MED439
KING SAUD UNIVERSITY

Acute Leukemia I & II

Objectives:

- *To understand the general concept of cancer pathogenesis
- *To understand the basis of acute leukemia classification
- *To appreciate the role of molecular in diagnosis ang
- *Treatment of acute leukemia
- *To recognize the clinical presentation of acute leukemia
- *To differentiate between AML and ALL

Dr's notes
Important
Extra notes
** Only in girls slide
** Only in boys slide

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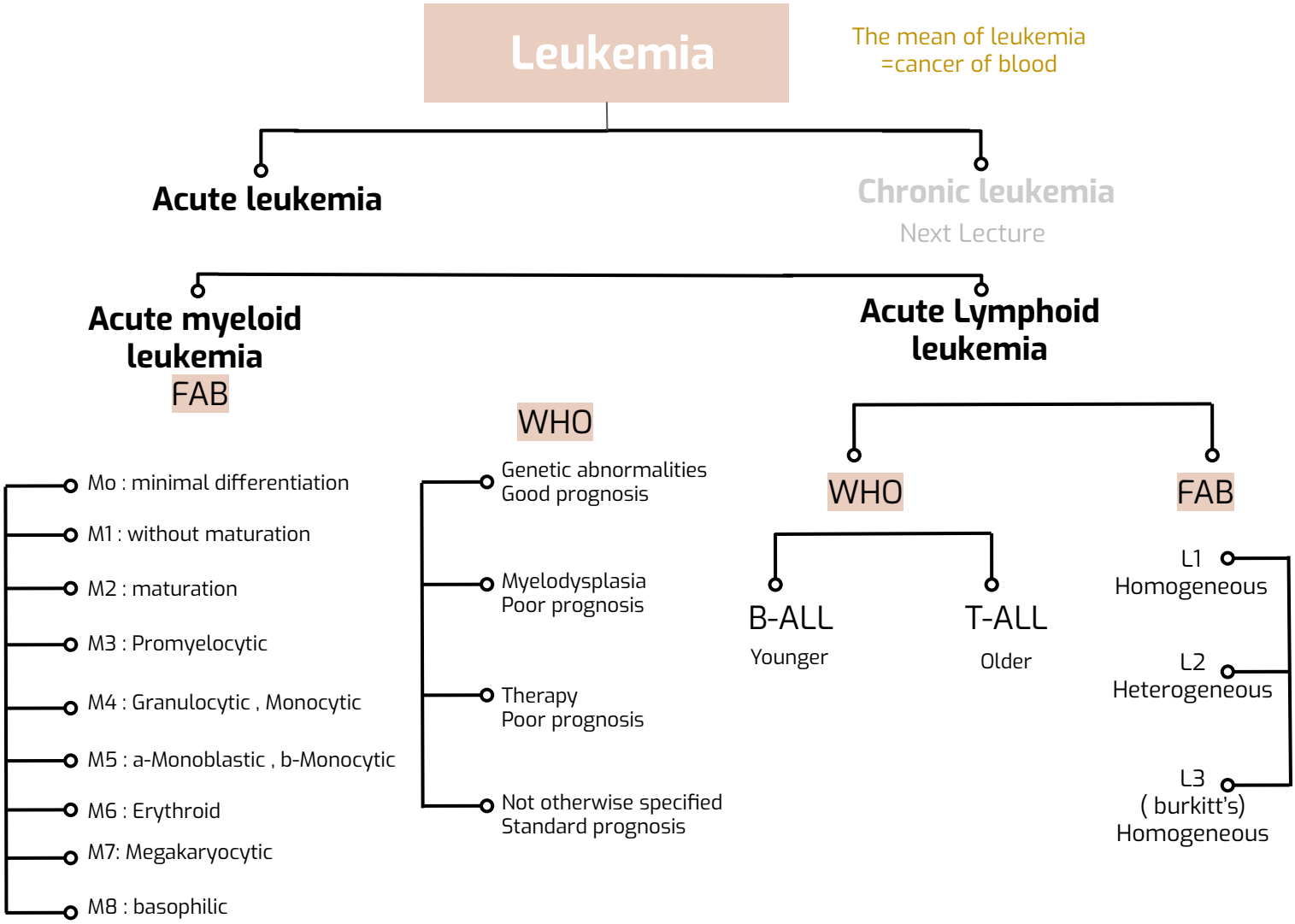
Editing file

Acute leukemia

Medicosis



HISTORY

It means White blood in greek , it was named by pathologist Virchow in 1845 , and was Classified by FAB(French - American - British) classification system in 1976 , then REclassified again by World health organization in 2001 and 2008

Definition

Acute Leukemia is: an Aggressive malignant hematopoietic disorders Caused by Accumulation of abnormal blasts (Immature precursors of WBCs)decreased mature forms and increased immature forms called blasts,in bone marrow and blood leading to:

1-Bone marrow failure (anemia , neutropenia , thrombocytopenia)

Acute symptoms are secondary to marrow failure, which can produce decreased erythrocytes (causing anemia and fatigue), decreased leukocytes (permitting infections and fever), and decreased platelets (inducing bleeding).

2-Organ infiltration (hepatosplenomegaly , lymphadenopathy)

Infiltration is the diffusion or accumulation (in a tissue or cells) of foreign substances or in amounts in excess of the normal. The material collected in those tissues or cells is called infiltrate.

Acute leukemia

PATHOGENESIS

Is unknown but it can be these causes (infection...etc) which all lead to a sequence of genetic alterations of immature precursor in bone marrow or the hematopoietic cells

- Genetic predisposition
  Like : P53 mutation
- Environmental factors
- Infection
- Previous therapy
- Other hematological disorders
  End by Acute leukemia

Unknown mechanism

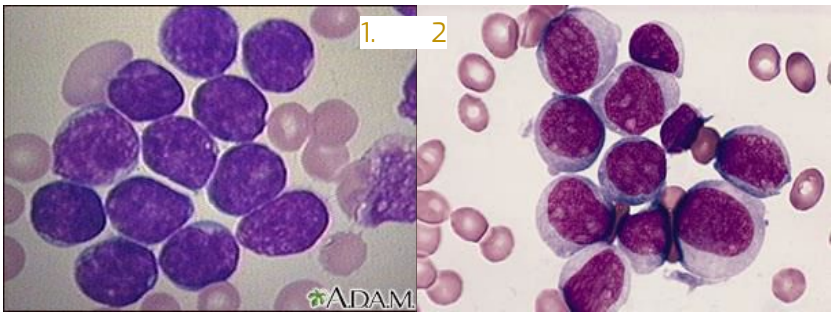
will cause Genetic alteration in the immature precursors ,
which will causes the following(sequence of malignancy of leukemia):-

★ Block of differentiation
Still immature and not maturing

★ Enhanced proliferation

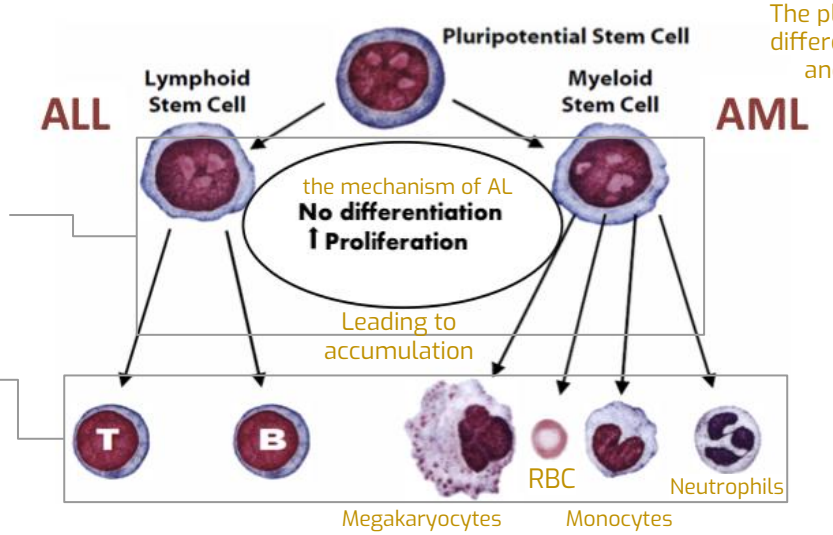
★ Decreased apoptosis

Normally the cells differentiate and proliferate (لعدد معين) and undergo normal cell death (بعد وقت معين)



(1-2) =
This abnormal malignant cells in Bone marrow in Acute leukemia called blast(حصلو ال)
BM infiltrated by these precursors)

We have 2 types of leukemia:
- If the leukemia in the lymphoid cells (stop the maturation of B&T) = ALL
- If it in myeloid =AML



In this stage will be acute and undifferentiation

In this stage it will be chronic

The pluripotent stem cell differentiate into Myeloid and lymphoid cells

- If there were any abnormality with Lymphoid stem cells **acute lymphoblastic leukaemia will occur**
- If there were any abnormality with Myeloid stem cells **acute myeloid leukaemia will occur**

Acute leukemia

EPIDEMIOLOGY

- Acute Leukemia represent about 8% of neoplastic disease & cause about 4% of malignancy related deaths (but highly curable)!
- AML has an incidence of 2 – 3 per 100 000 per year in children, rising to 15 per 100 000 in adults.(more in adult and less in children
- ALL has an incidence of 30 per million & represent about 76% of **childhood** leukemia

GENERAL CLASSIFICATION

Acute leukemia is classified into :

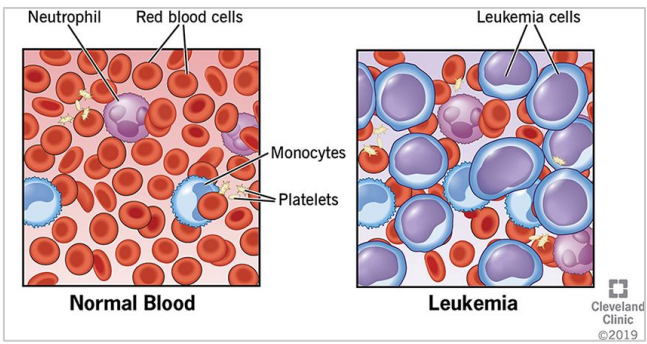
ما قدروا يحددون هي myeloid ولا lymphoid فسموها كذا
- carry both bio phenotype of the 2 (تكون بينهم)

BASIS OF CLASSIFICATION

How we recognize the blast or diagnose (patient)

- Clinical history (Previous therapy)
- **Morphology** .(important to identify the type and the features of cells)
- Flow cytometry.
- Chromosomal Karyotyping
  (study the chromosome of the patient)
- Molecular study
  (latest genetic studies- very important to recognize and know what is the change in the genetic level)

EXTRA



Acute leukemia

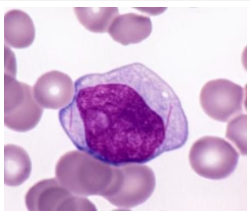
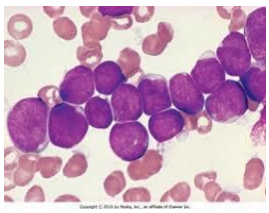
BASIS OF CLASSIFICATION

1- Light microscopy :

(blood smear, bone marrow aspirate & biopsy)

First of all we will use the light microscopy and and blood smear above the requested CBC
And take a piece of the bone marrow to aspirate it and stain it then read it the microscopy

The definition of AL is **Blast count** : it should be > 20 % out of the total cells
normally 3-5%
Is important to let you know what you deal with

Blast **Morphology**	**Myeloblast**	**Lymphoblast**
	 لو تلاحظ في الصورة فيه needle-like bodies هذي هي المقصودة	
Size	medium-Large	small medium
Nucleus	round, oval or irregular	round
Nucleolus	prominent	Not prominent
Cytoplasm	abundant, granular	**Scanty agranular** Almost no cytoplasm
Notes	**Auer rods** (when the cell can release its lysosomes they stick together forming Auer)	may be vacuolated

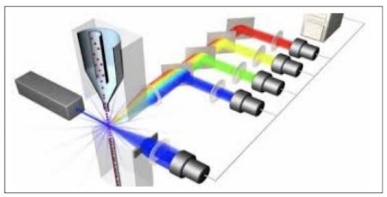
You have to know the morphology so when you go to the clinical you can know the types of AL and the diagnosis of the patient

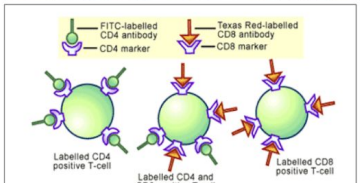
2- flow cytometry

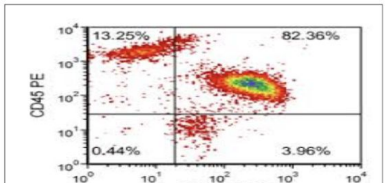
Laser based technology allows for cells counting & detecting of their surface & cytoplasmic markers by suspending them in a stream of fluid followed by analysis through electronic system

It reads the idea of the cell

ينحط على markers عبارة عن
Cell and give you % and the light dosage
And the markers and the you see what's the Marker is +ev which means Ag of the marker is present







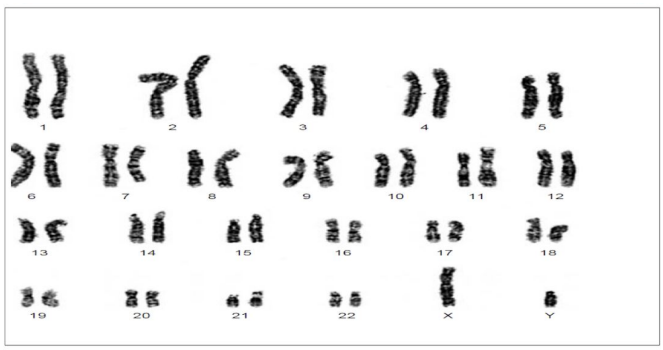
Acute leukemia

BASIS OF CLASSIFICATION

Stem cell markers: (CD34 & TDT) terminal deoxynucleotidyl transverse		
Myeloid	**B-lymphoid**	**T-lymphoid**
MPO ★ **CD13** **CD33** **CD14** **CD64** **CD41** **CD235a**	**CD10** **CD19** **CD22** **CD79a**	**CD3** ★ **CD4** **CD5** **CD7** **CD8**

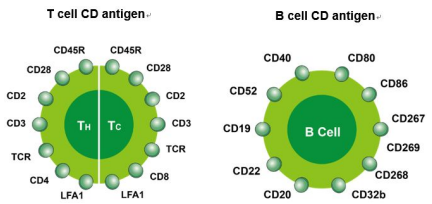
3- Chromosomal karyotype

Set of the chromosomes from one cell during **metaphase** to study the numerical (deletion & trisomy) and structural (translation & inversion) abnormality



Study the number of chromosomes to look if there's trisomy or deletion and the structure
23 chromosomes from each parent

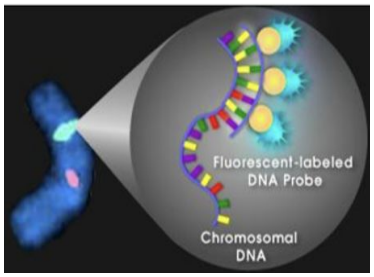
The most common leukemia biomarkers are CD (cluster of differentiation) markers, an extremely diverse series of membrane proteins predominantly expressed on the leukocyte surface.



4- Molecular studies

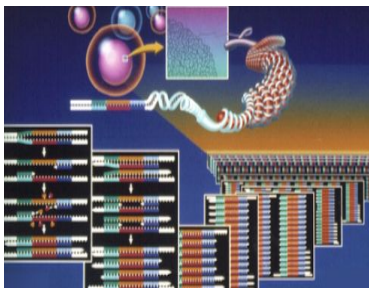
Male dr : you have to understand it .

Several techniques used to detect and localize the presence or absence of specific DNA sequences on chromosomes



Fluorescent In-situ hybridization (FISH)

Is the test that "maps" the genetic material in a person's cells. This **test** can be used to visualize specific genes or portions of genes



Polymerase Chain Reaction (PCR)

It is fundamental to much of genetic testing including analysis of ancient samples of DNA and identification of infectious agents. Using PCR, copies of very small amounts of DNA sequences are exponentially amplified in a series of cycles of temperature changes.

RECURRENT GENETIC ABNORMALITIES

Finding of chromosomes and molecular study

Female dr : (karyotype only)
Common and important

AML

- **Common recurrent genetic finding in patient with AML**

Molecular	★ Karyotype
AML 1-ETO	t (8;21)
CBFB-MYH11	t (16;16) or inv (16)
PML-RARA	t (15;17)
MLLT1-MLL	t (9;11)

ALL

Molecular	★ Karyotype
BCR-ABL1	**t (9;22)**
AF4-MLL	t (4;11)
ETV6-RUNX1	t (12;21)
IL3-IGH	t (5;14)

لا تحفظ الجدولين اهم شيء تعرف t(8;21) معناها ان
في segment من كروموسوم 8 يروح للكروموسوم 21 والعكس

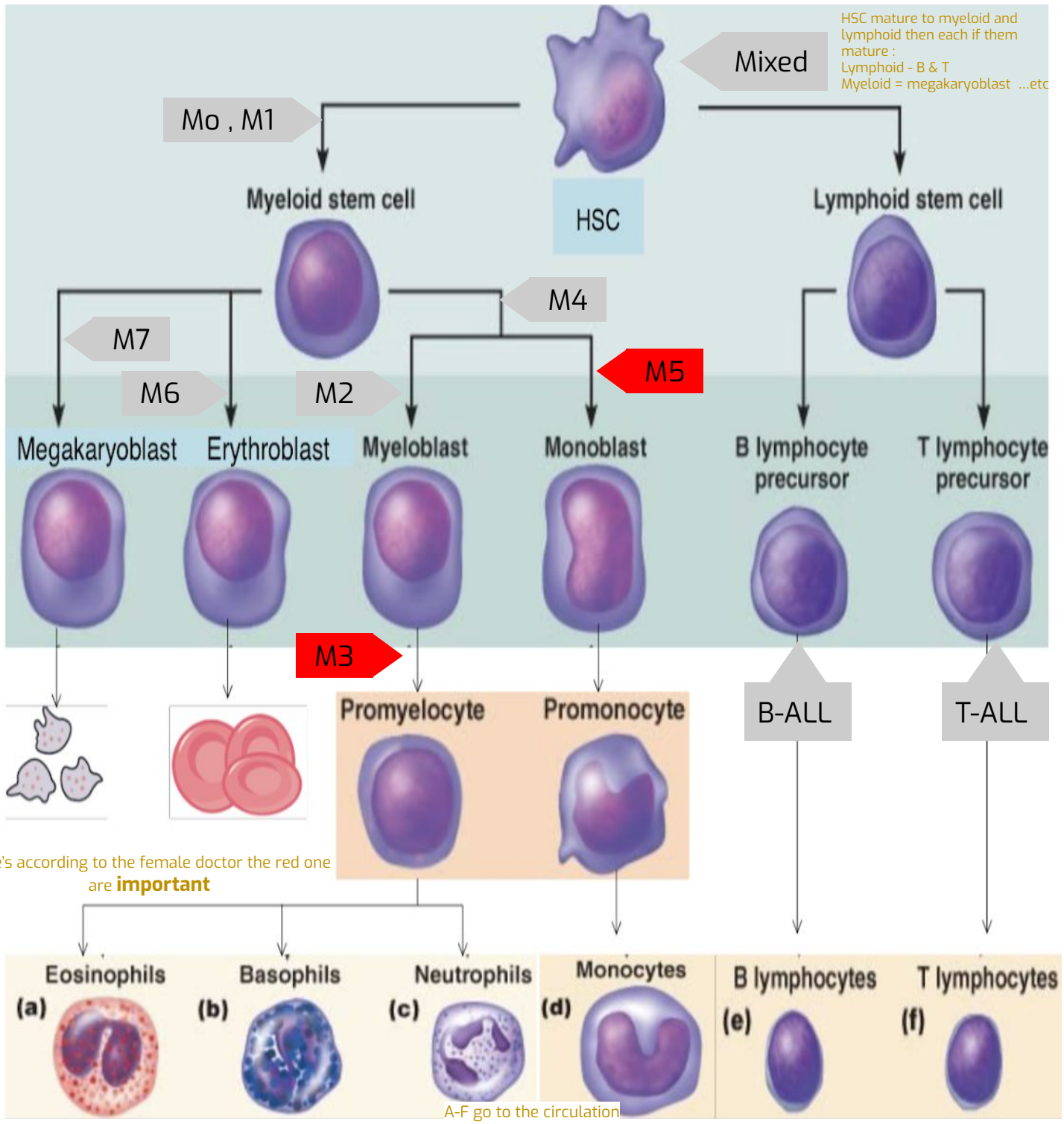
t(15;17) الاخطر بينهم واذا مابديت علاج خلال 24 ساعة المريض راح يموت بسبب intracranial hemorrage
في المقابل اذا بديت العلاج خلال 24 ساعة بنسبة 90% راح يتعالج

ACUTE MYELOID LEUKEMIA (AML)

Medicosis

- Group of hematopoietic neoplasms caused by proliferation of malignant myeloid blasts(so it's the main abnormally of AML) in bone marrow and blood
- **The blast is equal to 20% or more, or t(8;21) t(16;16) or t (15;17)**
- More in adults (do occur in infants) , **Worse than ALL**

Male dr:
if you understand it you will not need to memorize it .
لكنه شرحها بشكل مفصل فانت المفروض تعرف كل شيء في الصورة فبتحتاج تحفظ يعني :)
Female Dr:
These cells اللي بالمربع الاخضر
never leave the BM
All you have to know is the where is the defect and what's giving to you
Ex : B lymphocyte precursor cells = give you B-ALL



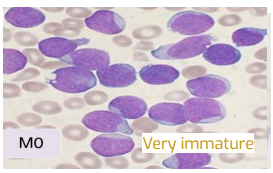
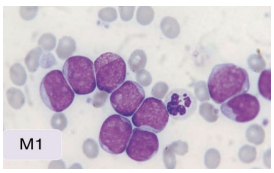
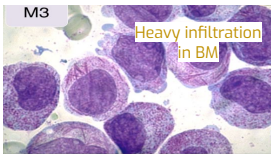
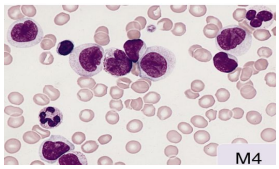
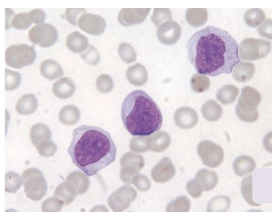
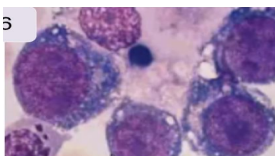
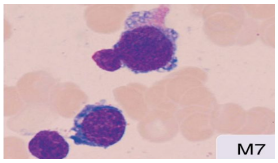
Note's according to the female doctor the red one are **important**

A-F go to the circulation

ACUTE MYELOID LEUKEMIA (AML)

FAB CLASSIFICATION
Based on morphology and flow cytometry

Dr's female: very important Q =
لما اقولك يعتمد ال
WHO in the classification of AML is genetic abnormalities while the FAB on the morphology and flow cytometry markers

Subtype Male's dr **Imp**	**Features**	**Genetics in WHO**	**Notes**	**Pics** Male's + female's dr : no need to memorize them
M0	Minimal differentiation	-		
M1	Without maturation	-		
M2	With maturation	t(8;21)		
M3	Promyelocytic	t(15;17)	DIC	
M4	Granulocytic and monocytic	t or inv(16;16)	Gum hypertrophy	
M5	**Monoblastic (M5a)** **Monocytic (M5b)**	t(9;11)	Gum hypertrophy	
M6	Erythroid	-	CD235a	
M7	Megakaryocytic	-	CD41	
M8	Basophilic	-		

ACUTE MYELOID LEUKEMIA (AML)

AML CLASSIFICATION (WHO)

Based on molecular and cytogenetic

Male's dr:
Molecular and cytogenetic مستقبل الطب ولازم تقوي نفسك في الـ

AML with recurrent genetic abnormalities	Myoplasia related AML	Therapy related AML	AML, not otherwise specified (FAB)
• t(8;21) • t(16;16) • t(15;17) Prognosis: **Good**	• 20% • Significant dysplasia Prognosis: **Poor**	• Blasts is 20% or more • Previous chemotherapy for any malignancy Prognosis: **Poor**	• Blasts are 20% or less • Genetic: normal • No dysplasia Prognosis: **Standard**

CLINICAL FEATURES

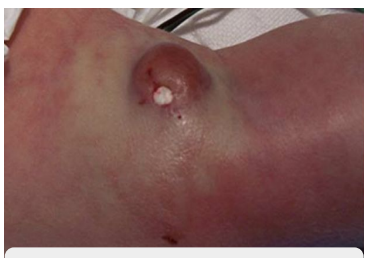
1- Pancytopenia: (acute onset)

In AML have BM infiltration so it's cause pancytopenia

↓WBC→Infection,Fever,Septic shock (المقصود هنا ال functional WBC)

↓Hb→ Anemia(fatigue, headache,pallor,SOB...)

↓platelets→bleeding(bruisis,epistaxis,menorrhagia...)



Myeloid sarcoma

2- Organ infiltration:

Lymphadenopathy(rare)

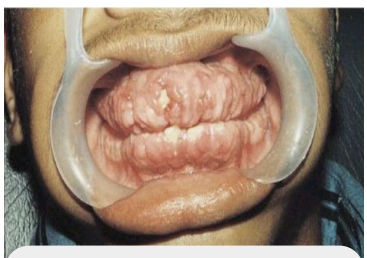
Hepatosplenomegaly

Myeloid sarcoma

Gum hypertrophy

CNS disease

} More With Acute **M5** Monoblastic leukemia



Gum hypertrophy

gum increased

لدرجة الاسنان ماتنشاف

3- Leukostasis (increase blood viscosity)

4- Disseminated Intravascular Coagulation (DIC)
(more with Acute promyelocytic Leukemia M3)

Widespread activation of coagulation system leading to intravascular fibrin deposition and consumption of platelet and coagulation factors which can be manifested as bleeding (85%) or Thrombosis(15%)

ACUTE MYELOID LEUKEMIA (AML)

CASE STUDY

- 65 yo Male presented to ER with Fatigue, Fever, and nose bleeding for 2 weeks
- O/E : moderate hepatosplenomegaly & multiple bruises.
- **CBC** : WBC40*109L **HB** : 7g/dl **PLT**: 51*109/l

Blood smear & bone marrow:

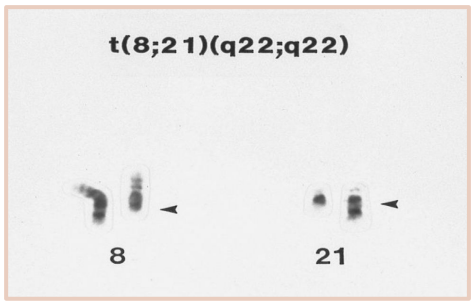
AML with maturation (M2) FAB

Flow cytometry :

The blast are positive for CD34,CD13.CD33,CD117 and **MPO**(character of myeloid)
THEY are negative for CD3 T cell, 'CD10,CD19,CD79a B cell'

Karyotype :

The final diagnosis: AML with t(8,21) (WHO)



PROGNOSIS :

Better prognosis

- Genetics t(8;21). inv(16;16) or t(15;17)
- Age<60
- Primary better than secondary(is from other disease so it has Poor prognosis)

TREATMENT :

Chemotherapy

- AML: M0-M8 but not M3 (same protocol)
- AML: M3 (ATRA(all trans retinol Vitamin A) or arsenic)
- Stem cell transplantation

ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

Medicosis

- Acute leukemia characterized by proliferation of malignant lymphoid blast in bone marrow and blood
- B and T cells
- More common in children is the most common malignancy of childhood.
- Better than AML (has better prognosis even if it's in the adult)

CLINICAL FEATURES

1- Pancytopenia[1]:

↓WBC⟶Infection,Fever,Septic shock المقصود هنا ال funcional WBC

↓Hb⟶ Anemia(fatigue, headache,pallor.SOB...)

↓platelets⟶bleeding(bruisis,epistaxis,menorrhagia...)

Acute onset

2- Organ infiltration:

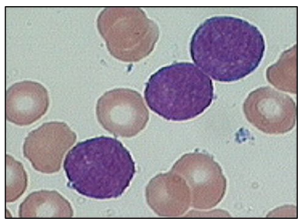
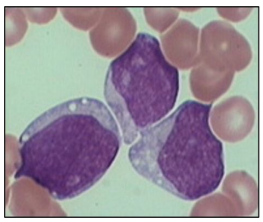
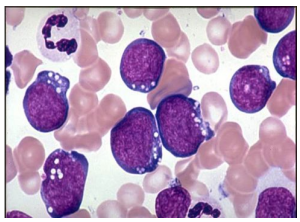
- **lymphadenopathy**(Very common) and less in AML
- Hepatosplenomegaly Morebin AML
- **Testicular involvement** In male
- **Mediastinal MASS (characteristic of T-ALL)**
- CNS disease

Characteristic for T-ALL

1- because there's heavy infiltration of lymphoblasts occupying all BM production
So the BM يصير متجهة اكثر for lymphoblastic production which result in =
A-no matureation
B- decreased other cells
بالتالي ماحيصير عندي انا
Normal WBC & platelets production and even more sometimes the bone marrow lake of megakaryoblast (the mother of platelets

ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

MORPHOLOGICAL SUBTYPES (FAB)

	L1	L2	L3 (Burkitt's)
Morphology	Homogenous	Heterogeneous	Homogenous
Size	Small	Variable(larger than L1)	Small
Cytoplasm	Little	More	**Vacuolated**
Nucleoli	Not Prominent	Prominent	Prominent
Genetics	Variable مافي شيء يميزه بالذات	Variable مالها شيء مخصص لها بس ممكن انها Associated with any chronic abnormalities	**t(8;14) c-myc**
			 L3 (Burkitt's): mature lymphoid neoplasm -a type of lymphoma NOT Acute lymphoblastic leukaemia حصلوه more with lymphoma

IMMUNOPHENOTYPIC SUBTYPES (WHO)

	B cell	T cell
Markers I have to see these marker +ev to tell is it B or T	CD19 CD10 CD79a	CD3
Percentage	Most of ALL is 80% B cell	20%
Age	More in Younger	More in Older
Clinical	–	• **Mediastinal mass** More character to T • CNS relapse
WBC count	Less	If I have Higher WBC is more T cell
Prognosis	Better	Most T cell have Worse
Genetics	T(9;22) T(4;11) T(12;21)	–

ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

TYPE OF ALL ' how we differentiate between the mature and precursor cells

Both are + CD3 then we look is is C or S CD3

	**B-ALL			**T-ALL	
Cell stage	precursor		mature	precursor	mature
Markers	Stem cell markers (CD34 + TDT)	CD34 TDT **CD10**	Surface immunoglobulin	Completely Positive or negative both CD4+CD8	CD4+CD8 One positive and other negative
	باختصار لو كان عندي-1 +ev CD10 and +ev CD19,20,79a or +ev CD34 TDT and +ev CD19,20,79a is more likely to be or you're dealing with B-ALL (precursor B cell)	CD19 CD20 CD79a	باختصار لو كان عندي-2 -ev precursor B cell and +ev surface immunoglobulin and +ev CD19,20,79a is more likely Burkett's (you're dealing with mature cell)	**+cCD3** C=cytoplasm	**+sCD3** S=surface
				CD2 CD5 CD7 Dan-T cell marker	
Disease	B-ALL[1]	Common B-ALL	Burkitt[2] lymphoma	T- Acute lymphoid leukemia	T-cell lymphoma

PROGNOSIS

	Better	**Worse**
Age	**2-10 yrs**	Less or more
Gender	Female	Male
WBC count	Low	High
Cell type	B cell	T cell
B-ALL phenotype	Common(CD10)	Others
B-ALL genetics	Hyperdiploidy[3] t(12;21) 3-number of chromosomes is more than **50**	Hypodiploidy[4] t(9;22) اقل من 46 chromosome 4-number of chromosomes is less than 45
CNS involvement	No	Yes

TREATMENT

- **Chemotherapy** high cure rate
- **Stem cell transplantation**

REMEMBER !

- Acute leukaemia is a fatal neoplastic condition
- 20% or more blasts = Acute leukaemia
- Diagnosis requires special investigations is not easy you have to make steps to reach it
- Auer rods = AML (diagnostic character)
- AML M3 = DIC &target therapy
- Gum hypertrophy = mostly M4 or M5,
- Mediastinal = T-ALL
- Subtypes of AML (M0-M8) + cytogenetic abnormalities
- Subtypes of ALL (T or B cell)
- Main lineages markers are MPO 'myeloid', CD19 'B cell' and CD3 'T cell'
- 'Very immature marker" Stem cell markers are CD34,TDT
- **FAB** classification based mainly on morphology
- **WHO** classification focused more on genetics

Summary

Acute Myeloid Leukemia (AML)

Definition	A group of hematopoietic neoplasms caused by proliferation of malignant myeloid blasts in bone marrow and blood. **Primary** AML has **better** prognosis than Secondary AML
Diagnosis	**Microscopy shows >20% Blast cells OR t(8;21) t(16;16) or t (15;17)**

FAB Classification of AML
based on **flow cytometry and morphology**

AML Type	Cell Type	Karyotype	Character
M2	With Maturation	(8,21)	
M3	**Promyelocytic**	**(15,17)**	**DIC (Emergency leukemia)**
M4	Granulocytic + Monocytic	(16,16)	Gum Hyperplasia
M5	**Monoblastic + Monocytic**	**(9,11)**	**Gum Hyperplasia, CNS Disease**

WHO Classification of AML
based on **molecular and genetic studies**

- AML with recurrent genetic abnormalities either **t(8;21), t(16;16), or t(15;17) = good prognosis**
- AML with significant dysplasia = poor prognosis
- AML with previous chemotherapy = poor prognosis

Basis of Classification
Clinical history, Morphology, Flow cytometry, Chromosomal Karyotyping, Molecular study

Morphology	**Microcopy: Auer rods present**	**Signs & Sym**	1- Pancytopenia: ↓**WBC**⟶Infection, ↓**Hb**⟶ Anemia 2- Hepatosplenomegaly 3- Leukostasis 4- Disseminated Intravascular coagulation **(with Acute Promyelocytic Leukemia, M3)**
Flow cytometry	**Positive for:** **Stem cell marker (CD34)** and Myeloid marker **(MPO)**		
Age Group	Adult (good prognosis if **less** than 60)		
Treatment	1-Chemotherapy (For M3 Atra/ Arsenic) 2- Stem cell replacement		

Recurrent Genetic Abnormalities

Molecular	Karyotype
AML 1-ETO	t (8;21) good prognosis
CBFB-MYH11	t (16;16) or inverted (16) good prognosis
PML-RARA	**t (15;17)** good prognosis
MLLT1-MLL	t (9;11)

Summary

Acute Lymphoid Leukemia (ALL)

Definition	A group of hematopoietic neoplasms caused by proliferation of malignant **lymphoid blasts** (B and T cells) in bone marrow and blood. Better prognosis than AML.
Diagnosis	**Microscopy shows >20% Blast cells.**
FAB Classification	Based on **flow cytometry and morphology** (1) L1: Homogeneous (2) L2: Heterogeneous (3) L3: Homogeneous (Burkitt's Lymphoma)
WHO Classification	Based on **molecular and genetic studies** (1) B- ALL, better prognosis (2) T-ALL, worse prognosis

Basis of Classification

Clinical history, Morphology, Flow cytometry, Chromosomal Karyotyping, Molecular study

Morphology	Vacuolated	**Signs & Sym**	1- Pancytopenia ↓WBC⟶Infection,, ↓Hb⟶ Anemia 2- Hepatosplenomegaly 3- **lymphadenopathy**(Very common) 4- **Testicular involvement** **5- Mediastinal mass (T-ALL)**
Flow cytometry	**Positive for:** in T-cell ALL (CD3) in B-cell ALL (CD19)		
Age Group	Children		

Recurrent Genetic Abnormalities

Molecular	Karyotype
BCR-ABL1	**t (9;22), worse prognosis** (Philadelphia chromosome)
AF4-MLL	t (4;11)
ETV6-RUNX1	**t (12;21), better prognosis**
IL3-IGH	t (5;14)

Disease Depends on cells type and stage

	T-ALL		B-ALL		
Cell stage	Precursor	Mature	Precursor		Mature
Markers	Completely Positive or negative both CD4+CD8	CD4+CD8 One positive and other negative	Stem cell markers (CD34 + TDT)	CD34 TDT **CD10**	Surface immunoglobulin **t(8;14) c-myc**
	+CD3,2,5,7		**+CD19,CD10 CD79a**		
Disease	T- Acute lymphoid leukemia	T-cell lymphoma	B-ALL	Common B-ALL	**l3-Burkitt** lymphoma
Signs	**1-Mediastinal Mass** 2- Pancytopenia 3- Lymphadenopathy	1- Pancytopenia 2- Lymphadenopathy			

Quiz

Q1) Lymphadenopathy more common in :							
A	AML	B	ALL	C	BOTH	D	 شدعوه واضحه
Q2) Auer rods **characteristic for**							
A	AML	B	ALL	C	BOTH	D	 شدعوه واضحه
Q3) FAB CLASSIFICATION based on :							
A	Morphology and cytogenetic	B	Cytogenetic and molecular	C	Molecular and flow cytometry	D	None :)
Q4) we will see gum hyperplasia in which subtype ?							
A	m3	B	M4 - M5	C	m5	D	M3 -M4
Q5) B- ALL genetics in Better prognosis:							
A	Hyperdiploidy t(12;21)	B	Hypodiploidy t(9;22)	C	Hypodiploidy t(12;21)	D	Hyperdiploidy t(9;22)
Q6) L3 Burkitt's is:							
A	mature lymphoid neoplasm	B	More common in Acute lymphoblastic leukaemia	C	Precursor lymphoid neoplasm	D	Less common with lymphoma
Q7) FLOW CYTOMETRY was positive to CD3 and negative to CD10 , CD19 , and MPO .Those results will indicate which type of AL?							
A	AML	B	B-ALL	C	T-ALL	D	CML
Q8) Mediastinal mass characteristic for :							
A	AML	B	B-ALL	C	T-ALL	D	CML

Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
B	A	d	b	A	A	C	C

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