Hematology

Medical Committee
The University of Jorda

- Histology
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- lecture number : 6
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WBC'S Diseases

You should understand the differences between the 3 categories: myeloproliferative neoplasm, myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML)
We will begin with:

1- Myeloproliferative neoplasm:

*a **chronic disorder** which means it stays for a long time, persists for years

*defined by **hyperproliferation of neoplastic myeloid progenitors that retain the capacity of terminal differentiation**.
This means that the bone marrow is hypercellular (increase number of cells within it) and this is affecting the stem cells (myeloid progenitors) which differentiate normally, so we see the terminal cells of each cell line, a lot of RBC's, neutrophils, eosinophils , platelets and monocytes (they are the last mature cells which present in the peripheral blood). This is in contrast to AML (acute myeloid leukemia) which is a hyperprolifiration of neoplastic myeloid progenitors that can't differentiate and stop at early stage (myeloblast), so here in myeloproliferative neoplasm you don't see stem cells(myeloblast), you see terminal cells.

*patients will have **persistent peripheral blood cytosis**, increase the number of blood cells so they have in peripheral blood leukocytosis, polycytosis or thrombocytosis in addition to the hypercellularity in the bone marrow (it is the opposite of MDS, here we have increase cells in the bone marrow but they can't migrate to peripheral blood. So the patients will have cytopenia)

*splenomegaly, because the neoplastic blast (stem cell) like to migrate due to increase in numbers, they escape from the bone marrow and go to the spleen and liver and they start proliferating there. This will cause splenomegaly and sometimes hepatomegaly.

We have 4 main types of myeloproliferative neoplasm:

1- **Chronic myelogenous leukemia (CML) :**
*It is the most common type
* chronic means it stays for years
*myelogenous means it involves mainly the myeloid line
* leukemia because of increased number of WBC's in the blood

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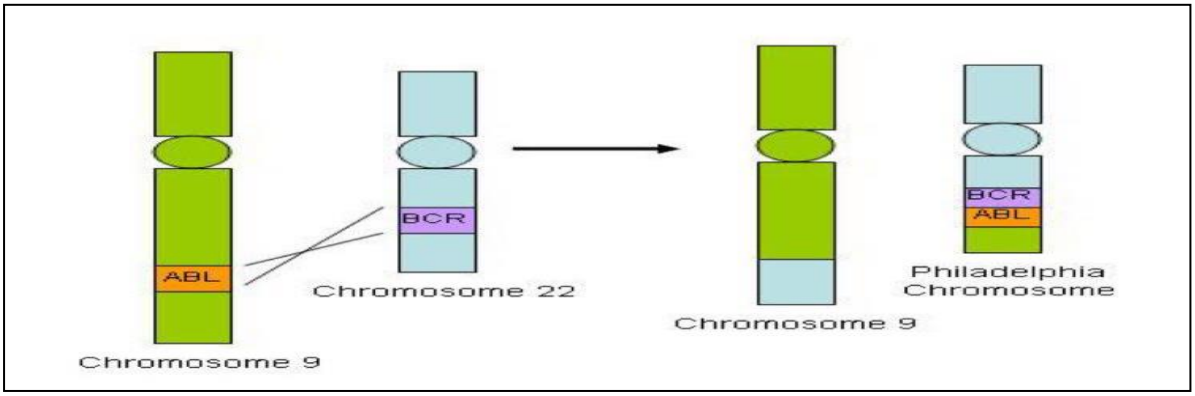
*the most important to know is the genetic mutation :
they always have **a translocation between chromosome 9 and 22**, normally on chromosome 9 we find ABL gene, and on chromosome 22 a BCR gene. So in this mutation the chromosomes exchange parts so on chromosome 22 there will be a fusion between ABL and BCR which creates a new gene **(BCR-ABL fusion gene)** which is functioning and produces a tumor.
this new chromosome since it was discovered in the seventies it was called **Philadelphia chromosome**



again, the translocation 9,22 that moves the gene ABL from chromosome 9 to chromosome 22 to adjacent to the gene BCR
the BCR-ABL fusion gene has tyrosine kinase activity . tyrosine kinase is general protein (enzyme) which activates cell proliferation and survival.

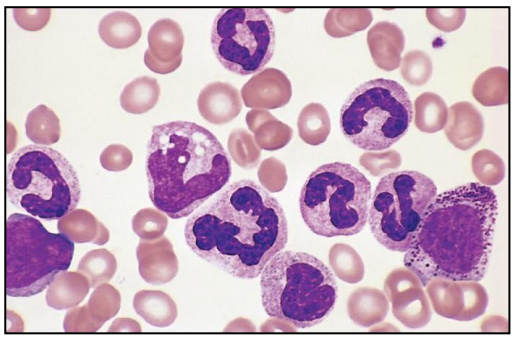
So these cells (stem cells) have this mutation and their progenitor cells (neutrophils, myeloid, and even megariocyte) have the ability to stay a longer time than the normal, they live long and increase in number. So at the end the patient will have hypercellular bone marrow and increased number of cells in the peripheral blood. But for some reason erythroid line is not markedly affected, it mainly affects the myeloid line (myeloid cells) and megakaryocytes.

Manifestations :

1- Peripheral blood shows markedly increase in WBC's , sometimes they exceed 100,000 cells/microliter and reach very high numbers.
2- Most cells are mature and they mature normally so we have increase number in neutrophils, eosinophils, basophils (all the terminally differentiated cells (neutrophils, metamyelocytes, myelocytes) but NOT the blasts)
Remember from the last lecture we said in leukimoid reaction in severe stress we have high production in neutrophils(severe neutrophilia) . cells are active and toxic but the cells in CML are neoplastic (increase in number) and they don't have toxic granules and in CML you see eosinophils, basophils, metamyelocytes and large number of platelets but in leukimoid reaction we mainly see neutrophils.
3- Thrombocytosis , megakaryocytes have a mutation so they increase in number

4- Anemia, RBC line is not affected, iron deficiency is common because WBC's will take the iron and because of the tumor we have large numbers of cells which means high amounts of iron will be taken and that cause iron deficiency and anemia
5- Bone marrow is hypercellular , increased numbers of granulocytes and mekagranulocytes
6- Spleen is enlarged due to secondary extramedullary hematopoiesis and this is a neoplastic one NOT a hemolytic anemia

In the blood film:
Increased number of WBC's ,
you see them compact and next to each other and this is not normal
You see all the cell lines of cells in peripheral blood, and myeloblasts.

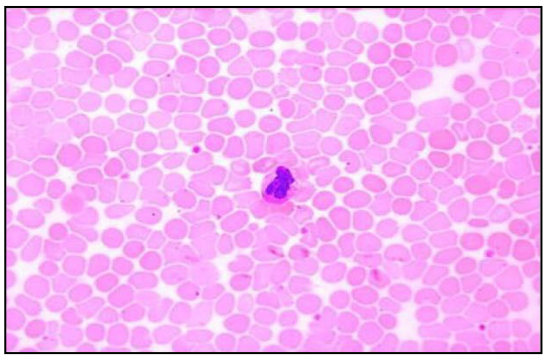


2- Polycythemia vera

we talked about it , this is neoplastic erythroid, they have a mutation in a gene called Janus kinase-2 (JAK-2) gene, we will take about this gene in the next types but here it is the essential mutated gene.
in this mutation erythroid stem cells become very sensitive to erythropoietin, so we will have a production of large numbers of erythroid cells even with very low level in erythropoietin . erythropoietin is suppressed due to negative feedback inhibition but the stem cells can still produce RBC's
also stem cells are sensitive to growth factors which are still unknown, so we will have high erythropoiesis and the bone marrow is hypercellular and we see a lot of normoblasts.
-Granulocytes and megakariocytes also increased but the dominant one is erythroid and we call this increase **(panmyelosis: the 3 cell lines are increased, but the predominant one is the erythroid line).**
-Erythropoietin is low
-Patients have **spleenomegaly**, because there is extramedullary hematopoiesis a neoplastic one.

Manifestations :
In CBC (complete blood count):
1- there is increased haemoglobin consentration, in men above 18, in women above 16
2- there is erythrocytosis, RBC count is high more than 6,000 and commonly as we said the other cell lines are also affected, patient have leukocytosis and thrombocytosis.
3- the bone morrow is hypercellular and we see sheets of normoblasts increased in number

clinically, patients have :
plethora (redness of the skin)
cyanosis (hypoxia)
thrombosis due to increased number of erythrocyte and they move slowly not like in the normal blood
itching
hypertension because
uric acid accumulation causing gout



In the blood film :
we see compact RBC's, they stuck to each other

Secondary polycythemia: we talked about it , please refer to the previous lecture

3 - primary myelofibrosis :

As the name implies there is a dense fibrosis in the bone marrow
This disease is similar to CML but it has differences
It is similar to CML in increased numbers of myeloids and megakariocytes
BUT the difference is the predominant type of cells in each disease,
myeloids are predominant in CML
megakariocytes are predominant in primary myelofibrosis

also in primary myelofibrosis megakaryocytic secrete transforming growth factor β, so they are active. This will activate the fibroblast in the bone marrow, when they are activated they secrete a lot of collagen, and the bone marrow will become fibrotic, while in CML they are not.

At early stage like any myeloplroliferative neoplasm there is proliferation of megakaryocytes and myeloid cells, bone marrow is hyper cellular and in peripheral blood there is leukocytosis and thrombocytosis but with disease progression the fibroblast will increase and overcome the myeloid cells itself, so the bone marrow will become fibrotic (no cells only fibrous (collagen)), so there will be cytopenia (leucopenia, anemia and thrombocytopenia) and this is the difference between myelofibrosis and chronic myelogenous leukemia (CML)

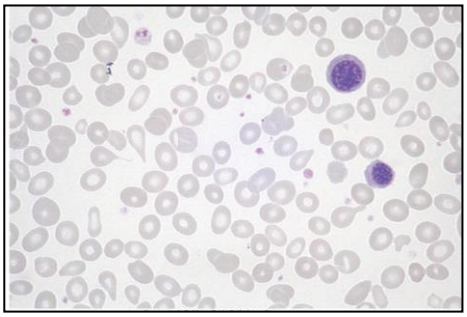
Extramedullary hematopoiesis is also active in this disease, spleen is producing erythroid and liver will have hematopoiesis, and this will cause spleenomegaly.

Jak-2 mutation (the gene which is affected in polycythemia vera) can be positive here.

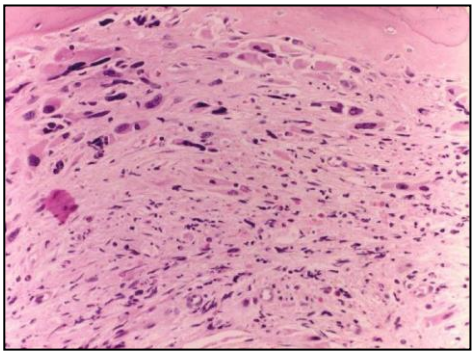
When the RBC's exit the bone marrow they will have change in the shape because of fibrosis , they become like tear drop (مثل الدمعة) and this secondary to fibrosis

*Early bone marrow is hyper cellular(increased megakaryocyte) , and in perephiral blood patients have leukocytosis like CML
***(shift to left)** is presence of early immature cells like: metamyelocyte and myelocytes which will appear in peripheral blood
*thrombocytosis (the most important one in this disease), platelets normally up to 450 but in these patents they exceed 1000 and more
*nucleated RBC's for some reason appear in the blood
* RBC's has tear drop shape

Later after disease progression :
*fibrosis in the bone marrow
*bone marrow will become hypocellular
*in peripheral blood there is cytopenia
*spleen is enlarged



In blood film you can see tear drop RBC's , and other nucleated RBC's

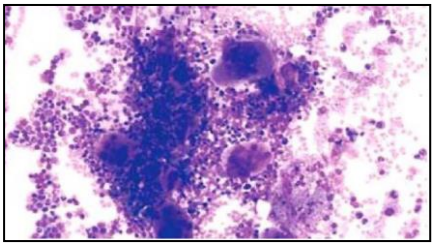
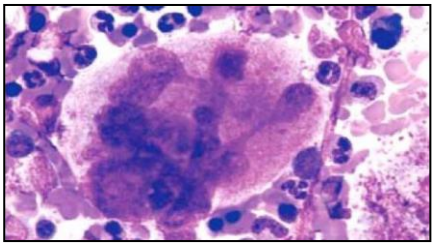


In the Bone marrow is hypercellular, cells are spindle and stretch and this is secondary to fibrosis, the larg dark cells are megakaryocytes and they are proliferating and stuck between collagen fibers

4-Essential thrombocythemia

this is the easiest one ☺
essential = primary neoplastic not reactive

thrombocythemia = increased numbers of megakaryocytes and platelets
* chronic myeloprolefirative neoplasm that affects only megakaryocytes
* sustained thrombocytosis , number of platelets is more than 450 x10^9 /L in peripheral blood
*increase number of large mature megakaryocyte in the bone marrow but there is NO fibrosis as in Primary myelofibrosis
* JAK-2 mutation present in 50% of the patients
* patients tend to have thrombosis, due to increased number of platelets, and sometimes they may have hemorrhage because platelets are not functioning like in normal ones.
*splenomegaly can happen in these patients, but because the disease is mild it can be absent specially in the early disease

***(the left picture one at low magnification)** In the picture you can see the megakaryocytes, they are large, crowded, close to each other. so in peripheral blood we see a lot of platelets(thrombocytosis)

***(the right picture at high magnification)** one megakaryocyte has so many nuclear lobes **(hyperlobulated nucleus)**

important
**The genetic causes for each diseas:

Chronic myelogenous leukemia	Philadelphia chromosome

	(translocation 9,22) which result in BCR-ABL fusion gene
Polycythemia vera	JAK-2 gene (in all patients)
Primary myelofibrosis	JAK-2 gene can has an effect (NOT in all patients)
Essential thrombocythemia	50% JAK-2 gene

You can notice that we have the same gene affects in 3 diseases with different manifestations for each one which indicates other mutations in other genes are involved

**Types of cells affected in each disease

Chronic myelogenous leukemia	Mainly myeloid and megakaryocytes
Polycythemia vera	Dominant one is erythrocyte but myelocytes and megakaryocytes also increase
Primary myelofibrosis	Megakaryocytes and myeloid cells with fibrosis
Essential thrombocythemia	Only megakaryocytes

2- Myelodysplastic syndrome (MDS):

A group of clonal stem cell disorders (clonal means neoplastic) characterized by maturarion defect associated with ineffective hematopoiesis (ineffective means that peripheral blood is not normal).
patients have neutropenia, anemia and thrombocytopenia although the bone marrow is hypercellular.

REMEMBER: myeloprolefirative which is intact maturation with NO defects which is opposite to myelodysplastic syndrome

Hematopoietic stem cells and their progenetors have abnormal morphology, when you see them there is prominent abnormality. Also the cells have abnormal function, they can't move normally and get outside so they stay in the bone marrow, so you see a lot of neutrophils in the bone morrow

which means **the bone marrow is hypercellular but in peripheral blood patients will have cytopenia(neutropenia, anemia and thrombocytopenia)**

*REMEMBER: in myeloprolefirative there is increased in quantity only

To sum up:
The patient will have: (hallmark):
*persistent peripheral cytopenia(for example anemia , we can't treat it, and it has NO specific cause, he also has thrombocytooenia)

*morphologic dysplasia (bone marrow shows abnormal morphology).

How to diagnose it ?
It is different from myeloprolefirative which have cytosis and normal morphology .

Phatogenesis:
We have cytogenetic abnormality (which means we have strong mutations not only in genes, chromosomes are involved too) so we have abnormal chromosomes in these patients
Mostly patients are **idiopathic** like in cancer they manifest without previous factors. So they have the primary disease suddenly.

*we use the terms **primary , idiopathic, essential** to describe unknown reason*

It can be **secondary** in minority of patients and we talked about risk factors. the most important one in MDS is chemotherapy

For example: a patient has a cancer in different formula and they take chemotherapy, after years they can have myelodysplastic syndrome and even acute myeloid leukemia.

Chemotherapy is also given to rheumatoid arthritis not only in cancer cases so even those patients can be complicated with secondary MDS or AML

We said that myeloprolerative (all 4 types) and myelodysplastic (with chemotherapy) can gain more mutations and transform into AML

Classification of MDS :

As we said it is a group of diseases, heterogenous

In classification we take 2 factors :

1-the number (count) of blast

in the last lecture we said blast count normally doesn't exceed 5% of all cells in the bone marrow
in MDS we must count the blast if it is normal or increased , and we make categories

< 6 %
6-10 %
11-19 %
Above 19% (20% or more) ⟶ means acute myeloid leukemia (AML)

2-morphologic dysplasia

is it present in one cell line or more?

we mean by cell lines: erythroid, myeloid, megakaryocyte

*if the number of blast between 6 – 10 % we call it : **refractory anemia with excess blast 1 (RAEB-1)**
[refractory because MDS has persistent neoplasm]
[excess blast, they are more than 5%, as we mentioned above 6-10%]

* if the number of blast between 11-19 % we call it : **refractory anemia with excess blast 2 (RAEB-2)**

We use this classification because the disease course is different for example when the blast is 19% is much worse than when it is 6%, so the treatment will be different too.

if the blast count is normal (less than 6%) , we look at the lines of dysplasia is it in one cell line or more

*If it is one cell line we call it : **refractory cytopenia with unilinage dysplasia**
[blast count is normal and we have dysplasia in one cell line , means the

patients will have isolated anemia or isolated neutropenia or isolated thrombocytopenia]

* If it is more than one cell line (2 or 3) we call it: **refractory cytopenia with multilinage dysplasia**

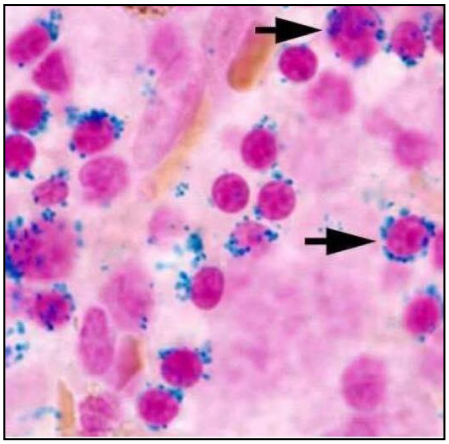
We use this classification because the disease course is, so the treatment will be different too

In unicellular linage dysplasia we have one special type called **refractory anemia with ring sideroblast,** dysplasia present only in erythroid , the cells (normablast) are coated with iron . The prognosis in this disease is the best one so patients will live for a long time (disease course is excellent)

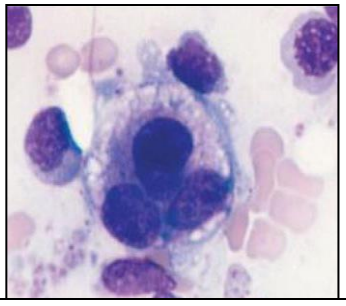
Those patients commonly have cytopenia (anemia, neutropenia, thrombocytopenia or a mixture of them).

features of normoblast dysplasia:

in ring sideroblast (sidro means iron) , we use iron stain so you case see in the picture cells are red and this stain stained the iron with blue color . normally normoblast doesn't condensate iron but in this case normoblast are not normal and they can't mature normally. they condensate iron in the mitochondria around the nucleus as a ring.
these patients will have persistent anemia



multinucleation: multinucleated erythroid cells which is abnormal.
you see in the picture normoblast with multiple nuclei (feature of dysplasia).



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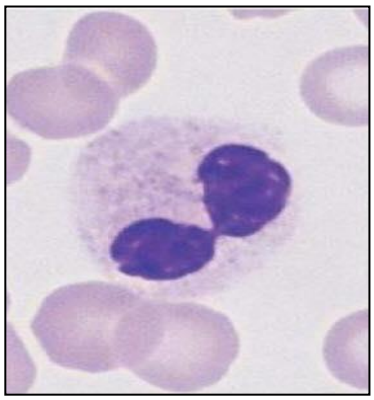
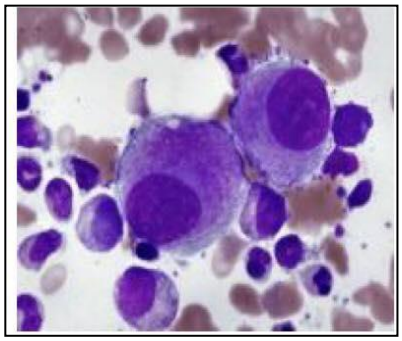
neutrophils become hyposigmented (opposite to megaloblastic anemia which is hypersigmented) , with single or 2 lobes nucleus cytoplasm is very pale with NO granules, so the normal function is not present.



Megakaryocytes: dysplasia is small in size, nucleus is on the is only one and has NO lobules. They are abnormal because normally megakaryocytes are giant and multiple nuclear lobes, but in dysplasia, Megakaryocytes become shrunken, small in size, cannot grow and nucleus cannot segment.



THE END …

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