Dall'esame morfologico alla citofluorimetria

Esame morfologico del Midollo Osseo

Susanna Fenu
Coordinamento Board Morfologia e Board attività educazionale e divulgative per Lab.Net GIMEMA AML-MDS Roma



AIEOP
ASSOCIAZIONE ITALIANA EMATOLOGIA ONCOLOGIA PEDIATRICA

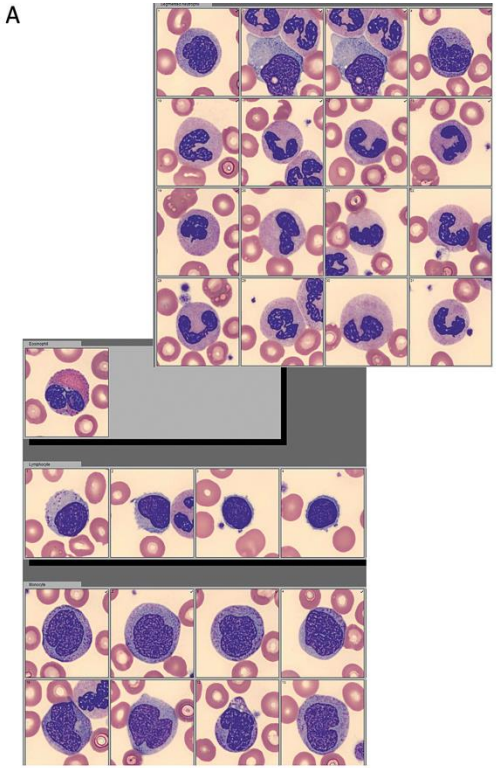
AIEOP GIOVANI
LECCE, 12 ottobre 2026
HILTON GARDEN INN

Disclosures of Name Surname

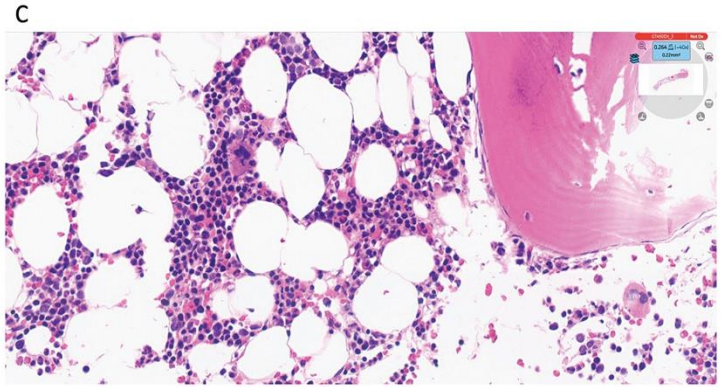
Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Jazz							

Elizabeth L. Courville, MD, Technology Spotlight
Digital Microscopy and Artificial Intelligence in Bone Marrow Evaluation: Where Are We Now and Where Are We Going?









REVIEW ARTICLE INTERNATIONAL JOURNAL OF LABORATORY HEMATOLOGY

ICSH guidelines for the standardization of bone marrow specimens and reports

S.-H. LEE*, W. N. ERBER†, A. PORWIT‡, M. TOMONAGA§, L. C. PETERSON¶ For The International Council for Standardization In Hematology

Table 1. Indications for bone marrow examination

Investigation of unexplained anaemia, abnormal red cell indices, cytopenias or cytoses
Investigation of abnormal peripheral blood smear morphology suggestive of bone marrow pathology
Diagnosis, staging and follow-up of malignant haematological disorders (e.g. acute and chronic leukaemias, myelodys… mastocyt…
Investigation of suspected bone marrow metastases
Unexplained focal bony lesions on radiological imaging
Unexplained organomegaly or presence of mass lesions inaccessible for biopsy
Microbiological culture for investigations of pyrexia of unknown origin or specific infections, e.g. military tuberculosis, leishmaniasis, malaria
Evaluation of iron stores
Investigation of lipid/glycogen storage disorders
Exclusion of haematological disease in potential allogeneic stem cell transplant donors

QUANDO FARE UN ASPIRATO MIDOLLARE??????

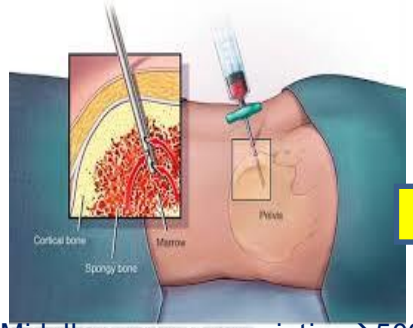
Table 2. Collection and processing of bone marrow aspirate and core biopsy specimens

Specimen	Test	Anticoagulant or media	Fixative	Further processing	Staining (no. of slides or sections)*
Aspirate	Smear (6 slides)	None/EDTA	Air dry, methanol-fix	–	MGG or Wright stain (2 slides), Prussian Blue (1 slide), cytochemistry
Aspirate	Squash (≥2 slides)	None/EDTA	Air dry, methanol-fix	–	MGG or Wright stain (1 slide), Prussian Blue (1 slide)
					…, IHC,
Aspirate	Molecular genetics	EDTA	to specific protocols		
Aspirate	Cytogenetics, FISH	Sterile tissue culture media e.g. RPMI with 10% bovine fetal serum			
Aspirate	Microbiology	Sterile plain or heparinized tubes, lysis centrifugation tubes or culture media			
Core biopsy	Histology	–	NBF, AZF, B5, Bouin's, etc.	Decalcify, paraffin embed, cut sections	H&E (2-4 sections), reticulin (1 section), Giemsa, IHC, histochemistry, FISH
Core biopsy	Touch imprint (≥2 slides)	–	Air dry, methanol-fix	–	MGG or Wright stain (1 slide), cytochemistry

*Several smears and imprints should be left unstained for possible immunostains, cytochemical stains, FISH or DNA extraction. Additional sections of particle clots and BM biopsy specimens should be cut as required.

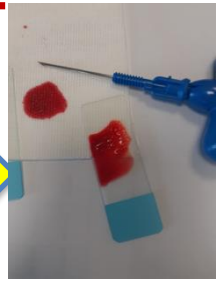
AZF, acetic acid–zinc–formalin; B5, mercuric chloride, sodium acetate and formalin; EDTA, ethylenediamine tetra-acetic acid; FISH, fluorescent *in-situ* hybridization; H&E, haematoxylin and eosin; IHC, immunohistochemistry; MGG, May–Grünwald Giemsa; NBF, neutral buffered formalin.

ASPIRATO MIDOLLARE



Midollo osseo emopoietico→500-700g
Cellule emopoietiche 10^{12} dell' organismo

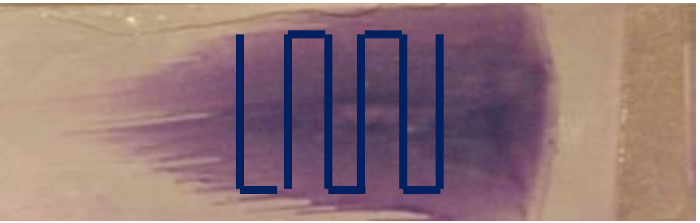
- Aspirare 02-0,3 ml per morfologia
- per altri indagini cambiare sede / siringa
- Evitare diluizione con sangue periferico
- Strisciare i frustoli di sangue midollare
- Strisciare almeno 6 vetrini







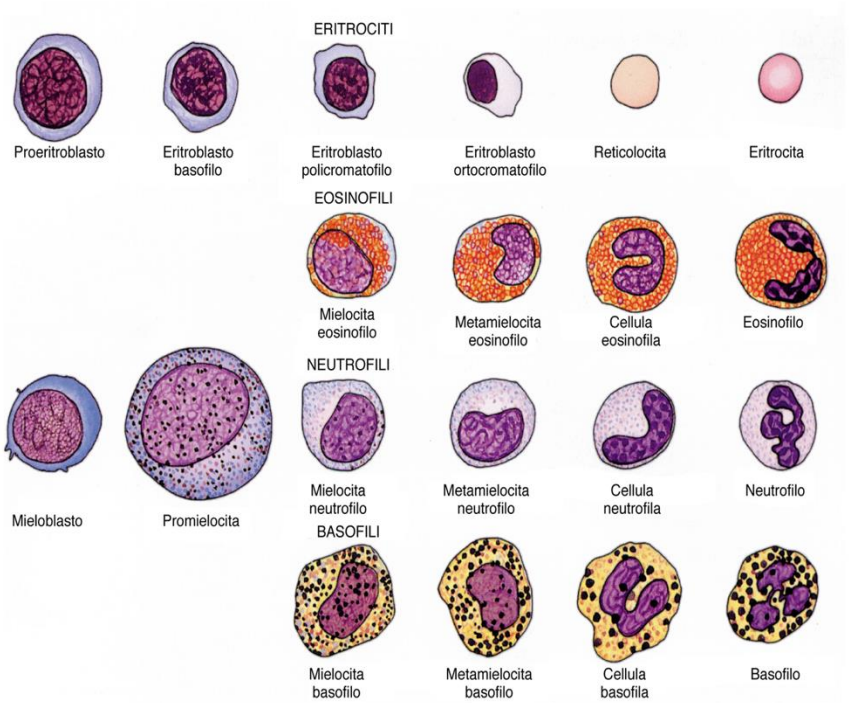
Scelta dell' area più rappresentativa



La valutazione del vetrino al microscopio ottico è un processo **MULTISTEP**:

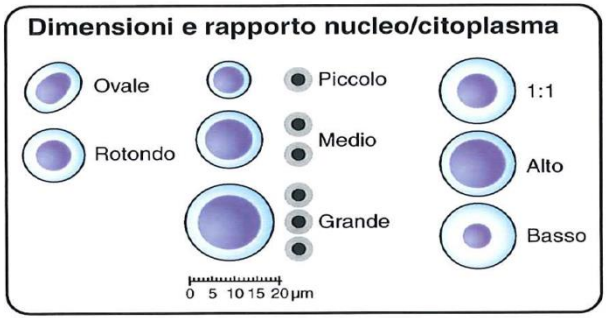
- Obiettivo 10x (low power field high dry - total magnification100 x)
  VALUTAZIONE DELLA QUALITA **Cellularità** Mkegac. Istiociti parassiti mieloftisi ecc'
- Obiettivo 40-50x(high dry total magnification 400-500 x)
  distribuzione delle filiere emopoietiche scelta della migliore zona
- Obiettivo100x (high power field –oil.high magnification 1000x) **definizione delle caratteristiche morfologiche conta differenziale**

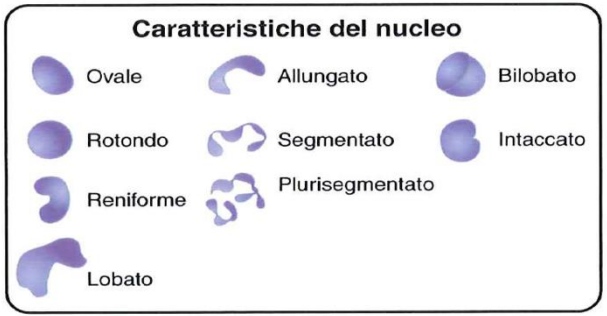
Valutazione morfologica midollo osseo

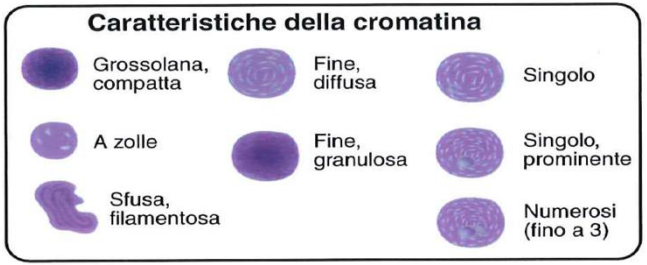


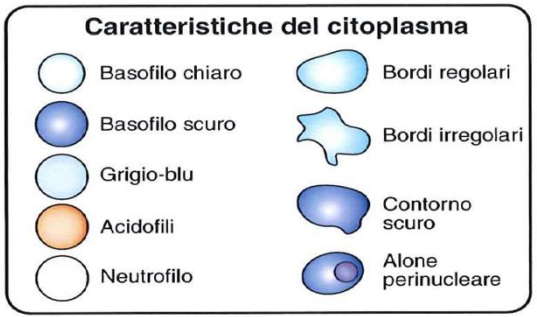
- In ogni serie cellulare tra il BLASTO (cellula immatura) e la cellula definita MATURA esiste una TRASFORMAZIONE GRADUALE verso i vari stadi di differenziazione
- In ogni cellula vi sono componenti (nucleo/citoplasma), ciascuno dei quali va incontro ad una sua trasformazione :
- In condizioni normali essa è simultanea (SINCRONISMO)
- In condizioni patologiche essa è ASINCRONA

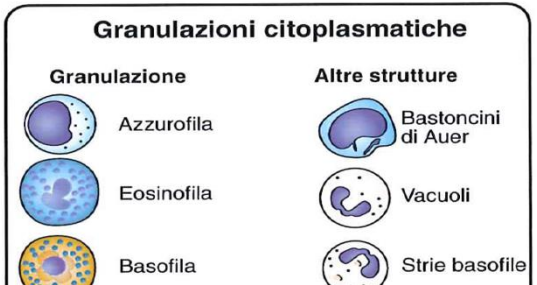
CARATTERISTICHE MORFOLOGICHE DELLA CELLULA EMATOLOGICA





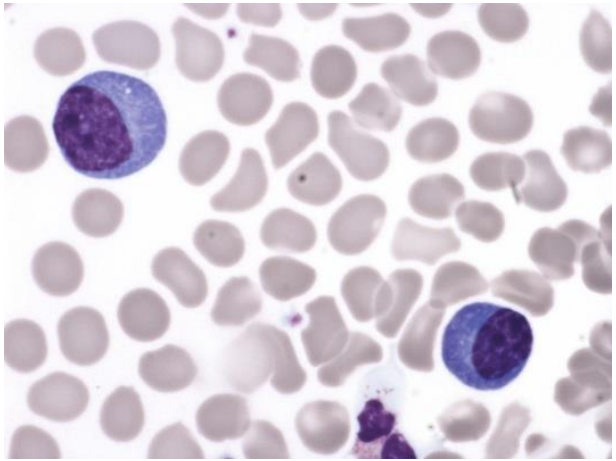


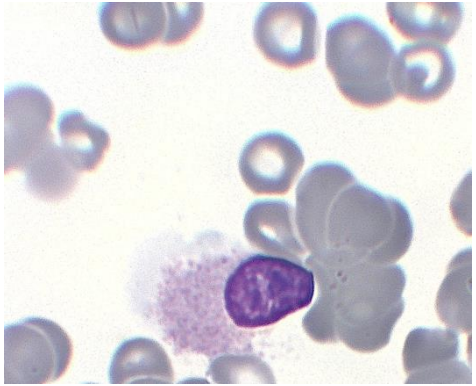


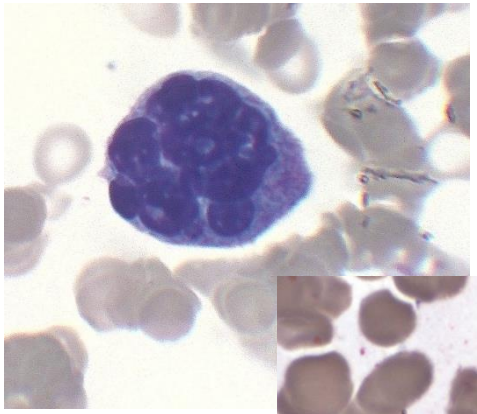


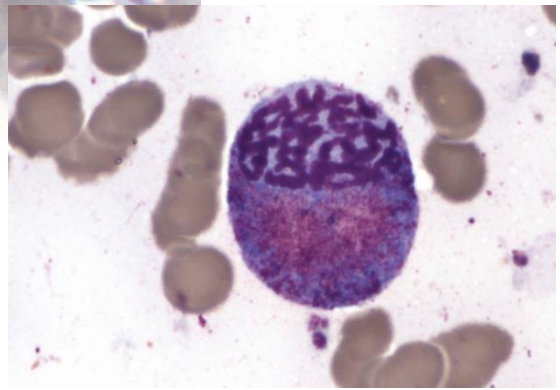
MORFOLOGIA
ISTRUZIONI PER L'USO

??????





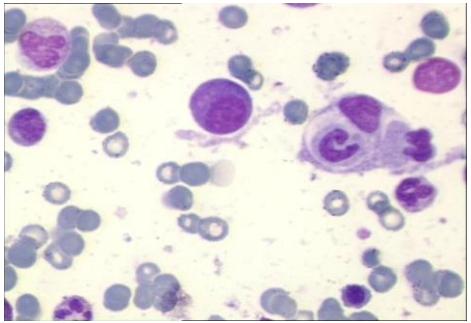
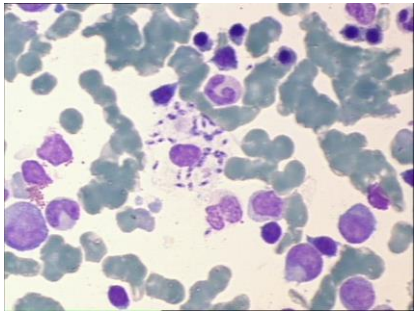
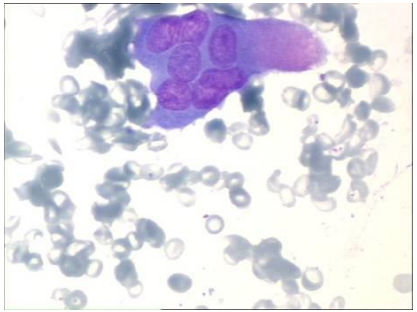
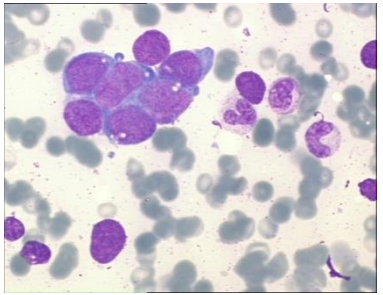




VALUTAZIONE DEL MIDOLLO OSSEO A LPF (100X)

CELLULARITA'
POLIMORFISMO-MONONORFISMO (FILIERE EMOPOIETICHE)
MEGACARIOCITI IN NUMERO:
NORMALE RIDOTTO AUMENTATO ASSENTI
ISTIOCITI-OSTEOCLASTI OSTEOBLASTI
MIELOFTISI
PARASSITI

Lo studio morfologico del midollo ci permette di comprendere lo stato di differenziamento o maturazione delle filiere ematopoietiche, il rapporto equilibrato tra di esse o, nelle condizioni patologiche, la prevalenza di una linea rispetto all'altra ed ancora l'eventuale invasione da parte di cellule atipiche o neoplastiche ematologiche e non

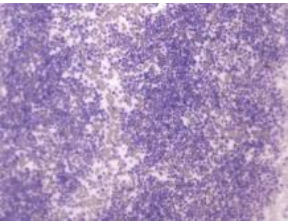
VALUTAZIONE MORFOLOGICA DEL MIDOLLO OSSEO

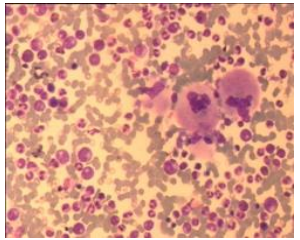
Tabella 6.7. Valutazione quantitativa della cellularità del sangue del midollo osseo ematopoietico

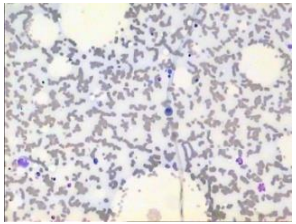
Simbologia	N° di cellule per campo visivo (ingrandimento 10X)	Indicatore numerico	Grado di cellularità del midollo	Criterio di valicabilità
+	15-<100	0,5-1	Ipocellulare	Non valutabile
++/+++	100 – <500	1,5-3	Normale	Valutabile
++++	>500	4	Aumentata	valutabile

- **Istologia: volume %**
- La cellularità è una valutazione semiquantitativa
- Rapporto tra emopoiesi-sroma reticolare –stroma adiposo

ipercellulare **normocellulare** **ipocellulare**



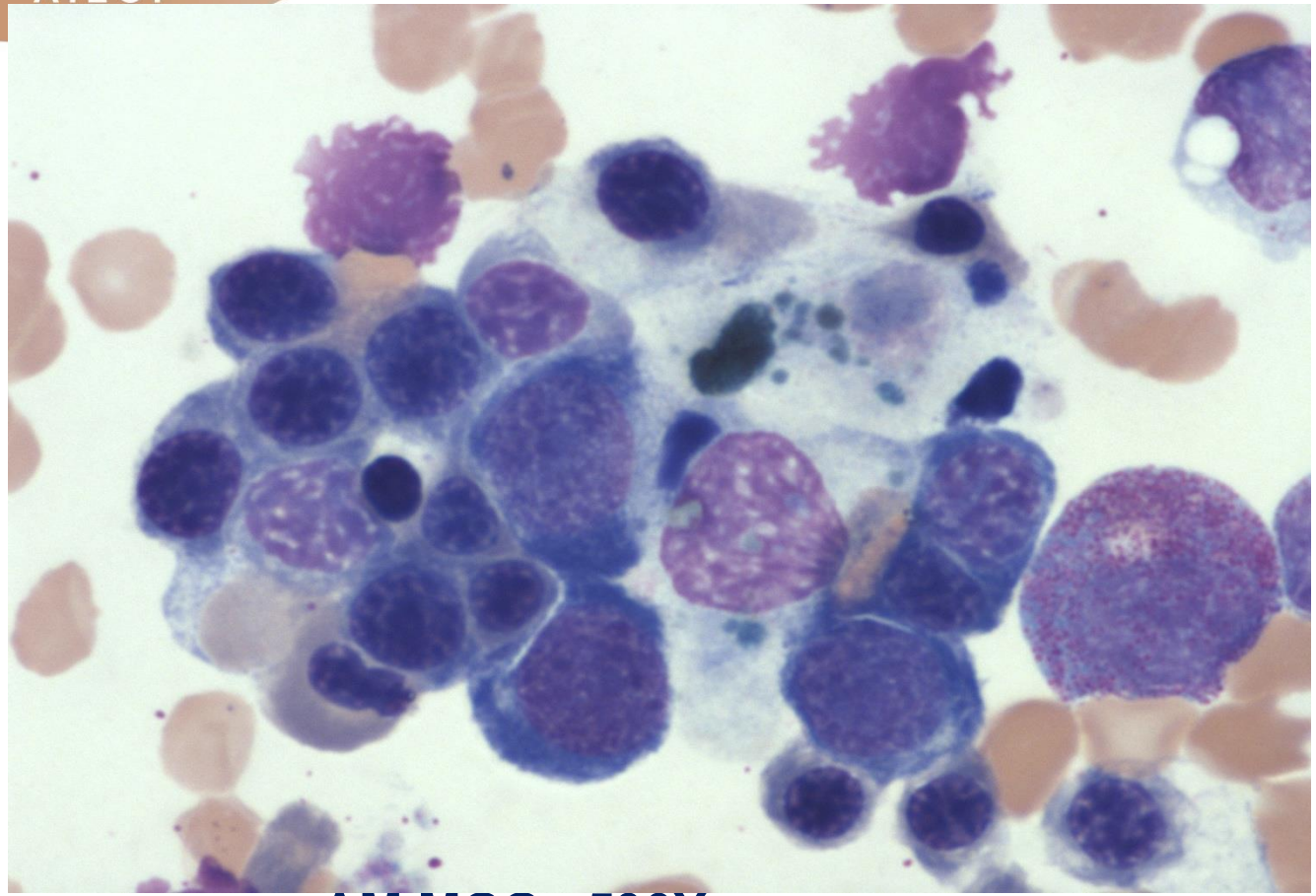




Mielogramma
Conta su 500 cellule nucleate

TIPO CELLULARE	Range di normalità (%)
Serie granuloblastica	**60-70**
Mieloblasti	1-3
Promielociti	1-7
Mielociti Neutrofili	5-20
Metamielociti Neutrofili	10-30
Neutrofili Band -Segmentati	15-25
Eosinofili	0-2
Basofili	0-1
Serie Eritroblastica	**15-35**
Proeritroblasto	0-3
Eritroblasto Basofilo	1-5
EritroblastoPolicromatofilo	5-15
Eritroblasto ortocromatico	5-15
Rapporto L/E*	**1,5-4,5**
Altre cellule	
Linfociti	5-20
Monociti	0-3
Plasmacellule	0-4
Istiociti	<2
Serie megacariocitaria	**1-2 Per campo**

*Rapporto L/E = rapporto tra tutte le cellule della serie granuloblastica e tutte quelle della serie eritroblastica



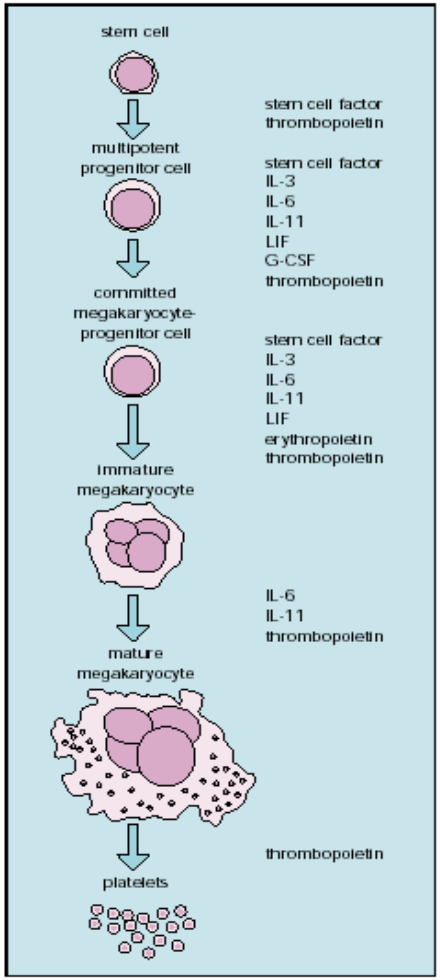
AM MGG 500X

??????????

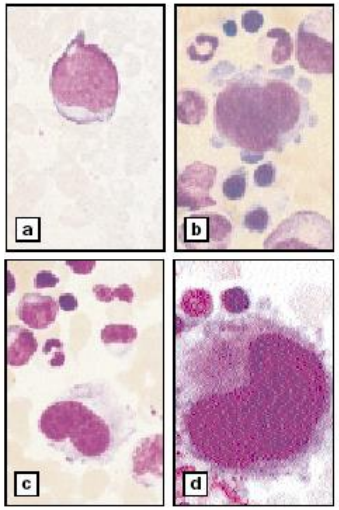
Micrometastasi

TROMBOCITOPOIESI

Fig. a



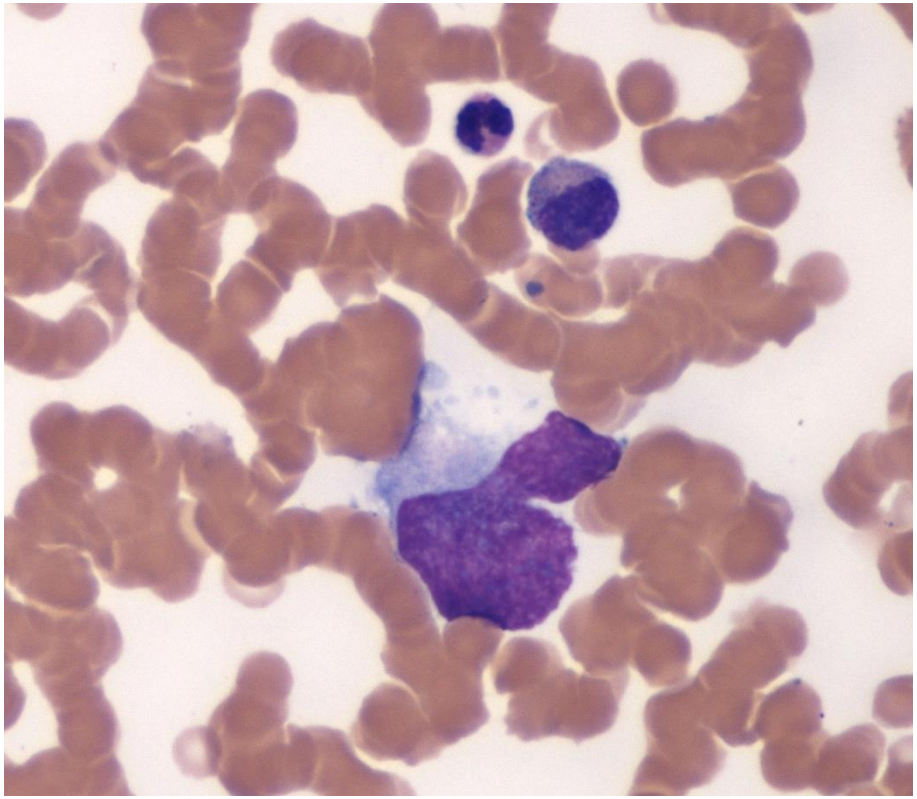
b,c



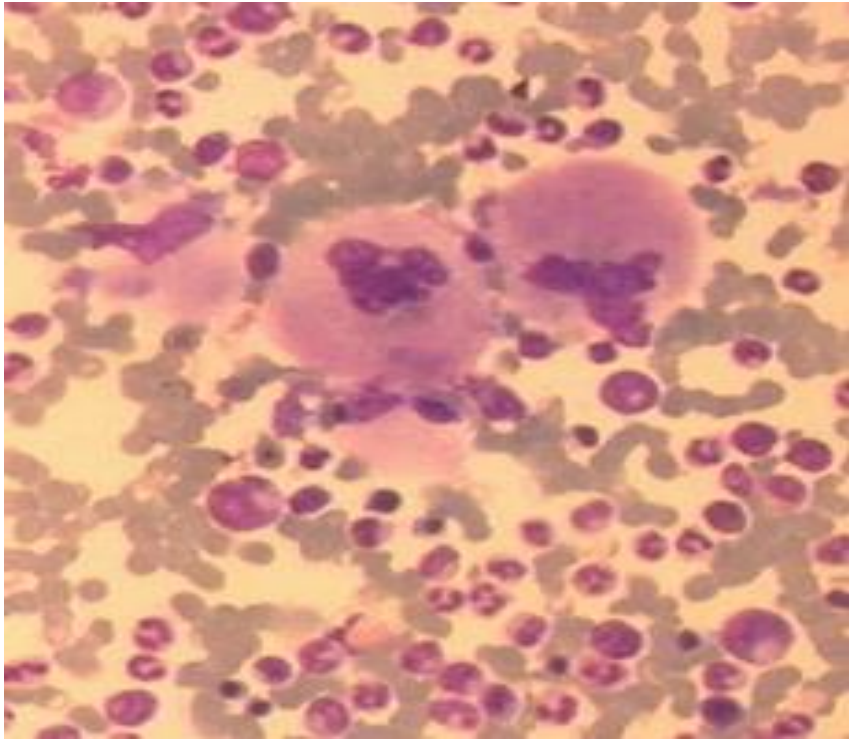
d,e



- **ogni megacariocita genera circa 4.000 piastrine**
- **le piastrine vivono in media 9-12 giorni**
- **ogni giorno sono prodotte 30.000-40.000 piastrine per mL;**
- **in caso di necessita', la produzione di piastrine puo' aumentare** 8 volte;



AM MGG 500x Megacariocita immaturo

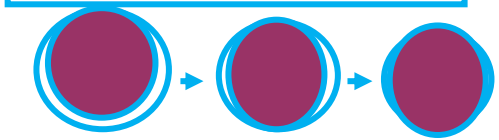


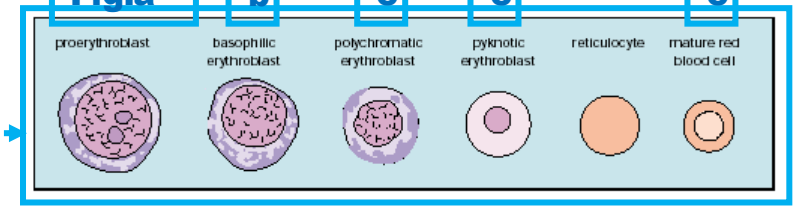
AM MGG 400x Megacariocita maturo

IL-3, GM-CSF

EPO

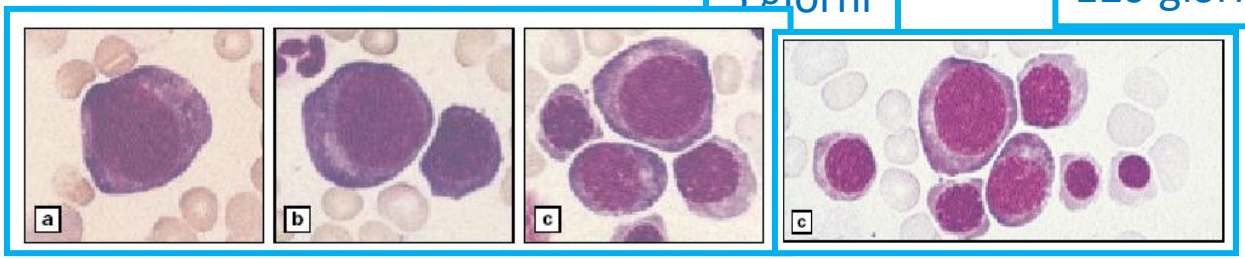
CFU-GEMM BFU-E CFU-E





5 giorni

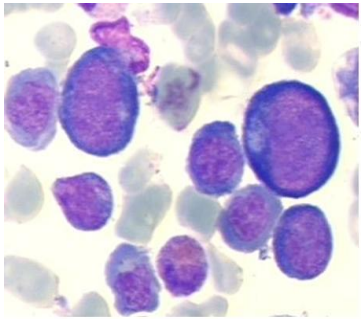
120 giorni



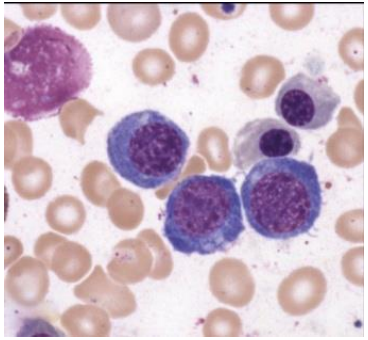
CELLULE DELLA SERIE ERITROBLASTICA

La serie eritroblastica si modifica durante la fase maturativa presentando due principali caratteristiche:
modificazioni cromatiche del citoplasma per la produzione delle catene globiniche;
espulsione del nucleo dall' eritroblasto ortocromatico che si trasforma in globulo rosso maturo privo di nucleo.

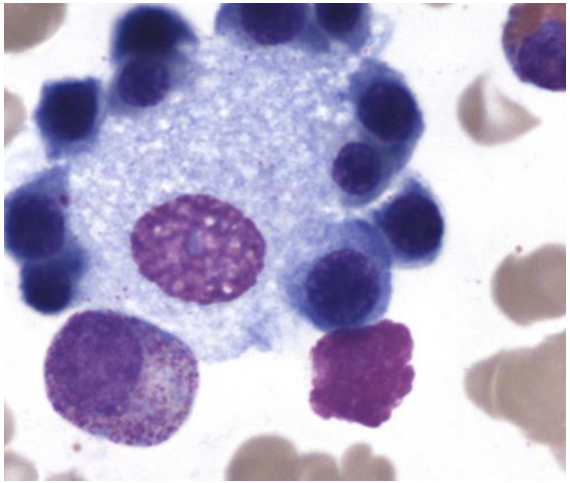
Proeritroblasto-er.basofilo



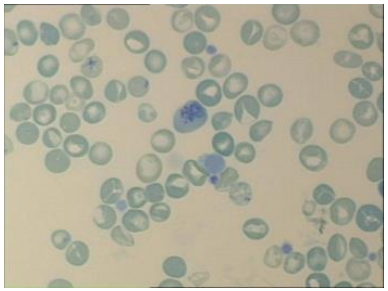
er.basofilo-ortocromatico

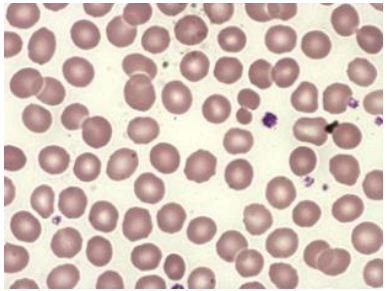


Isola eritroide
Cellula nutrice



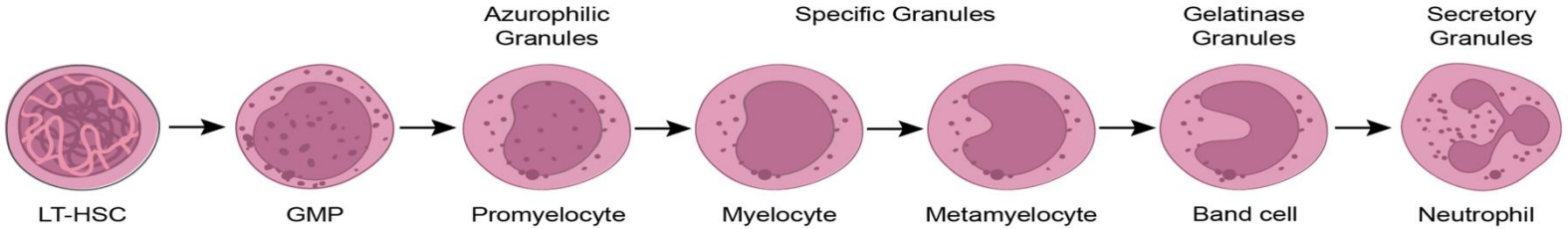
reticolociti

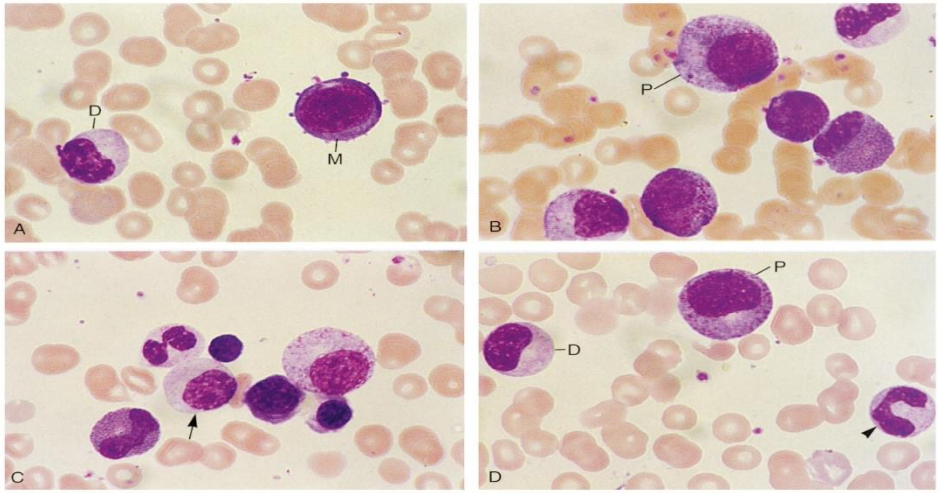




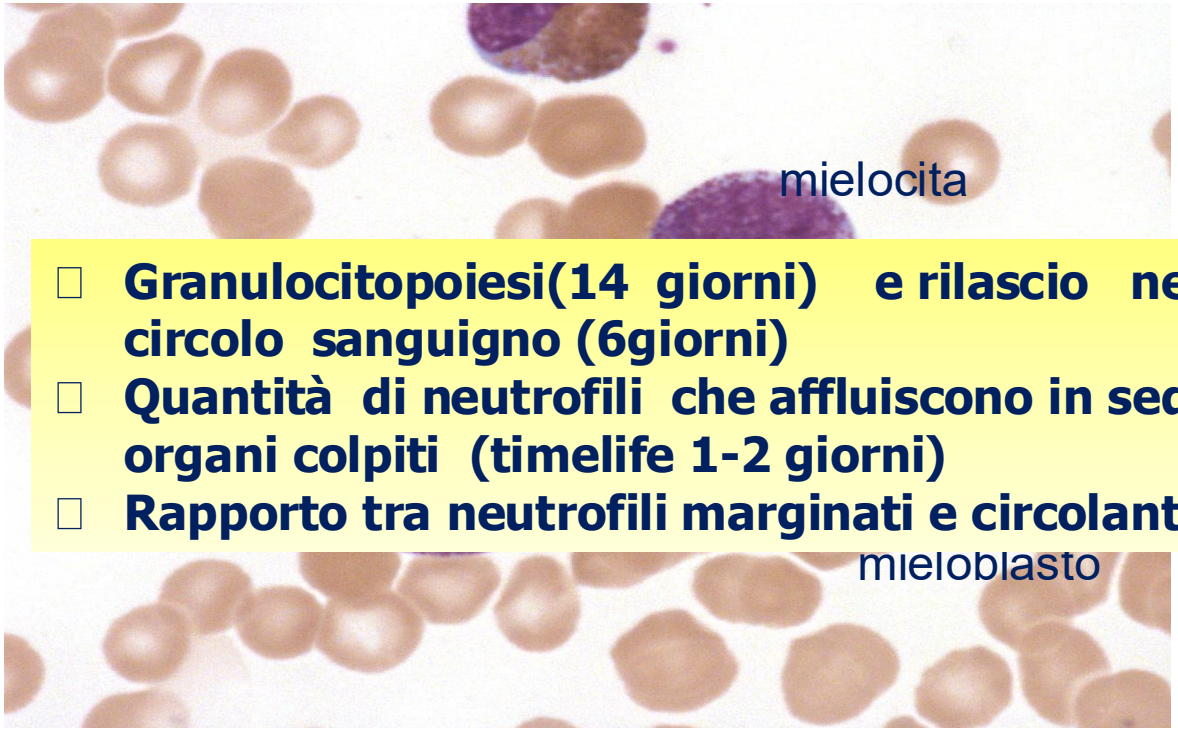
Emazie

GRANULOPOIESI





Differenziazione granuloblastica→ Precursori mieloidi
AM MGG 1000x



- **Granulocitopoiesi(14 giorni) e rilascio nel circolo sanguigno (6giorni)**
- **Quantità di neutrofili che affluiscono in sedi od organi colpiti (timelife 1-2 giorni)**
- **Rapporto tra neutrofili marginati e circolanti**

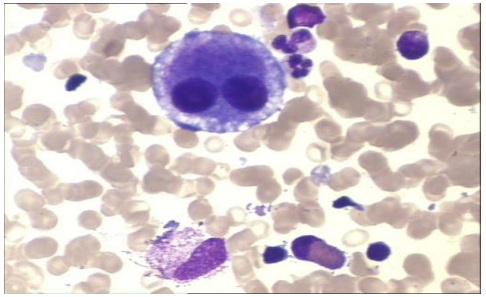
Granulazioni specifiche Mieloperossidasi, Esterasi, Fosfatasi Elastasi, Proteasi

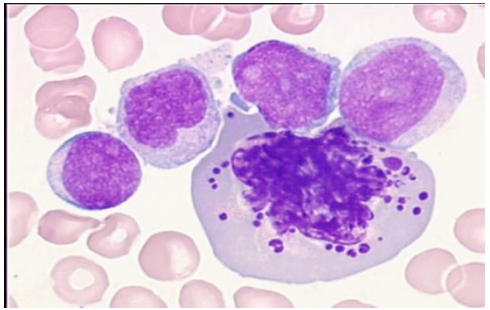
MDS: CRITERI DIAGNOSTICI

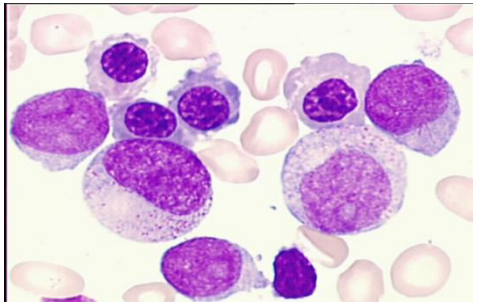
Briito-Babapulle F.,Catovsky D.,Galton D.,Br.j.Haemat1987;66:445-454
Goasguen,Matsuo,Benett: Leukemia 6(6) 1992,520-525
WHO 1999-2001-2008

Sangue midollare e Periferico

- Diseritropoiesi
- Disgranulopoiesi
- Dismegacariopoiesi







Percentuale di Blasti (AC)):

- Mieloblasti agranulati (Tipo I)
- Mieloblasti granulati (TipoII)
- Blast equivalents (Promonociti ,micromegacarioblasti)

Definizione displasia >10% per filiera emopoietica

Morphology remains the cornerstone of diagnosis:Well-stained blood and BM smears; well-processed BM biopsies

DISPOIESI IN ETA' PEDIATRICA



CLINICA

ORGANOMEGALIA FEBBRE RUSH CUTANEO ALTRO

CITOPENIA

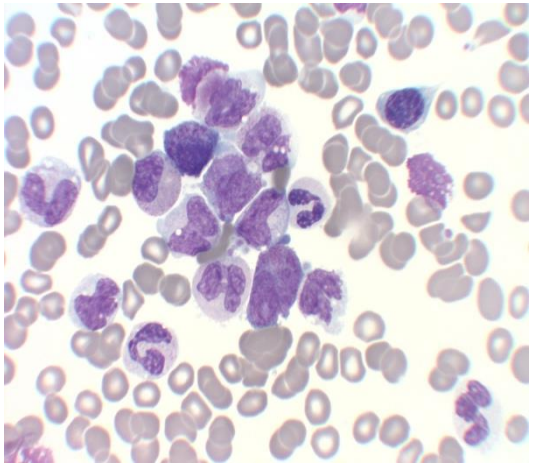
TRAPPOLA NELLA DIAGNOSI DELLE MDS

- ……**morphologic dysplasia is not necessarily synonymous with an MDS; morphologic mimickers of myelodysplasia could result in a misdiagnosis**.

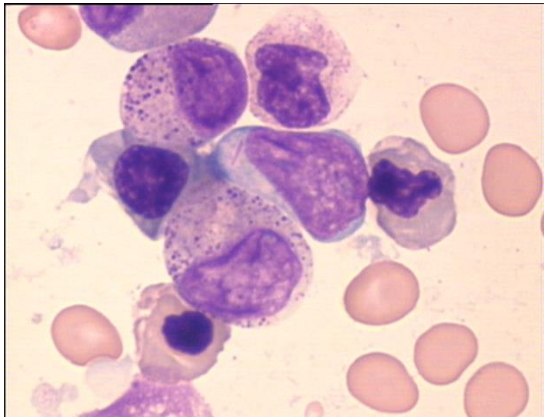
MDS PRIMITIVE	MDS PROLIF.	MDS SEC.
JMCL	S.Down	
Costituzionali		
S.Mon. 7 infantile	MDS +eosinofilia	Therapy-related
A.refrattaria	LMC atipica	
ARS	MDS familiare	
AREB	Miscellanea	
AREB-t		

MDS iEb1/iEb2

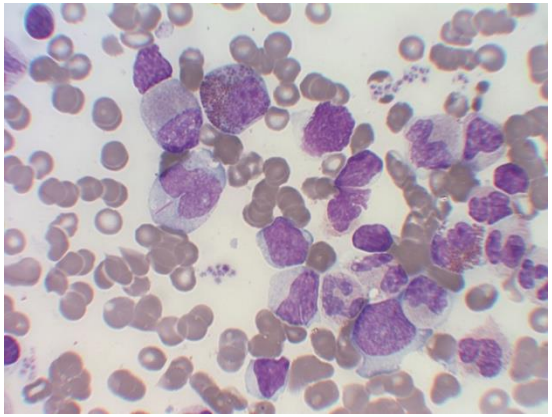
MDS -iB1
MDS iB2
MDS/AML ??????



CORPI DI AUER



AML MR



Pediatric Hematopathology in the Era of Advanced Molecular Diagnostics: What We Know and How We Can Apply the Updated Classifications

Yen-Chun Liu[a] Julia T. Geyer[b]

[a]Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN, USA; [b]Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York, NY, USA

Table 10. Subtypes of myeloid neoplasms
predisposition.

Myeloid neoplasms with germline predis
existing platelet disorder or organ dysfu
• Germline *CEBPA* P/LP variant (CEBPA-asso
• Germline *DDX41* P/LP variant[a]
• Germline *TP53* P/LP variant[a] (Li-Fraumeni syndrome)
Myeloid neop
platelet disor
• Germline *RU*
associated my
• Germline *AN*
• Germline *ET*
Myeloid neop
organ dysfun
• Germline *GA*
• Bone marrow
○ Severe co
○ Shwachm
○ Fanconi a
• Telomere bio
• RASopathies
syndrome or
• Down syndr
• Germline *SA*
• Germline *SA*
Syndrome)[c]
• Biallelic germ
[a]Lymphoid neo
[b]See respective
[c]Ataxia is not a
P pathogenic, *L*

lasms with germline
redisposition without a
ple organ systems
mutation
mline *DDX41* mutation
mline *TP53* mutation
Hematologic neoplasms with germline predisposition

Table 7. ICC guideline for features associated with MDS progression in patients with germline predisposition (adapted from ref. [26])

Two or more out of the following:
- Acquired pathogenic genetic alteration(s)
- Monosomy 7/del(7q), monosomy 5/del(5q), multi-hit *TP53* mutations (defined as ≥2 distinct *TP53* mutations each with VAF ≥10%, or a single *TP53* mutation with (1) 17p deletion, (2) VAF of ≥50%, or (3) copy neutral LOH at the 17p*TP53* locus), *TP53* mutation (VAF ≥10%) and complex karyotype (often with loss of 17p), or *SF3B1* mutation (VAF ≥10%)[a]
- Cytopenia in a new lineage(s) or progressive cytopenia[b], particularly in the context of increasing marrow cellularity
- Multilineage dysplasia[c]

Or:
- Increased blasts: ≥5% in bone marrow; ≥2% in peripheral blood

[a]Genetic alterations should be interpreted in the context of the specific germline condition. [b]In children, cytopenia is defined according to age-adjusted values. [c]Dysplasia is defined as dysplastic cytologic changes in ≥10% of erythroid cells, ≥10% of granulocytic cells, and/or ≥10% of megakaryocytes. Bone marrow in patients with germline mutations may show evidence of dyspoiesis; caution should be made not to overinterpret dysplasia as criteria for MDS in the absence of additional supporting evidence.

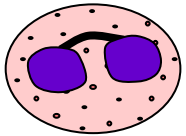
Patients with genetically defined hereditary myeloid malignancy syndromes (HMMSs) constitute an increasingly recognized group that warrants further evaluation
Firas El Chaer, Christopher S. Hourigan, and Amer M. Zeidan Blood ,June 23

Dysplasia in Myelodysplastic Syndrome

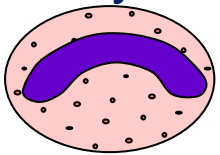
Dysgranulopoiesis



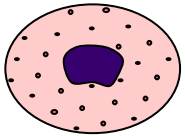
Normal segmented neutrophil



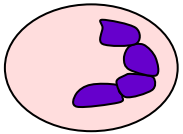
Pseudo-Pelger-Hüet anomaly



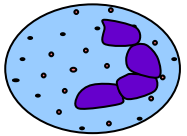
Macrocytosis



Chromatin clumping



Hypo-, agranulation of cytoplasm



Asynchr. maturation nucleus - cytoplasm

Dyserythropoiesis

MIELOGRAMMA ESEGUITO SU 500 CELLULE NUCLEATE
SCORE SYSTEM(of cells) 0%,2=%,3=>%
Total score 1-4x% abnormal /100

erythroblast

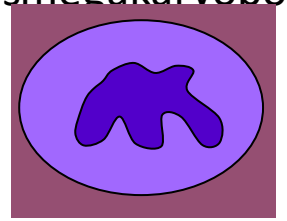
lobulation

nuclei

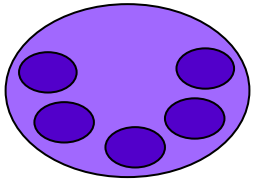
granules

egalo-blastic changes

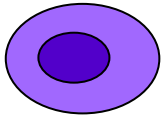
Dysmegakaryopoiesis



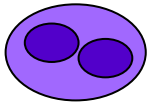
Normal megakaryocyte



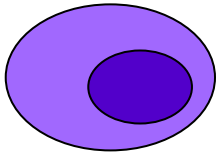
Separated single Nuclei



Mikromega-karyocyte



Small binucleated megakaryocyte



Rund, non-lobulated megakaryocyte

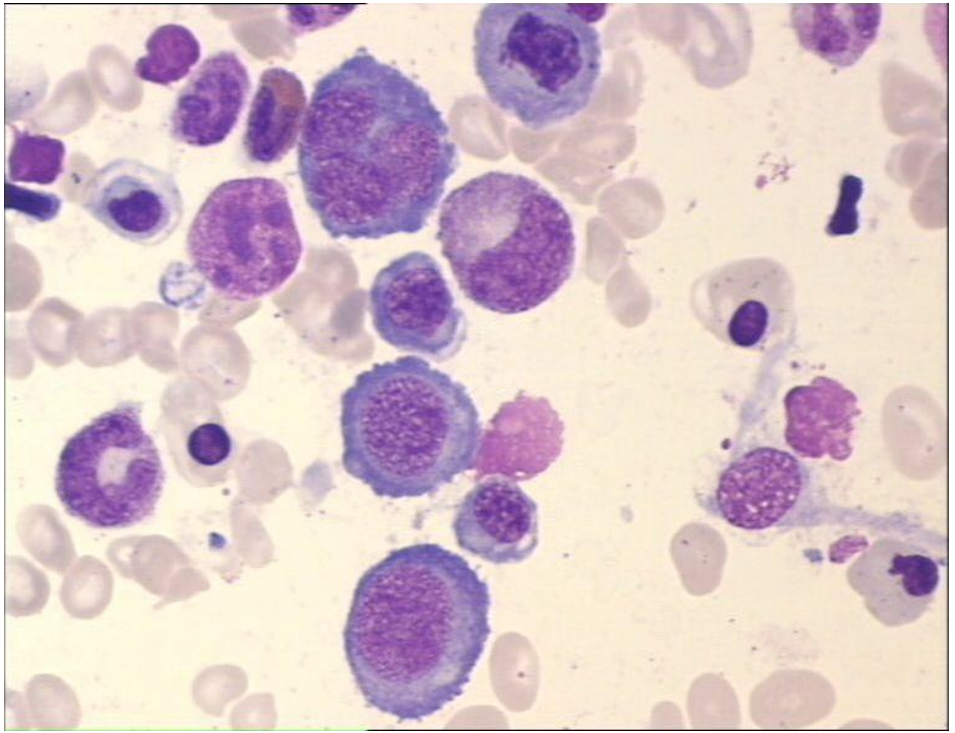
Cantù Rajnoldi et al. Ann Hematol 2005;84:429-33

DISERITROPOIESI

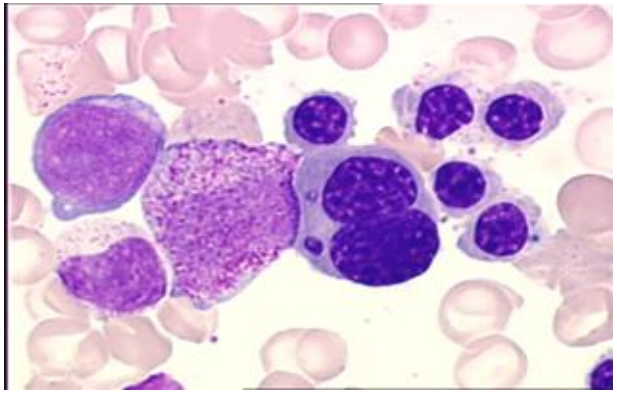
- *Midollo osseo*

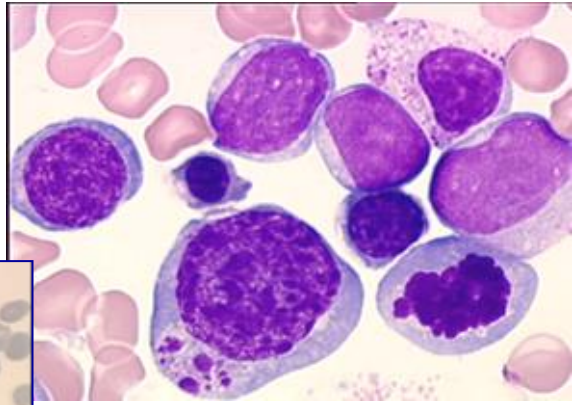
- *ERITRODISPLASIA 50% degli eritroblasti*
- *Megaloblastosi (pernicioso-simile)*
- *Macroblastosi (eritroblasti poli-ortocromatici)*
- *Lobulazioni nucleari*
- *Multinuclearita'*
- *Inclusi citoplasmatici*
- *CITOHIMICA:*
- *PAS : eritroblasti*

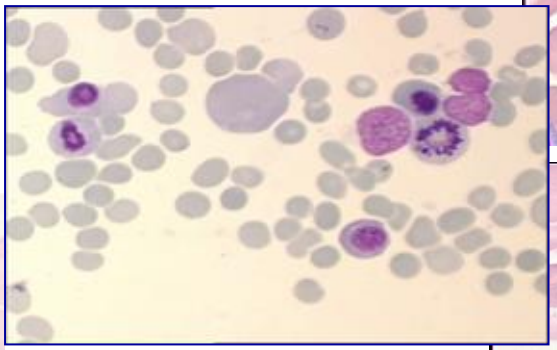
PERL'S : negativo

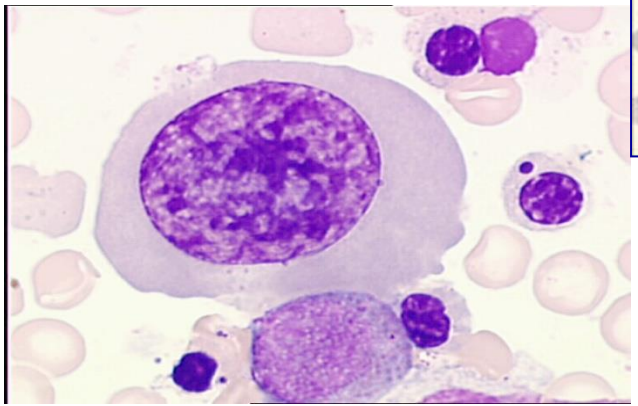


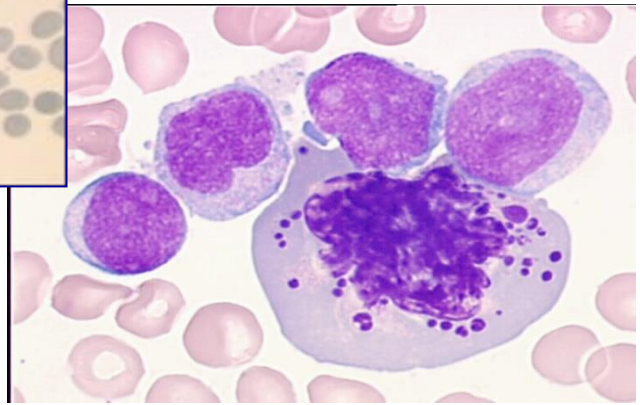
ERITRODISPLASIA→eritropoiesi inefficace

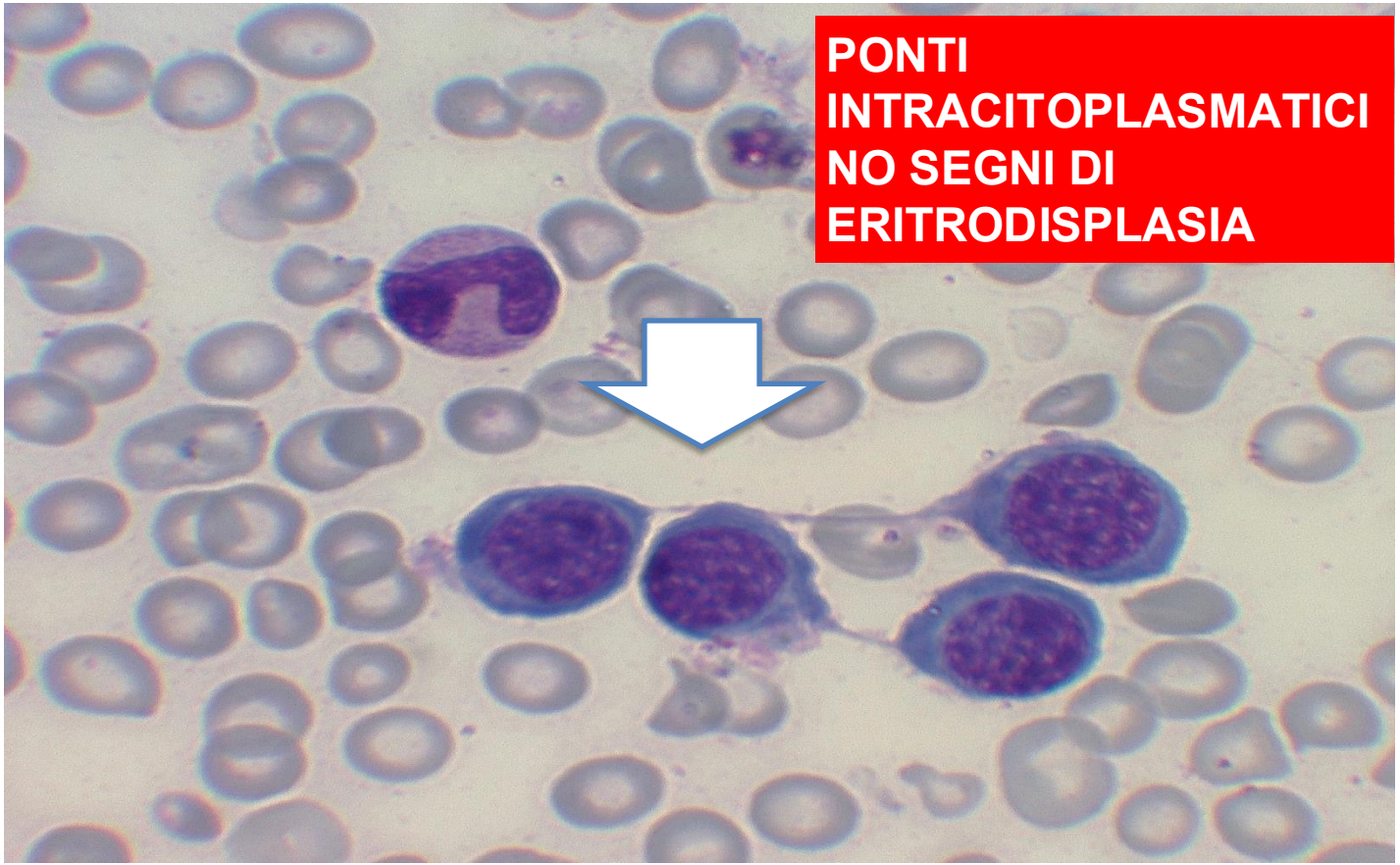




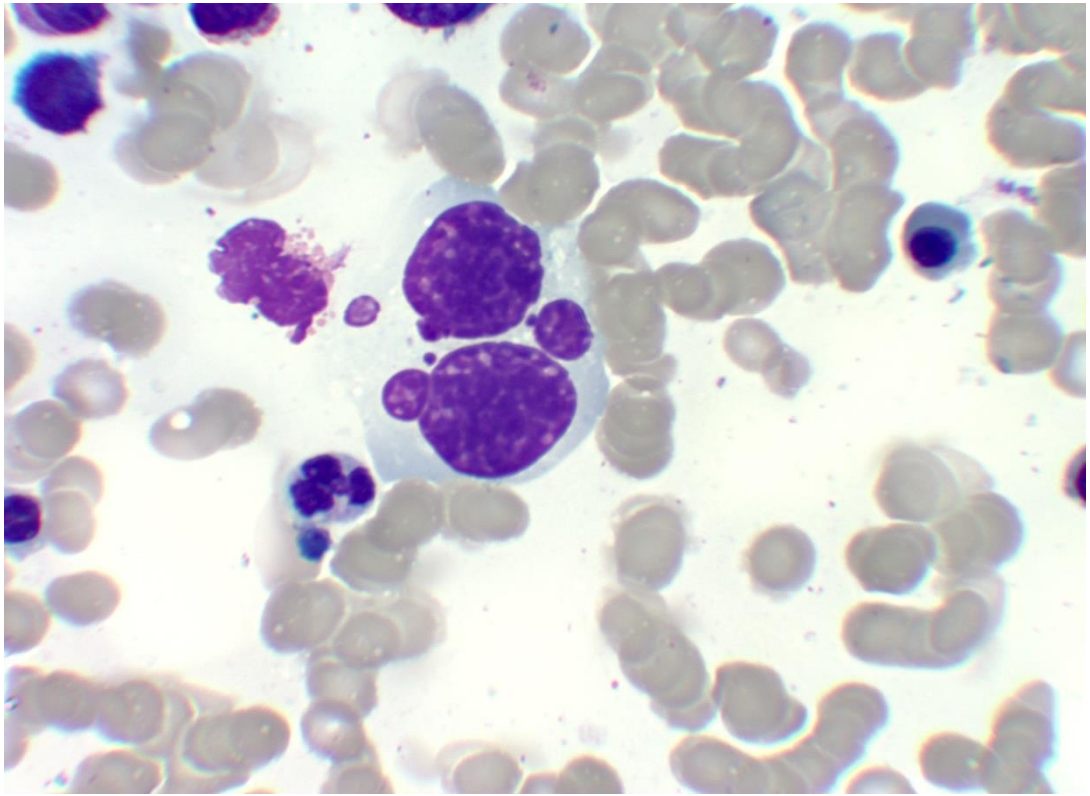







PONTI
INTRACITOPLASMATICI
NO SEGNI DI
ERITRODISPLASIA

??????????

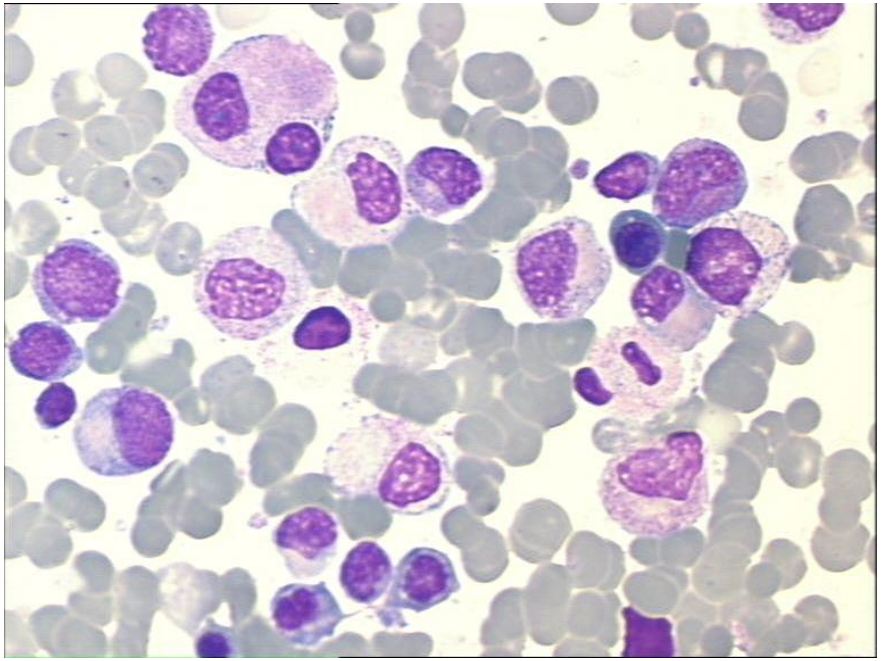


macronormoblasto

Criteri morfologici
DISGRANULOPOIESI

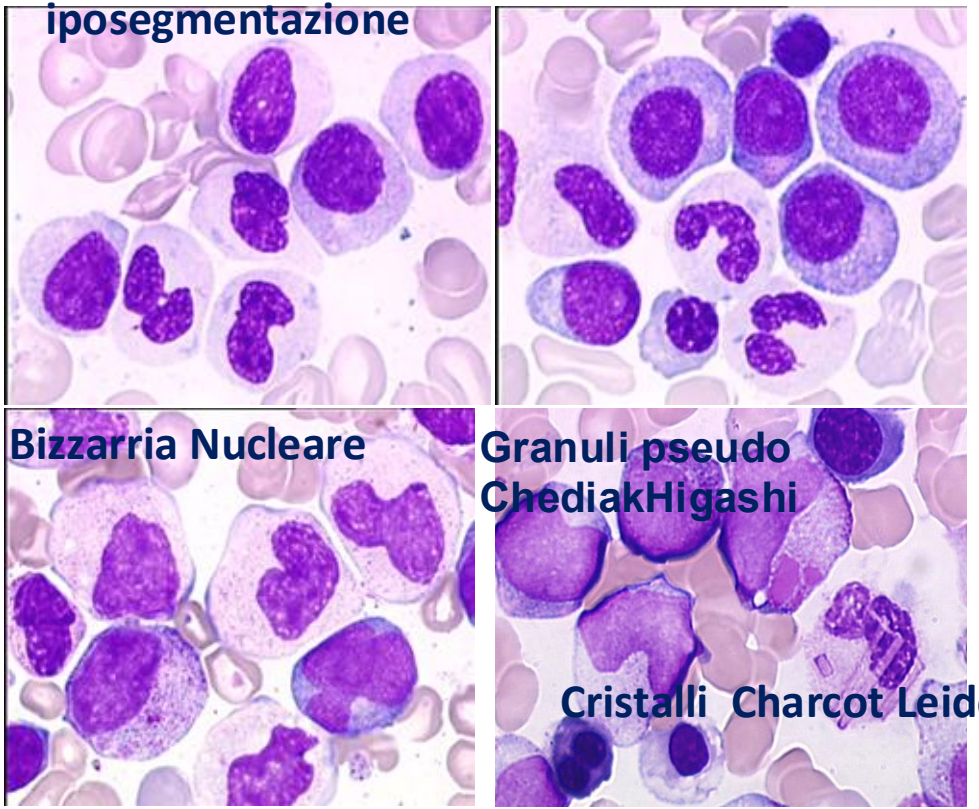
- ***Midollo osseo***
- ***Bizzaria nucleare (dal promielocita in su)***
- ***Agraulo/ipogranulosita'***
- ***Asincronia nucleo/citoplasmatica***
- ***(Basofilia citoplasmatica,nucleo mielocita simile)***
- ***Eosinofili con granulaziozioni basofile***

- ***Sangue periferico***
- ***Pseudo- Pelger***
- ***Clumping chromatin***
- ***Corpi di Dohle***



AM MGG 500x

DISGRANULOPOIESI



Courtesy of prof Daniel

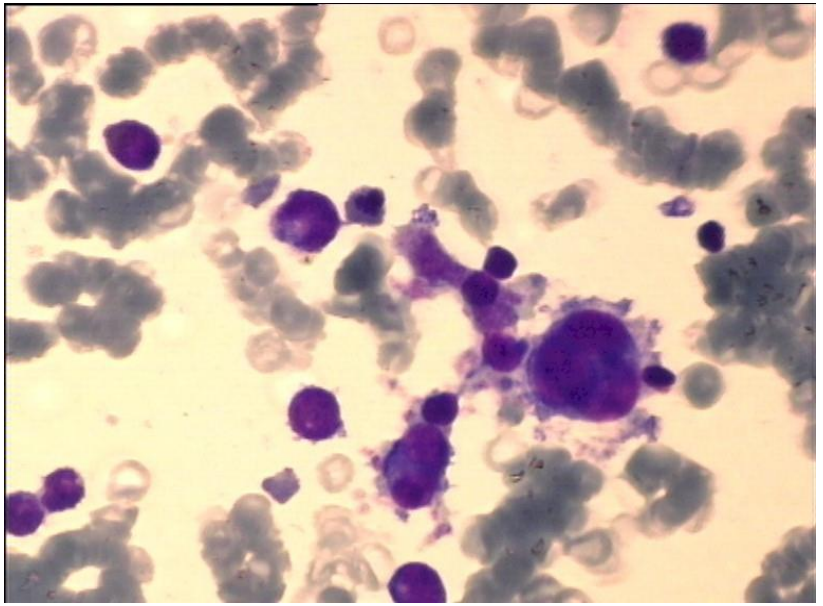
AM MGG 1000x

Criteri per la valutazione della DISMEGACARIOCITOPOESI

MEGACARIODISPLASIA

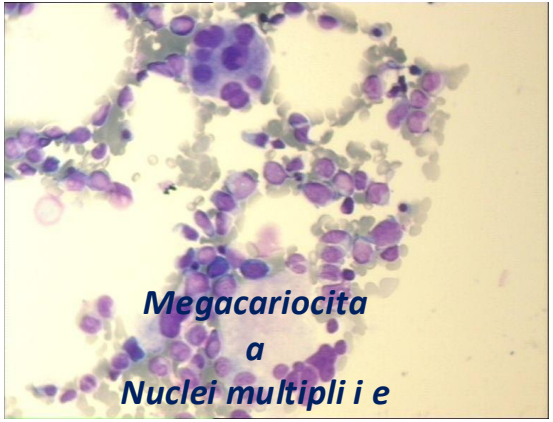
- ***Midollo osseo***
- ***Numero di Megacariociti(ridotti-aumentati)***
- ***Megacariociti piccoli e binucleati***
- ***Megacariociti a piccoli nuclei separati***
- ***Ipolobulati***
- ***Micromegacarioblasti***
- ***Micromegacariociti***

- ***Sangue periferico***
- ***megatrombociti***



DISMEGACARIOCITOPOIESI

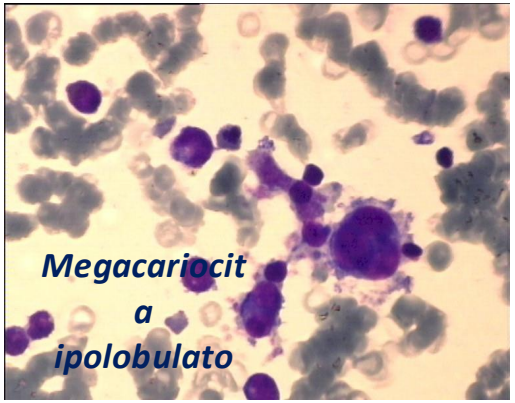
Numero ridotto:<1megacariociti x 10 campi



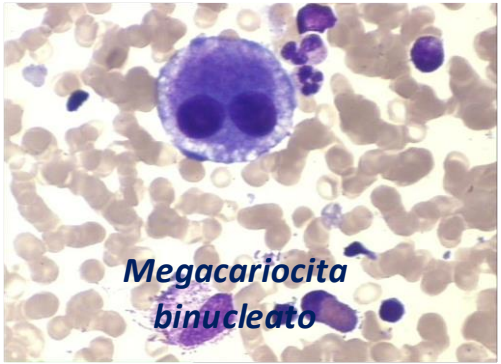
Megacariocita a Nuclei multipli i e separati



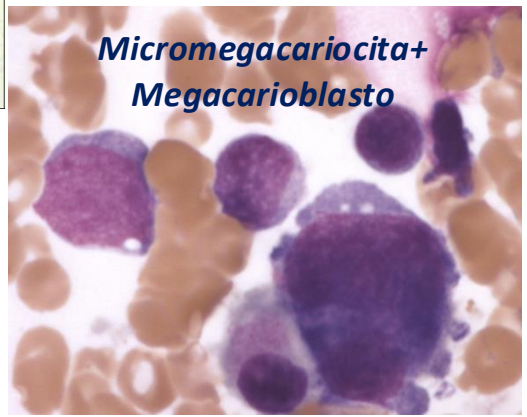
Emperipolesi



Megacariocit a ipolobulato



Megacariocita binucleato



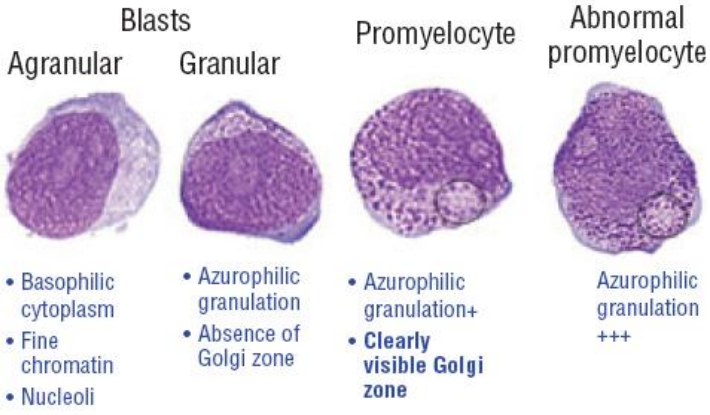
Micromegacariocita+ Megacarioblasto



??????

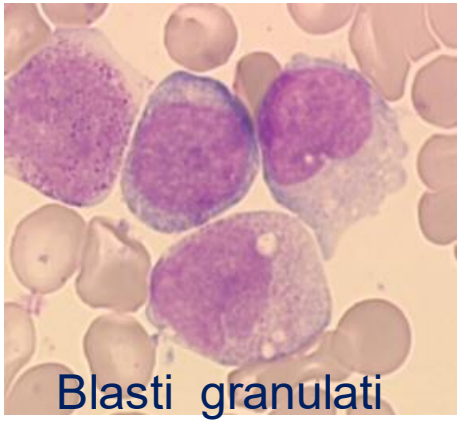
osteoclasto

Diagnosis and classification of myelodysplastic syndrome: International Working Group on Morphology of myelodysplastic syndrome (IWGM-MDS) consensus proposals for the definition and enumeration of myeloblasts and ring sideroblasts

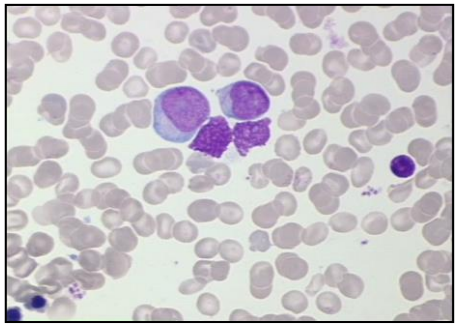


| 1714 | haematologica | 2008; 93(11)

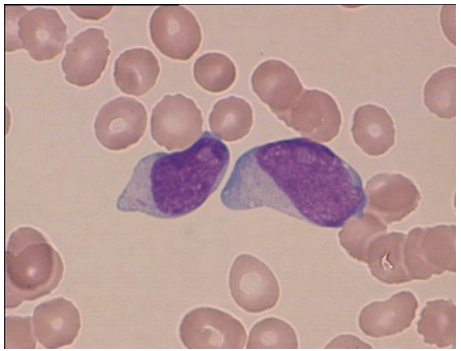
Mufti et al,2009



Blasti granulati



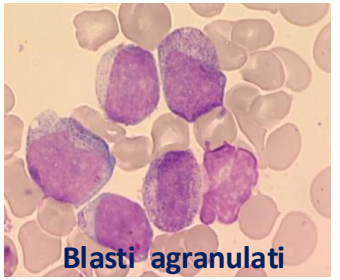
Blasti agranulati



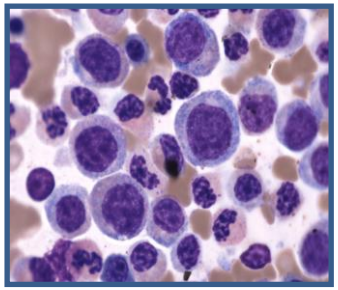
BLASTI VS PROMIELOCITI ATIPICI

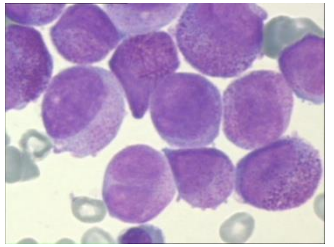
CRITERI MORFOLOGICI per la DEFINIZIONE dei BLASTI nelle MDS

MORFOLOGIA	Bl. agran	Bl.granulati	Prom.anormale	Promi. Displastico	Promi.normale
Dimensioni	15	30	25-30	25	25-30
N/C	alto	medio	baso	basso	basso
Basofilia citopl.	++	++		irregolare	+
Nucleo	rotondo centrale	rotondo centrale	eccentrico ovale	eccentrico ovale	eccentrico ovale
Cromatina	fine	fine	fine	fine grossolana	fine intermedia
Nucleoli	1-2	1-2	01-feb prominenti	1 prominente	01-gen prominente
Golgi	--	--	presente oco sviluppat	presente poco sviluppato	presente visibile
Granulazioni	---	<20	grossalani numerose	distribuzione irregolare	azurofili disperse
Corpi di Auer	+/-	+	fagoot's	--	-



Blasti agranulati





Promielociti atipici

Evalutation of Dysplasia
BAM vs BMT

BAM

SCORE	0(%)	1(%)	2(%)	3(%)	ND
	Absent	Mild	Moderate	Severe	
Dismyel	9(28)	9(289	10(31)	3(9,3)	1
Diseryth	13(40)	8(25)	8(25)	28(6,2)	1
Dysplasia					
N° Tot	22	18	20	4	
%	73	60	66	13,30	

BMT

SCORE	0(%)	1(%)	ND	Tot
	Absent	Present		
Dismyelo	2	30 (93,7%)	0	32
Diserythr	0	32(100%)	0	32

No grading of Dysplasia

	DYGR	DYSERY	DYSMEK	Micrk
absent	2	0	1	5

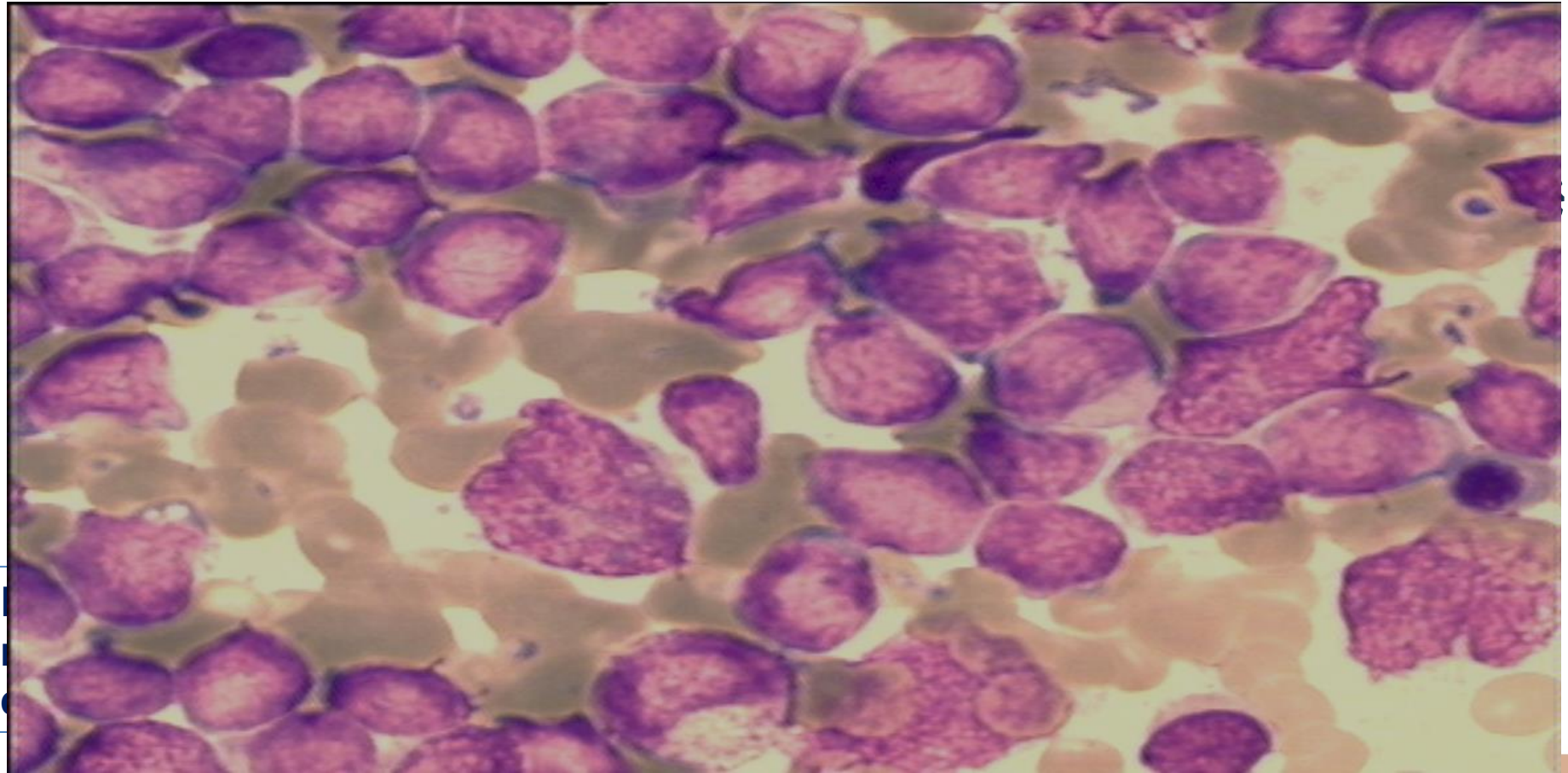
SCORE	0	1	ND
	Absent(%)	Present(%)	
Dismegak	1	31 (100)	0
Micromeg	5 (16,1)	26 (83)	1

Dismek >in BMT

SCORE	0	1	ND
	Absent(%)	Present(%)	
Dismegak	28 (87)	4(12,5)	/
Micromegak	30 (93)	2(6,2)	/

Courtesy of dr De Vito &Fenu

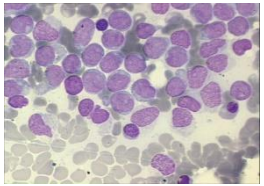
LEUCEMIA ACUTA -- >quali filiere →quali sottotipi?
Quale cutoff dei blasti ???? BLAST EQUIVALENTS'??



Diagnosi morfologica Leucemia mieloide Acuta FAB 1976-2000 (blasti>30%)

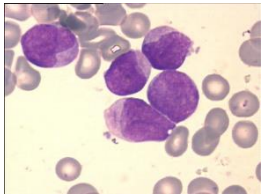
CITOCHIMICA

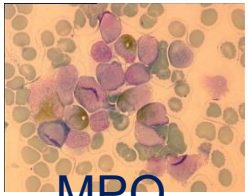
M0



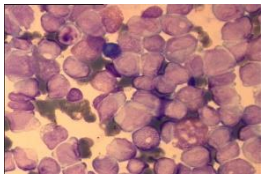


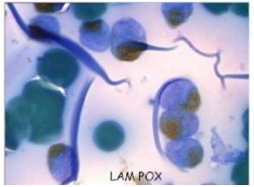
M1



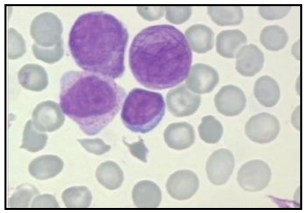


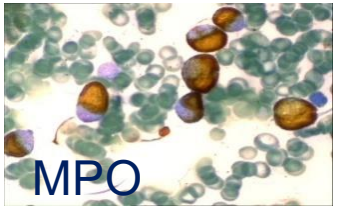
M2



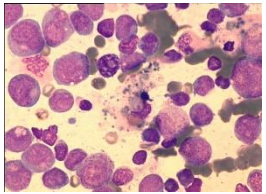


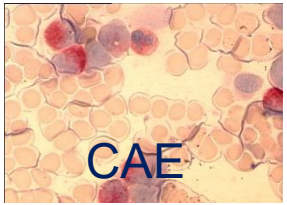
M3



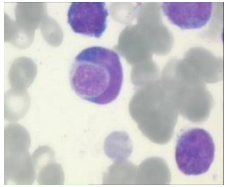


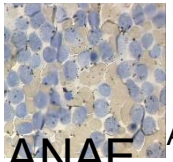
M4

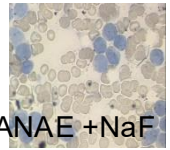




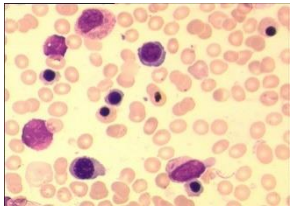
M5



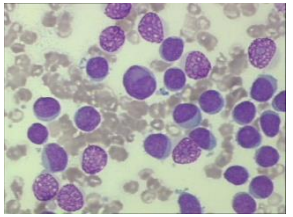




M6



M7



AML: Blast cutoff

WHO 2022

- AML with defining genetic abnormalities

Blast cut off 20% no required for diagnosis of AML
(AML KMT2A/ AML NPM1 9

- cutoff ≥20%
- AML with CEBPA mutation
- BCR::ABL1

AML defined by differentiation (previous NOS)
Blasts ≥20%

AML MR (specifiche alterazioni genetiche MDS correlate)
NO DISPLASIA MORFOLOGICA
Blasts ≥20% (comprende trasformazione da MDS – MDS/MPD)

Secondary myeloid neoplasm

- AML MN pCT MDS pCT TP53 multi Hit >2 mutazioni concomitanti 17p/TP53 ecc
- MN germline predisposition

ICC 2022

- AML with defining genetic abnormalities

Blasts >10%

Myeloid Neoplasm →TP53mut (complex karyot).
MDS TP53 mut. Blasts 0-9% (VAF>10%)
MDS/AML TP53 Blasts 10-19%
AML TP53 Mut (VAF>10%) Blasts ≥ 20% (PEL)
AML t(9;11)(p21.3;q23.3) KMT2A::MLLLT3
AML other KMT2A rearrangment

AML -NOS Blasts ≥20%

MDS/AML NOS
°(WHO HAEM5→MDS iB2) Blasts 10-19%
AML-MDS (related mutation) blasts ≥20%
AML /MDS(cytogenetic abnormalities) ≥20%

AML –MRC HAEM4

- Germline predisposition with costitutonal
- disorder (Down syndrome .Bloom Syndrome ecc

ALL IKZF1-PAX5 –Li Fraumeni

Therapy related pCHT

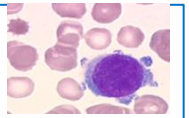


Blasti+Blasti equivalenti come definirli ….. quando utilizzarli

Indicazione→ La conta dei blasti mieloidi + blasti equivalenti
Diagnosi di LAM vs MDS/ MDS-MPD

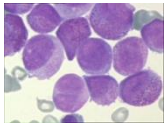
Blasti equivalenti sono :

Megacarioblasti



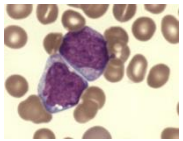
→ **Leucemia Acuta Megacarioblastica**

Promielociti anor



→ **Diagnosi di Leucemia Acuta Promielocitica**

Promonociti



→ **L.Acuta Monocitica/ mielomonocitica vs L.Mielomonocitica cronica**

Proeritroblasti (NO Blast Equivalents)

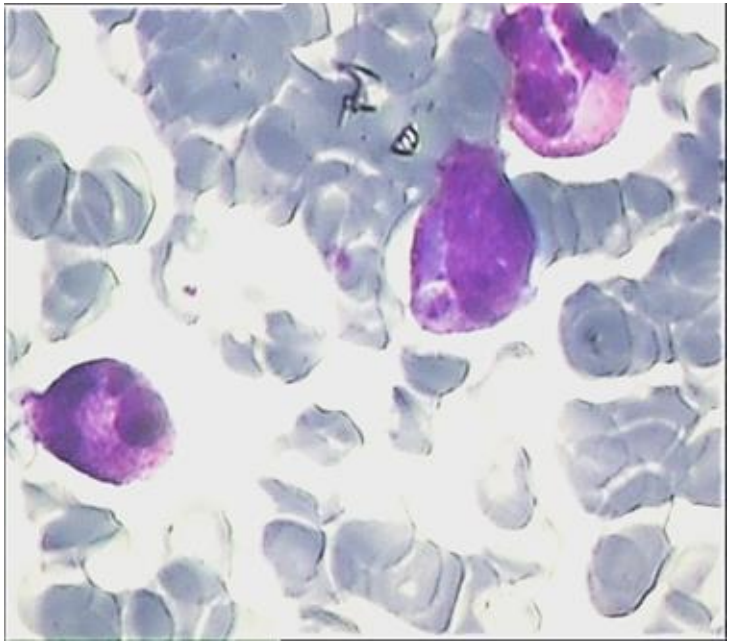
BLASTI → **Eritremia vs LAM –MR vs MDS-IB1**

NON SONO DA CONSIDERARSI BLASTI EQUIVALENTI .
MONOCITI MATURI ,MICROMEGACARIOCITI , PLASMACELLULE

Leucemia Megacarioblastica Acuta

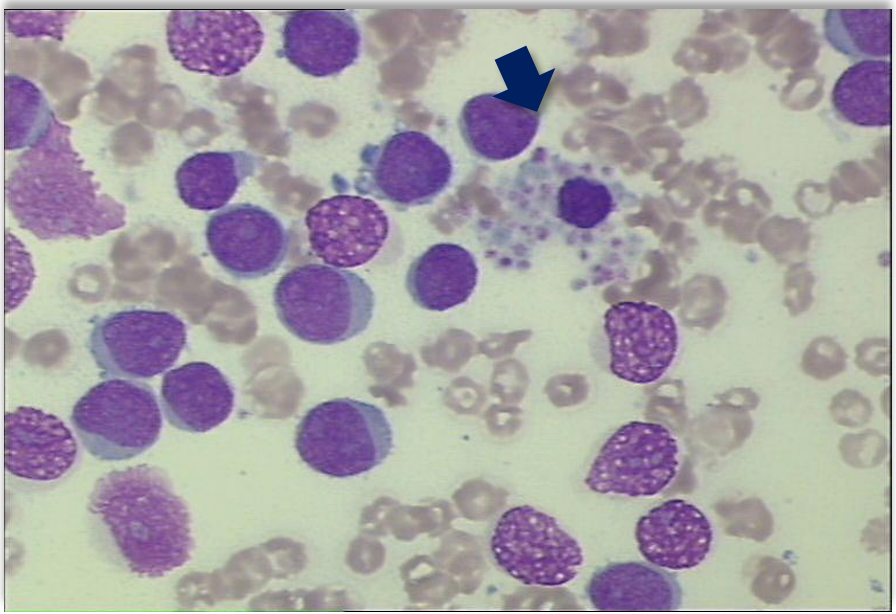
Diagnosi
Blasti >20% di questi > 50% sono megacarioblasti
Morfologia : media taglia 12-18mm
Nucleo irregolare/indentato fine cromatina reticolata 3 nucleoli
Citoplasma basofilo con blebs
lymphoblasts like
Citochimica : SBB / MPO neg
PAS FAC NSE possono essere positivi
Immunofenotipo CD 41 CD61 CD42b CD13
Citogenetica (1;22)(p13.3;q13.1) RMb15::MKL1 (età pediatrica - S Down)

Diagnosi Differenziale
AML –MR-AML Monoblastica
LAL CB di MPD

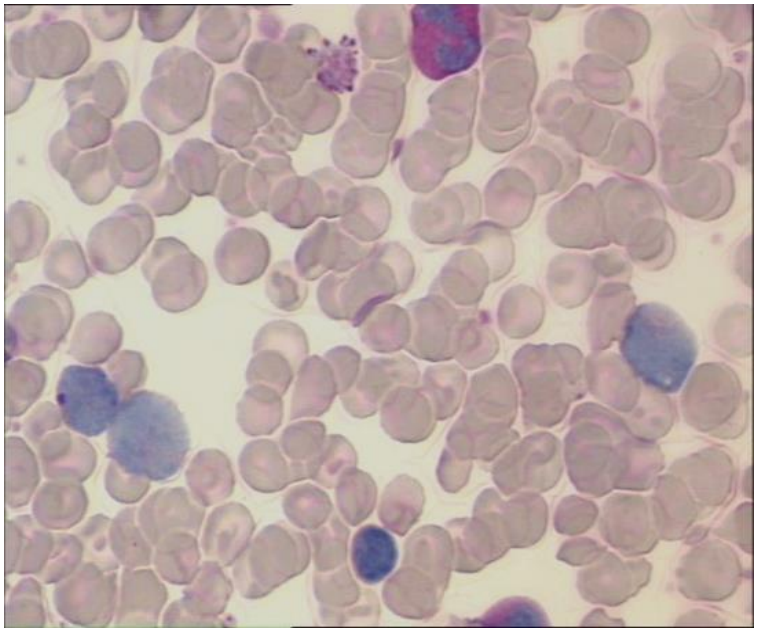


LAM megacarioblastica distinta in 3 gruppi:
1.AMKL Sindrome di Down
2.AMKL no Sindrome di Down(AML NOS-/ KMt2A definite anomalie citogenetiche)
3.AMKL nell' adulto

Leucemia Megacarioblastica Acuta
Aspirato Midollare 1000x MGG



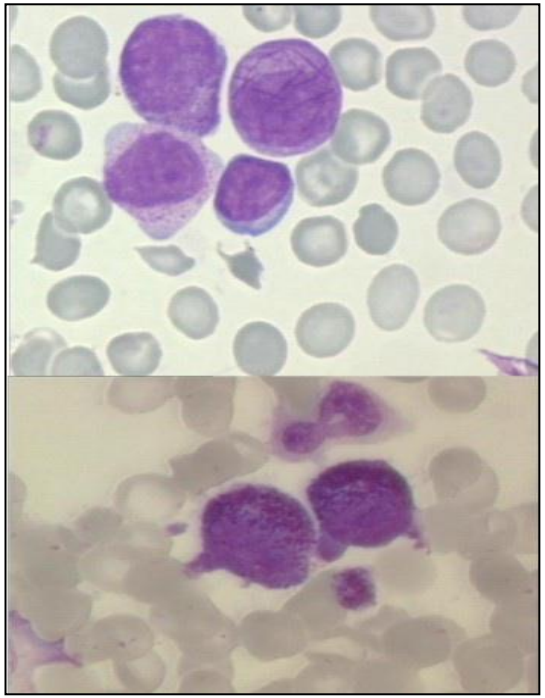
AM MGG 500x

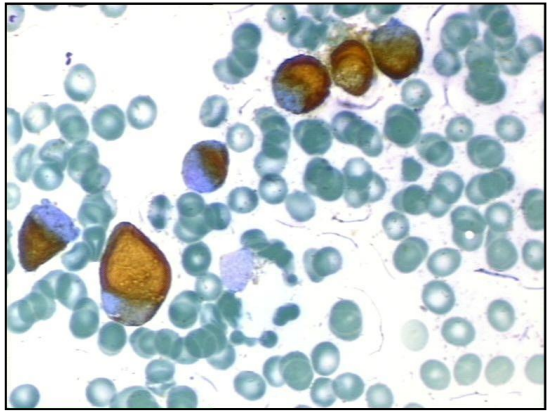


PAS

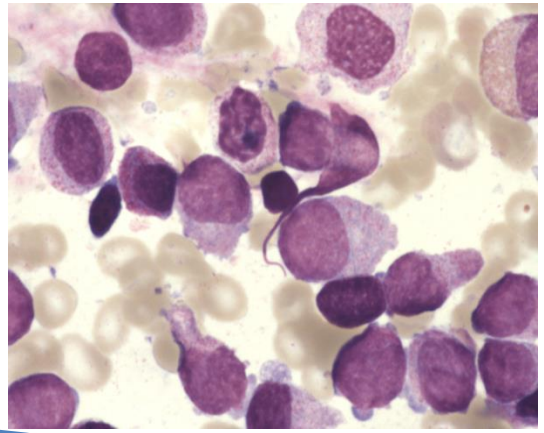
LEUCEMIA ACUTA PROMIELOCITICA

AM MGG hpf 1000x
LAP IPERGRANULARE



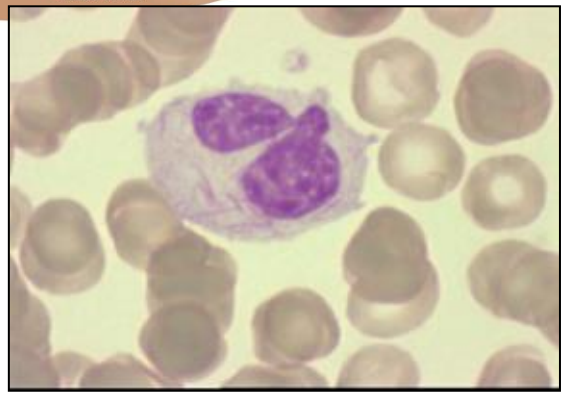


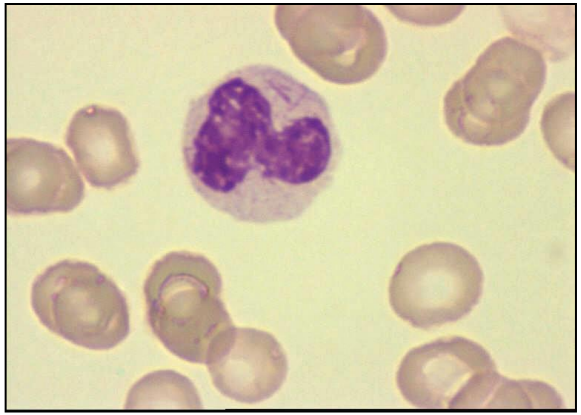
AM MGG hpf500x
LAP ipogranulare



Mieloperossidasi
AM hpf 500
Blasti positivi
90%++++

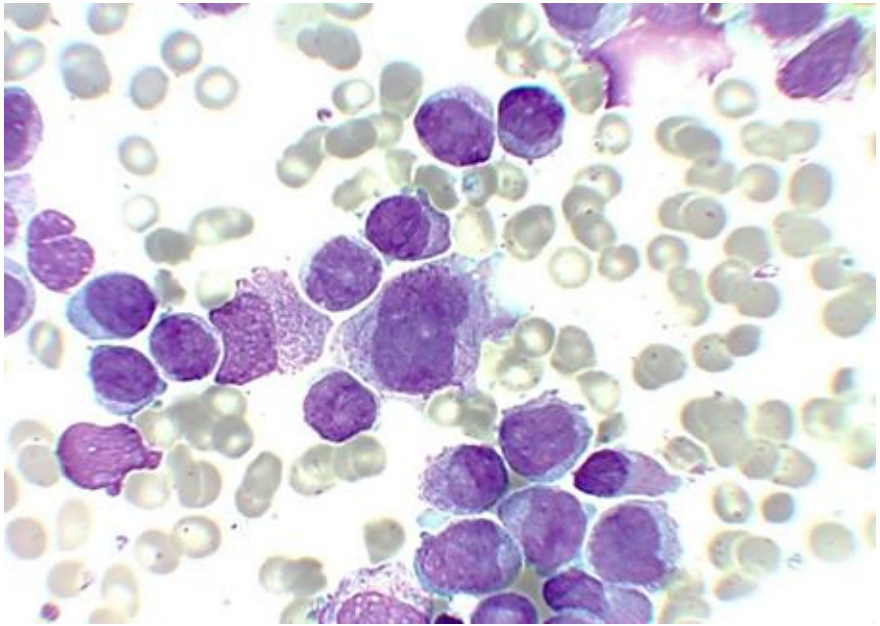
ATRA THERAPY



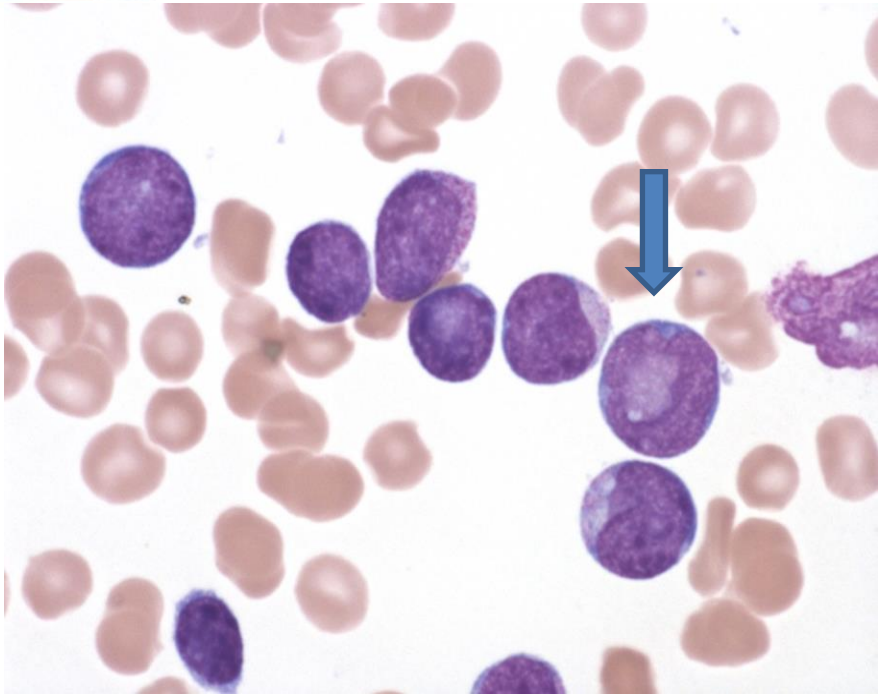


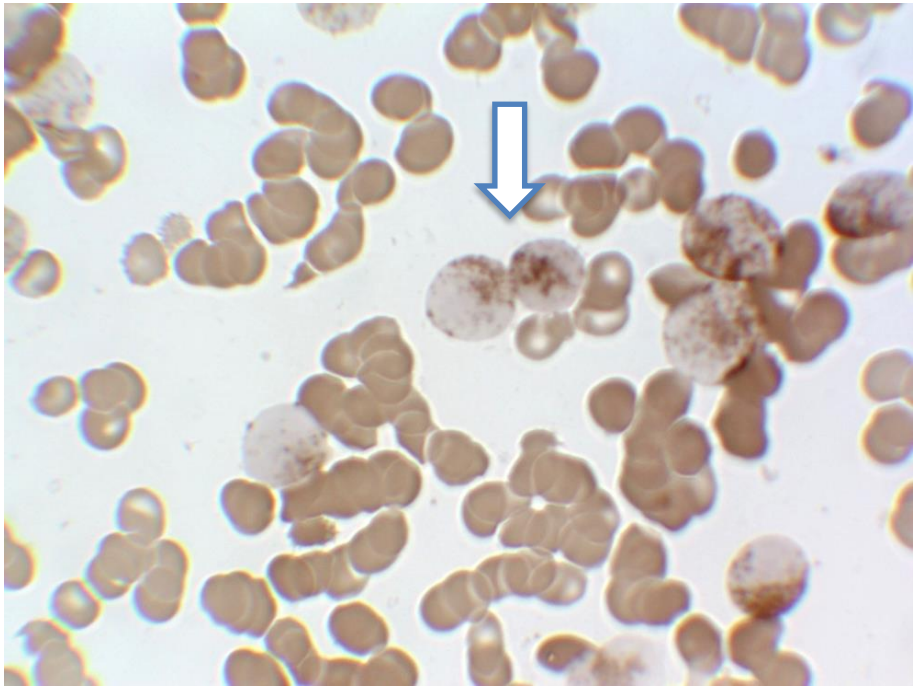
svo

Hpf 1000x MGG



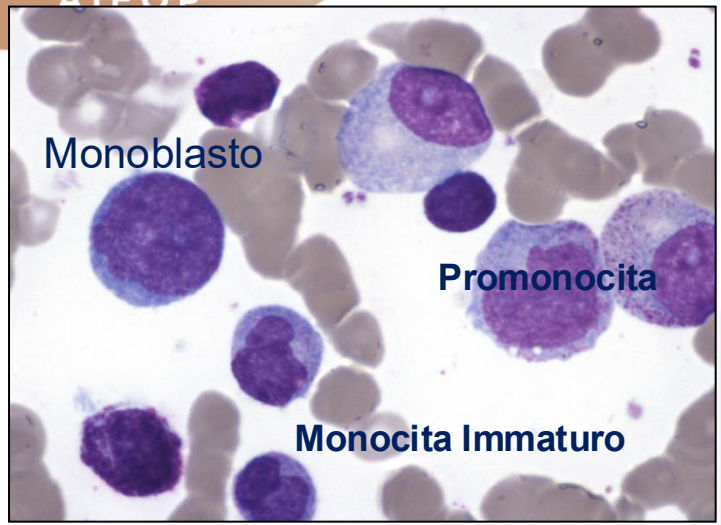
AM MGG Hpf 1000x





AM 1000x MGG
L.Mieloide Acuta cup like blast APL –Like NPM1 Mut
HLADR Neg CD34Neg CD2pos NO BLASTI EQUIVALENTI

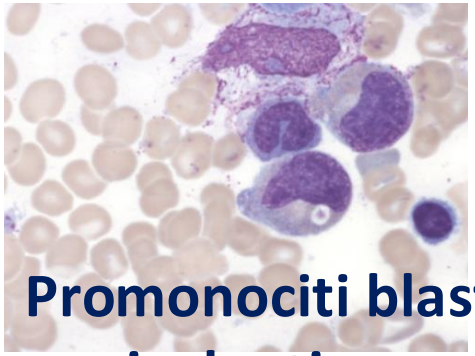
AM Sudan B 500x
Positività ++



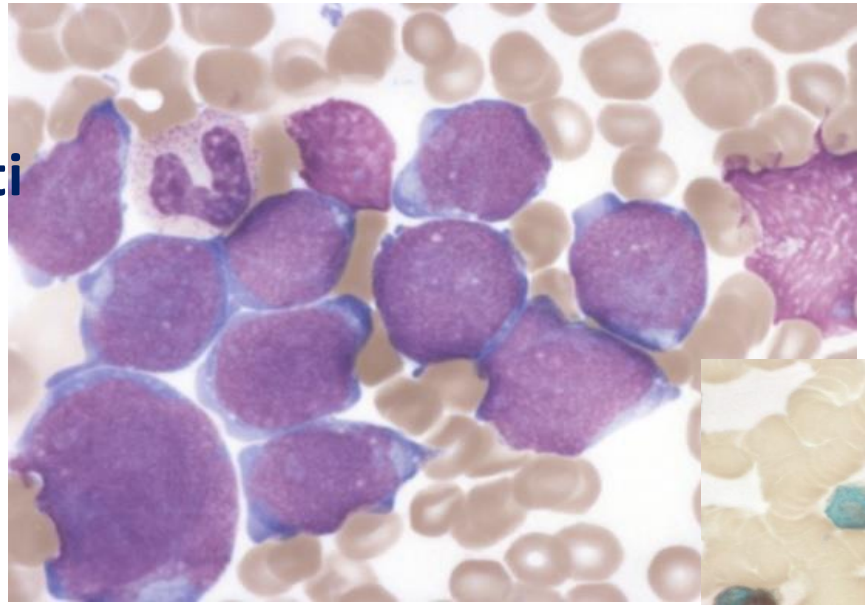
Criteri morfologici per la definizione dei monociti ed i sui precursori (IWG-MDS 2009)

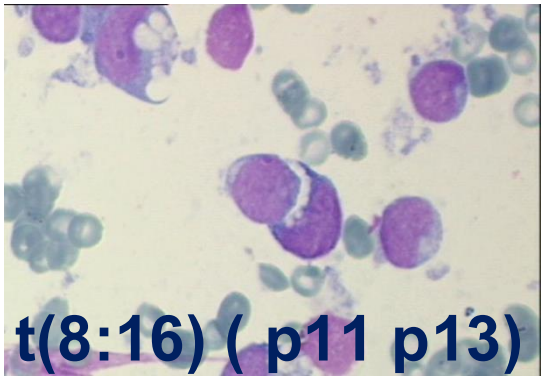
	Nucleo	Cromatina	Citoplasma	Definizione
MONOBLASTO	Rotondo/ ovale	Fine dispersa / reticolata Nucleolo prominente	Basofilo grabnu. Azur.+/-	Grandi dimensioni 20-3maturo 0mm
PROMONOCITA	Convoluto/ indentato	Fine dispersa /reticolata Nucleolo prominente	Basofilia variabile gran.Azurr. +/-	20-30 µm Simile al monoblasto ne differisce per il nucleo indentato
MONOCITA IMMATURO	Convoluto/ indentato	Più addensata Nucleolo visible	Tenue basofilia No granulazi.	<20µm Simile al monocita maturo
MONOCITA MATURO	Lobulato /indentato	Addendanta Assenza di nucleoli	Grigia Granulazioni +/-	20-25 µm

Leucemia Monocitica Acuta
>80% Monoblasti+promonociti
<20% di preucursori mieloidi

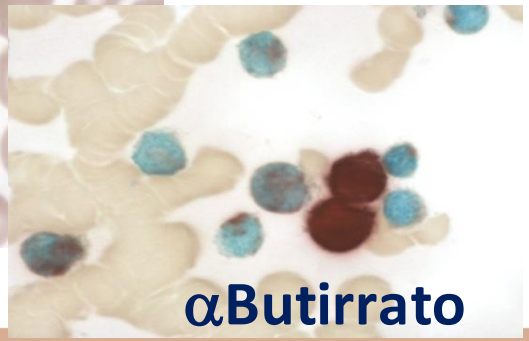


Promonociti blasti equivalenti



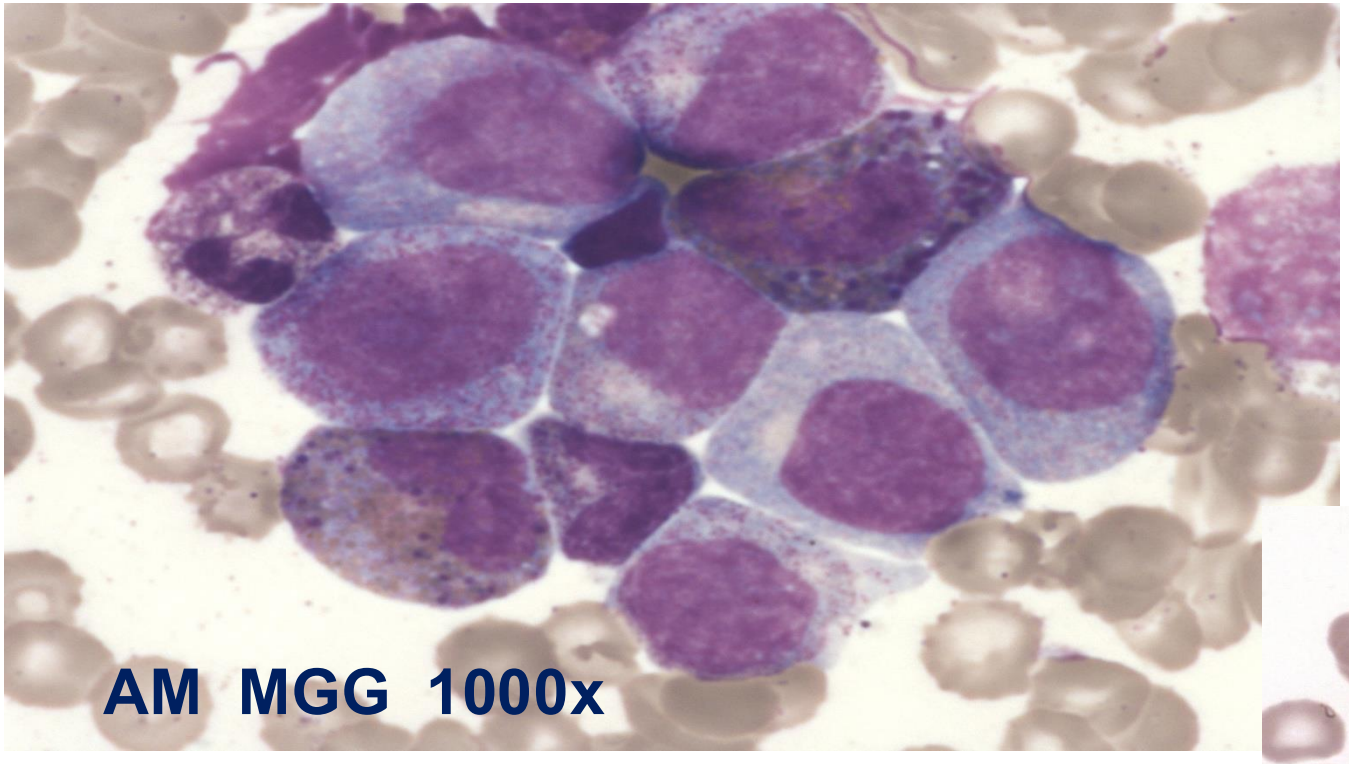


t(8:16) (p11 p13)

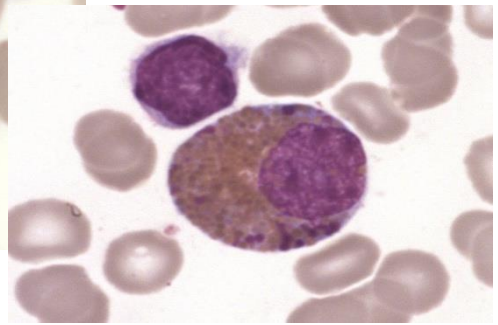


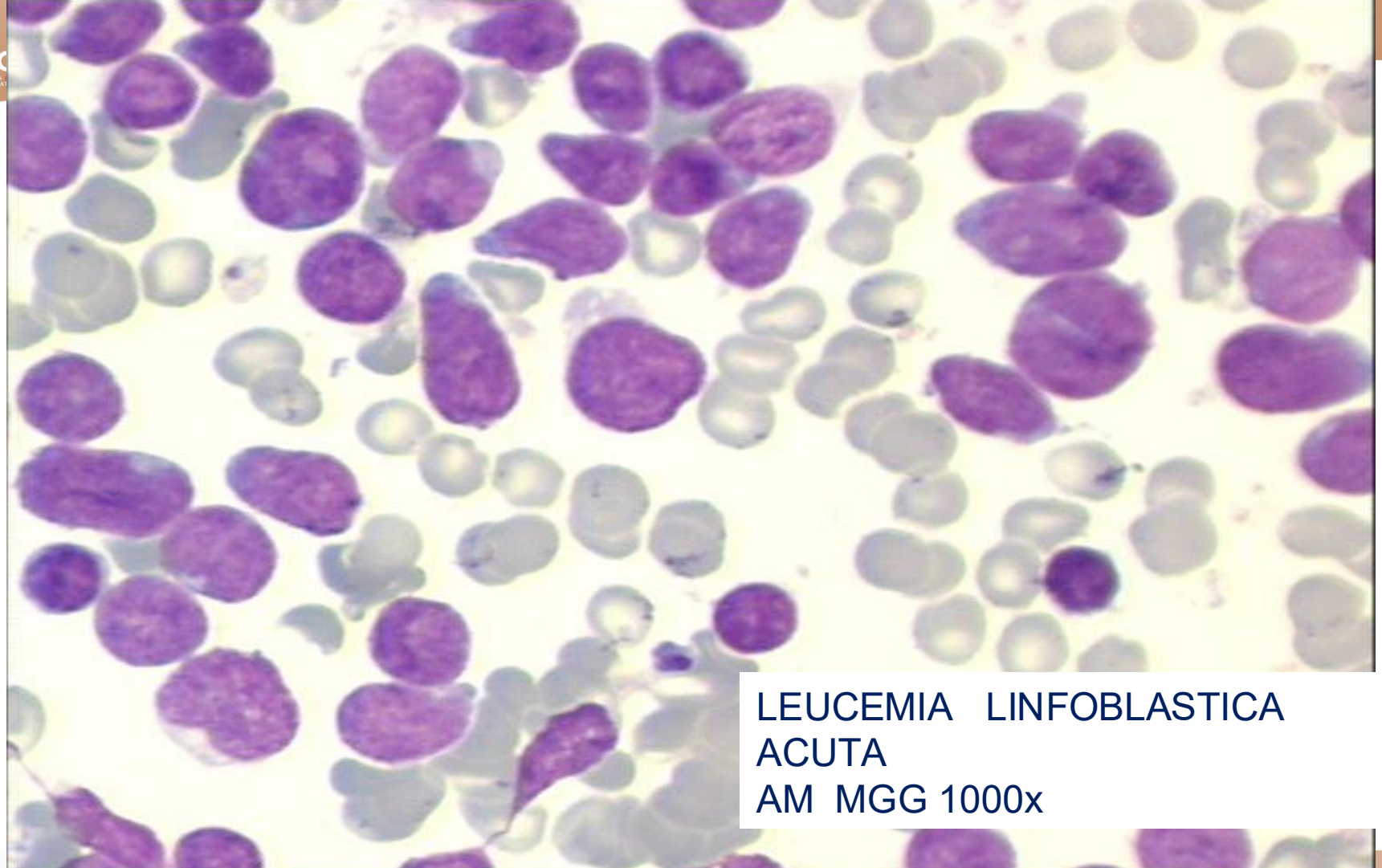
αButirrato

LAM Mielomonocitica con eosinofilia Inv 16(p13.1;1q22)



AM MGG 1000x





LEUCEMIA LINFOBLASTICA ACUTA
AM MGG 1000x

- The diagnosis of ALL generally requires demonstration of ≥20% bone marrow lymphoblasts on hematopathology review of bone marrow aspirate and biopsy materials. A value of ≥25% marrow blasts is often used in treatment protocols to define leukemia.
- Unlike with myeloid leukemia, there is no clear lower limit for the proportion of blasts required to establish an ALL diagnosis. In general, it is uncommon to observe presentations of ALL with low blast counts and the diagnosis of ALL should be avoided when there are <20% marrow blasts. In addition, there is no compelling evidence that not treating a patient when there are <20% marrow blasts has an adverse effect on outcome. I
- When the disease is restricted to a mass lesion primarily involving nodal (including thymus for T-ALL) or extranodal sites with no or minimal involvement in blood or bone marrow (generally defined as <20% lymphoblasts in the marrow), the case would be consistent with a diagnosis of LL.
- In clinical situations that preclude bone marrow aspirate and biopsy, such as hyperleukocytosis (eg,> 100,000 leukocytes per microliter) and/or mediastinal mass, peripheral blood may be substituted for bone marrow provided there is a significant amount of circulating disease with the NCCN Pediatric ALL Panel suggesting a general guide of >1,000 circulating lympho blasts per microliter or 20% lymphoblasts
- **However, based on morphologic, genetic, and immunophenotypic features, LL is indistinguishable from ALL. Patients with LL generally benefit from treatment with ALL-like regimens versus traditional lymphoma therapy[26,27] and should be treated in a center that has experience with LL.**

REVIEW ARTICLE OPEN

Check for updates

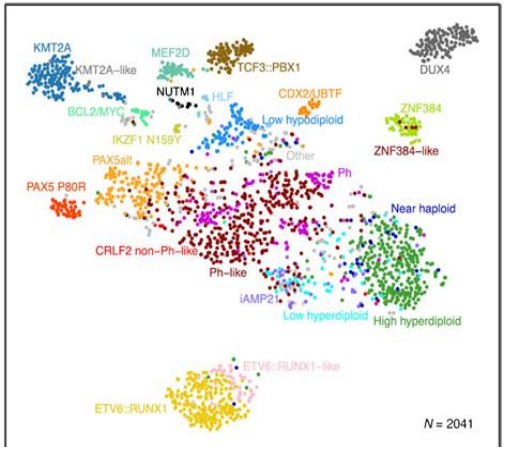
LYMPHOMA

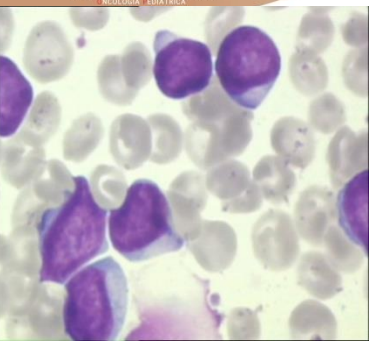
The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms

Precursor B-cell neoplasms
B-cell lymphoblastic leukaemias/lymphomas
B-lymphoblastic leukaemia/lymphoma, NOS
B-lymphoblastic leukaemia/lymphoma with high hyperdiploidy
B-lymphoblastic leukaemia/lymphoma with hypodiploidy
B-lymphoblastic leukaemia/lymphoma with iAMP21
B-lymphoblastic leukaemia/lymphoma with *BCR::ABL1* fusion
B-lymphoblastic leukaemia/lymphoma with *BCR::ABL1*-like features
B-lymphoblastic leukaemia/lymphoma with *KMT2A* rearrangement
B-lymphoblastic leukaemia/lymphoma with *ETV6::RUNX1* fusion
B-lymphoblastic leukaemia/lymphoma with *ETV6::RUNX1*-like features
B-lymphoblastic leukaemia/lymphoma with *TCF3::PBX1* fusion
B-lymphoblastic leukaemia/lymphoma with *IGH::IL3 fusion*
B-lymphoblastic leukaemia/lymphoma with *TCF3::HLF* fusion
B-lymphoblastic leukaemia/lymphoma with other defined genetic abnormalities
Mature B-cell neoplasms

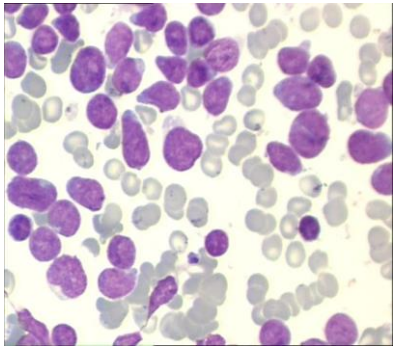
Precursor T-cell neoplasms
T-lymphoblastic leukaemia/lymphoma
T-lymphoblastic leukaemia / lymphoma, NOS
Early T-precursor lymphoblastic leukaemia / lymphoma
(Entity deleted)

Burkitt lymphoma
Burkitt lymphoma
KSHV/HHV8-associated B-cell lymphoid proliferations and lymphomas
Primary effusion lymphoma

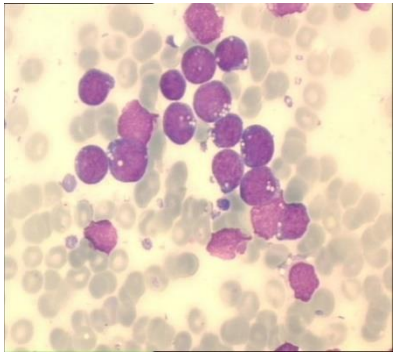




L1 Omogeneo



L2 Eterogenea

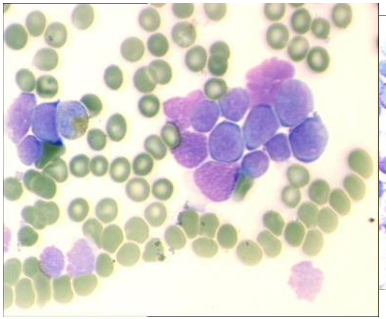


L3

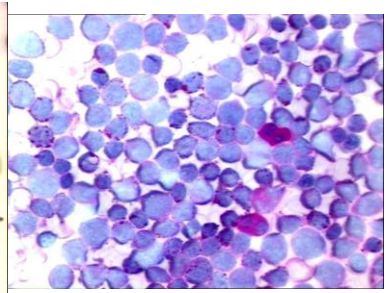
Linfoblasto
N/C alto / medio
- Diametro 8–10 μm.

Nucleo rotondo ovale , Covoluto(forme T)
- Cromatina fine omogenea

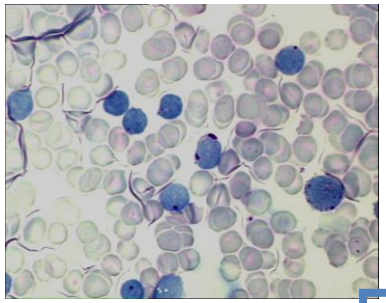
Nucleoli scarsamente visibili
Citoplasma.scarso /ampio da tenuamente basofilo ad intensamente basofilo (Burkitt like)
presenza di pseudopodi(Hand Mirrow Cells)
Granulazioni rare forme LAL granulate (rare)



MPO negativa

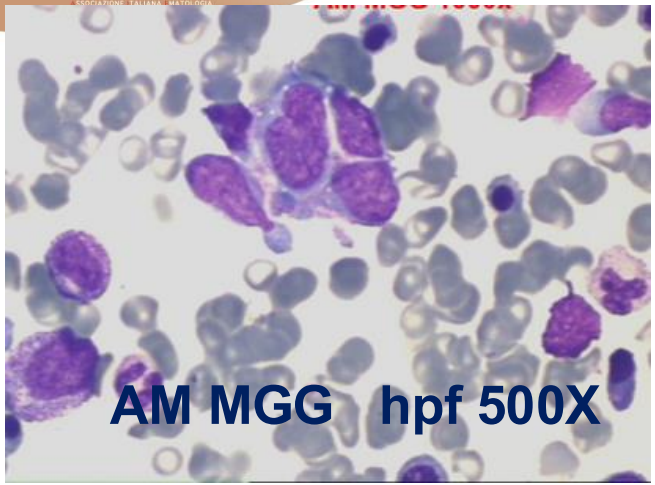


PAS Granulare

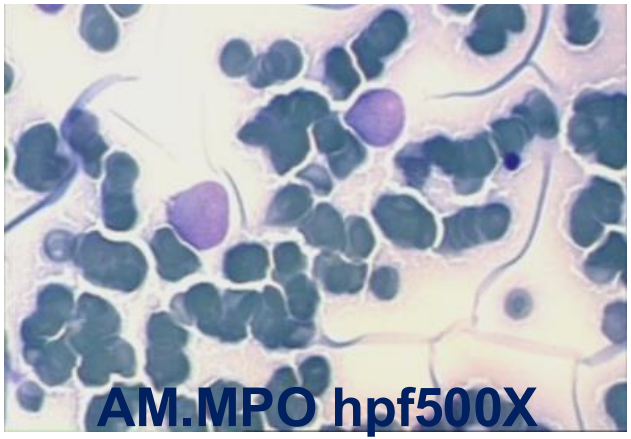


PAS a spot

→It cannot be reliably distinguished from some types of undifferentiated or minimally differentiated myeloblasts and therefore should be counted as a blast

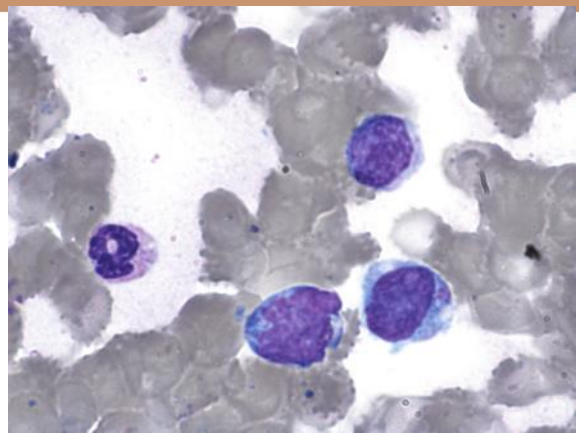


AM MGG hpf 500X

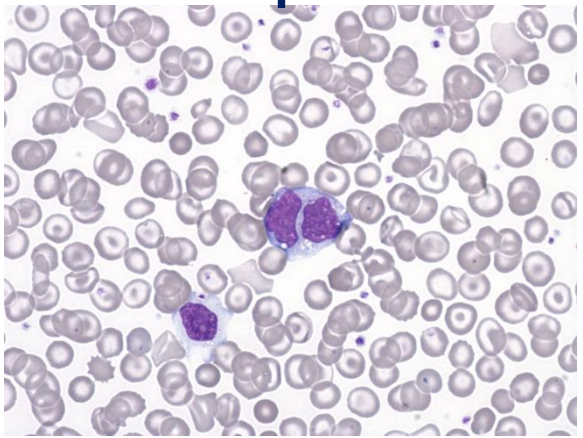


AM.MPO hpf500X

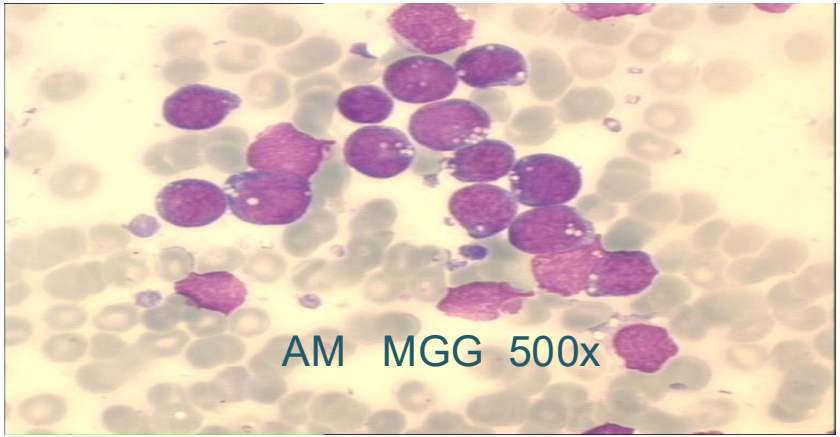
Leucemia / Linfoma Linfoblastico T
Morfologia eterogenea

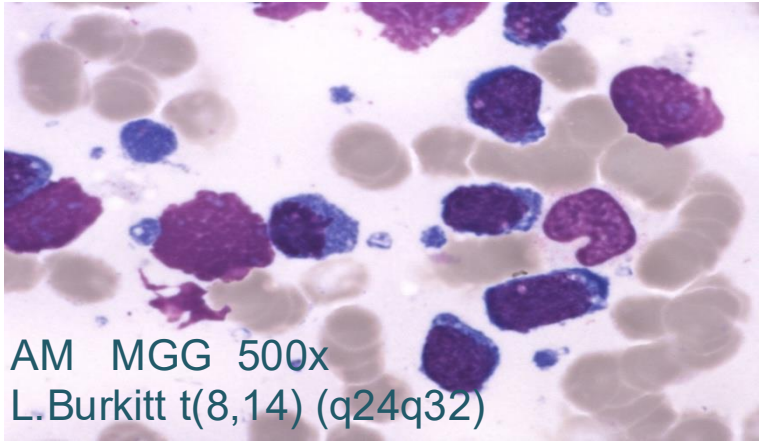


AM MGG hpf 500X



Leucemia / Linfoma Linfoblastico B (morfologia Burkitt)
TdT pos CD34+ No riarrangiamento MYC





Infiltrazione blastica >20%
Caratteristiche morfologiche : High grade morphology
Intermediiate → Dimensioni **8–10 μm**
N/C medio
Nucleo rotondo ovale membrana nucleare regolare
Cromatina finemente dispersa 1.2 nucleoli prominenti simili alle cellule del Linfoma di Burkitt
Cromatinaaddensata
Citoplasma ampio intensamente basofilo se rima citoplasmatica squadrata → L.Burkitt
Possono essere presenti vacuolizzazioni→**NON Specifico**
Numerose mitosi (Ki67 95%)

CONCLUSIONI



Asger Jorn, *Raphael's Angels*
Cobra: a creative utopia

- *La MORFOLOGIA ha ancora un ruolo fondamentale nell' indirizzare le indagini di secondo e terzo livello → diagnosi integrata →armonizzazione tra clinica e laboratorio*
- *L' identificazione continua di nuove mutazioni geniche, necessita spesso di una stretta correlazione con gli aspetti morfologici utili nella defizione diagnostica nella* ***clinical pratice***



La medicina è un processo in continua evoluzione



ROME EWOG –MDS MORHOLOGY BOARD

International Journal of Molecular Sciences
MDPI

Review

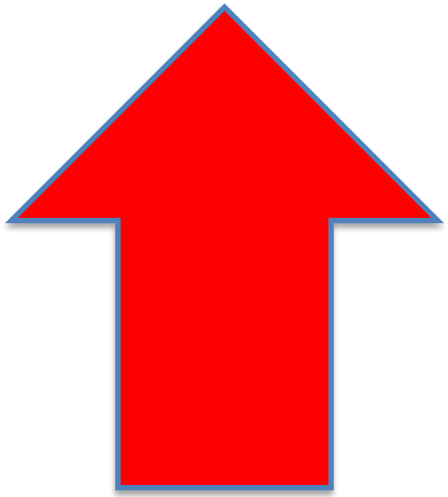
Molecular Landscape of Acute Myeloid Leukemia in Pediatric Patient-Age-Related Correlations: A Systematic Review

Katarzyna Cencelewicz [1], Barbara Pieniążek [1], Joanna Chajec [1], Jakub Buziak [1], Aleksandra Ozygała [2], Julia Sochaczewska [3], Monika Lejman [2,†] and Joanna Zawitkowska [4,*,†]

Table 4. Prognostic implications depending on genetic changes in different

Genetic Alteration	Age Group	Prognostic Implications
KMT2A rearrangements	infants	Poor; 5-year OS 35–50%, EFS 30–40%; outcome depends on fusion partner. Requires intensive chemotherapy and strict Minimal Residual Disease (MRD) monitoring.
CBFA2T3::*GLIS2* fusion	infants	Very poor; 5-year OS < 20%, EFS 15–20%, frequent relapses. Characteristic aggressive course of AMKL in infants. High resistance to standard chemotherapy schemes
t(7;12)/*MNX1*::*ETV6* fusion	infants	Poor; 5-year OS < 30%, EFS 20–25%, high relapse in infancy. Standard chemotherapy regimen weakly effective.
RBM15::*MKL1* fusion	infants	Intermediate-poor; 5-year OS 30–50%, EFS 35–40% Prognosis depends on response to induction.
CBF fusions (t(8;21), inv(16))	children	favorable; OS 75–85%, EFS 60–75%. The prognosis is worsened by the presence of *KIT*, *RAS*, *FLT3-ITD*.
NUP98 rearrangements	children	Poor; OS < 40%, EFS < 30%, high risk of relapse. Co-occurrence of *FLT3-ITD* or *WT1* worsens prognosis.
FLT3 mutations (*ITD*/*TKD*)	adolescents	Intermediate-poor; OS 40–50%, EFS 30–40%. High risk of relapse, especially ITD with high allelic ratio.
CEBPA mutations	adolescents	Favorable; 5-year OS 80–90%, EFS 70–80%. Co-occurrence of *FLT3-ITD* worsens prognosis.
NPM1 mutations	adolescents	Favorable; OS 75–85%, EFS 70–75%. The co-occurrence of *FLT3-ITD* worsens the prognosis.
Trisomy 8	adolescents	Variable: neutral to moderately favorable; OS 60–70%, EFS 55–65%. Prognosis significantly dependent on additional aberrations.

,------AND THE MAIN THING TO LEARN



CAREFUL PHYSICAL EXAMINATION IS STILL IN !

AND MORPHOLOGY EVALUATION

(S.Fenu M.Veltroni)

(….specially if you want o use the genome…..)

C.Niemayer Rome morphology board 2006

Perspective

What's New in the Classification, Diagnosis and Therapy of Myeloid Leukemias

Marco Pizzi [1,*], Carmela Gurrieri [2] and Attilio Orazi [3]

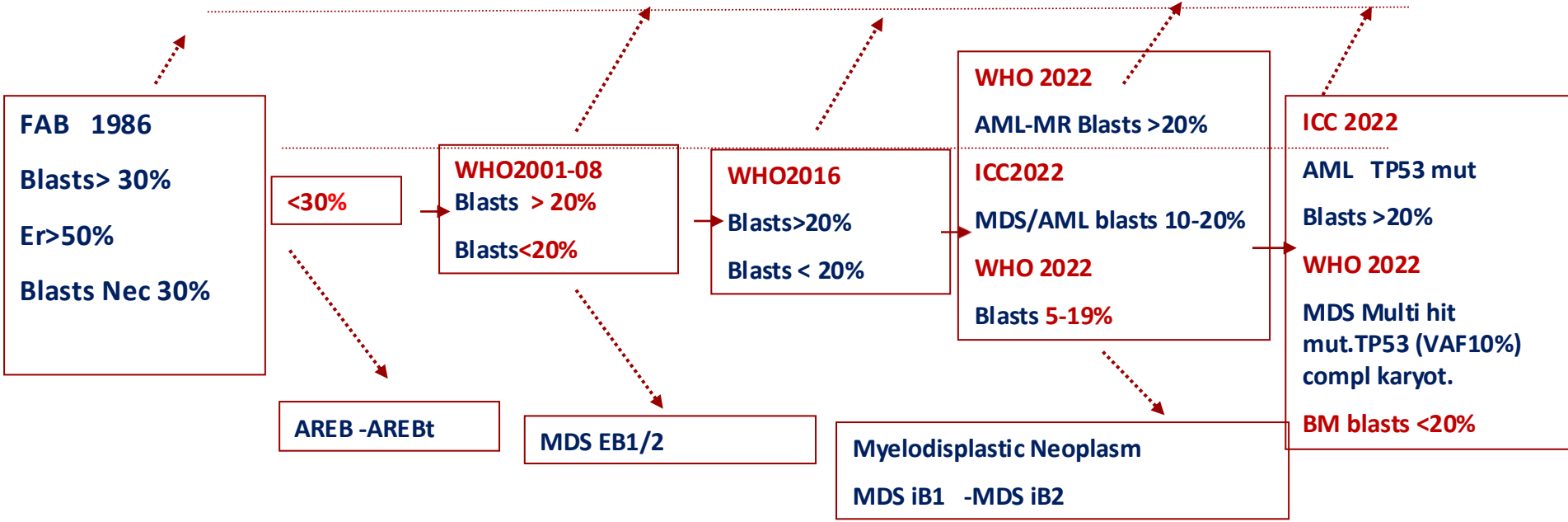
International Consensus Classification (ICC)		2022 WHO Classification	
AML subtypes	*Blasts* *	*AML subtypes*	*Blasts* *
AML with recurrent genetic abnormalities		**AML with defining genetic abnormalities**	
Acute promyelocytic leukemia with t(15;17) (q24.1;q21.2)/*PML::RARA*	≥10%	Acute promyelocytic leukemia with *PML::RARA* fusion	no threshold
Acute promyelocytic leukemia with other *RARA* rearrangements			
AML with t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1*	≥10%	AML with t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1* fusion	no threshold
AML with inv(16)(p13.1;q22) or t(16;16) (p13.1;q22)/*CBFB::MYH11*	≥10%	AML with *CBFB::MYH11* fusion	no threshold
AML with t(9;11)(p21.3;q23.3)/*MLLT3::KTM2A*	≥10%	AML with *KTM2A* rearrangement	no threshold
AML with other *KMT2A* rearrangements			
AML with t (6;9)(p22.3;q34.1)/*DEK::NUP214*	≥10%	AML with *DEK::NUP214* fusion	no threshold
AML with inv(3)(q21.3q;26.2) or t(3;3)(q21.3;q26.2)/*GATA2::MECOM*	≥10%	AML with *MECOM* rearrangements	no threshold
AML with other *MECOM* rearrangements			
AML with other rare recurring translocations	≥10%	AML with other defined genetic alterations	no threshold
AML with t(1;3)(p36.3;q21.3)/*PRDM16::RPN1*		AML with *NPM1::MLF1*	
AML with t(3;5)(q25.3;q35.1)/*NPM1::MLF1*		AML with *KAT6A::CREBBP*	
AML with t(8;16)(p11.2;p13.3)/*KAT6A::CREBB*		AML with *MNX1::ETV6*	
AML with t(1;22)(p13.3;q13.1)/*RBM15::MRTF1*		AML with *FUS::ERG*	
AML with t(5;11)(q35.2;p15.4/*NUP98::NSD1*		AML with *RUNX1T3(CBFA2T3)::GLIS2*	
AML with t(11;12)(p15.4;p13.3)/*NUP98::KMD5A*			
AML with NUP98 and other partners			
AML with t(7;12)(q36.3;p13.2)/ETV6::MNX1			
AML with t(10;11)(p12.3;q14.2)/PICALM::MLLT10			
AML with t(16;21)(p11.2;q22.2)/FUS::ERG			
AML with t(16;21)(q24.3;q22.1)/RUNX1::CBFA2T3			
AML with inv(16)(p13.3q24.3)/*CBFA2T3::GLIS2*			
AML with t(9;22)(q34.1;q11.2)/*BCR::ABL1*	≥20%	AML with *BCR:: ABL1* fusion	≥20%
AML with mutated *NPM1*	≥10%	AML with *NPM1* mutation	no threshold
AML with in-frame bZIP *CEBPA* mutations	≥10%	AML with *CEBPA* mutation	≥20%
AML with mutated *TP53* **	≥20%	-	
AML with myelodysplasia-related gene mutations [§]	≥20%	**AML, myelodysplasia-related**	≥20%
AML with myelodysplasia-related cytogenetic abnormalities [#]			
AML, not otherwise specified	≥20%	**AML, defined by differentiation**	≥20%
Myeloid sarcoma	n.a	**Myeloid sarcoma**	n.a

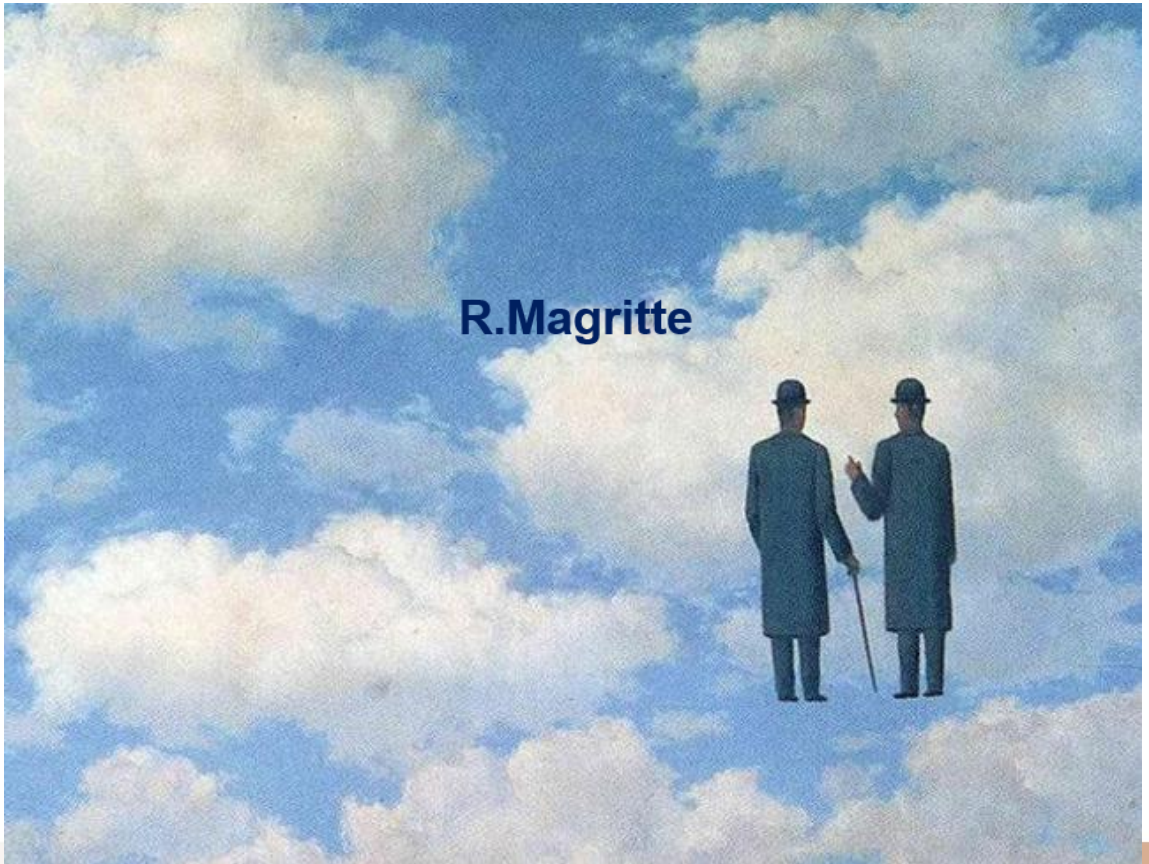
Notes: (*) blast threshold refers to either peripheral blood or bone marrow samples; (**) a separate diagnostic category of AML with mutations in TP53 is not included in the 2022 WHO Classification; (§) defined by mutations in *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, *ZRSR2*; (#) defined by presence of complex karyotype, del(5q)/t(5q)/add(5q), -7/del(7q), +8, del(12p)/t(12p)/add(12p), i(17q), -17/add(17p) or del(17p), del(20q), idic(X)(q13). The WHO category of AML, myelodysplasia related does not include mutations in *RUNX1* and relies on slightly different cytogenetic alterations [9].

- Tipo e gerarchia della alterazioni genetiche nella diagnosi di LAM
- **Ridefinizione dei criteri diagnostici delle LAM**
- **Inserimento di nuove categorie molecolari**

- Morfologia non più il solo criterio diagnostico MDS/AML-AML-MR
- **Utile il cutoff dei blasti per definire alcuni specifici sottotipi di LAM ??**

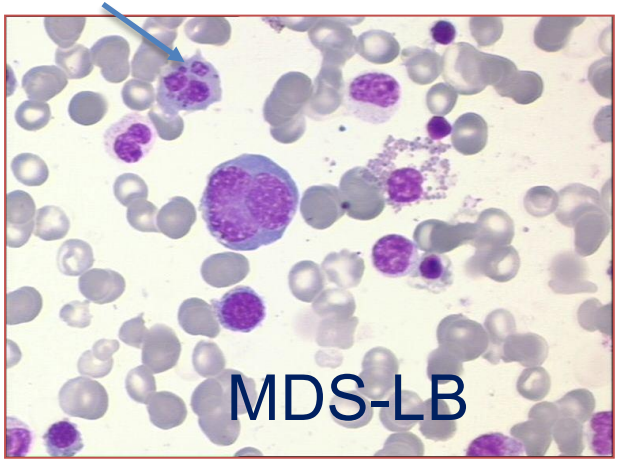
Algoritmo enumerazione dei blasti midollari nella diagnosi di Leucemia Acuta Mieloide
cosa è cambiato
AML

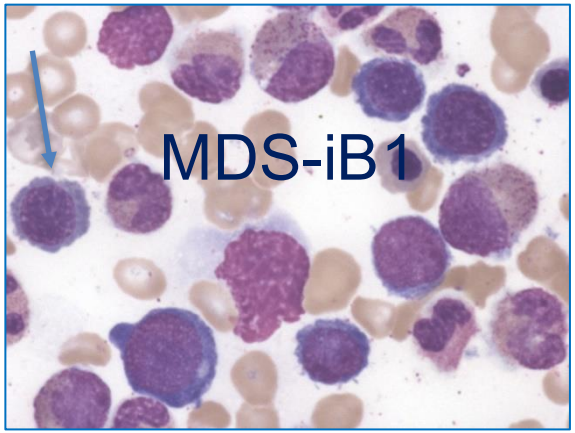


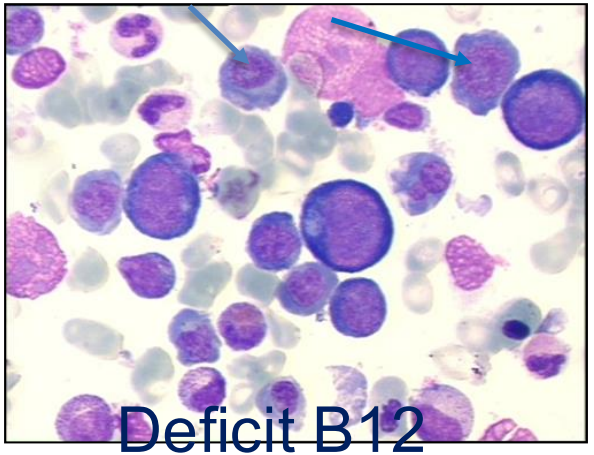

R.Magritte

ERITRODSPLASIA

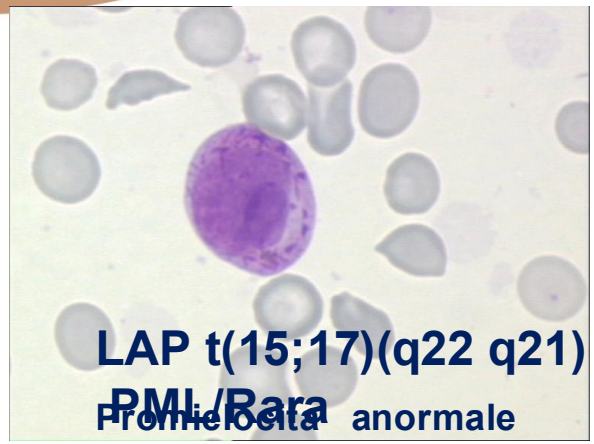
Difficoltà morfologiche Asincronia Nucleo/ Citoplasma disallineamento nella maturazione eritroide nucleo immaturo rispetto al citoplasma che presenta difetti di emoglobinizzaione



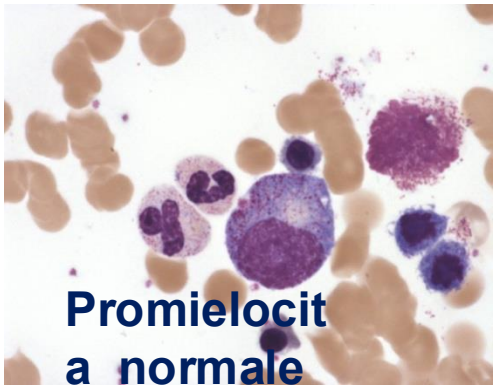




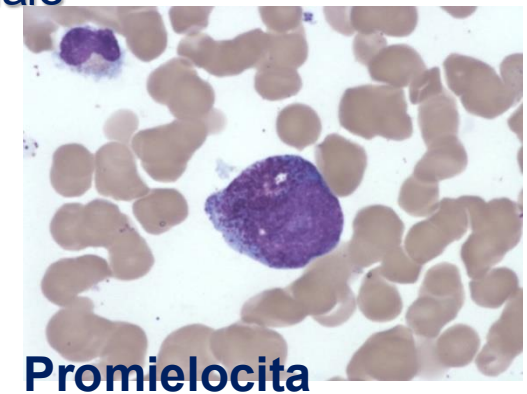
Diagnosi differenziale
LAM? MDS?



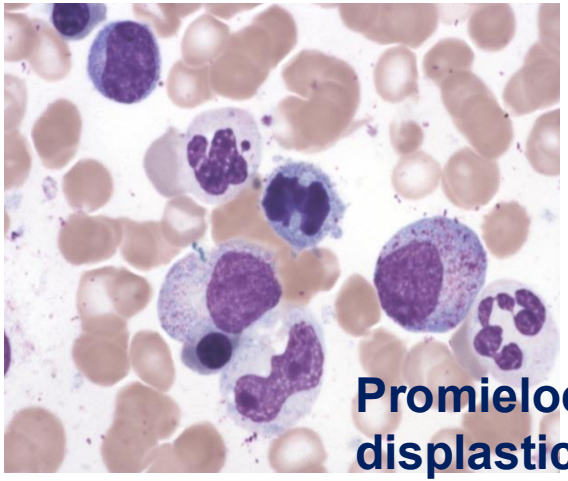
LAP t(15;17)(q22 q21)
PML /Rara
Promielocita anormale



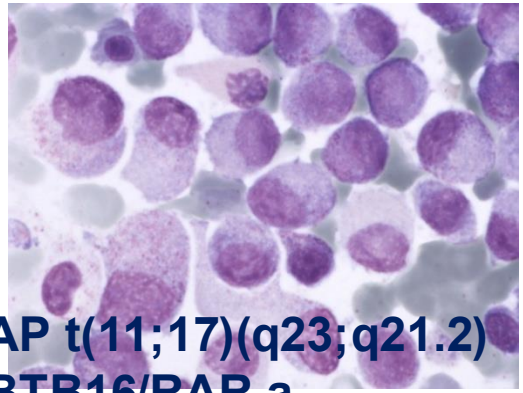
Promielocit
a normale



Promielocita
G-CSF



Promielocita
displastico



LAP t(11;17)(q23;q21.2)
ZBTB16/RAR a

Advances in molecular characterization of pediatric acute megakaryoblastic leukemia not associated with Down syndrome; impact on therapy development

Jixia Li[1,2*] and Maggie L. Kalev-Zylinska[1,3*]

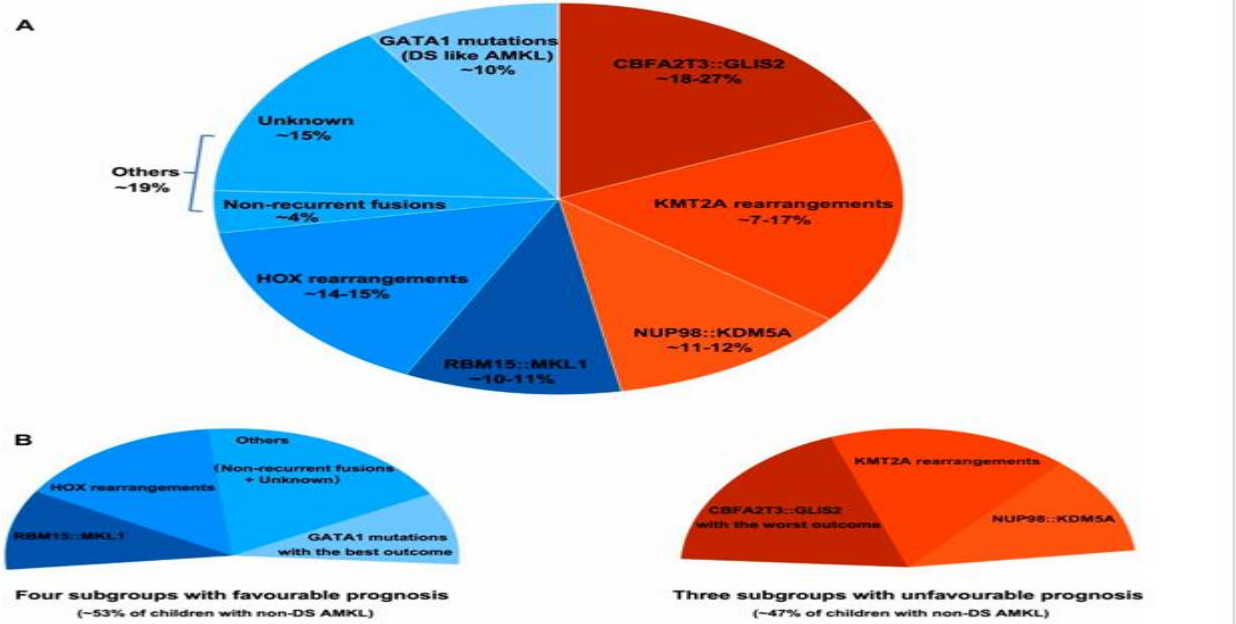
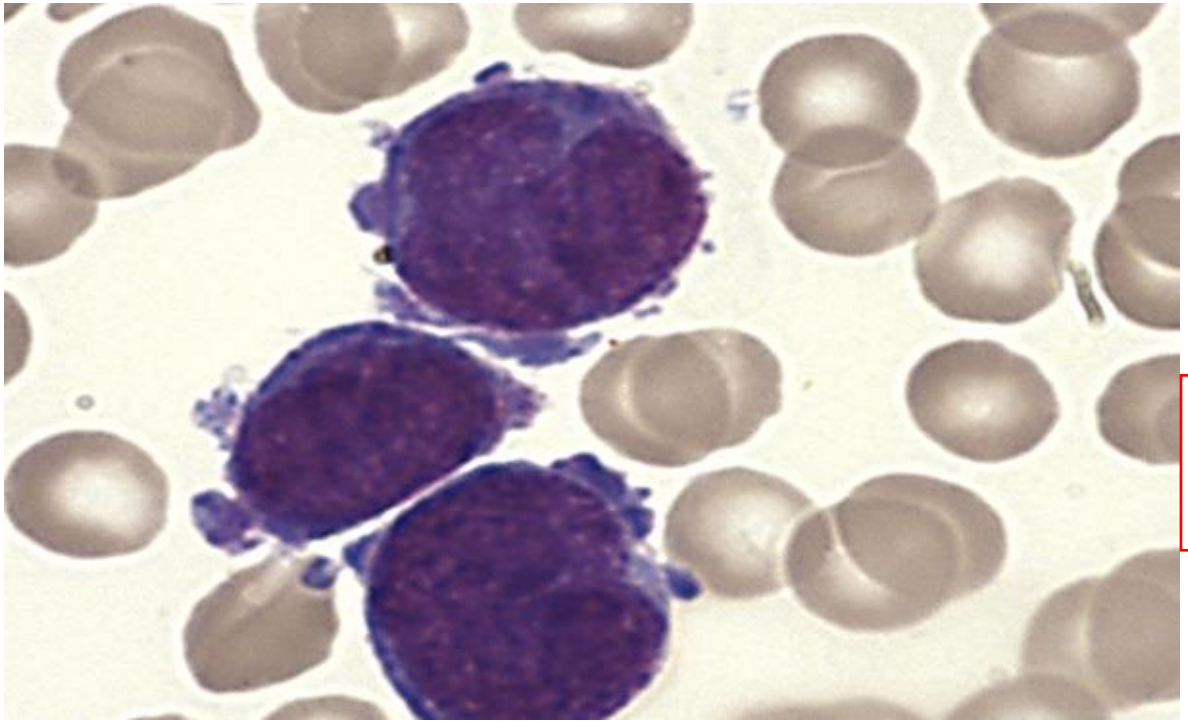


FIGURE 1
Distribution of distinct molecular subgroups and their prognostic impact in pediatric non-DS AMKL. **(A)** Distribution of distinct molecular subgroups in pediatric non-DS AMKL. Pediatric non-DS AMKL is characterized by recurrent and mutually exclusive chromosomal abnormalities, primarily including *CBFA2T3::GLIS2* (18%–27%), *KMT2A* rearrangements (*KMT2Ar*) (7%–17%), *NUP98::KDM5A* (11%–12%), *RBM15::MKLI* (10%–11%), and *HOX* rearrangements (*HOXr*) (14%–15%) (de Rooij et al., 2016; de Rooij et al., 2017; Hara et al., 2017; Lalonde et al., 2021). Further 4% of patients have non-recurrent fusions engaging hematopoietic transcription factors and epigenetic regulators (de Rooij et al., 2017). Other cases do not have known oncogenic fusions, but *GATA1* mutations are detected in about half of this population (overall found in approximately 10% of pediatric non-DS AMKL) (de Rooij et al., 2017). **(B)** Prognosis of distinct molecular subgroups in pediatric non-DS AMKL. Based on molecular aberrations, three subgroups (*CBFA2T3::GLIS2*, *KMT2Ar*, and *NUP98::KDM5A*) have an unfavorable prognosis, amounting to nearly half of patients; other three subgroups (*RBM15::MKLI*, *HOXr*, non-recurrent fusions and unknown drivers) have an intermediate prognosis, accounting for approximately 40% of patients; and patients with *GATA1* mutations represent an excellent prognostic group, making up for roughly 10% of children with non-DS AMKL (de Rooij et al., 2017). Among the unfavorable subsets, *CBFA2T3::GLIS2* has the worst outcome, with the 5-year overall survival rate being 14% (de Rooij et al., 2017). In contrast, DS-like AMKL has the best outcome, with



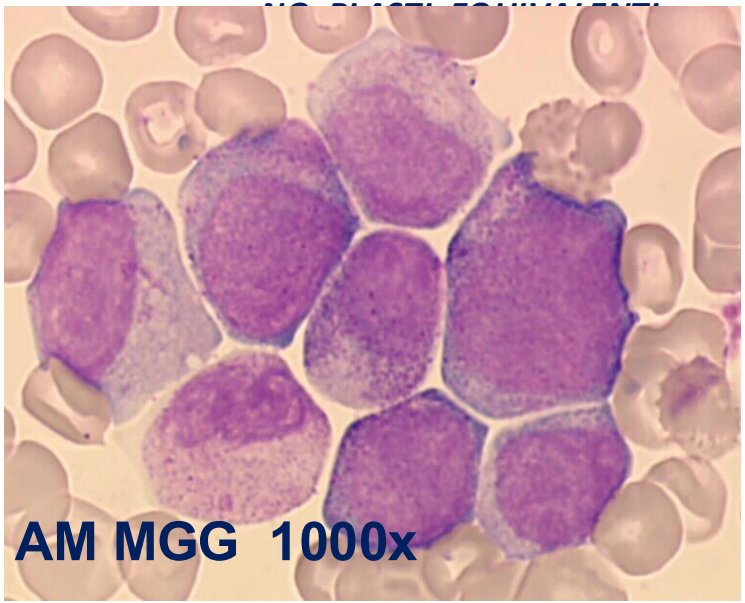
Aspirato midollare MGG 1000x

LAM

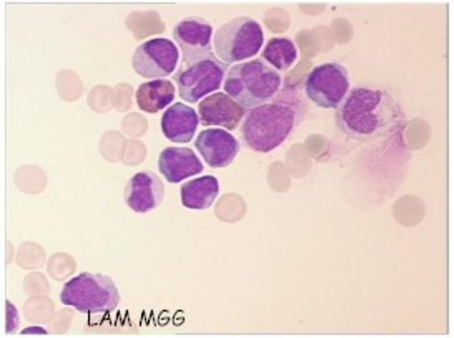
Megacarioblastica ???

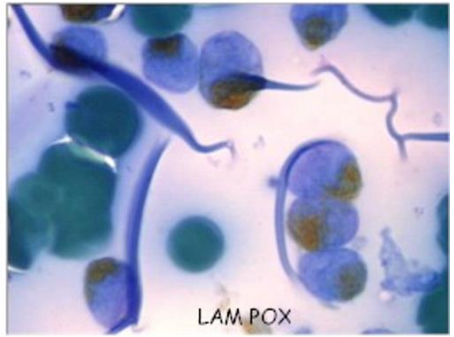
L.Promielocitica criptica

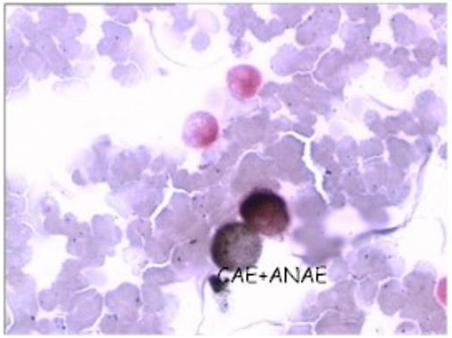
LAM t(8;21)(q22;q22.1)RUNX1::RUNX1T1) 20% dei casi di AML pediatriche
Immunofenotipo CD34+CD13+CD15+
espressioni abberranti CD19+ PAX5 CD79a

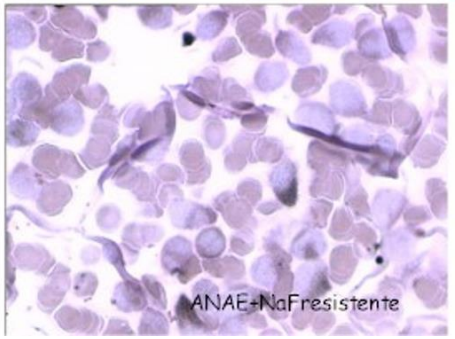


≥20% blasti svp/AM
<20% cellule monocitarie

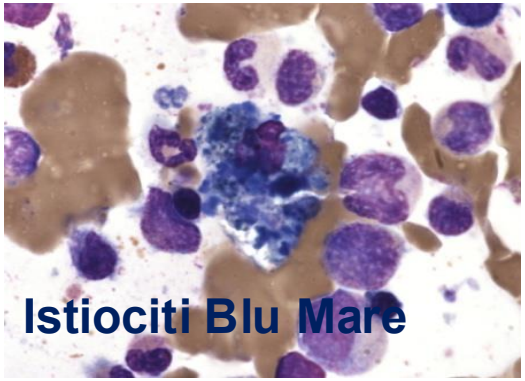






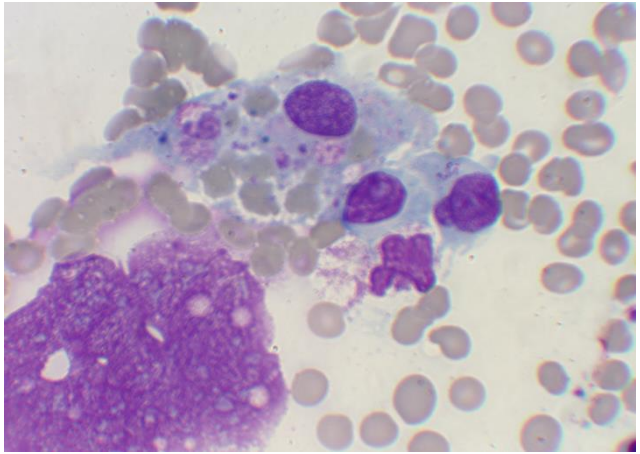


CITOCHIMICA



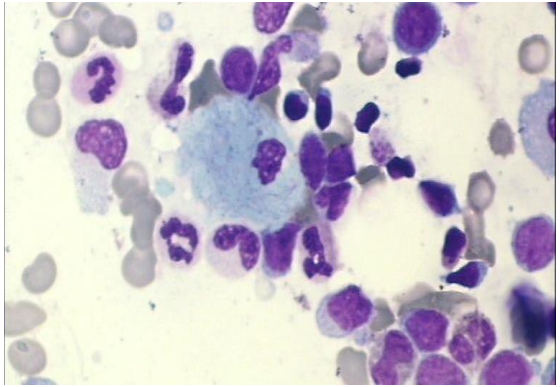
Istiociti Blu Mare

Istiocita emofagocitosi

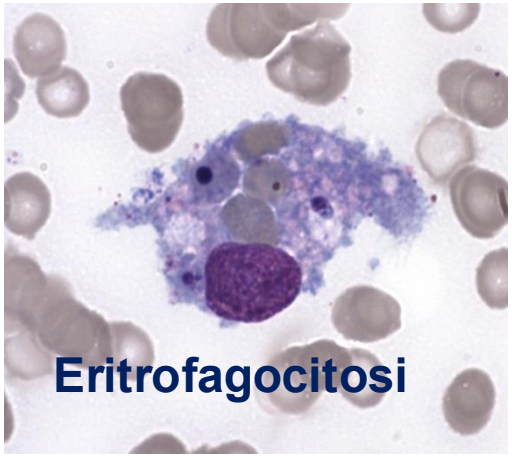




Deposito di ferro blu di Prussia

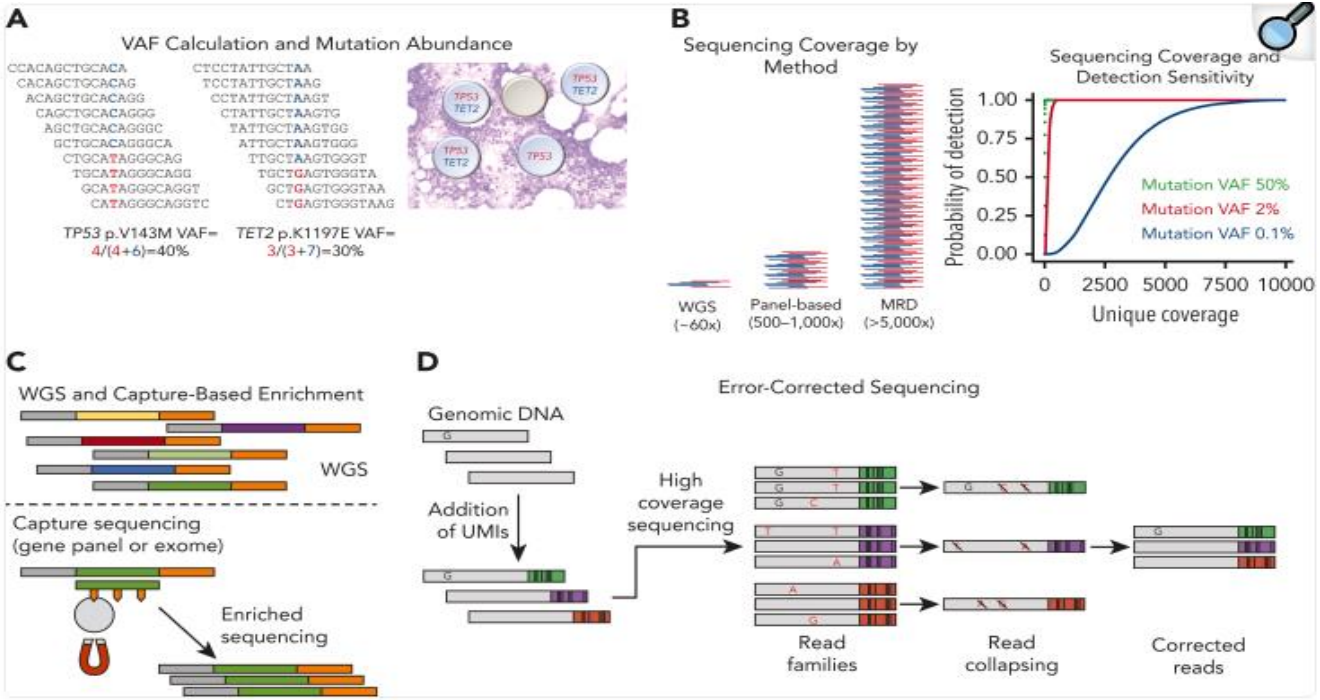


Istiociti Pseudo-Gaucher



Eritrofagocitosi

NEOPLASIE MIELOIDI/LINFOIDI
QUALI PERCORSI DIAGNOSTICI NELLA REAL LIFE ???
MORFOLOGIA ??? HA ANCORA UN RUOLO??



Blood. 2022 Nov 24; 140(21): 2228–2247.

CASOpPEDIATRICO
M 4 anni

- **Febbre 39 °C**
- **Es.emocromo**

Hb 8.4 gr/dl MCV 83/fl WBC . 36000 /mmc

Pmn 22% L 58% Mc 1% Mmc 5% Mono 14%

(monociti :2570/mmc)PLTS 83000/mmc

◊ Reticolociti 0.1%

- **Organomegalia**

◊ Milza 2cm

◊ Fegato 3cm

- Aspirato midolare

Cellularità Normale

Eritroblasti 46 %

Granuloblasti 33 %

Monociti 5%

Lymphociti 13%

Blasti 3%

G/E 0,6%

Megacariociti norm.

Conclusione: JMML

Diseritr. score 1.8

Dismiel. score 0.4

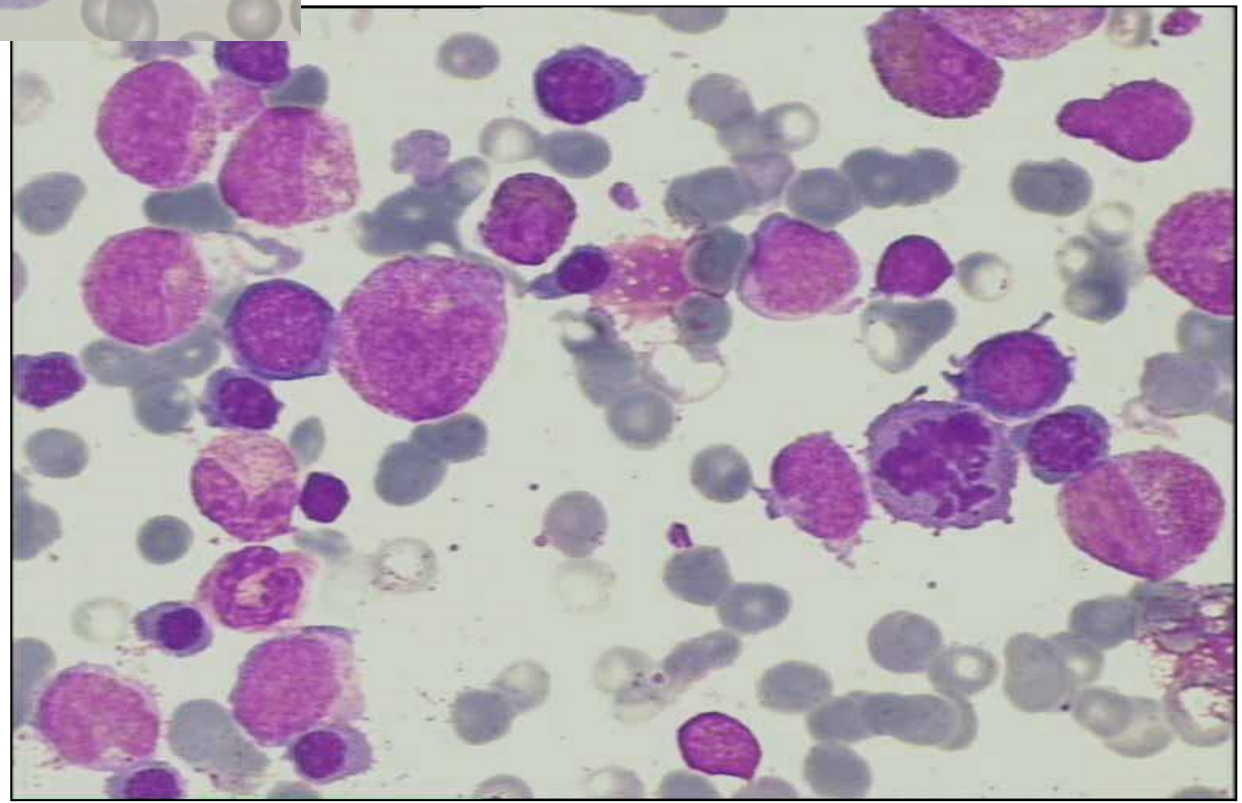
Dismegac. score 0.4

Displasia* tot. score 2.6

***according EWOG-MDS score**

ASPIRATO MIDOLLARE
MGG hpf 500X





Citogenetica: Negativa

Terapia: osservazione

2 mesi dopo

es emocromo nella norma

Studi virologici: infezione Parvovirus B19 (IgM positive)

Blood 91(1): 365-367. 1998

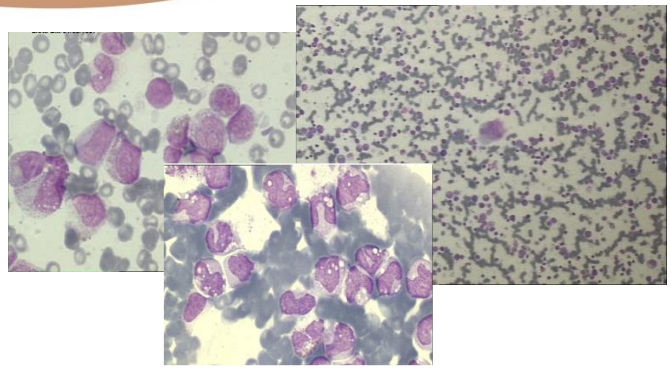
INFEZIONE

Principali meccanismi della pancitopenia

RIDOTTO *bone marrow output*:

- RIDUZIONE DELLA PROLIFERAZIONE
- DIFETTI DI MATURAZIONE
- EMOFAGOCITOSI

Normale produzione midollare ma distruzione periferica



Sangue periferico

(GB25-100.000/mmc)

Emazie-macrocitiche

Eritroblastemia

trombocitopenia

Blasti<20%

Eosinofilia rara

Citochimica: FAL elevata 50%

Midollo osseo

Ipercellulare

Megacariociti ridotti

Iperplasia granuloblastica

Blasti+promonociti<20%

Displasia trilineare moderata

BOM→fibrosi reticolinica

HAEM 4R	HAEM 5	ICC 2022
JMML => MDS/MPN	JMML => MPN	JMML => Pediatric and/or germline associated disorders

Clinical, hematological, and laboratory criteria (all 5 required)	Genetic criteria (any 1 criterion is sufficient)

• Peripheral blood monocyte count ≥ 1 x 10^9/L
• Blast and promonocyte in PB and BM < 20%
• Clinical evidence of organ infiltration, mostly splenomegaly
• No Ph chromosome or *BCR-ABL1* fusion
• No *KMT2A (MLL1)* gene rearrangement
	• Mutation in a component or a regulator of the canonical RAS pathway:
	  ◦ Clonal somatic mutation in *PTPN11*, *KRAS*, or *NRAS*[a]
	  ◦ Clonal somatic or germline *NF1* mutation and LOH or compound heterozygosity of *NF1*
	  ◦ Clonal somatic or germline *CBL* mutation and LOH of *CBL*[b]
	• Non-canonical clonal RAS pathway pathogenic variant[c] or fusions causing activation of genes upstream of the RAS pathway, such as *ALK*, *PDGFR-B*, *ROS1*, among others.

Other criteria

Cases not meeting any of the genetic criteria listed above (or when genetic testing is not available) must meet following criteria in addition to aforementioned clinical, haematological, and laboratory criteria: ≥2 of the following

- Increased haemoglobin F for age
- Myeloid (promyelocytes, myelocytes, metamyelocytes) and erythroid precursors on peripheral blood smear
- Thrombocytopenia with hypercellular marrow often showing decreased number of megakaryocytes. Dysplastic features may or may not be evident.
- Hypersensitivity of myeloid progenitors to GM-CSF as tested in clonogenic assays in methylcellulose or by measuring STAT5 phosphorylation in the absence or with low dose of exogenous GM-CSF.

HAEM 4R	HAEM 5	ICC 2022
JMML => MDS/MPN	JMML => MPN	JMML => Pediatric and/or germline associated disorders

Clinical and hematological features (the first 2 features are present in most cases; the last 2 are required)	Genetic studies (1 finding required)
• PB monocyte count ≥ 1 × 10^9/L* • Splenomegaly† • Blast percentage in PB and BM < 20% • Absence of *BCR::ABL1*	• Somatic mutation in *PTPN11* or *KRAS* or *NRAS* or *RRAS* • Germline *NF1* mutation and LOH of *NF1* or clinical diagnosis of neurofibromatosis type • Germline *CBL* mutation and LOH of *CBL*§

*This monocyte threshold is not reached in approximately 7% of cases.
†Splenomegaly is absent in 3% of cases at presentation.
‡Germline mutations (indicating Noonan syndrome) need to be excluded.
§Occasional cases with heterozygous splice site mutations

Diagnostic features in paediatric MDS-EB with *UBTF*-internal tandem duplication: defining a unique subgroup

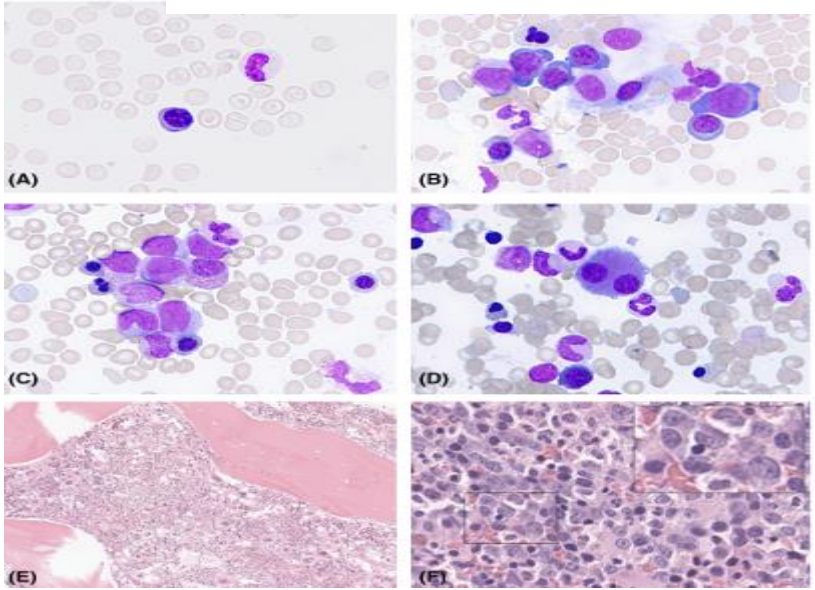


Figure 1. Cytomorphology and histomorphology of *UBTF*-TD. (A) Blood smear with normoblast. (B–D) Bone marrow smears showing dysplastic erythropoiesis with megaloblastoid changes (B), an increase of myeloid blasts (C), and hypolobulated megakaryocytes (D). Bone marrow biopsies are hypercellular (E, H&E) with effaced architecture. Immature myeloid precursors are increased centromedullary (F; H&E). EWOG ID-number D1113.

-----the bone marrow morphology of UBTF-mutated AML frequently displayed myelodysplastic changes, and in some cases was compatible with the diagnosis of AML-M6 according to the FAB classification….

Table 1. Morphological features of paediatric MDS with excess blasts and *UBTF*-TD

Cytology (bone marrow aspirate)		
Blast count (%)	Median (range)	11.2% (5%–19%)
EP to MP ratio	Median (range)	1.8 (0.3–12)
Dyserythropoiesis (>10%)	Number (%)	14 (100)
Severe immaturity in erythropoiesis	Number (%)	14 (100)
Dysgranulopoiesis (>10%)	Number (%)	14 (100)
Dysmegakaryopoiesis (>10%)	Number (%)	14 (100)

S. Schwarz Furlan et al
Histopathology 2025, 86, 603–610. DOI: 10.1111/his.15378