Hematology

Pathology

☑ Sheet ☐ Slide ☐ Handout

Number: 7

Subject: Myeloid Neoplasms

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Price:

- This sheet was written according to the record of section 3.

- Before you read this sheet, it would be very helpful to watch Dr. Sattar's Pathoma video on "Myeloproliferative Disorders".

Hematopoietic Malignancies

- Hematopoietic malignancies are classified according to the cell of origin into:
  a- Myeloid Neoplasms
  b- Lymphoid Neoplasms
  c- Histiocytic Neoplasms: these are very rare and complex, so we will not go through them in this course.

- Important Notes:
  - If there's neoplastic proliferation in:
  1- **Immature Cells**:
  Myeloblasts → Acute Myeloid Leukemia (AML)
  Lymphoblasts → Acute Lymphoblastic Leukemia (ALL)

  2- **Mature Cells:**
  a- Lymphocytes → Chronic Lymphocytic Leukemia (CLL)
  b- Granulocytes → Chronic Myelogenous Leukemia (CML)
  c- RBCs → Polycythemia Vera (PV)
  d- Platelets → Essential Thrombocythemia (ET)
  e- Megakaryocytes → Primary Myelofibrosis
- CML, PV, ET and primary myelofibrosis are called myeloproliferative neoplasms.

Myeloid Neoplasms:
Classified into three categories:
a- Myeloproliferative Neoplasms (MPNs)
b- Myelodysplastic Syndromes (MDS)
c- Acute Myeloid Leukemia (AML)

- The first two are chronic diseases that may progress into AML.

- Common Features:
  - Recurrent genetic mutations (i.e. these mutations are common and characteristic for these neoplasms).
  - Increased bone marrow cellularity.
  - Tendency to progress to AML (MPNs and MDS progress into AML).

- Risk Factors:
  1- Chemicals (Benzene, Pesticides)
  (e.g. benzene, which is a solvent used in industry, not that of gasoline. Benzene is toxic to the bone marrow.).
  2- Radiation (Nuclear Ionizing Radiation).
  3- Congenital Diseases (e.g. Fanconi Anemia)
  4- Smoking
  5- Paroxysmal Nocturnal Hemoglobinuria (10 % of patients encounter mutations in their lives and develop MPNs or MDS).
- Bone Marrow Examinations:
  - In acute leukemia, there's a neoplastic proliferation of immature blasts (myeloblasts or lymphoblasts). In this case, 20% of cells in the bone marrow would be blasts.
  - If blasts constitute 5% of cells → Normal
  - 5-20 % → Myelodysplastic Syndromes
  - >20% → Acute Leukemia

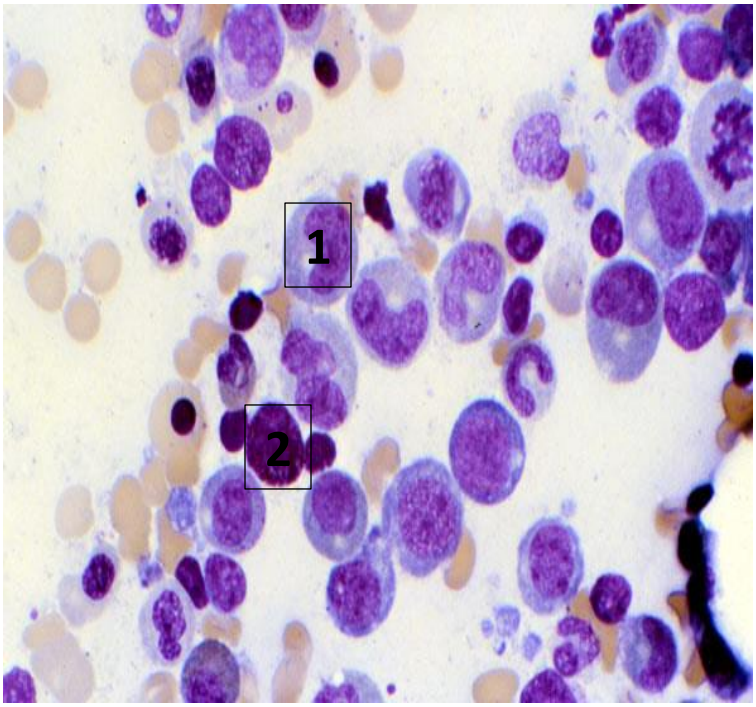
➔ The percentage of cells in the bone marrow is important for diagnosis, hence, in pathology, we care about **the morphology of the bone marrow**, as well as **the number of cells**.
- Normal numbers of cells in the bone marrow:

Blasts (immature cells) < 5%
Monocytes: <5%
Myeloid to erythroid ratio = 3-4 times

Plasma cells: <3%

- There are two ways of **bone marrow examination**:

1) Bone marrows aspirate: some bone marrow cells are sucked into a syringe, then it’s stained with H&E (a fluid specimen taken from the bone marrow, it just shows the cells)
   Note: the aspirate can also be taken from blood.
2) Bone marrow biopsy (Trephine biopsy) :1 or 2 cm core of bone marrow is removed in one piece, then it’s stained with H&E
   (It shows the structure of bone marrow inside the bone as well as the cellularity)



Aspirate smear from **Normal** bone marrow shows normal myelogenesis and erythropoiesis

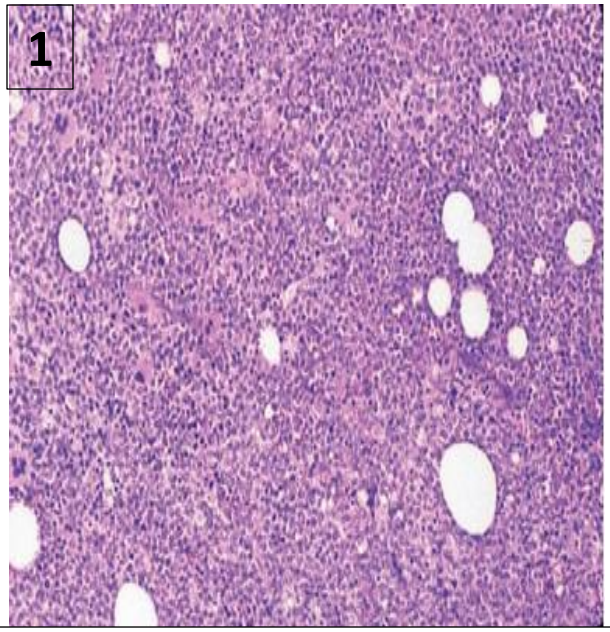
1: Myeloid :Larger

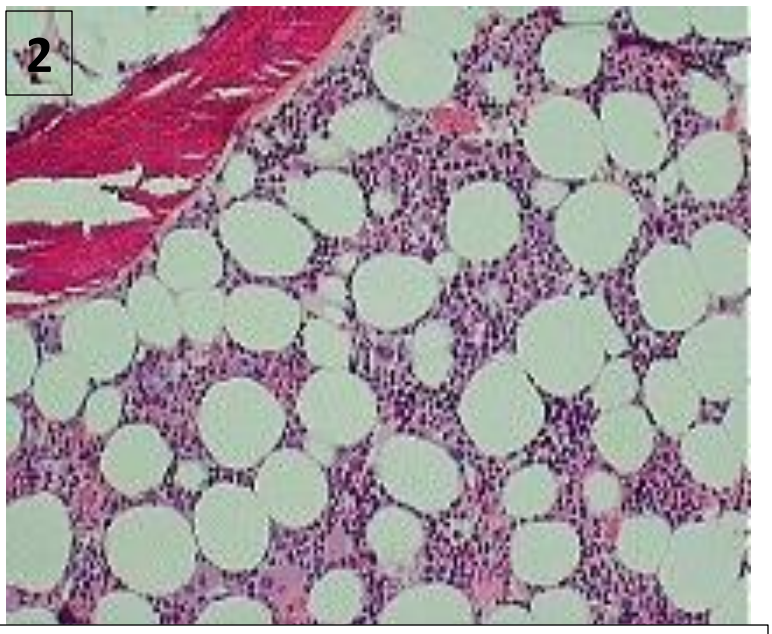
2: Erythroid : smaller

*Notice that the cells are present individually and they appear clearly.

*Notice that the myeloid cells are more abundant than the erythroid cells (M:E 3-4)

*You can see myeloblast, Myelocyte, Metamyelocyte and band cells)





Bone marrow biopsy (Trephine biopsy):

The first thing we care about in trephine biopsy is the **cellularity** of bone marrow.

-**Normal** bone marrow cellularity depends on age (cellularity =100-age)%

1: In early life, the hematopoietic cells are predominant (High cellularity)

(90% of the biopsy is hematopoietic cell, 10% fat, so this biopsy is expected to be from a ten-year old child)

2: In the elderly, the hematopoietic cells undergo atrophy, and fat become predominant

(50% of the biopsy is hematopoietic cell, 50% fat, so this biopsy is expected to be from a fifty-year old man

Myeloproliferative Neoplasms MPN (were also known as myeloproliferative disorders).

- Accumulation of **mature** myeloid cells.
- Although all myeloid cells increase, **one type is predominant**. So, MPNs are classified according to the dominant cell type:
  RBCs → PV
  Platelets → Essential Thrombocythemia
  Megakaryocyte → Primary Myelofibrosis
  Granulocytes → CML
- Don't forget this: **All cells increase but only one is dominant.**

Terminology Box:

Myelo- means myeloid cells, referring to granulocytes, erythrocytes, megakaryocytes and platelets.

-proliferative means that there's proliferation of all cells of the myeloid lineage.

Neoplasms → indicates that this proliferation is induced by mutations.

Before going into each neoplasm, it would be convenient to discuss their **general features** first:

- **Chronic Diseases**
- Being chronic increases the **possibility of transforming into AML**.
- There's **hyperproliferation of myeloid progenitor cells** that retain their capacity of terminal differentiation (i.e. if a mutation hits stems cells and made them unable to differentiate, we will end up with accumulation of immature blasts, which is acute leukemia. Whereas, in MPNs, the mutated cells are still able to differentiate, so they give us mature cells.)
  → MPNs are characterized by accumulation of mature myeloid cells (RBCs in PV, granulocytes in CML .. etc).

➔ **MPNs are associated with activating mutations in tyrosine kinases.**
- JAK2 (seen in all MPNs except CML).
- BCR-ABL fusion protein has potent tyrosine kinase activity (seen only in CML).

The frequency of JAK2 mutation:

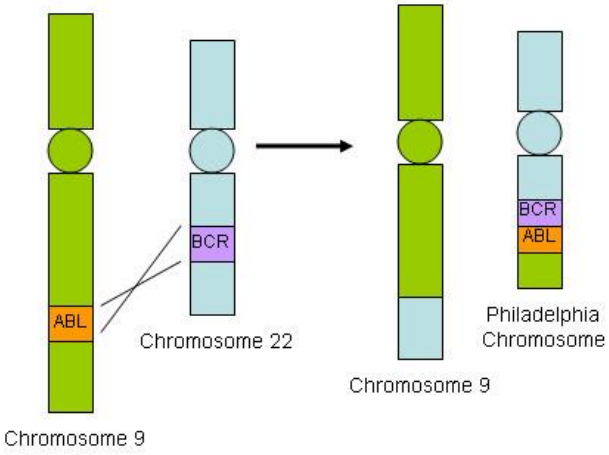
1) All PV cases have JAK2 mutation
2) 50% of Myelofibrosis and essential thrombocythemia case have JAK2 mutation

- Normally, hematopoietic growth factors act through **tyrosine kinase receptors.**
- Now, knowing that MPNs are associated with **hyperproliferation of myeloid progenitor cells**, where do you expect to find the mutations? In **tyrosine kinase receptors** (i.e. if a mutation results in constitutive activation of tyrosine kinases, myeloid progenitor cells will hyperproliferate).

- **Hyperproliferation** of myeloid progenitor cells makes the bone marrow **hypercellular** [Bone Marrow Hypercellularity].
- High WBC count (Persistent peripheral blood cytosis).
- Neoplastic progenitor cells tend to seed secondary hematopoietic organs (liver, spleen and lymph nodes). This results in **hepatosplenomegaly** (caused by extramedullary hematopoiesis).
- CML is associated with BCR-ABL fusion gene.
- Other MPNs are associated with activating JAK2 mutations

1- **Chronic Myelogenous Leukemia:**

- Neoplastic proliferation of myeloid cells, and the dominant cell type is the **granulocyte**.



- CML is always associated with **BCR-ABL fusion gene**. In most cases, this results from a **balanced translocation between chromosomes 9 and 22**.
- The chromosome that results from the translocation is a new chromosome, known as **Philadelphia chromosome**.
- All myeloid cells have the mutation, but for unclear reasons, mainly granulocytes and megakaryocytes increase in CML.
- **Why does this translocation cause hyperproliferation?** because the protein product of the fusion gene has a potent tyrosine kinase activity.
- Tyrosine kinase stimulate the proliferation and survival (prolong the half-life of cells)

Manifestations:

- Increased WBC count (may reach 100,000 and more).
- Most of the cells are neutrophils, band cells, myelocytes and metamyelocytes (Shift-to-the-left).
- Eosinophils and basophils may increase

- there's characteristic **basophilia.**

- BCR-ABL fusion protein mainly increases granulocytes and megakaryocytes. Cells of the erythroid lineage are less affected.

  This may be attributable to the increased iron needs of the hyperproliferating WBCs, that thus makes iron less available for erythroid precursors.

- Anemia and thrombocytopenia are common.

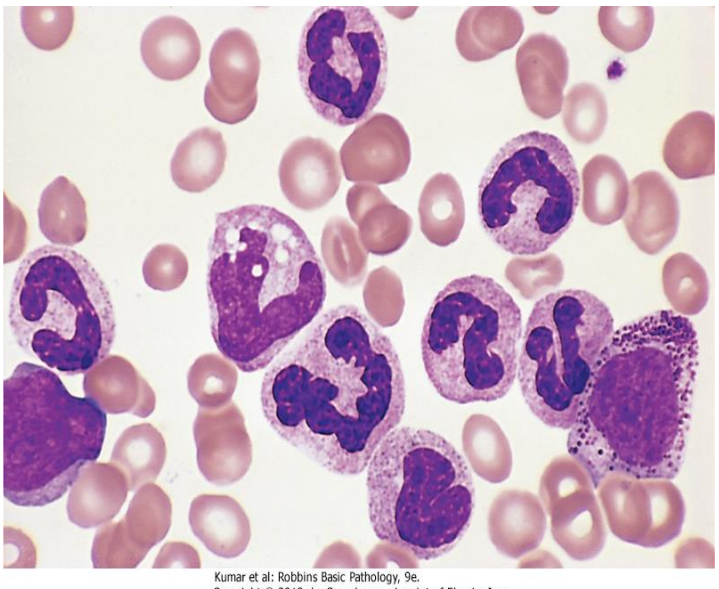
- The bone marrow is **hypercellular** owing to increased numbers of granulocytic and megakaryocytic precursors.
- **Splenomegaly**, due to extra-medullary hematopoiesis.

- How to distinguish between chronic myelogenous leukemia and leukemoid reaction?

CML	**Leukemoid Reaction**
Basophilia	Only increased neutrophils
t(9;22) or BCR-ABL fusion gene	No translocation
Mostly asymptomatic	Presents with signs of bacterial infection
Not mentioned but important: LAP (Leukocyte Alkaline Phosphatase) negative this is an enzyme that helps fight bacteria	LAP positive

- In CML, there's a risk of transformation into AML or ALL.

<u>Morphology of CML:</u>


Kumar et al: Robbins Basic Pathology, 9e.
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1- **Bone marrow:**
is hypercellular

2- **Peripheral blood:**

- Elevated WBC count that may reach 100,000.
  - These WBCs are mainly neutrophils and other less mature cells (band cells, metamyelocytes and myelocytes). Note that granulocytes at various stages of differentiation are present in this blood smear.
  - **Basophilia**
  - Eosinophils and basophils may increase.

- Note: Myeloblasts are only slightly increased in the blood (This is important to distinguish between acute leukemia and CML).

3- Spleen:

Grossly, the spleen is enlarged (**splenomegaly**)

Microscopically, the red pulp of the spleen resembles the bone marrow due to extramedullary hematopoiesis.

Primary Myelofibrosis

-the name can be deceiving; because when you hear "fibrosis" the first thing that comes to your mind is tissue repair, but actually (primary myelofibrosis) is not a normal repair. It's a neoplasm

- Neoplastic hyperproliferation of myeloid cells, especially megakaryocytes.
- There's a JAK2 mutation in myeloid cells that mainly affects megakaryocytes. This causes a **brief period of granulopoiesis and megakaryopoiesis**. This is followed by **fibrosis of the bone marrow.**
- As the bone marrow becomes fibrotic, hematopoiesis will be shifted to the spleen and liver (extra-medullary hematopoiesis). This results in **massive hepatosplenomegaly**.

Pathogenesis:

1- JAK2 mutations cause the neoplasm (50% of cases).

2-Neoplastic megakaryocytes release two important factors:
a- PDGF (Platelet-derived Growth Factor).
b- TGF-beta (Transforming Growth Factor-beta)
These two factors stimulate the proliferation of fibroblasts, that will deposit collagen and cause fibrosis.

- The fibroblasts are not neoplastic.
- This JAK2 mutation is also seen in polycythemia vera.

Morphology

1- **Bone marrow:**
Main theme: **Fibrosis and hypocellularity**

Note: At early stages, like any myeloproliferative neoplasm, there is proliferation of Atypical megakaryocytes and myeloid cells, bone marrow is hyper-cellular and in the peripheral blood there is leukocytosis and thrombocytosis. With disease progression, fibroblasts will increase and overcome the myeloid cells themselves, so the bone marrow will become fibrotic (no cells, only fibrous (collagen)), so there will be hypocellular blood (cytopenia (leucopenia, anemia and thrombocytopenia) and this is the difference between myelofibrosis and chronic myelogenous leukemia (CML)

Extra Note:
Although extramedullary hematopoiesis occurs in the spleen, the spleen is still too small to produce enough RBCs, WBCs and platelets. Consequently, the patient will develop cytopenia, with increased susceptibility for infections (due to decreased granulocytes) and bleeding (due to decreased platelets).

2- **Peripheral blood:**

From pathoma: "In the bone marrow, there's a reticulin gate that prevents immature cells from passing into the blood, that's why when normal hematopoiesis is going on, no immature cells are seen in the blood. On the other hand, the spleen lacks such a gate and thus allows immature cells to pass into the blood."

So, as extramedullary hematopoiesis is going on, both leukoblasts (immature myeloid and lymphoid cells) and erythroblasts (immature RBCs) are expected to leak in the blood and to be seen on a blood smear; hence the **leukoerythroblastic change seen in primary myelofibrosis.**

In a Nutshell:

Leukoerythroblastic Change is the appearance of immature WBCs and nucleated erythroid precursors in the blood.

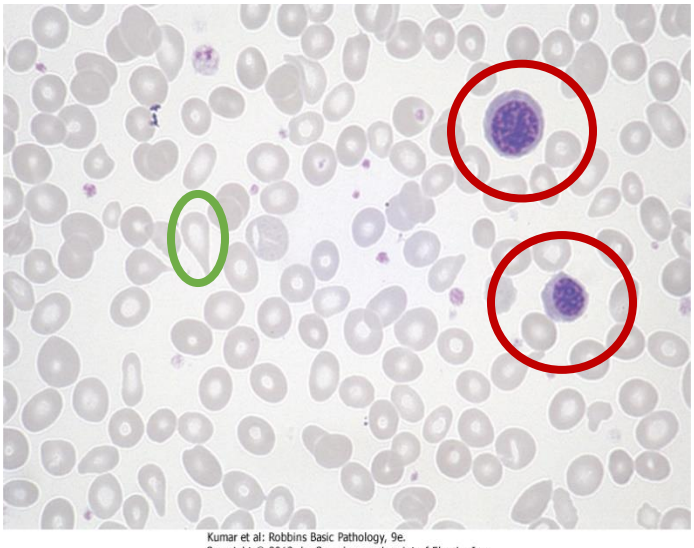
3- **Spleen and Liver:**
- The morbid feature in primary myelofibrosis: Marked enlargement of the spleen as it crosses the midline. (greater enlargement than other MPN). This marked splenomegaly is responsible for most of the complains by the patient

as the spleen feels the abdomen and exert pressure on other organs like the stomach.
(the spleen is normally not palpable, so when ever you become able to palpate it, you should conclude that there's splenomegaly)
- Hepatomegaly may also occur.

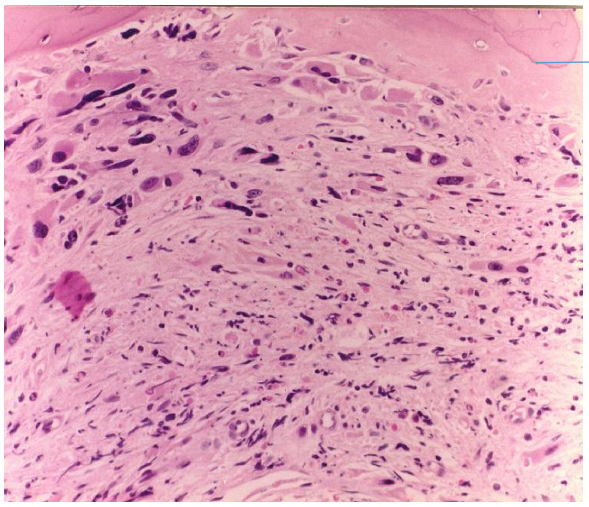
All these are due to the extensive extramedullary hematopoiesis.

- Red-circled cells are nucleated RBCs (immature RBCs).
  This is due to the leukoerythroblastic change.

- Green-circled cell is tear-drop RBC.
  Remember that tear-drop RBCs occur in myelophthisic anemia and primary Myelofibrosis.



Questions related to this figure:
1- In which myeloproliferative neoplasm would you expect to see this?
2- How many nucleated RBCs are there?
3- Are other immature myeloid cells expected to be seen in peripheral blood smear in this disease?
Yes.
4- What other disease would you expect to see such a histological picture in?



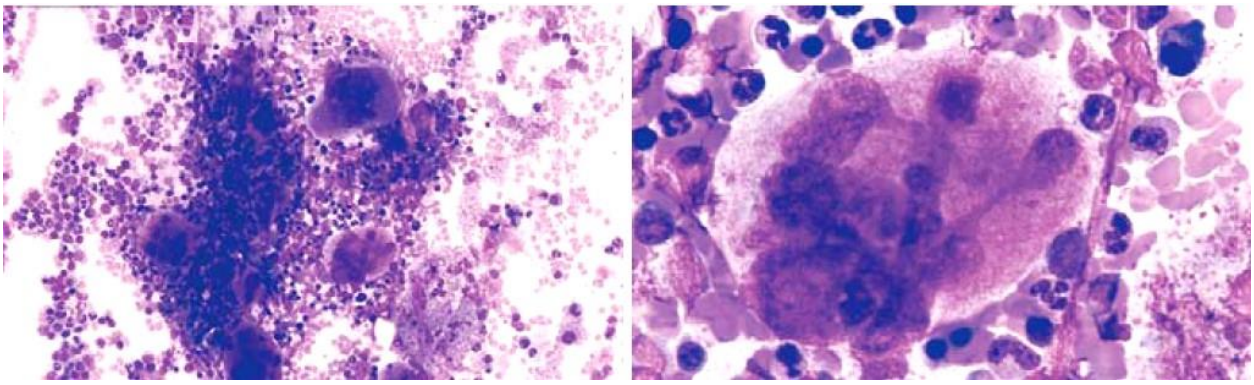
*The figure shows late stage of primary myelofibrosis
-BM biopsy shows hypocellular marrow, spindle shaped stroma and atypia of megakaryocytes
-No fat

Essential Thrombocythemia

- It's called essential because it's primary neoplastic not reactive.
- Chronic disease
- The mildest MPN
- There's neoplastic proliferation of only megakaryocytes, that give rise to platelets. So, in ET, we have increased megakaryocytes in the bone marrow and platelets in the blood.
- There's JAK2 mutations in 50% of cases.
- JAK2 mutations occur in non-CML MPNs (i.e. PV, primary Myelofibrosis, and ET).
  How to distinguish between them?
  PV → polycythemia
  Primary myelofibrosis → BM fibrosis (early in the disease)
  ET → No polycythemia and no BM fibrosis

- There's **sustained thrombocytosis**. Because of the increased number of platelets, there's a risk of thrombosis. However, in some cases, the platelets are not functioning well and thus there's a risk of hemorrhage.
- Elevated platelets is usually an incidental finding (i.e. accidentally on CBC, you find elevated platelets and you suspect ET. Then if you find elevated
- Splenomegaly may be seen but it's mild (ET is the mildest MPN).

Morphology



Left: Increased number of megakaryocytes.
Right: Large mature megakaryocytes with hyperlobated nuclei.

Myelodysplastic Syndromes

- Chronic diseases
- In myeloproliferative neoplasms, the problem was that there's a mutation in immature cells that makes them neoplastic, but they can still maturate normally. That's why in all MPNs, we see mature cells. Here, the problem is in proliferation.

- In myelodysplastic syndromes, there's a problem in the maturation of immature cells (i.e. there's a mutation in immature cells that makes them neoplastic and unable to maturate).
  Here, the problem is in maturation.

This actually makes sense.

Myeloproliferative Neoplasms = Neoplasms that increase proliferation of myeloid cells.

Myelodysplastic Syndromes
-dysplastic = bad growth

= Less maturation of normal myeloid blasts (myeloblasts, erythroblasts, megakaryoblasts).

- The myeloid cells are morphologically abnormal, and they remain in the bone marrow. Therefore, the patient will have cytopenia.
- The bone marrow is hypercellular.
- The hallmark of MDS is persistent , refractory cytopenia and dysplastic bone marrow (abnormal BM).
- When these patients present with anemia or leukopenia, treatment with iron, vitamin B12 or any other treatment would be useless because the BM is abnormal. This is what we mean by refractory.

In a Nutshell:
MDS:
- Abnormal cells in BM
- Peripheral cytopenia
- Hypercellular BM
- Refractory, persistent cytopenia.

-

Pathogenesis

- In MPNs, there's either one gene mutation (JAK2) or a single balanced translocation chromosome (BCR-ABL).
- In MDS, there are chromosomal aberrations (large segments of the chromosome are abnormal).
- **MDS is classified into**:

1- Primary MDS (idiopathic): They are more common.

2- Secondary MDS: It's therapy-related
The patient presents with a history of chemotherapy or radiotherapy.

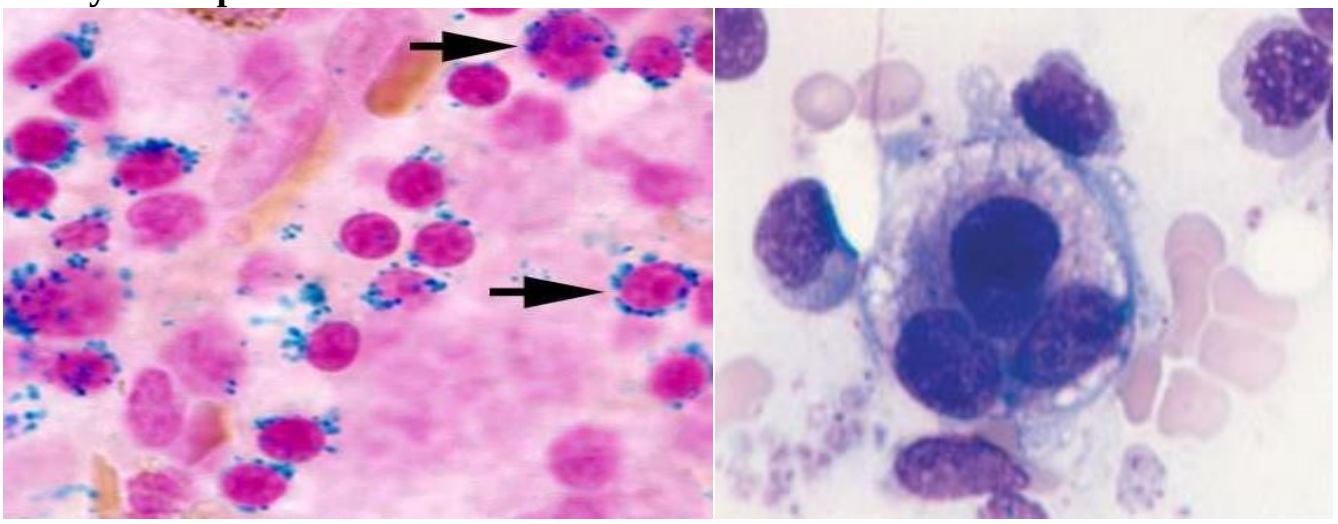
- Chemotherapy and radiotherapy cause mutations in stem cells in the bone marrow.
- Patients receiving chemotherapy for other cancers or rheumatological diseases are at risk of developing MDS.

- All forms of MDS can transform to AML, but transformation occurs with highest frequency and most rapidly in t-MDS (the secondary)

- Acute leukemia and MDS are characterized by accumulation of blasts in the bone marrow.
Less than 5% → Normal
5-20% → MDS
>20% → Acute leukemia

→ From this, we may think of MDS as pre-acute-leukemia stage (i.e. all types of MDS may progress into acute leukemia).
- MDS can be unilineage (arises in one cell lineage) or multilineage (more than one cell lineage).

Morphology:
In MDS, there's accumulation of immature blasts. We said that these cells show abnormal morphology, so now we will talk about these abnormalities in shape.
1- **Erythroid precursors:**



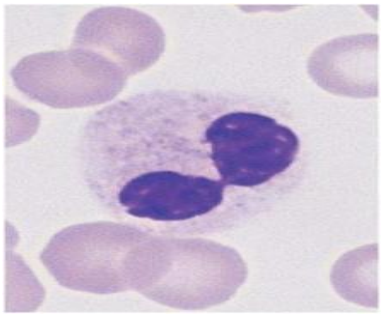
- Megaloblastoid nuclei (these are similar to what's seen in megaloblastic anemia)
- There's nuclear-to-cytoplasmic asynchrony.(late growth of the nucleus but normal growth of the cytoplasm)

-**Multinucleation** (seen in the left picture).

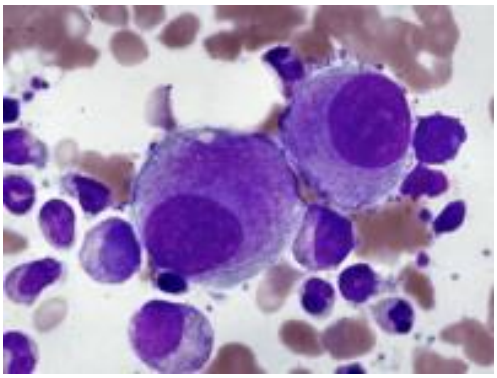
- **Ring sideroblasts** (iron is concentrated in the mitochondria (instead of the cytoplasm) around the nucleus, hence called "ring sideroblasts").
- Iron needs a special stain called Prussian Blue stain.

(Seen in the right picture)

2- **Granulocytes:**
a- Hyposegmented nucleus (2 lobes instead of 3-5)
b- Hypogranular cytoplasm (gray in color, instead of the normal pink)



3- **Megakaryocytes:**
a- small in size
b- hypolobated nuclei



This concludes our discussion on myeloproliferative disorders and myelodysplastic syndromes. Next lecture, we will talk about acute leukemia and lymphoid neoplasms.

Good Luck
And, sorry for any mistake.