notably, elevated hemoglobin and bone marrow fibrosis are absent.

- Clinical presentation: rare disease that presents with significant thrombocytosis. Due to the dysfunctional nature of the malignant platelets, thrombosis and bleeding may occur causing morbidity and mortality. The patients are usually above the age of 60.
- Morphology: the bone marrow is hypercellular with increased proliferation of megakaryocytes, which can attain large sizes. There is no fibrosis. The peripheral blood shows increased platelets with many large and giant platelets.



- Note the increase number of platelets (A simple way to estimate the increase is by remembering that roughly for every 10 RBCs there is one platelet, in this image, they are definitely more than that). Note also the large and giant forms.



- Note the hypercellular bone marrow with frequent large megakaryocytes.
- Prognosis: indolent disease with long asymptomatic periods punctured by thrombotic or bleeding events, median survival is 12-15 years.