DIAGNÓSTICO INTEGRADO DE LOS LOS SÍNDROMES MIELODISPLÁSICOS

Integrated diagnosis of myelodysplastic syndromes

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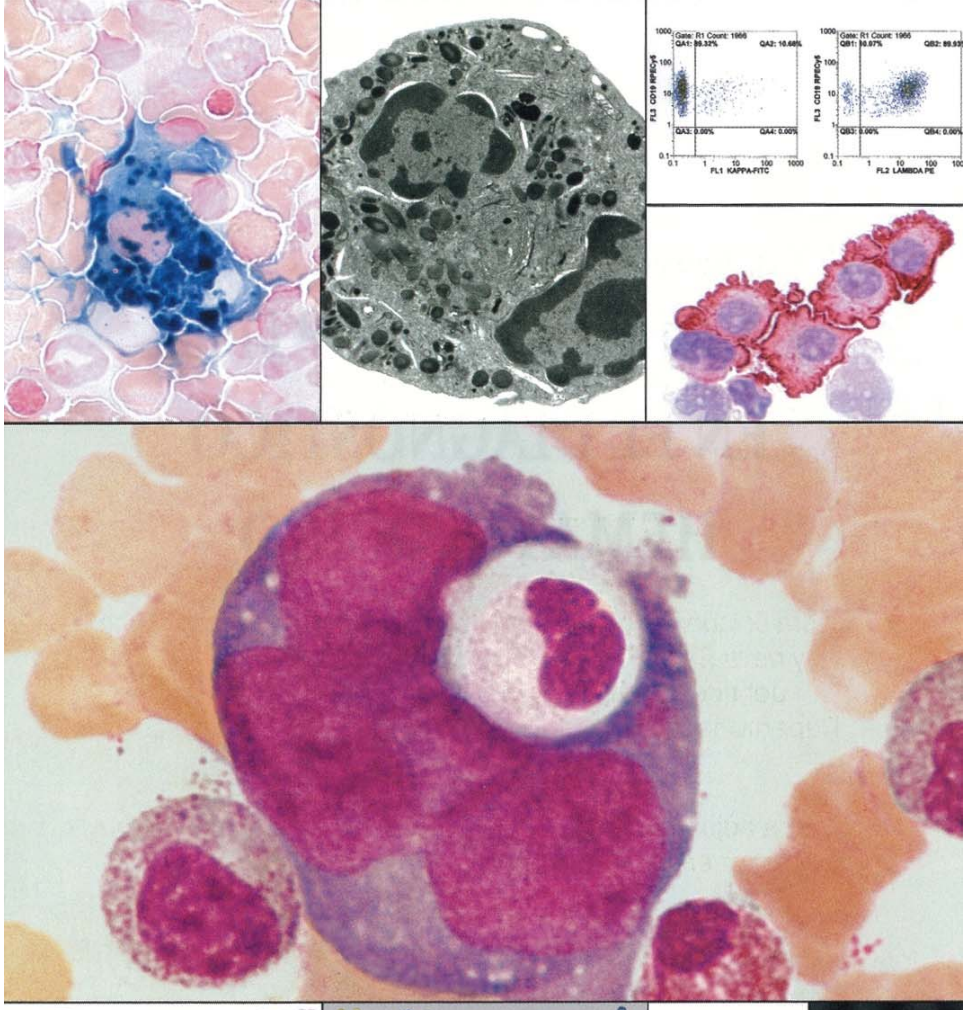
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QA1: 89.33%
QA2: 10.66%
QA3: 0.00%
QA4: 0.00%
FL3 CD19 RPE-Cy5
FL1 KAPPA-FITC
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QB1: 10.97%
QB2: 89.03%
QB3: 0.00%
QB4: 0.00%
FL3 CD19 RPE-Cy5
FL2 LAMBDA PE
5'
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Myelodysplastic syndromes (MDS)

- The myelodysplastic syndromes are a heterogenous group of clonal hematopoietic stem cell diseases characterized by cytopenia(s), dysplasia in one or more myeloid cell lineage and ineffective haematopoiesis

- They present:
  - A variable clinical course
  - Short survival (closely related to the subtype of MDS)
  - High risk of progression to acute leukemia

Classification of MDS: WHO 2008

7 SUBTYPES:

- Refractory cytopenia with unilineage dysplasia (RCUD)
- Refractory anemia with ring sideroblasts (RARS)
- Refractory cytopenia with multilineage dysplasia (CRDM)
- Refractory anemia with excess blasts 1 (RAEB-1)
- Refractory anemia with excess blasts 2 (RAEB-2)
- MDS associated with isolated del(5q)
- Unclassified MDS

1 PROVISIONAL:

- **ICUS**: Idiopathic cytopenia of undetermined significance

Vardiman 2008

Classification of MDS: WHO 2008

Subtype	Cytopenia	Blasts PB (%)	Blasts BM %	% ring SB in BM	Dysplasia
RCUD	1 or 2 cytopenias	<1	<5	<15	1 cell line
RARS	Anaemia	0	<5	≥15	Erythroid
RCMD	Cytopenia/s	<1	<5 No Auer R.	<15 o ≥15	≥2 lines
RAEB-1	Cytopenia/s	<5	5-9 No Auer R.	Indifferent	Indifferent
RAEB-2	Cytopenia/s	5-19 (+/- Auer R.)	10-19 +/- Auer R.	Indifferent	Indifferent
MDS associated with isolated del(5q)	Anaemia	<1	<5	Indifferent	Indifferent
Unclassified MDS	Cytopenias	=1	<5		<10% in ≥ 1 cell lines and CG abnormality
ICUS (provisional)	Cytopenias **> 6 months**	0	<5	0	NO displasia NO CG abnormality

Classification of MDS: WHO 2008

Recognizes *7 morphological subtypes* categorized into **three risk groups** based on duration of survival and the incidence of progression to acute leukemia:

Low: RCDU, RARS

Intermediate: CRDM, RAEB-1

High: RAEB-2

Minimal diagnostic criteria for MDS

Pre-requisites:

1. Marked prolonged (>6 months) cytopenia
2. Other diseases excluded

Additional diagnostic criteria (MDS related):

1. Morphologic dysplasia in ≥ 10% erythorid and/or neutrophil and/or megakaryocytic lineages
2. Blast cell number between 5% and 19%
3. Characteristic cytogenetic abnormalities (CG or FISH)(no: +8, del20q, -Y)

Diagnostic co-criteria:

1. Flow cytometry immunophenotypic abnormalities
2. Clonality (Chromosome X inactivation tests) and molecular tests (e.g. RAS mutations)
3. CFU-assays: Decrease in colonies formation in peripheral blood and bone marrow

Valent et al, Leuk Res, 2007

Diagnosis

- Myelodysplasia is not synonymous to MDS
- There is no pathognomonic data of MDS
- All cases of dysplasia and secondary cytopenia must be excluded

CLINICAL

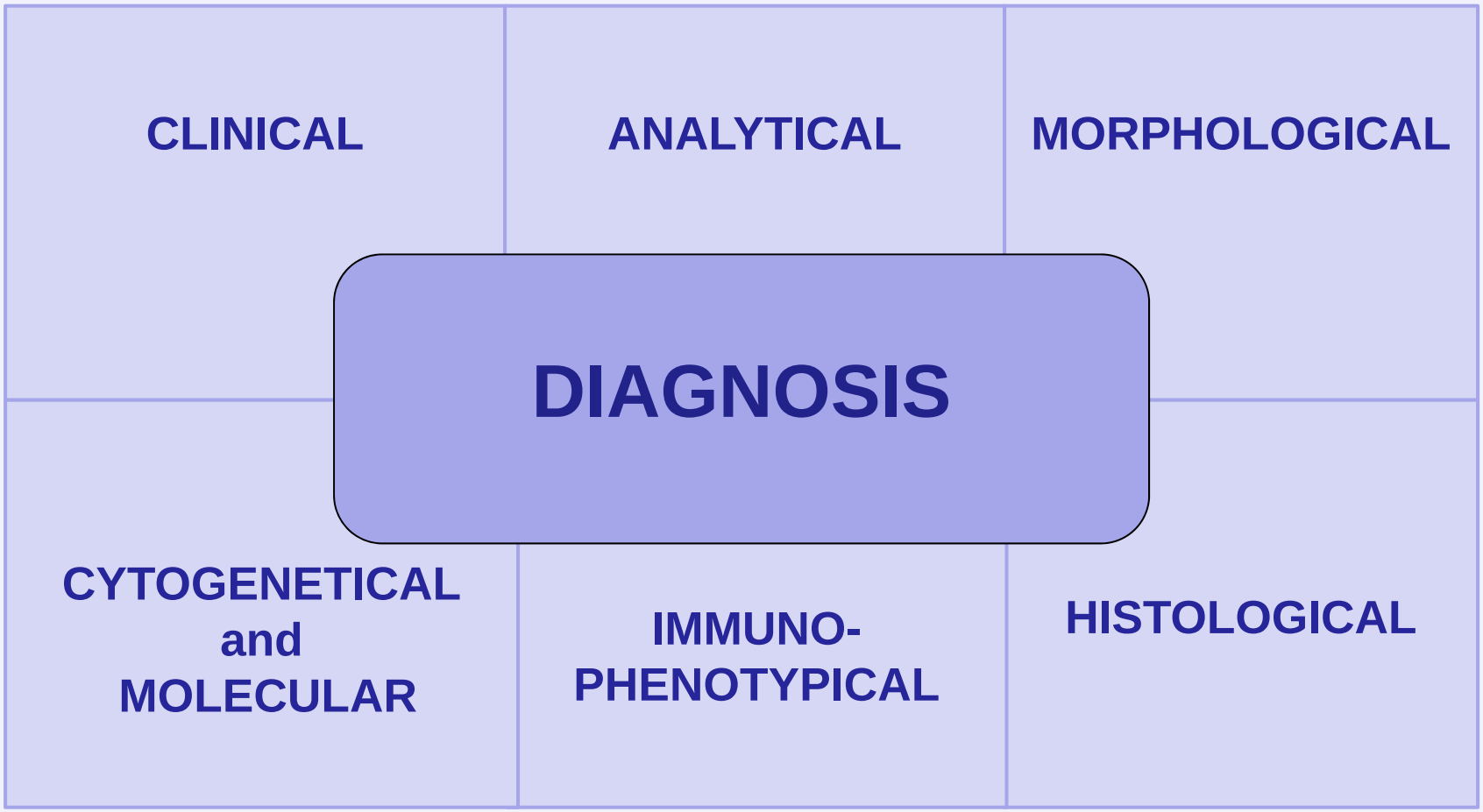
ANALYTICAL

MORPHOLOGICAL

CYTOGENETICAL and MOLECULAR

IMMUNO-PHENOTYPICAL

HISTOLOGICAL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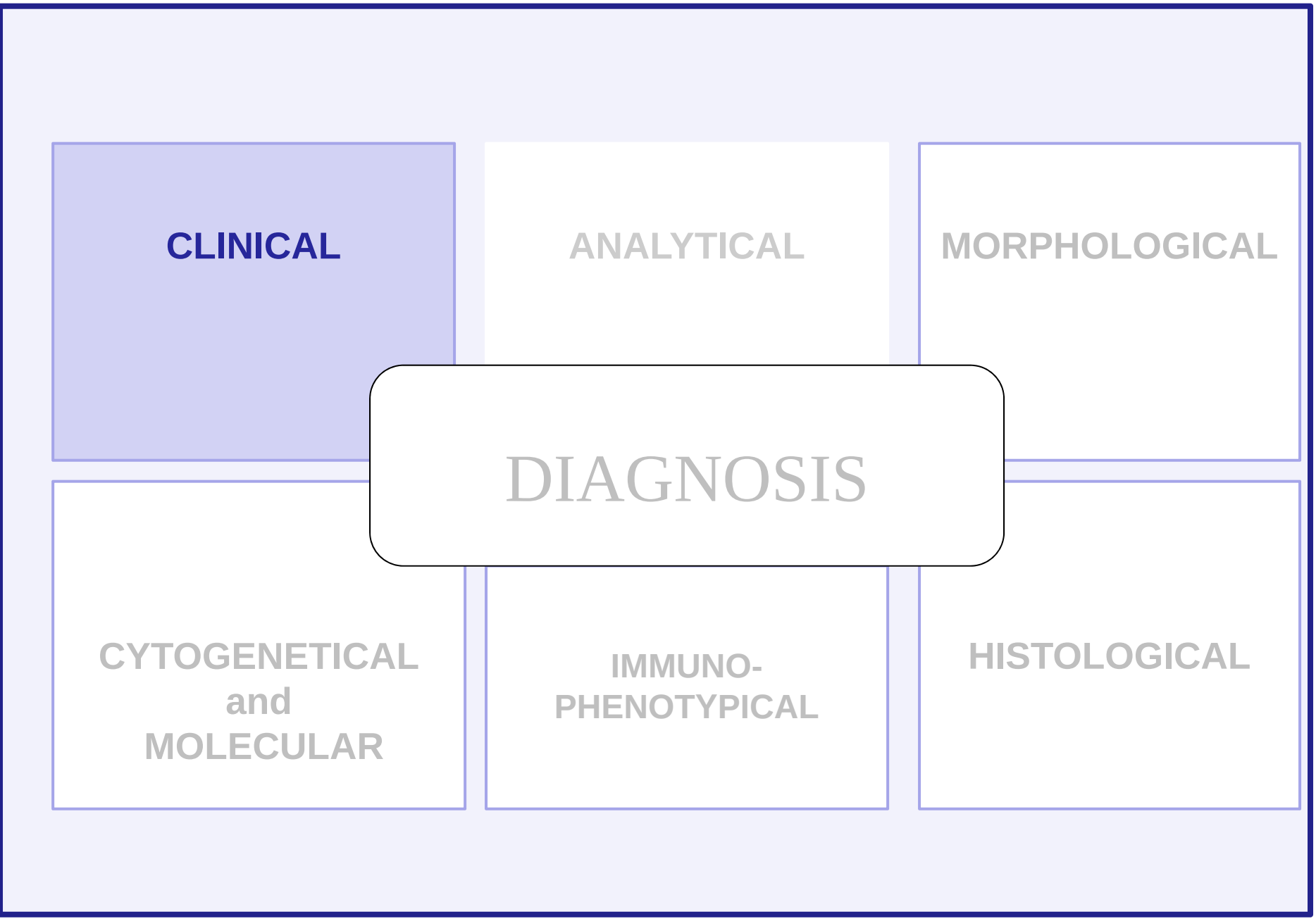
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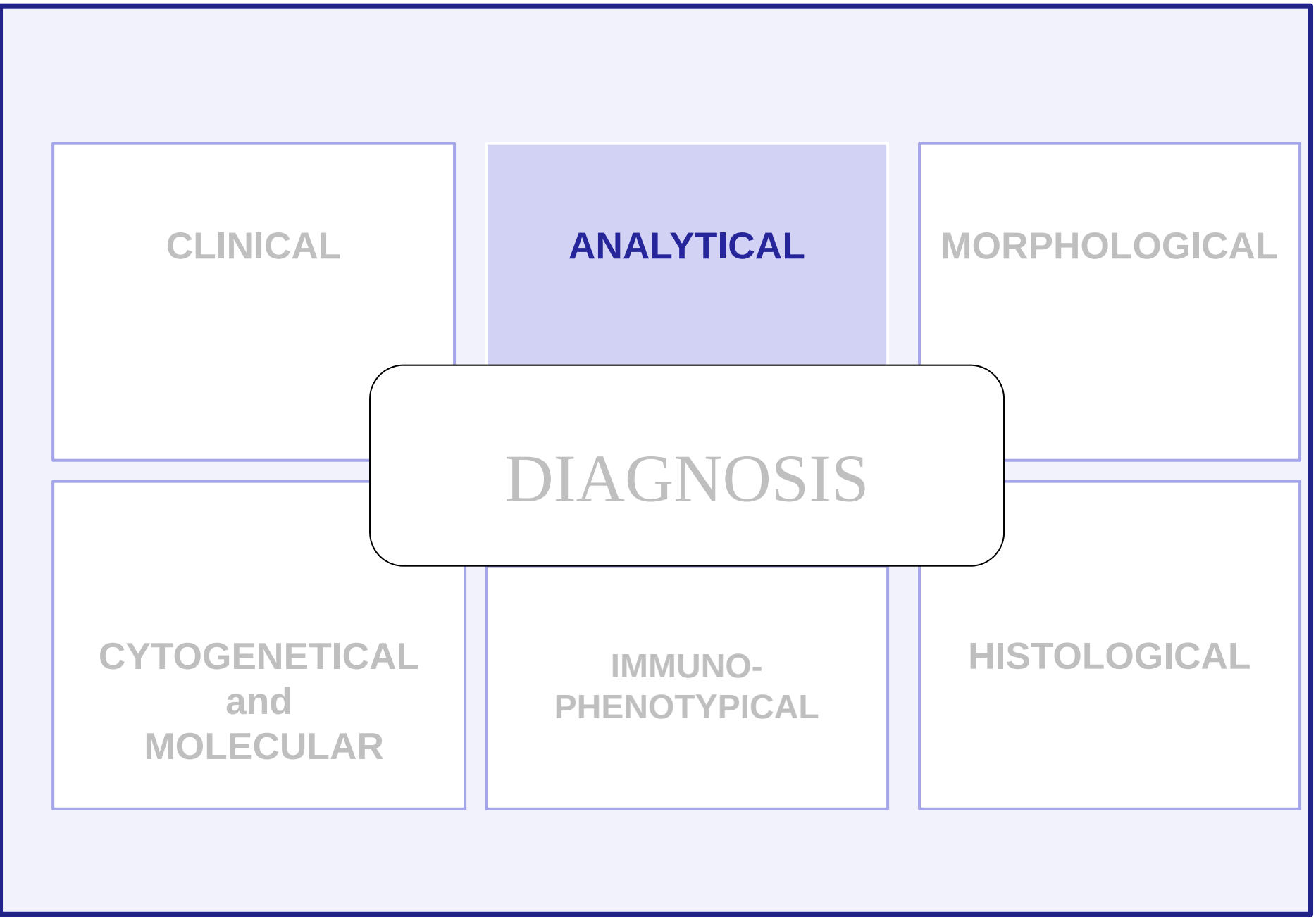
HISTOLOGICAL

Differential diagnosis: considerations (I)

To be excluded:

- Deficiencies of Fe, vitamin B12, folic acid and copper
- Anaemia of chronic disease
- Exposure to heavy metals: arsenic
- Chronic alcohol abuse
- Treatment with chemotherapeutic agents
- Viral infections (HIV, Parvovirus B19)
- G-CSF
- Inherited disorders, such as CDA
- Anaplastic anaemia
- PNH

As a result of all these possibilities, it is extremely important to be aware of the clinical history including exposure to drugs or chemicals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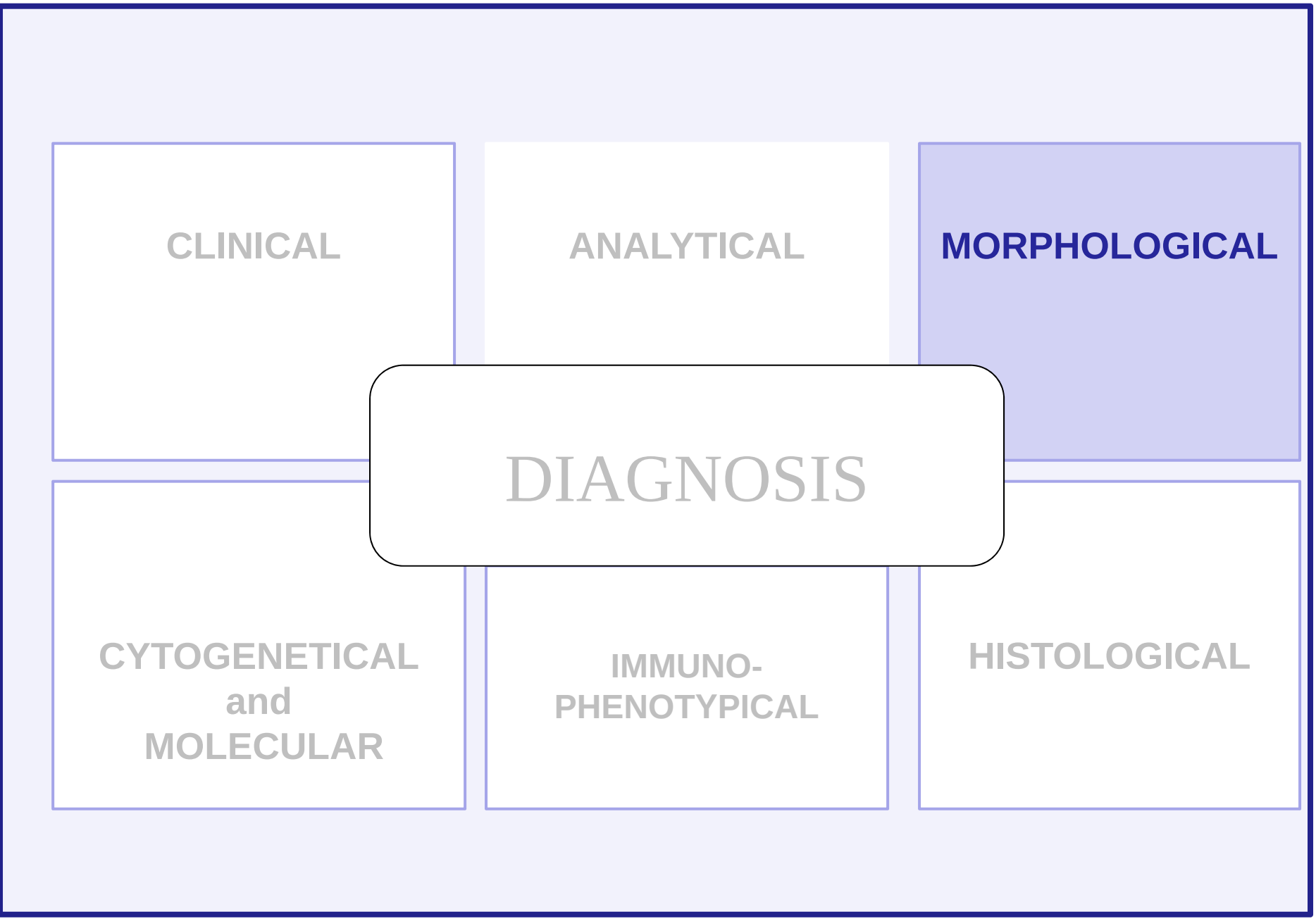
Analytical data

The initial laboratory procedures include:

- Blood cell counts*
- Reticulocyte counts
- Serum ferritin levels
- Total iron binding capacity and serum iron levels
- Levels of vitamin B12, red blood cell (RBC) folate
- Thyroidstimulating hormone

***Cytopenias (WHO 2008)** Hb: <10g/dL, Neutrophil: <1.8x10^9/L, Plat: <100x10^9/L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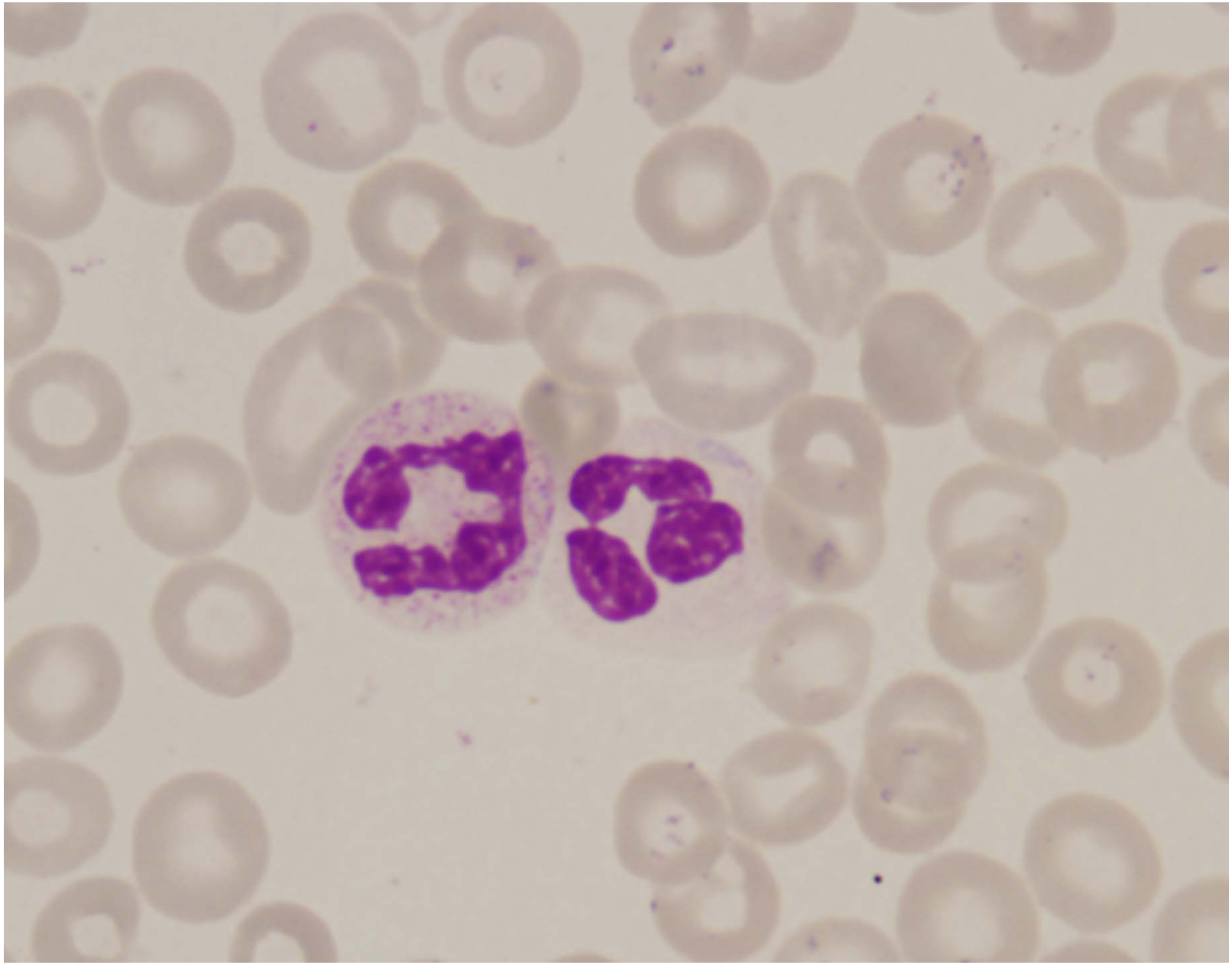
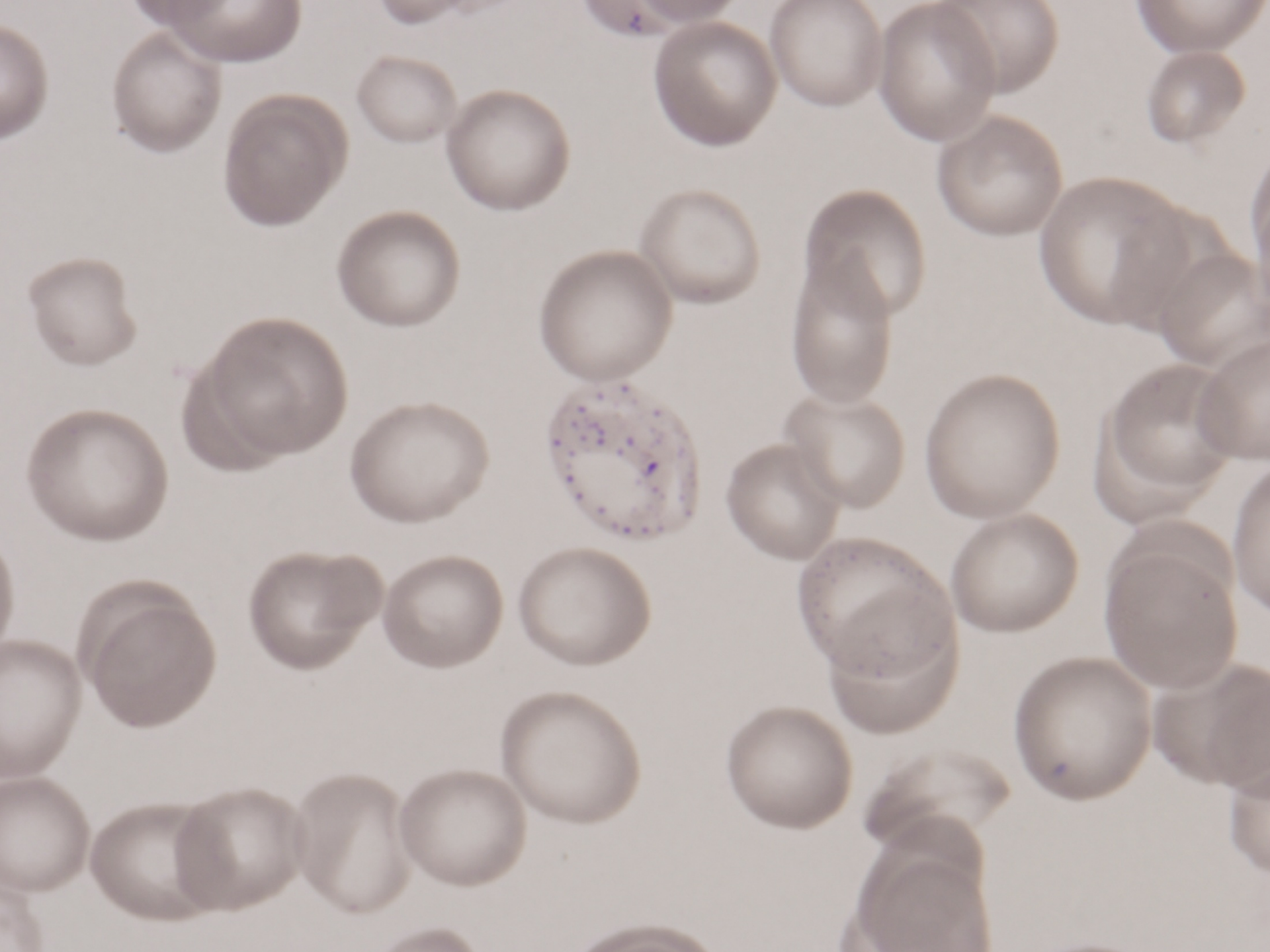
MDS: WHO diagnostic criteria

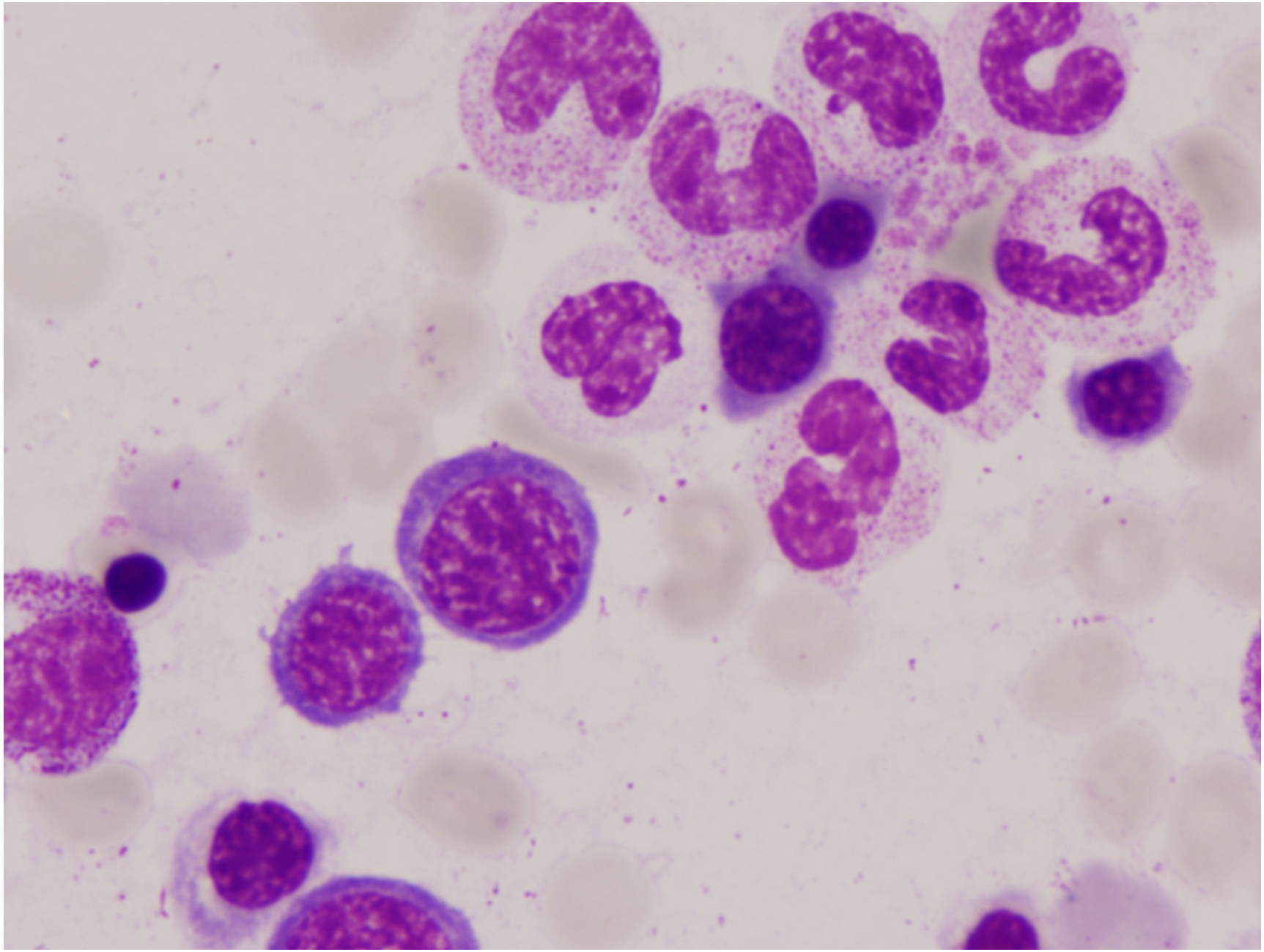
- Number of cytopenias
- The type and degree of dysplasia
- % of blasts in blood and bone marrow (≥**20**%: Acute L.)
- % of ring sideroblasts
- Bone marrow karyotype

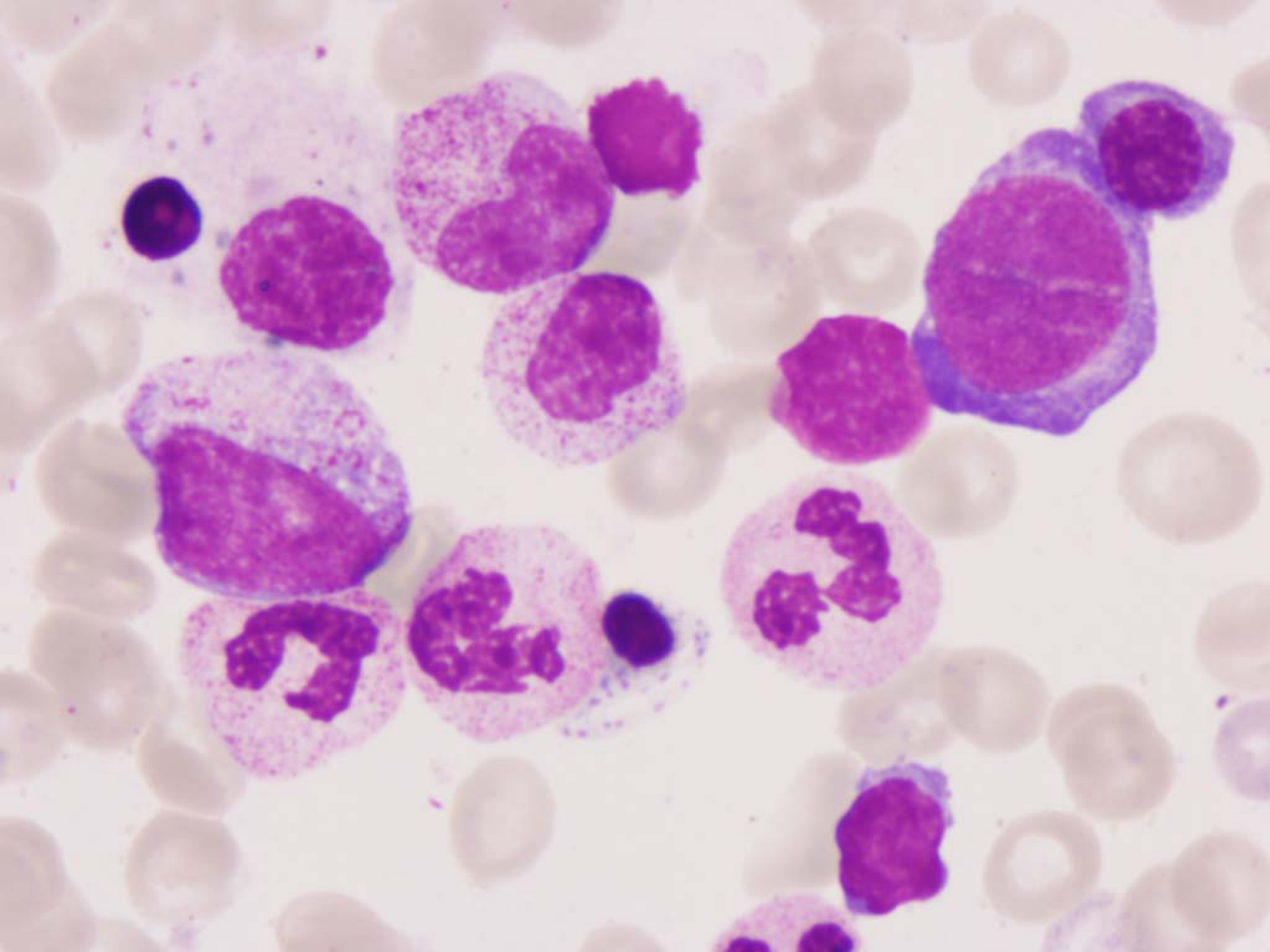
Peripheral blood

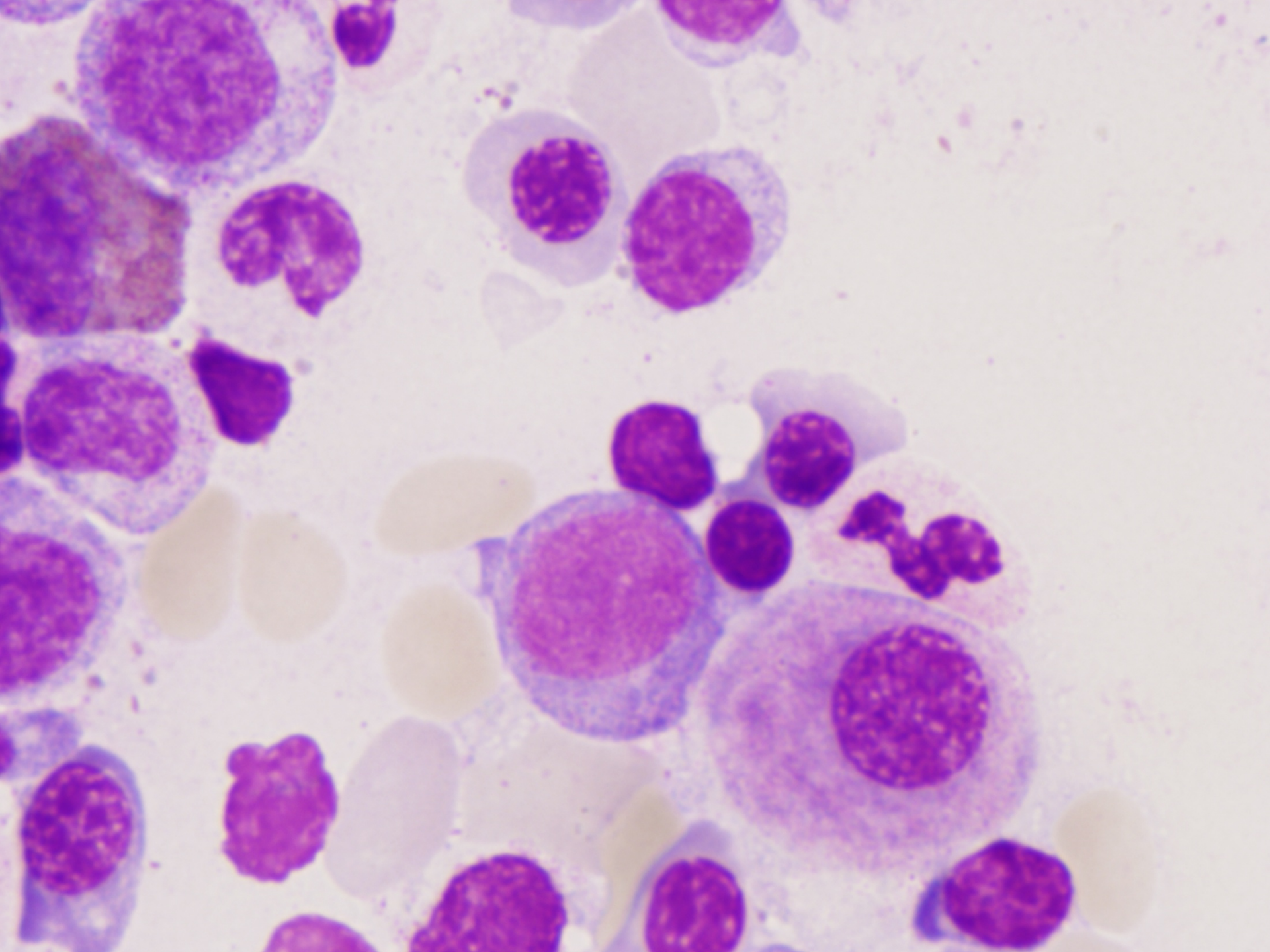


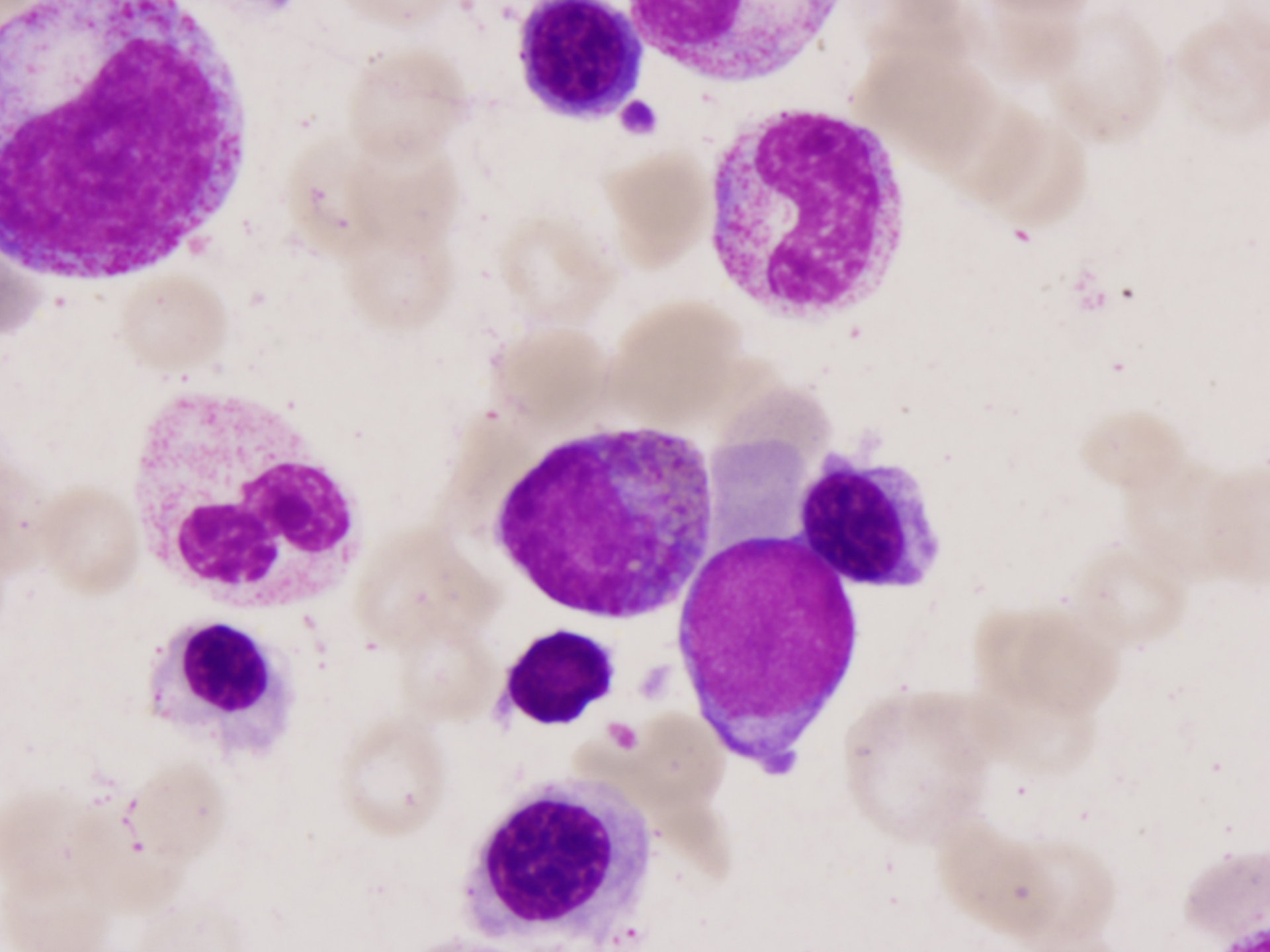


Bone marrow









Quantitative assessment (WHO)

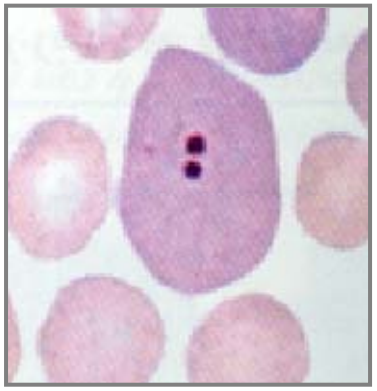
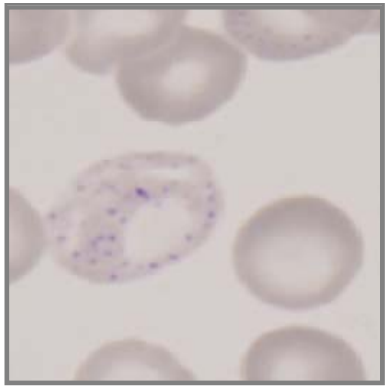
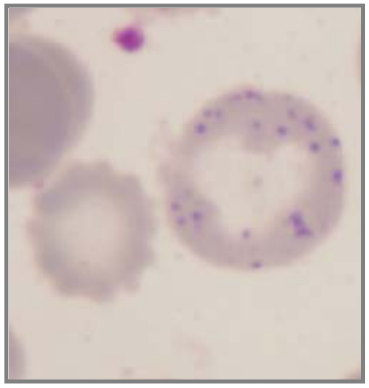
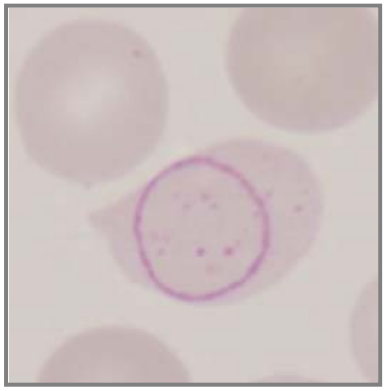
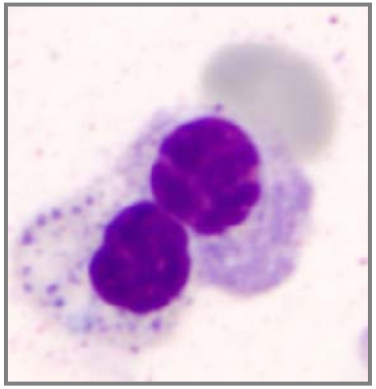
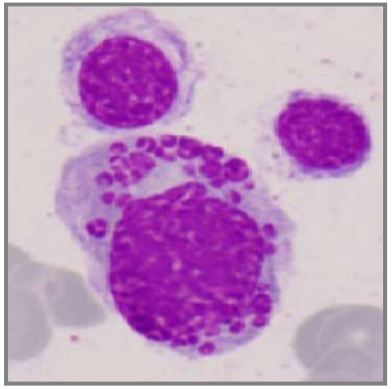
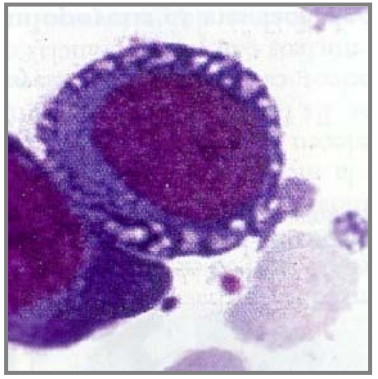
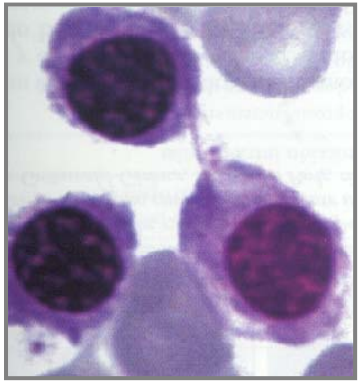
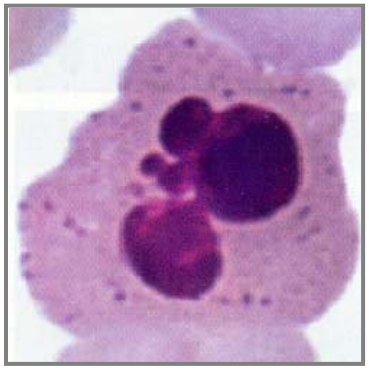
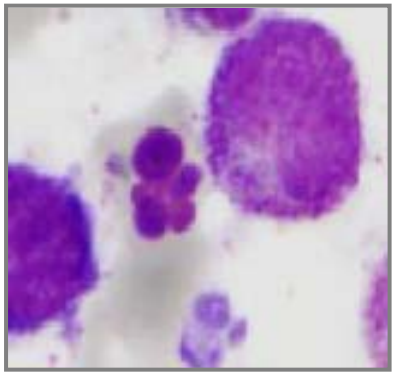
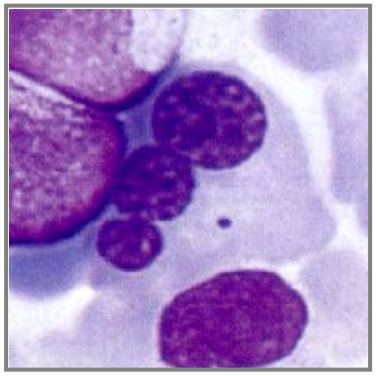
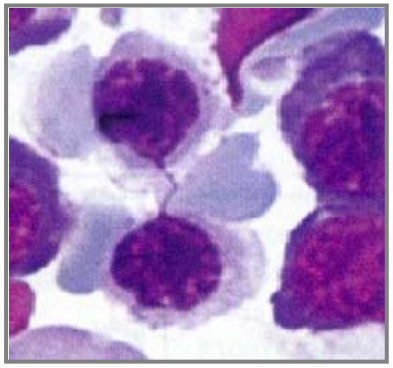
Keep in mind the concept of physiological dysmyelopoiesis

- **Dyserythropoiesis:** assessment of 200 erythroblasts
  ≥10% dysmorphic erythroblasts

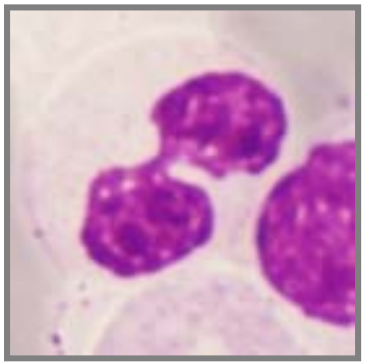
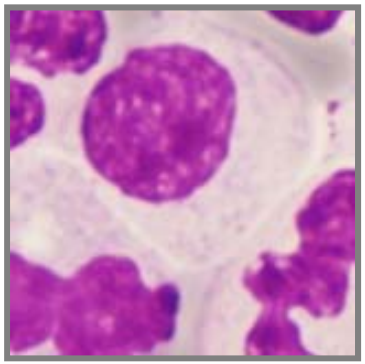
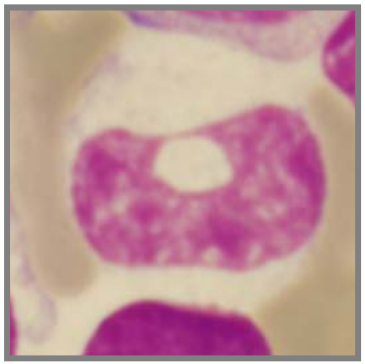
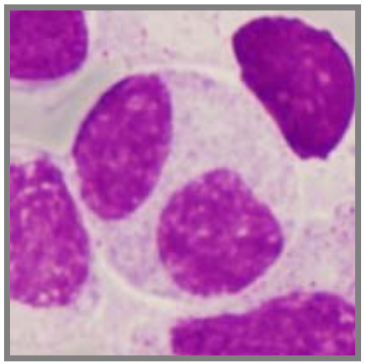
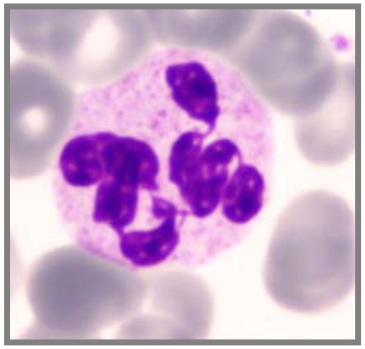
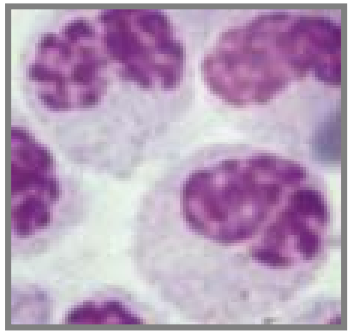
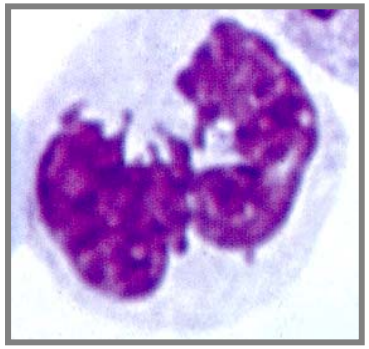
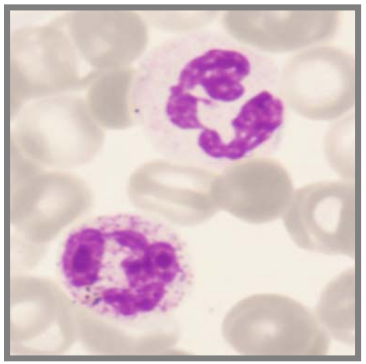
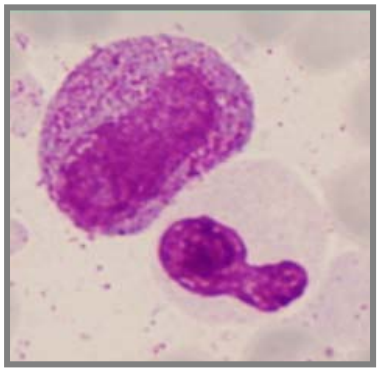
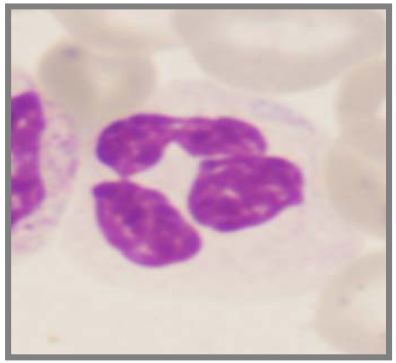
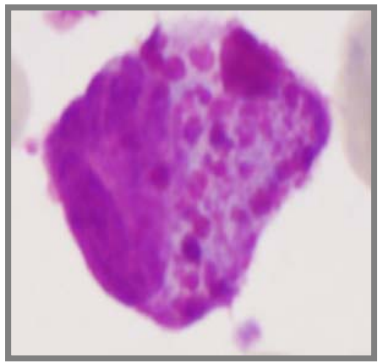
- **Dysgranulopoiesis**: assessment of 200 granulocytes
  ≥10% dysmorphic granulocytes

- **Dysmegakaryopoiesis**: assessment of 30 megakaryocytes
  ≥10% dysmorphic megakaryocytes

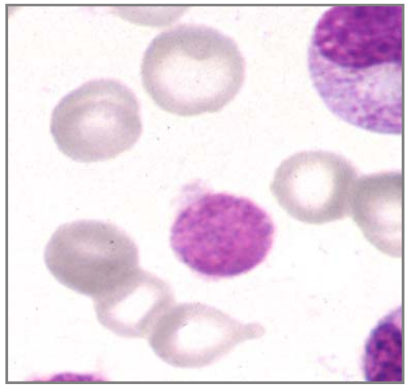
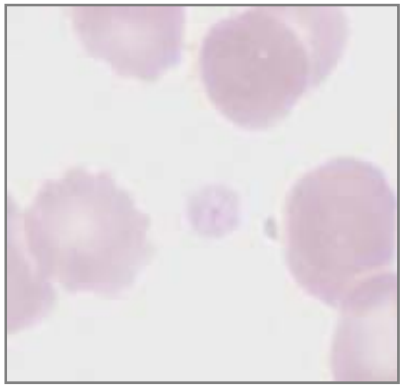
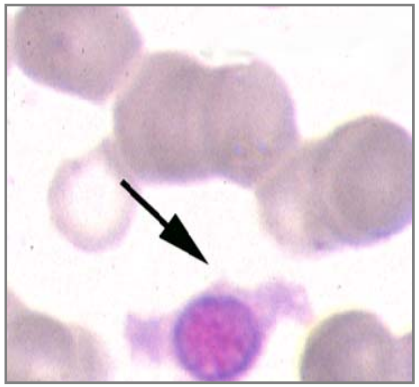
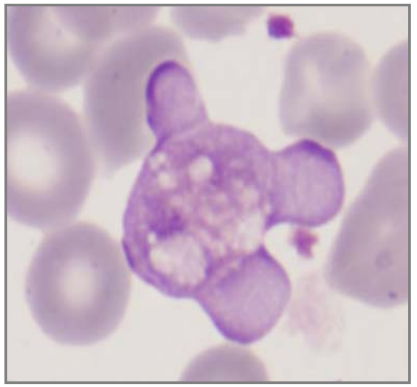
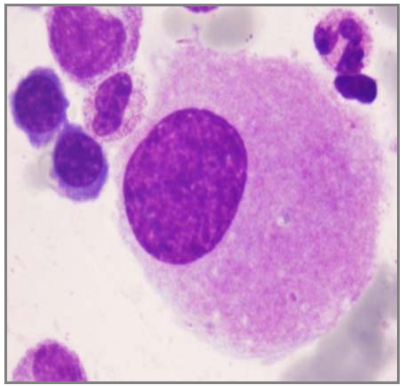
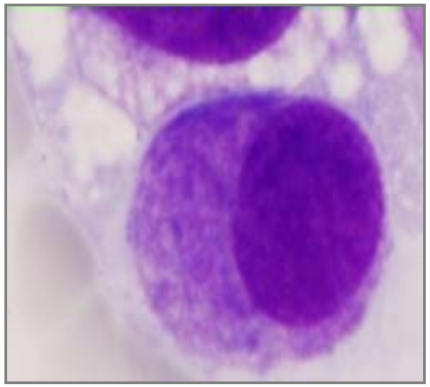
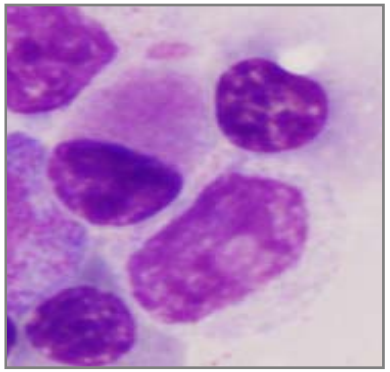
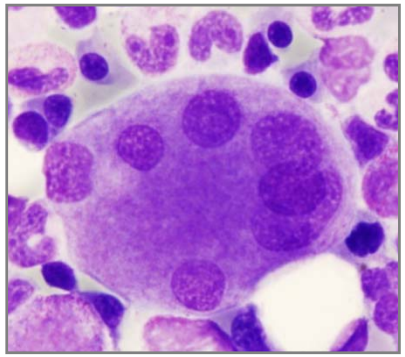
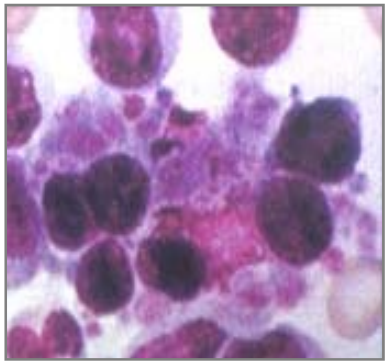
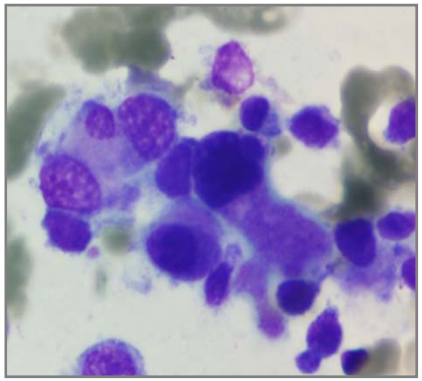
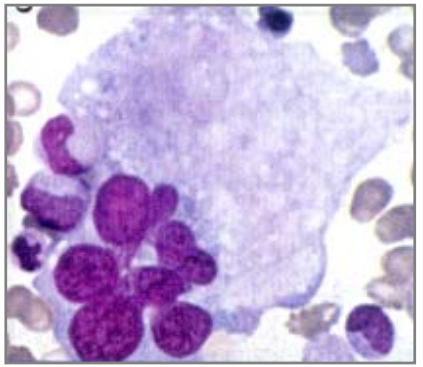
Qualitative assessment of the dyserythropoiesis

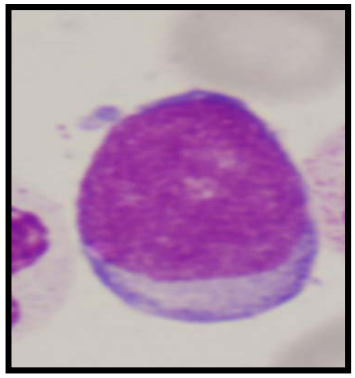
Qualitative assessment of the dysgranulopoiesis

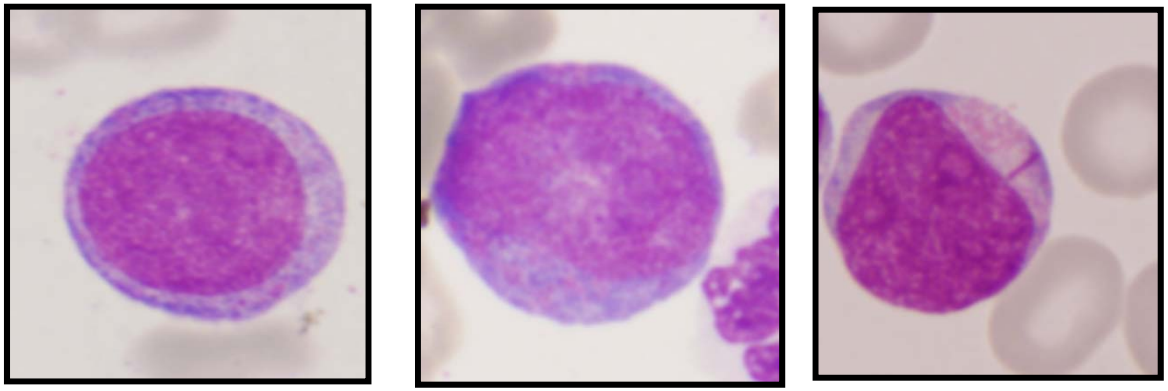
Qualitative assessment of the dysmegakaryopoiesis

MDS: Blasts

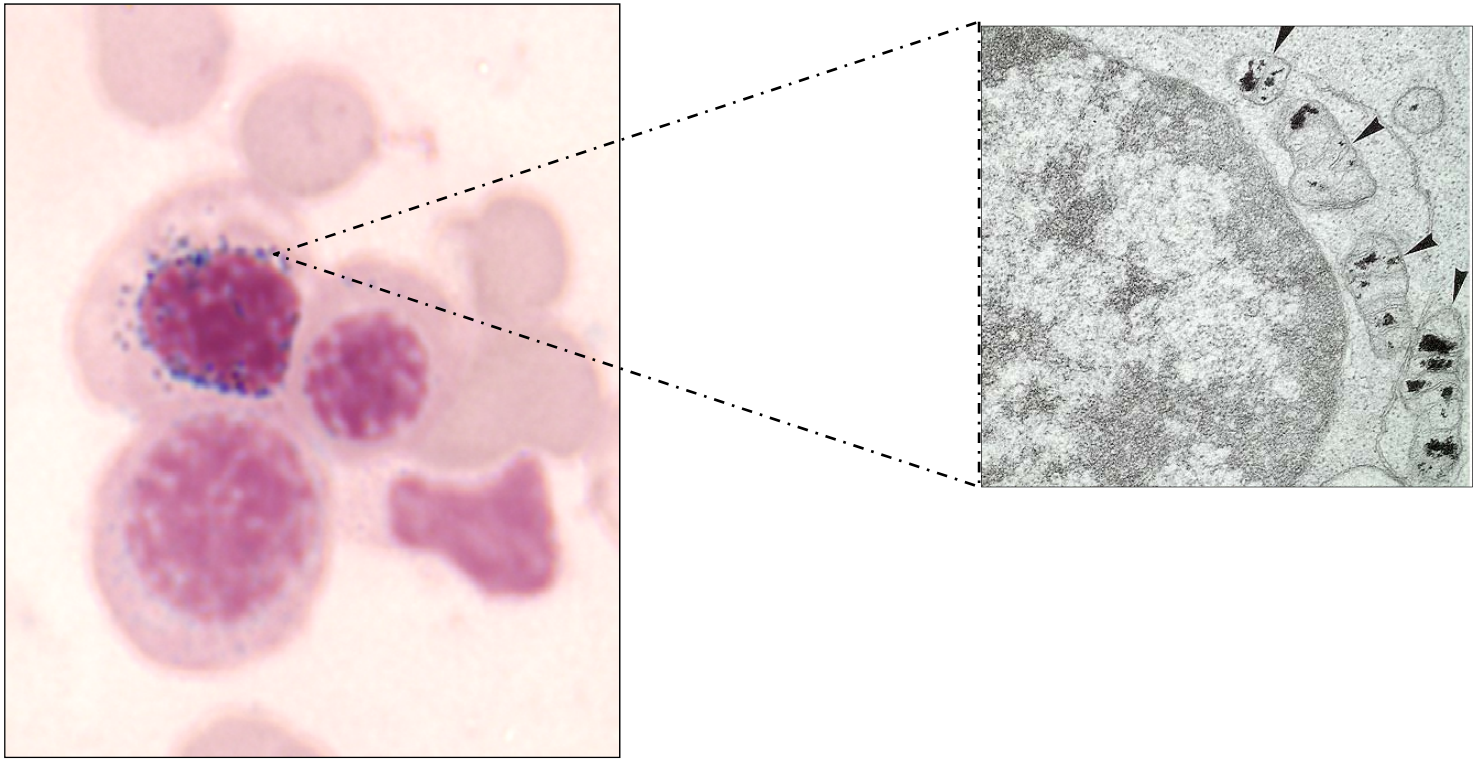


Agranular blast

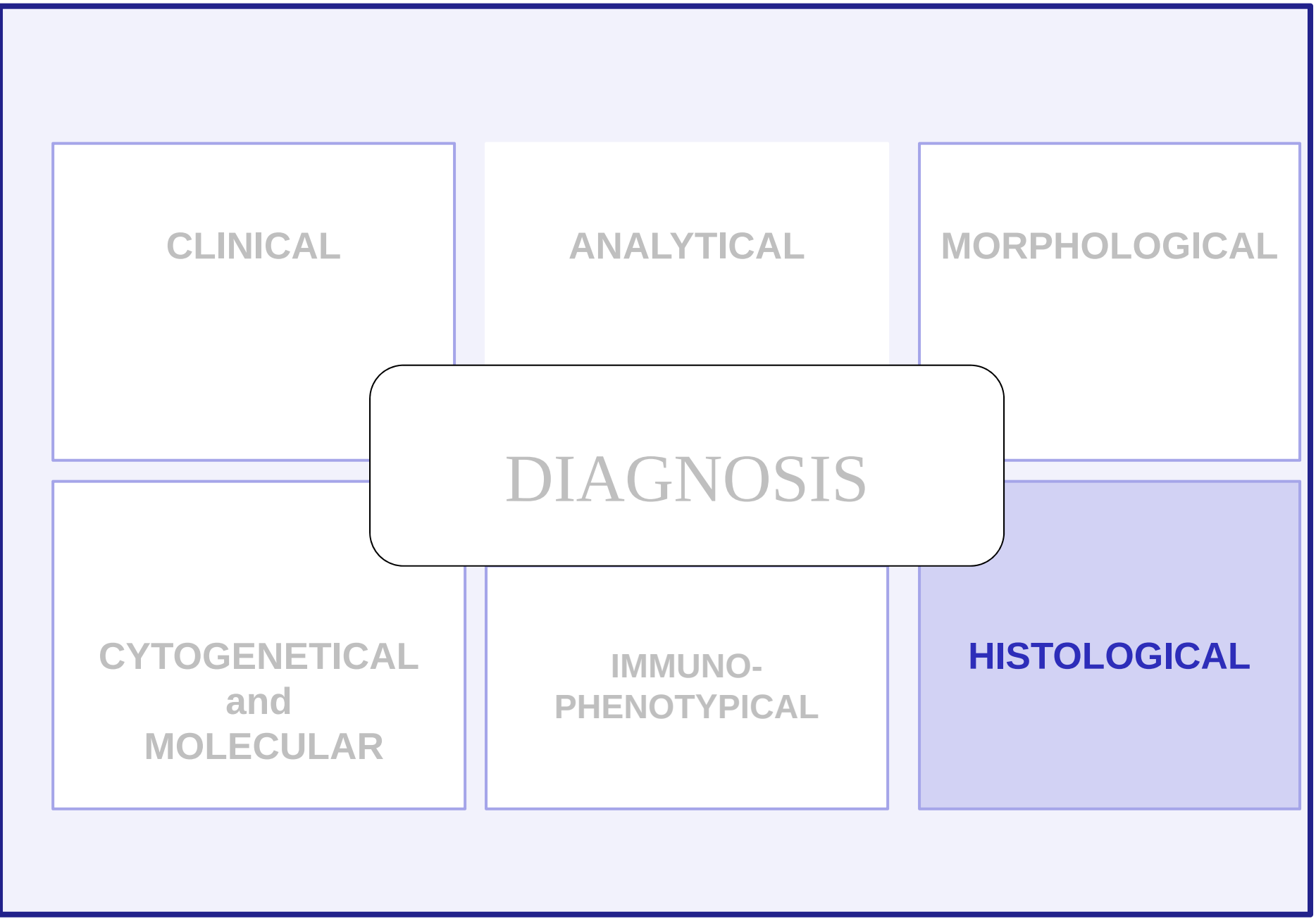


Granular blasts (Auer rods)

Iron stain: % of ring sideroblasts



THERE IS NO EXCLUSIVE ALTERATION OF MDS


CLINICAL
ANALYTICAL
MORPHOLOGICAL
DIAGNOSIS
CYTOGENETICAL and MOLECULAR
IMMUNO-PHENOTYPICAL
HISTOLOGICAL

MDS. Examination of the BM

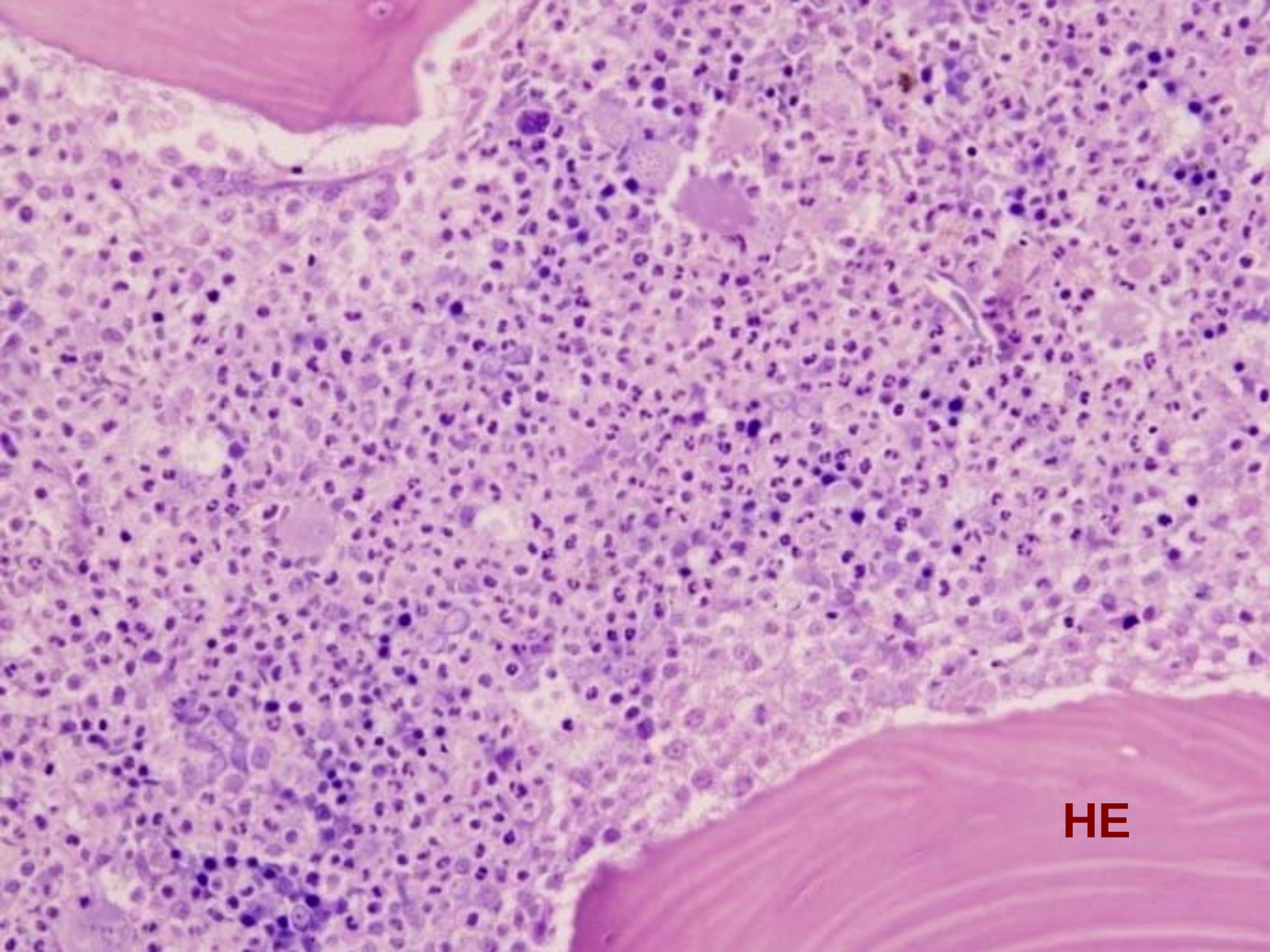
- Is **recommended** in
    - All patients with suspected MDS

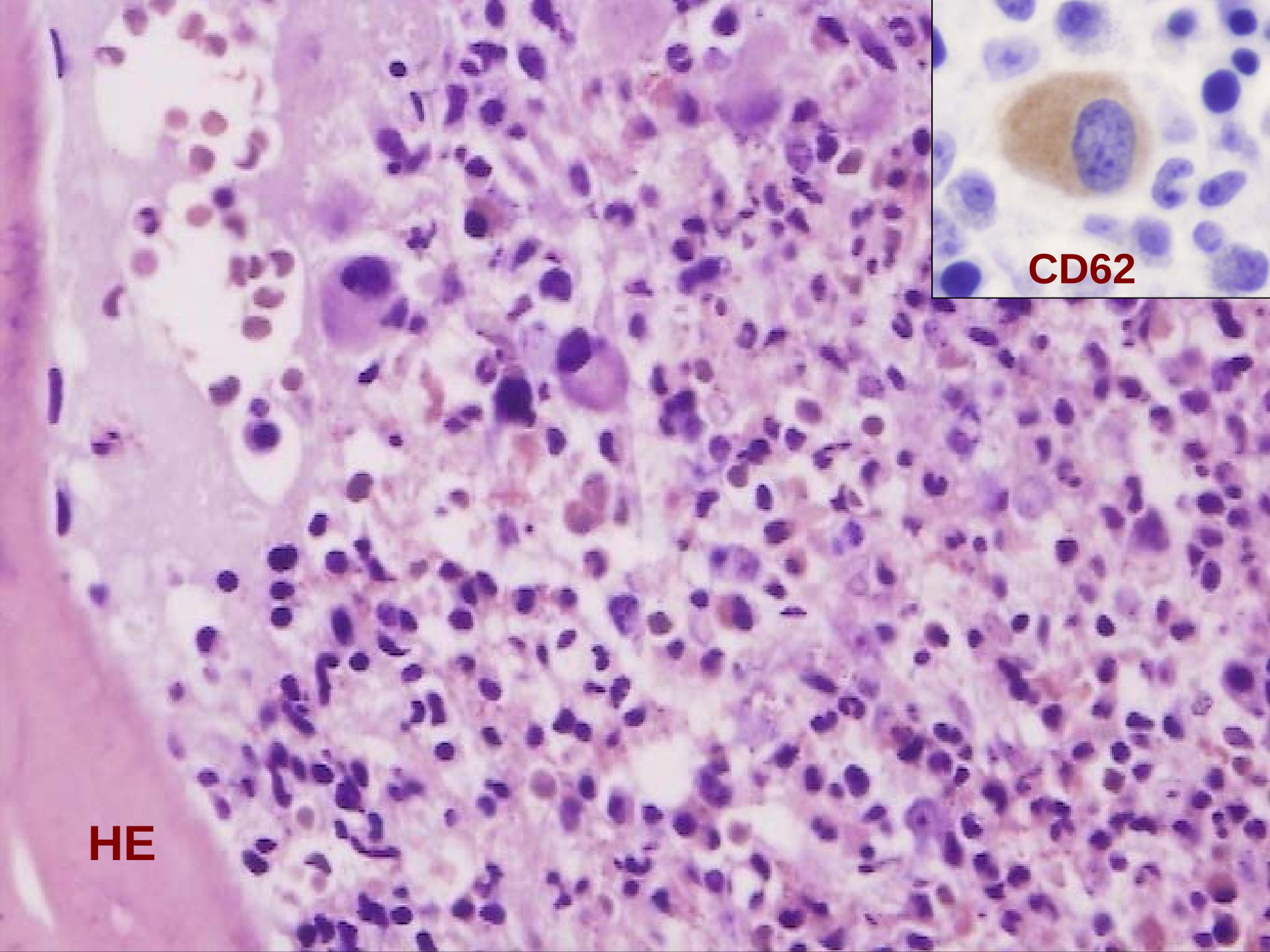
- Is **essential in:**
    - Hypoplastic MDS
    - MDS with myelofibrosis
    - ICUS to exclude an underlying hidden myelogenous neoplasm (mastocytosis) or other non-hematopoietic disorders
- Prognostic evaluation

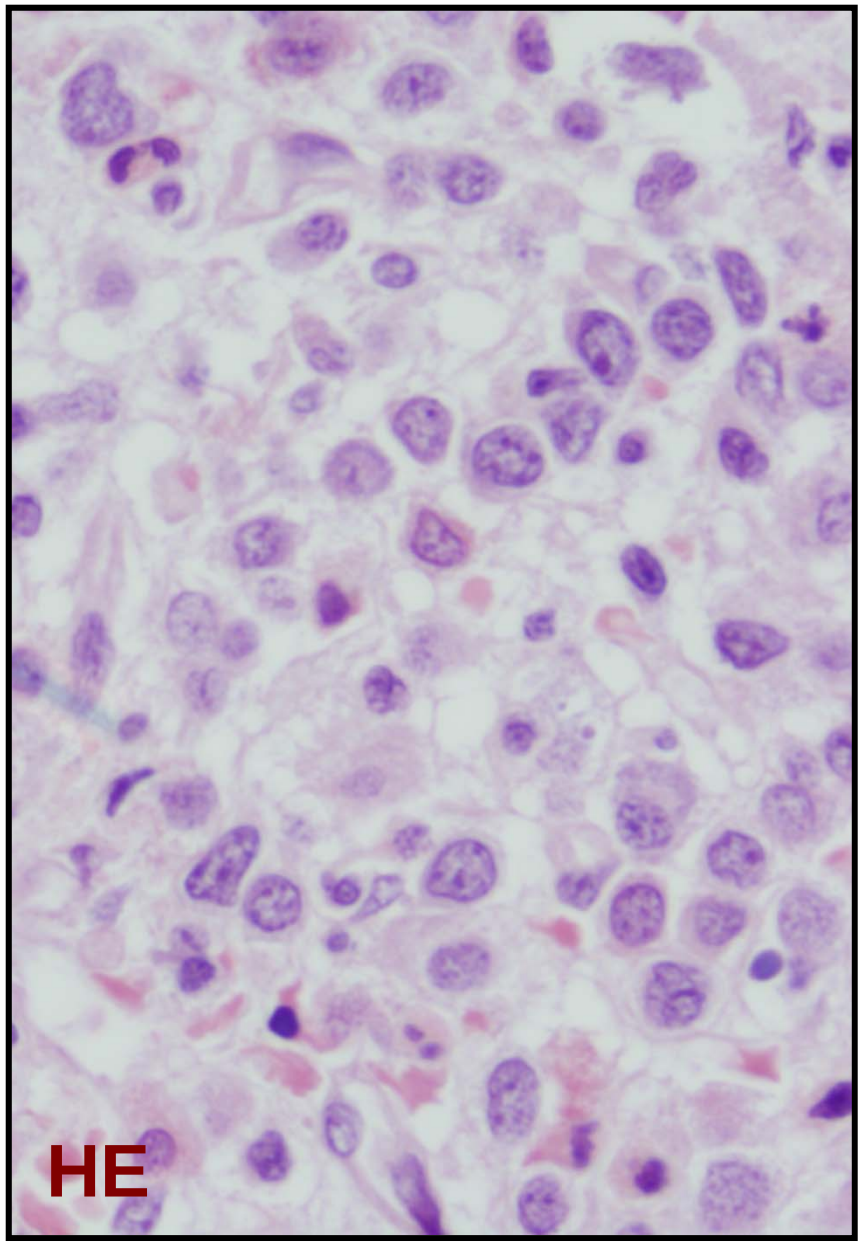
Value of bone marrow histology in MDS

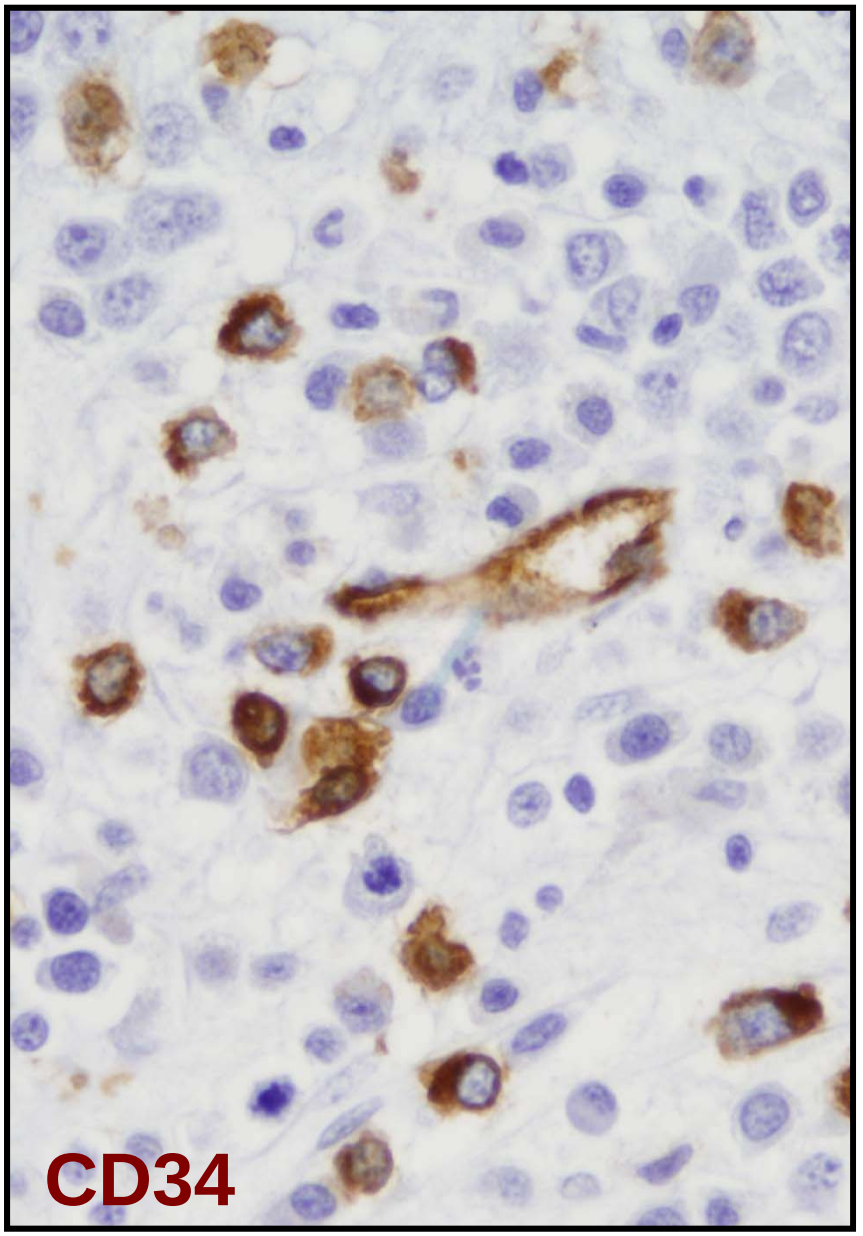
- Multifocal accumulations of (CD34+) progenitor cells (CD34-IHC)
- Abnormal distribution/localization of CD34+ progenitor cells, ALIP (CD34-IHC)
- Abnormal accumulation and morphology of megakaryocytes (IHC: CD31, CD42, or CD62)
- Evaluation of hypocellular bone marrow
- Evaluation of bone marrow fibrosis
- Evaluation of increased angiogenesis (CD34-IHC)
- Diagnosis of a second (concomitant) myelogenous neoplasm

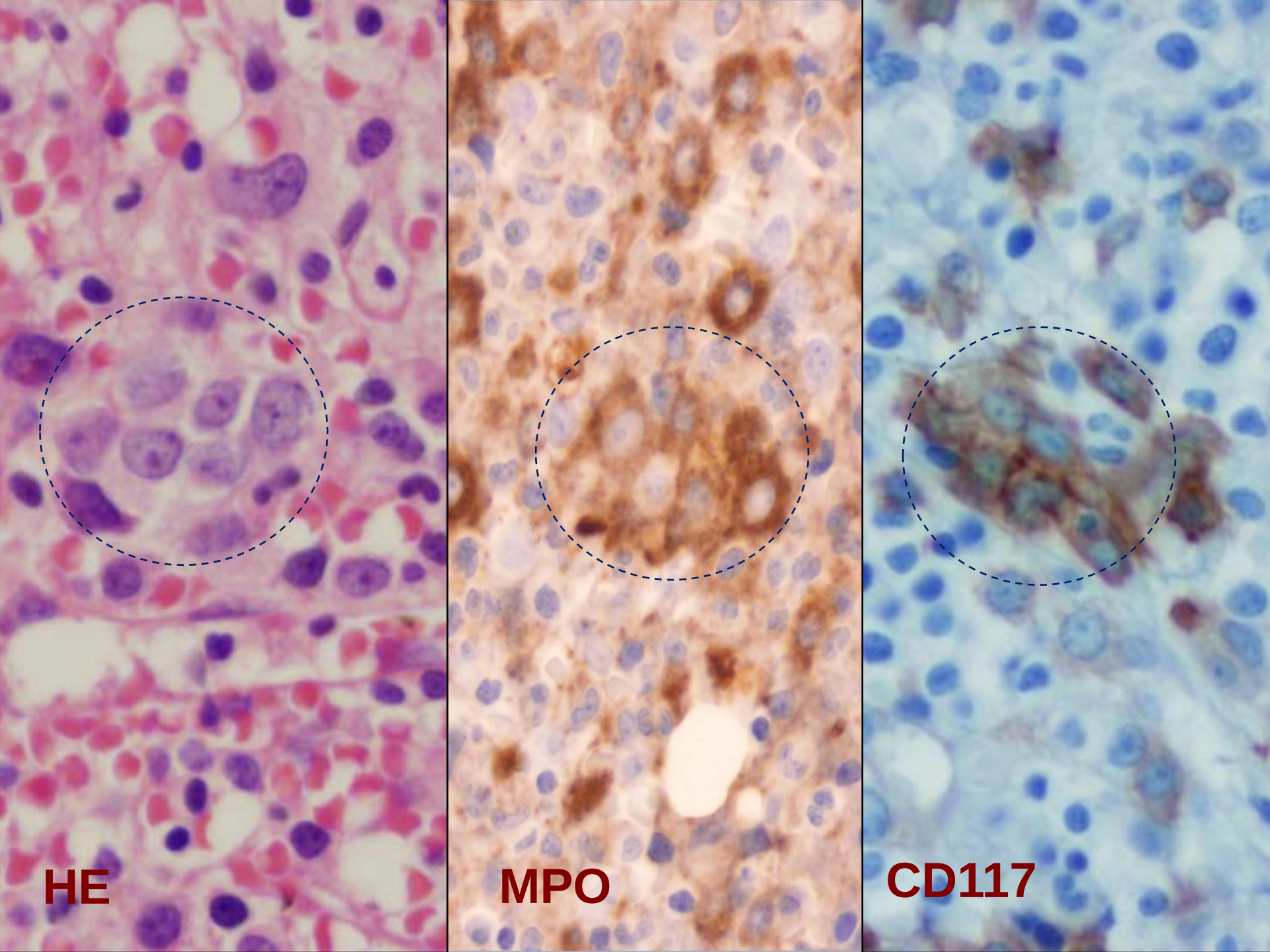
THERE IS NO EXCLUSIVE ALTERATION OF MDS


HE

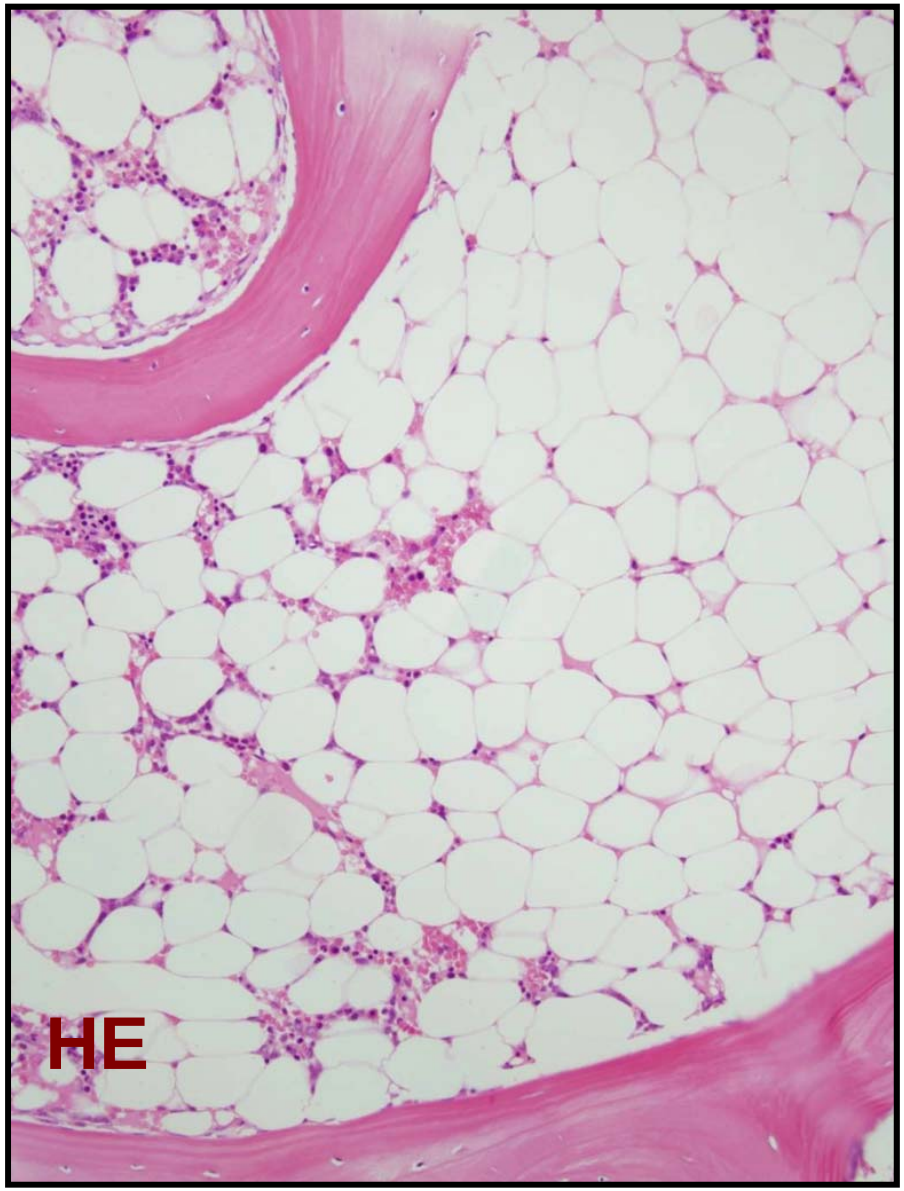

CD62
HE

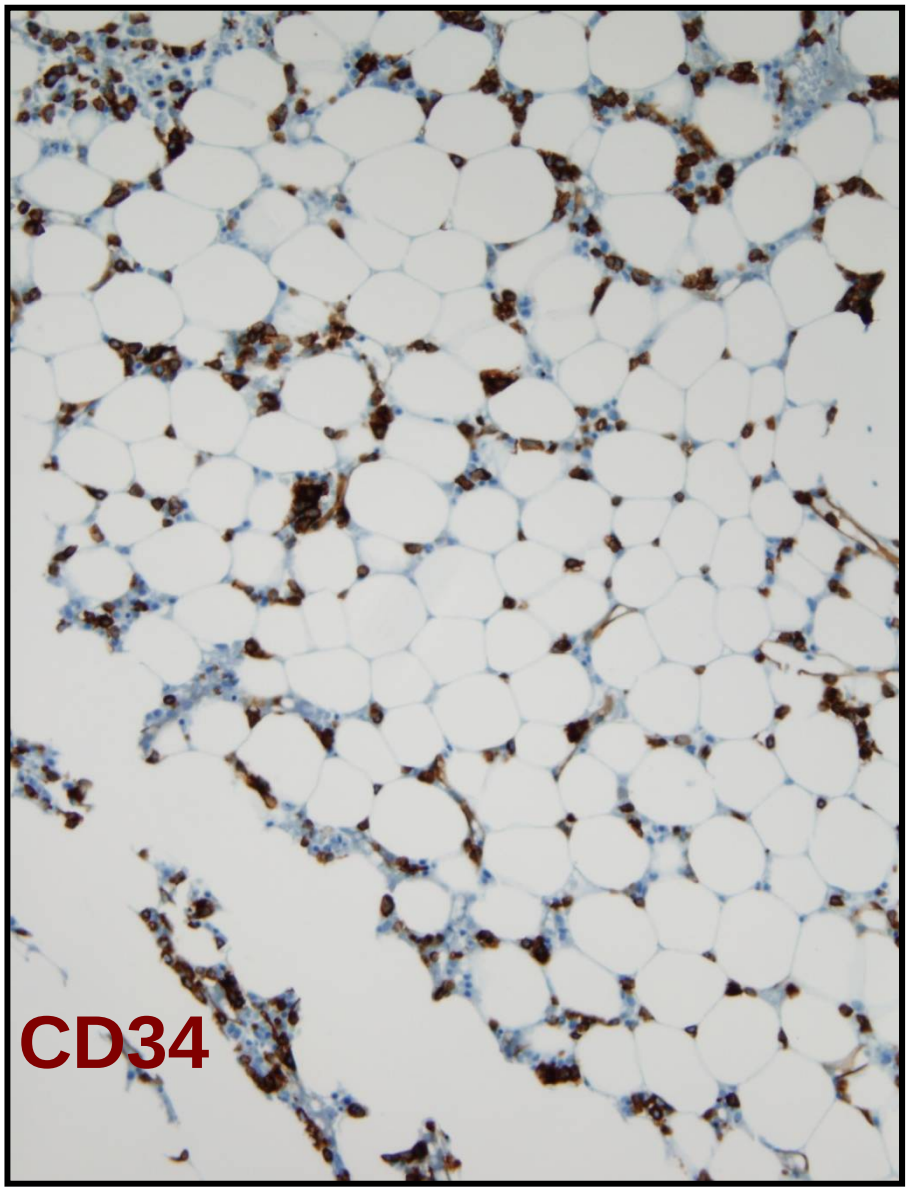

HE


CD34

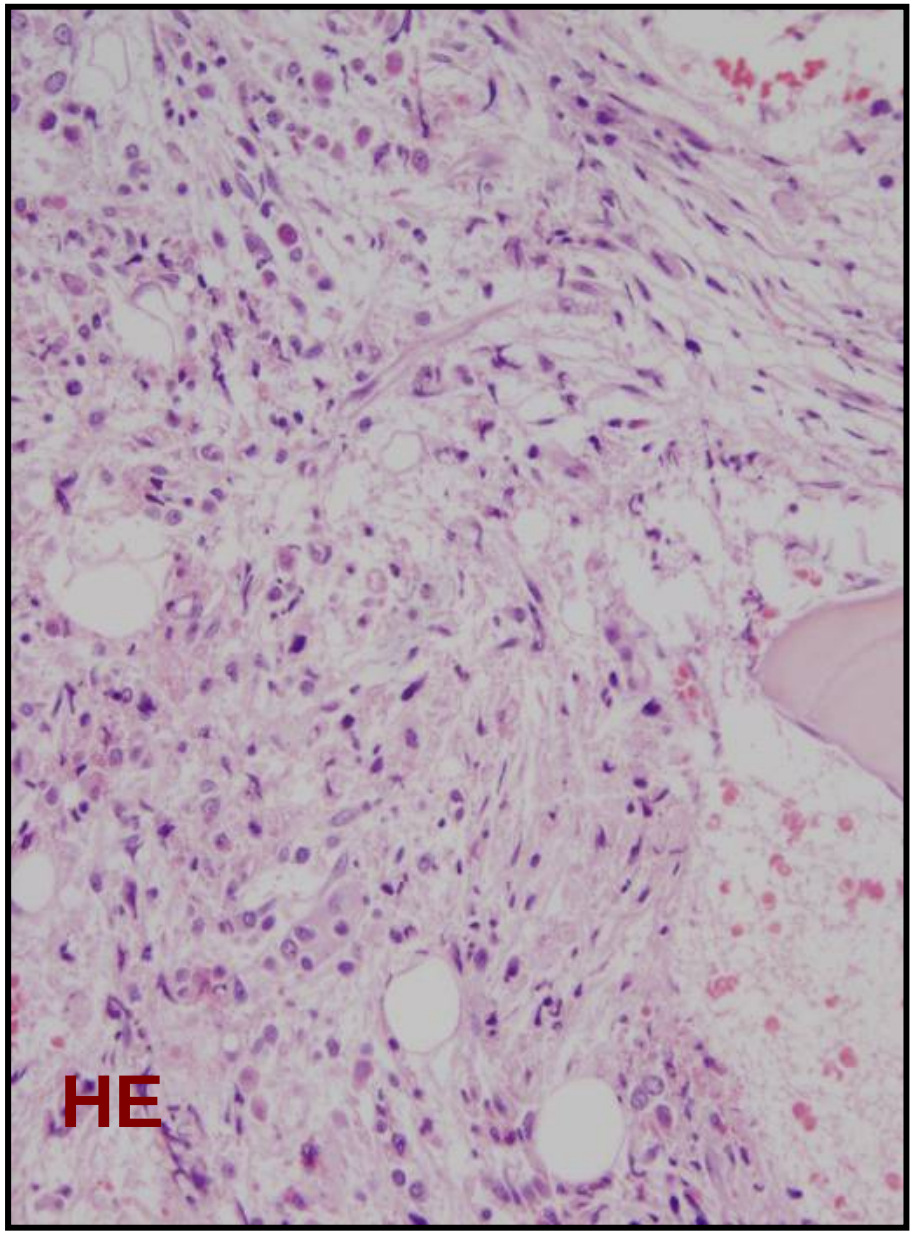

HE
MPO
CD117

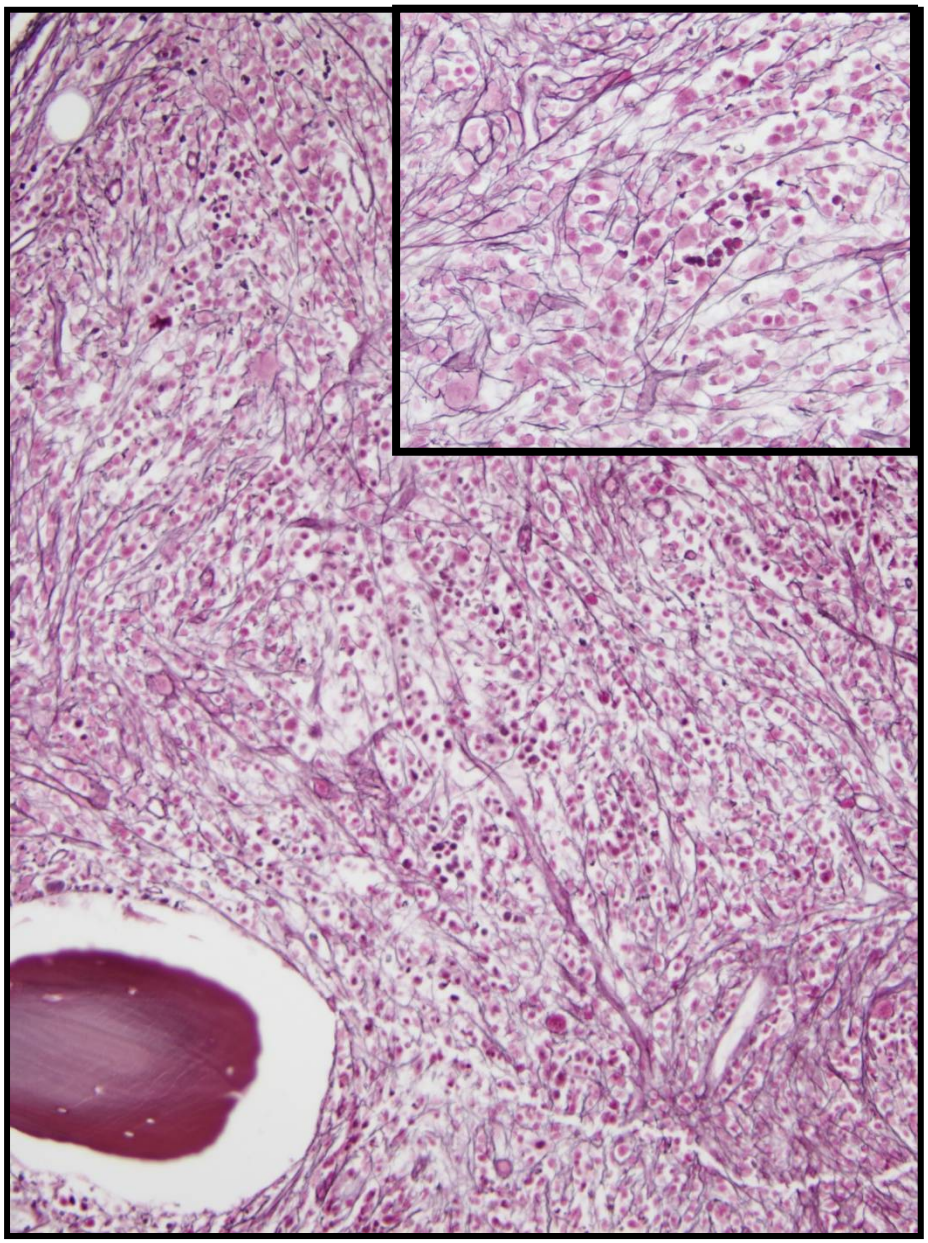
Hypoplastic MDS

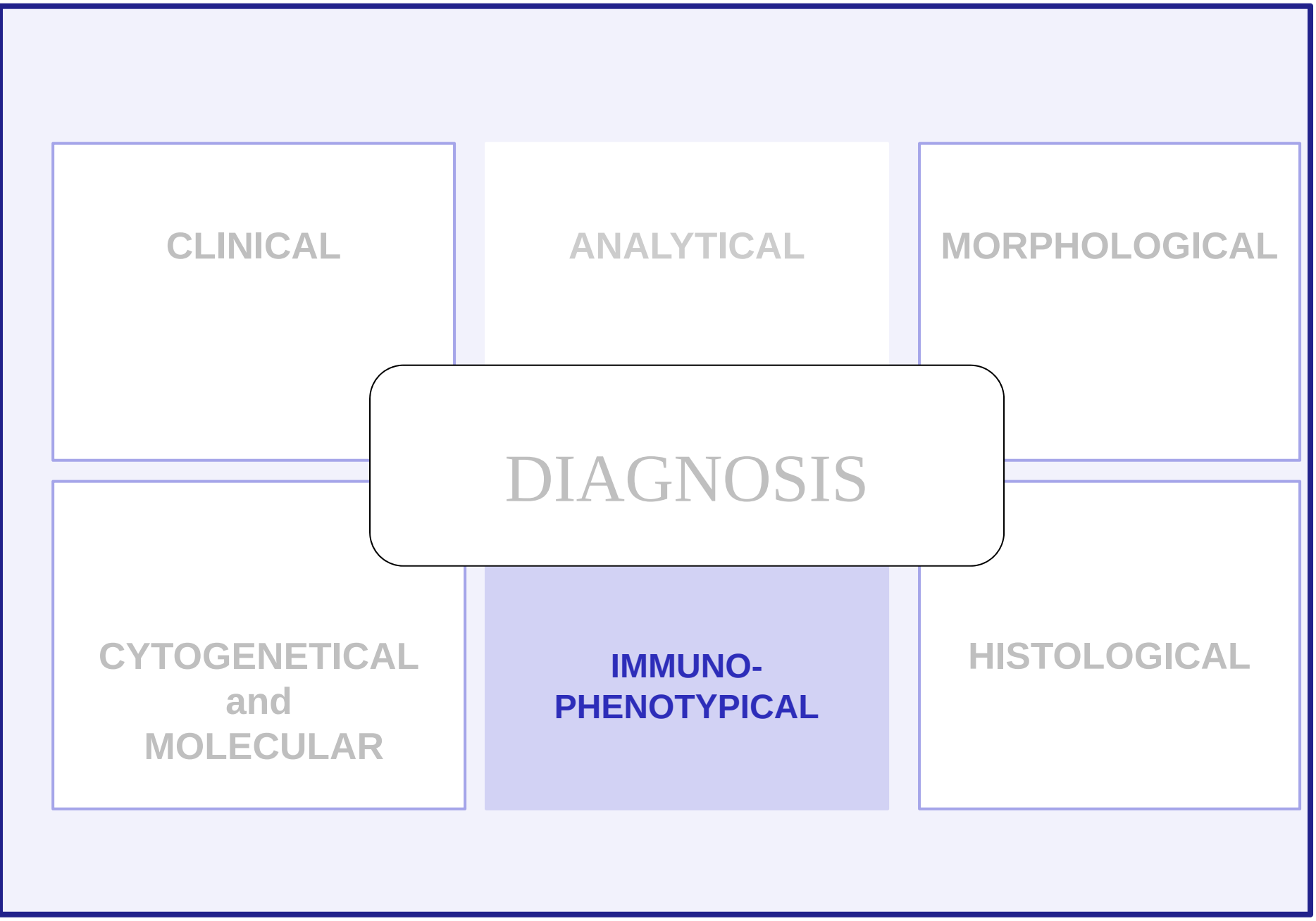




MDS with fibrosis






CLINICAL
ANALYTICAL
MORPHOLOGICAL
DIAGNOSIS
CYTOGENETICAL and MOLECULAR
IMMUNO-PHENOTYPICAL
HISTOLOGICAL

MDS: Potential contribution of immunophenotyping

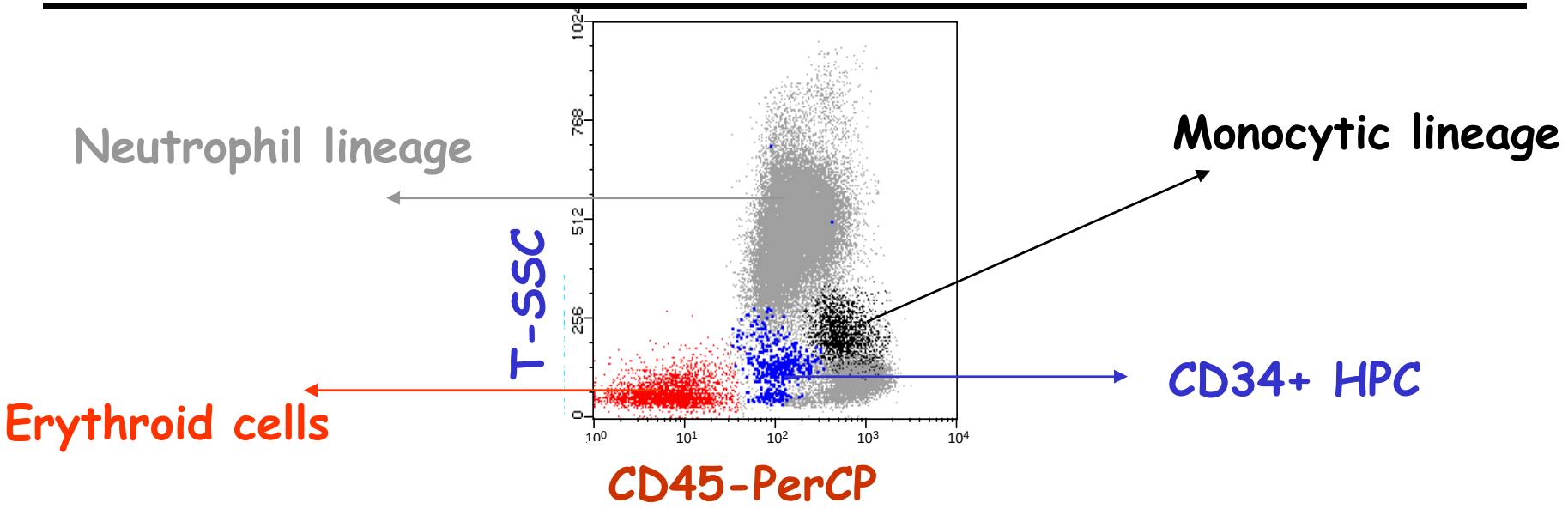
- **Diagnosis**
  - Suspected of MDS
  - ICUS
- **Diagnostic subclassification**
  - RA vs RCMD
  - RAEB (% of blasts)
- **Prognostic evaluation**
- **Disease monitoring**
  - Follow-up of flow abnormalities after therapy
  - MRD ?

van de Loosdrecht Haematologica. 2009
Orfao 2008

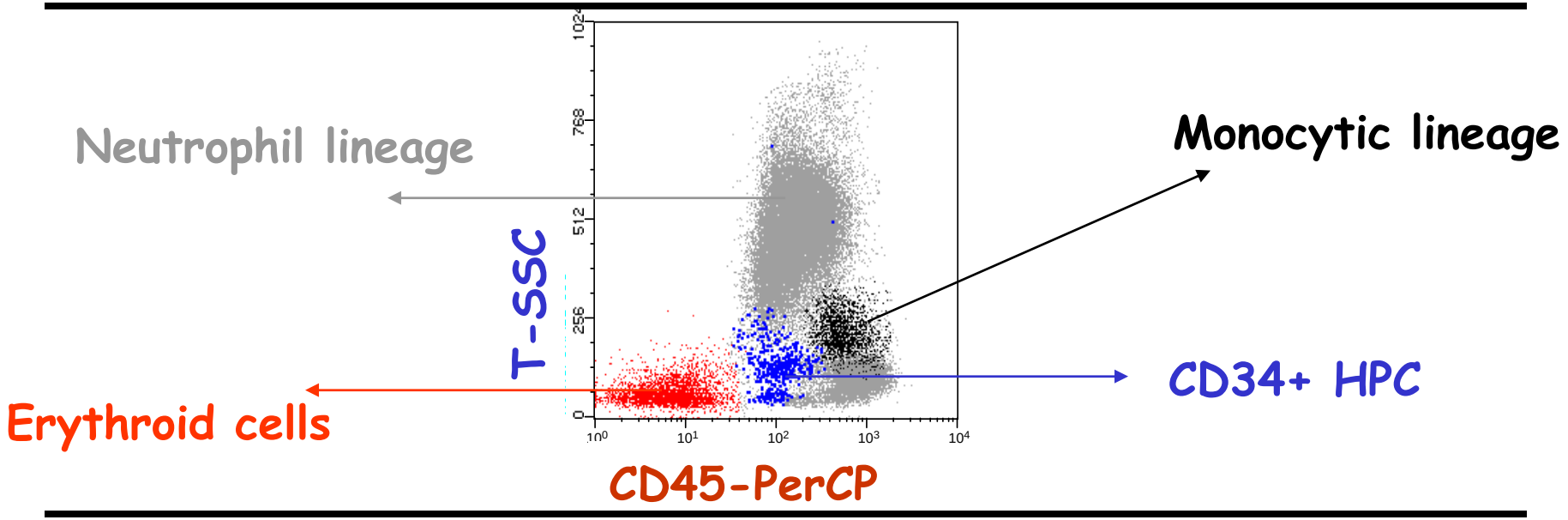
Flow cytometry in MDS

- Altered numbers of CD34+ precursors
- Aberrant expression of markers on myeloblasts
- Detection of immunophenotypically altered features in maturing neutrophil, monocytic and erythroid cells
- Expression of lineage infidelity markers

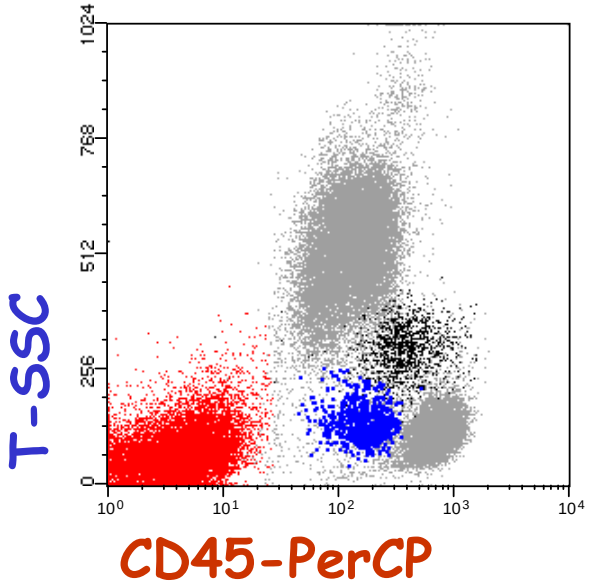
NORMAL BONE MARROW

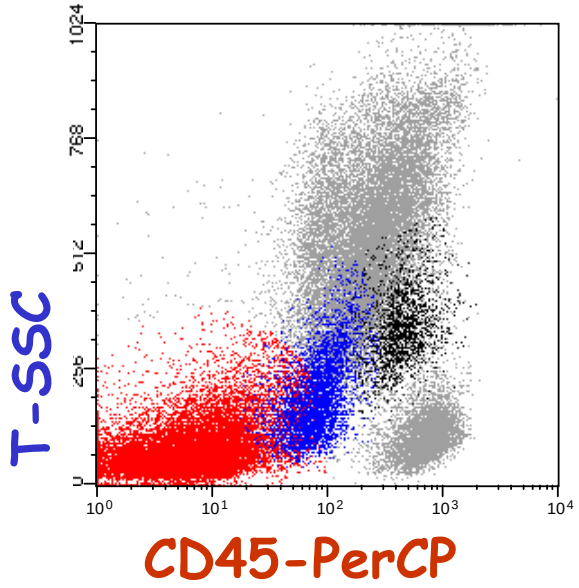


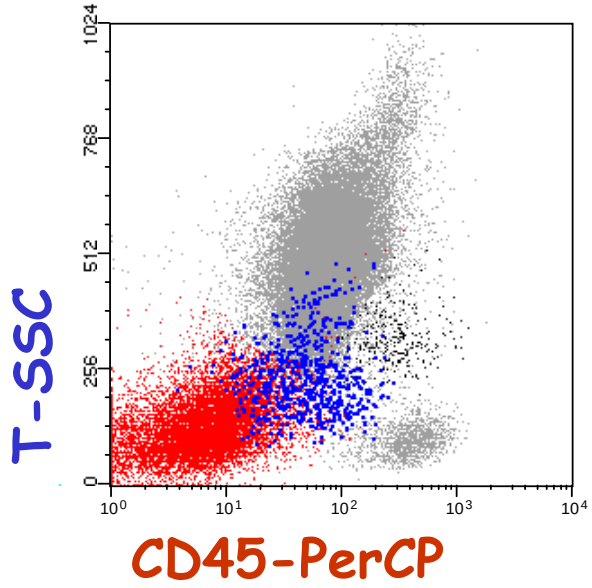
NORMAL BONE MARROW



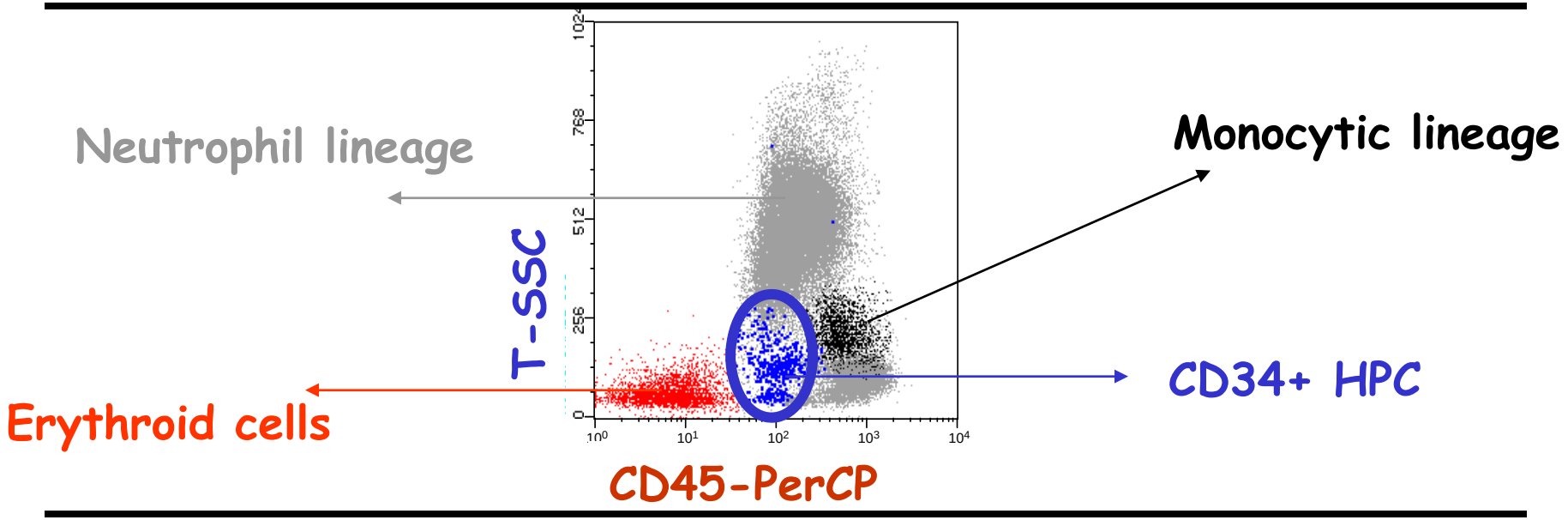
MDS: ALTERED PHENOTYPES



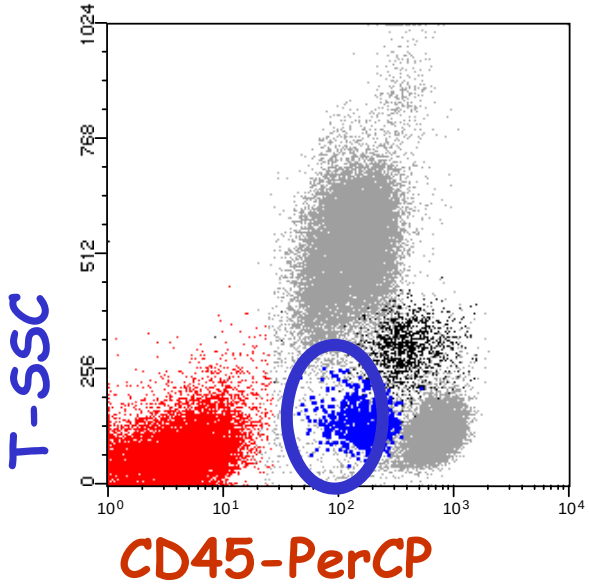


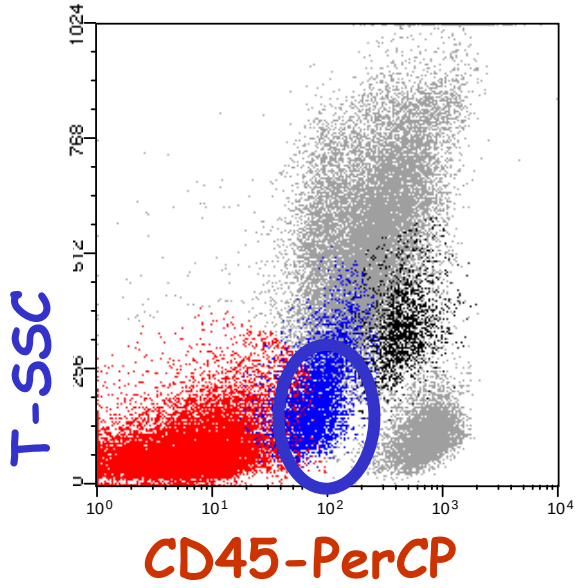


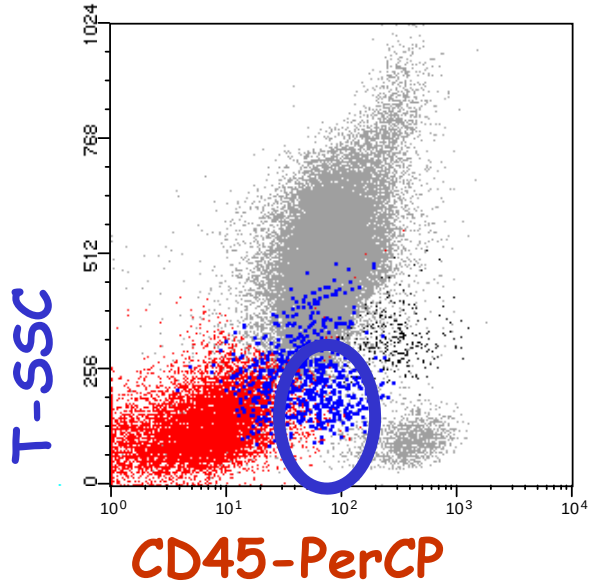
NORMAL BONE MARROW



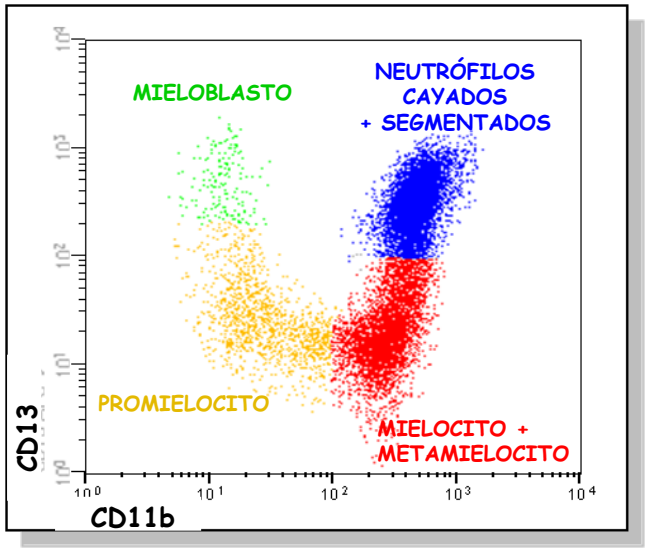
MDS: ALTERED PHENOTYPES

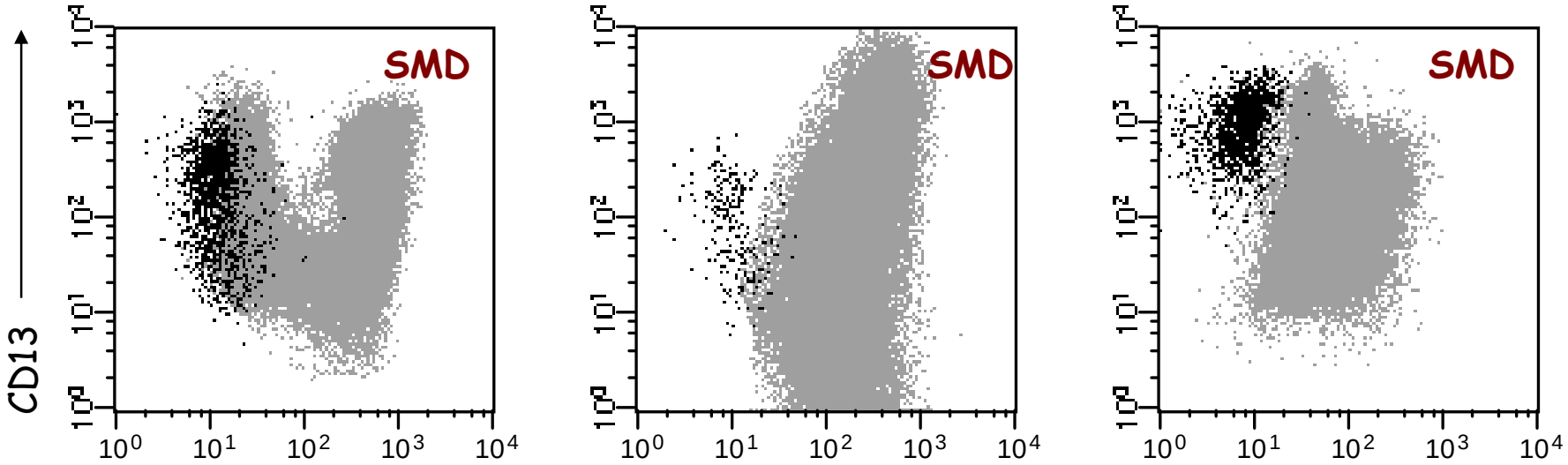






IMMUNOPHENOTYPIC CHARACTERIZATION OF NEUTROPHIL MATURATION IN MDS VS NORMAL BM





Orfao et al, Clin Cytometry, 2004

- Despite the IF data in MDS, a relatively high degree of heterogeneity and subjectivity exists with regards to the criteria for the definition of specific altered phenotypes
- There isn't any marker or phenotypic characteristic and unique pattern of MDS
- The simultaneous evaluation of multiple phenotypic parameters is required in order to ensure the existence of abnormalities characteristic of MDS
- The European group Flow Euro has been working on a proposal for a joint panel for the study of MDS with an innovative strategy for the analysis of results

van de Loosdrecht Haematologica. 2009

Flow cytometry in MDS

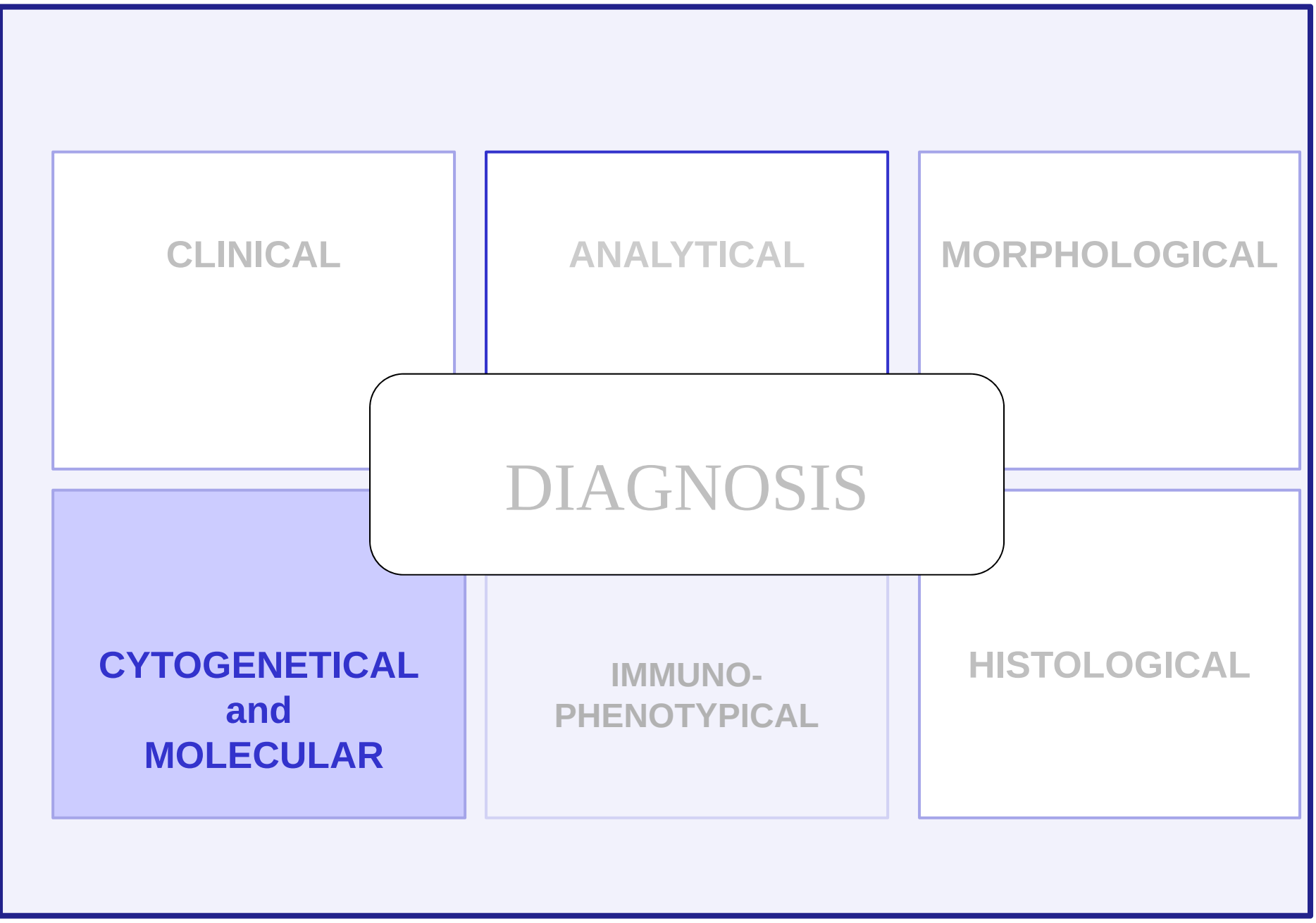
DECISION MAKING AND PROBLEM SOLVING

Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes.

van de Loosdrecht AA, Alhan C, Béné MC, Della Porta MG, Dräger AM, Feuillard J, Font P, Germing U, Haase D, Homburg CH, Ireland R, Jansen JH, Kern W, Malcovati L, Te Marvelde JG, Mufti GJ, Ogata K, Orfao A, Ossenkoppele GJ, Porwit A, Preijers FW, Richards SJ, Schuurhuis GJ, Subirá D, Valent P, van der Velden VH, Vyas P, Westra AH, de Witte TM, Wells DA, Loken MR, Westers TM. Haematologica 2009;94:1124-34.

- Flow cytometry is an increasingly important technology in the diagnosis and prognostication of haematopoietic neoplasms
- In MDS, FCM is also regarded as a new forthcoming standard, although several questions remain to be solved

THERE IS NO EXCLUSIVE ALTERATION OF MDS


CLINICAL
ANALYTICAL
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Cytogenetic abnormalities are major determinants in the pathogenesis, diagnosis, and prognosis, and, increasingly, the basis for selection of drugs in individual patients with MDS

Cytogenetics in MDS

- **Diagnosis:**
  - Recurring chromosomal abnormalities in the SMD

- Prognosis
  - **IPSS (1.997), GCECGH (2.005), WPSS (2.007), *GA Score* (2.007), New IPSS**

- Selection of drugs in individual patients with MDS **(ej. Lenalidomida and 5q-)**

Cytogenetics

- Chromosomal anomalies:
  - 50% of patients with de novo MDS
  - 80% of patients with MDS secondary to chemotherapy or other toxic agents
- Reciprocal translocations are uncommon in MDS (<1%)
- Unbalanced chromosomal abnormalities are more prevalent (Loss >Gain)

Chromosome abnormalities MDS primary

Most frequent alterations (30%):

del(5q)
-7/del(7q)
+8

Less frequent alterations (10)%:

del(20)(q11q13)
i(17)(q10)
del(12p)

Other:

1q, inv(3)(q21q26), t(1;7)(q10;p10)
+6, +11, 11q-, +13, -21, +21, -Y

Haase D. The 10th IS on MDS. Patras, Greece, May 6-9, 2009

Cytogenetic abnormalities suggestive of SMD

Cytogenetic abnormalities suggestive of MDS in cases with persistent cytopenia but in the absence of definitive morphological features of MDS:

-5/5q-
-7/7q-
i(17)(q10), del(17p)
-13, del(13q)
del(12p)
del(9q)
idic(X)(q13)
Complex with any of the above changes

t(11;16)(q23;p13)
t(3;21)(q26;q22)
t(1;3)(p36;q21)
t(2;11)(p21;q23)
inv(3)(q21q26)
t(6;9)(p23;q34)

NO

+8
20q-
-Y

Vardiman et al., Blood (2009)

The International Prognostic Scoring System (IPSS)

- The IPSS, which applies only to de novo MDS, assigns 4 categories of risk for death or transformation to AML (Low, Int-1, Int-2, and High) based on a numeric score that reflects the percentage of bone marrow blasts, number of cytopenias, and presence or absence and type of chromosomal abnormalities

- Cytogenetic risk groups are defined by the IPSS as:

  Good: normal, isolated Y, del(5q), and del(20q)

  Poor: complex, [3 abnormalities] and/or any chromosome 7anomalies

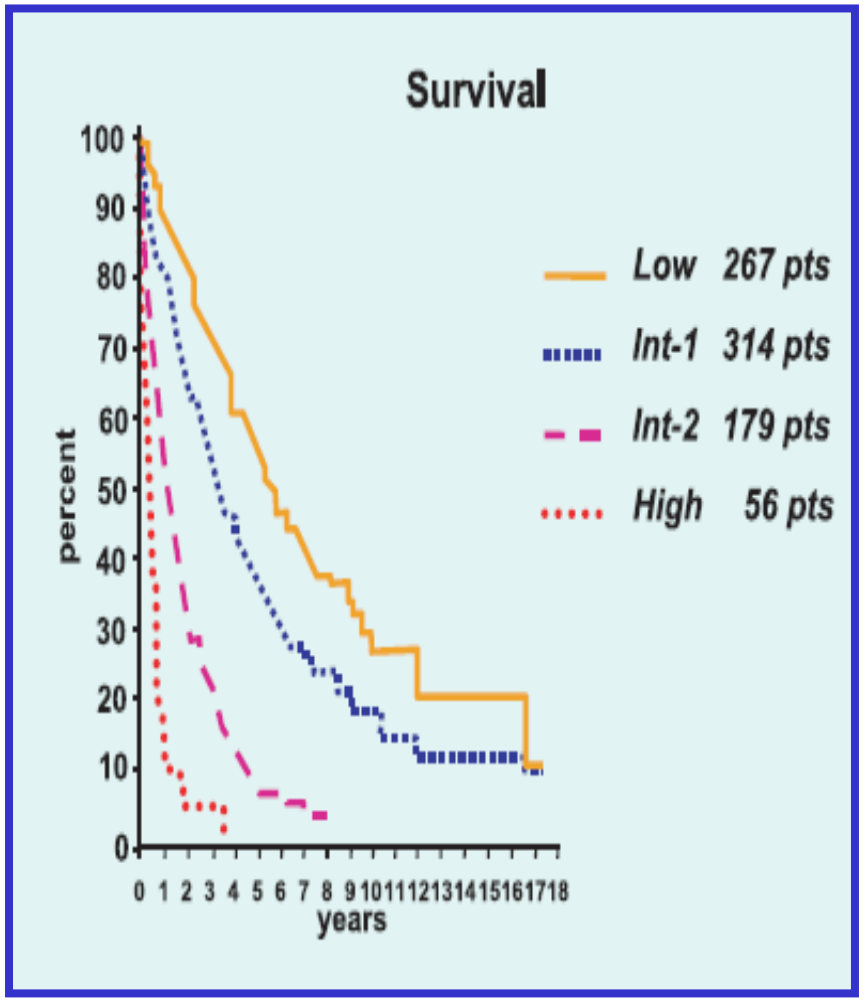
  Intermediate: all other abnormalities

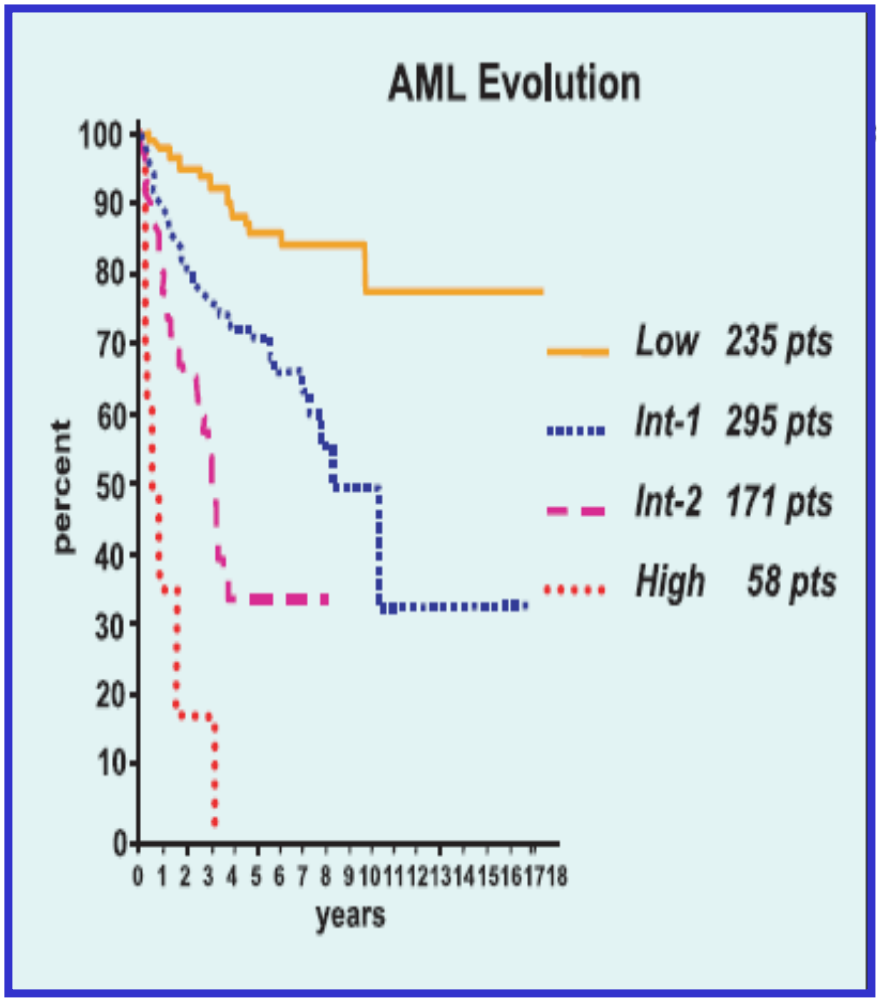
International Scoring System for Evaluating Prognosis in Myelodysplastic Syndromes

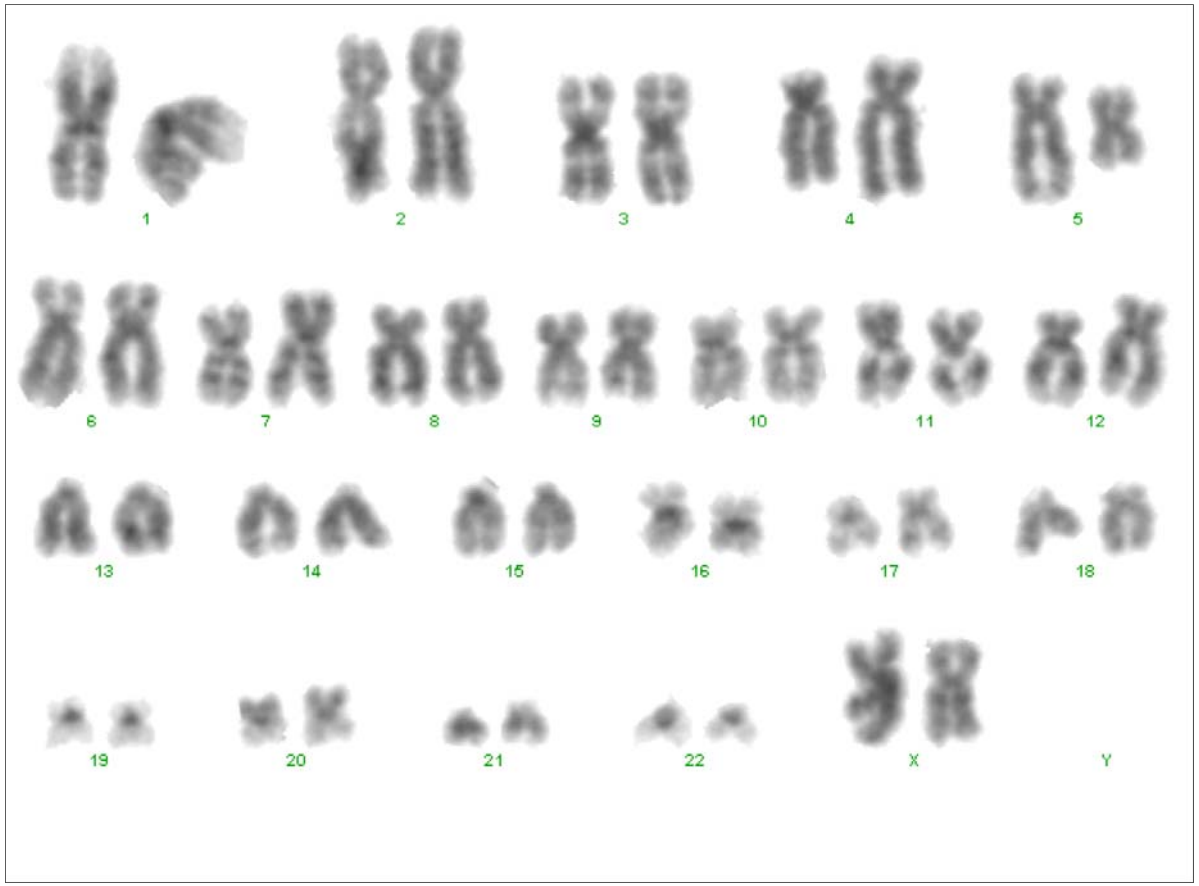
By Peter Greenberg, Christopher Cox, Michelle M. LeBeau, Pierre Fenaux, Pierre Morel, Guillermo Sanz, Miguel Sanz, Teresa Vallespi, Terry Hamblin, David Oscier, Kazuma Ohyashiki, Keisuke Toyama, Carlo Aul, Ghulam Mufti, and John Bennett

Blood 1997:89;2079-88

(Blasts, cytopenias and karyotype)

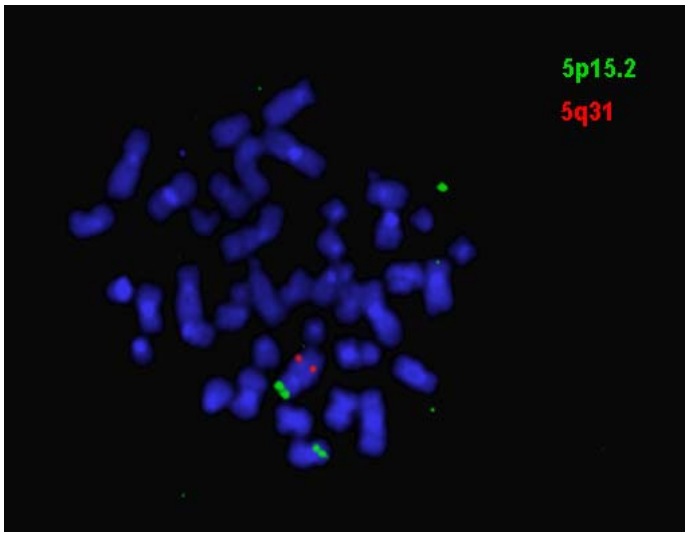


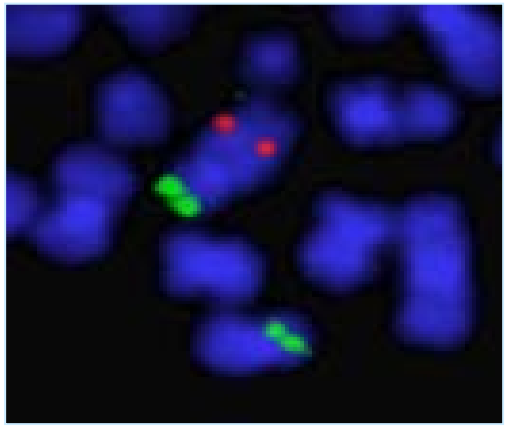




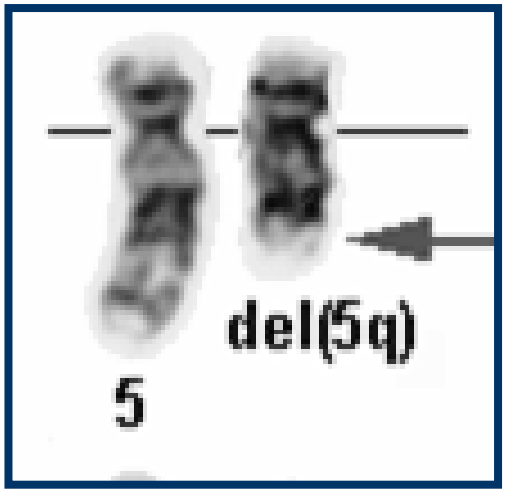
46,XX,del(5)(q13q33)

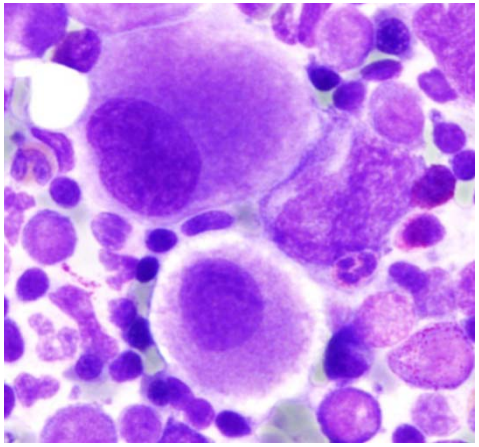
FISH with LSI 5q31 probe



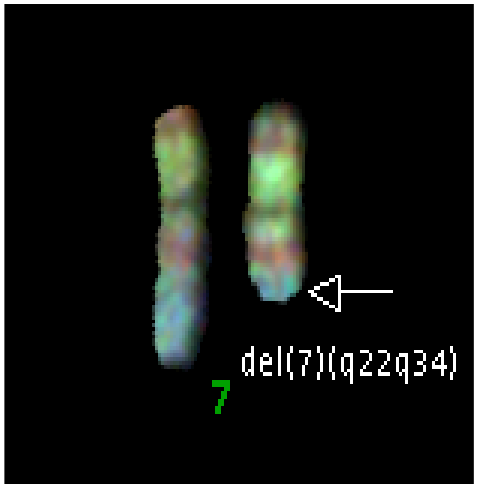


del (5q)





7/del (7q)



Recurrent infections

Delecion 12p

Eosinophilia
monocitosis

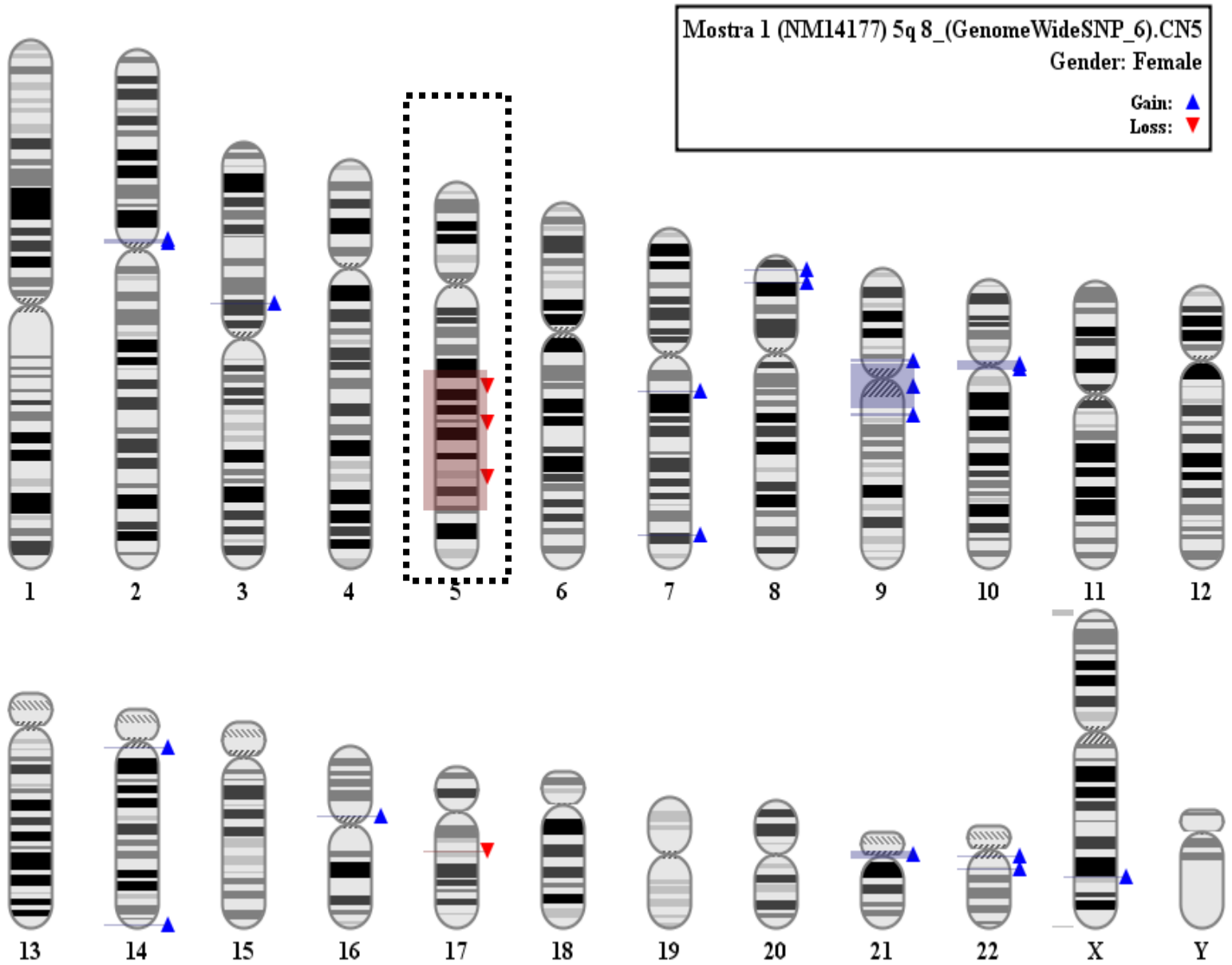
Molecular Karyotype: SNP arrays

- Presents major resolution vs CG, precise definition in breaking points and translocations, and detection of small size alterations
- Permits to study all the genome without the necessity of having divided cells
- Detects gains and losses frequent in MDS, information about the loss of heterozygosity (LOH) and UPD (uniparental disomy)

SNP arrays

- Doesn’t detect tumor cells inferior to 20%
- Doesn’t localise independent clones and doesn’t permit to quantify the cells with abnormal karyotype

Molecular Karyotype

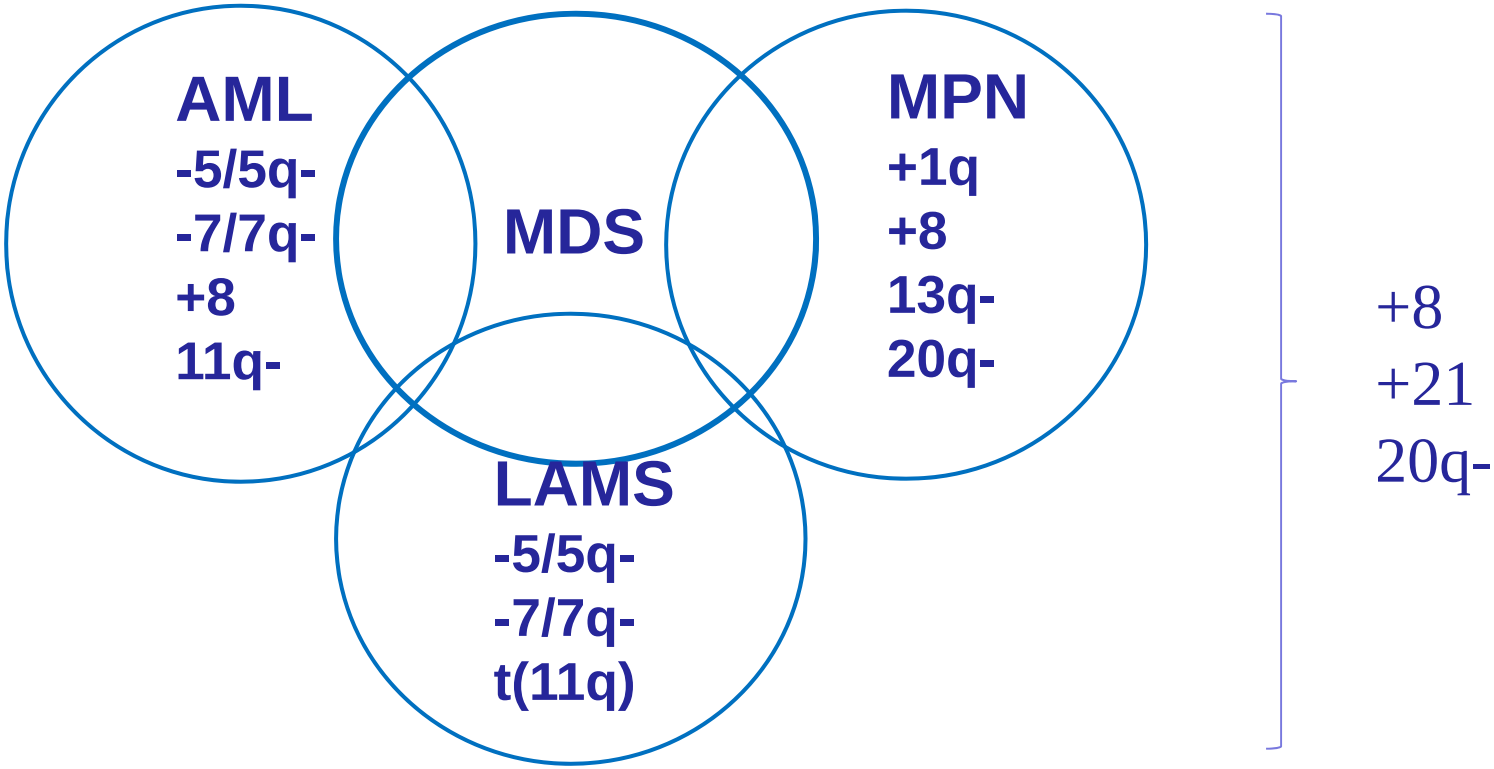


Loss of 5q

MDS: Detection of cytogenetic alterations

CG	FISH	SNP/CGH ARRAY	
50%	•60% (4 probes) • 5q- : Suspected 50% Without mitosis 20% Other alteration cr 5 80%	82% (without control tissue from the patient) 70% (with control from patient)	
	Mallo et al (2008) Haematologica	Gondek et al.(2007). Exp Hematol Heinrichs et al 2009. Leukemia	

MDS: Chromosome abnormalities



THERE IS NO EXCLUSIVE ALTERATION OF MDS

GENES IMPLICATED IN THE PATHOGENESIS OF MDS

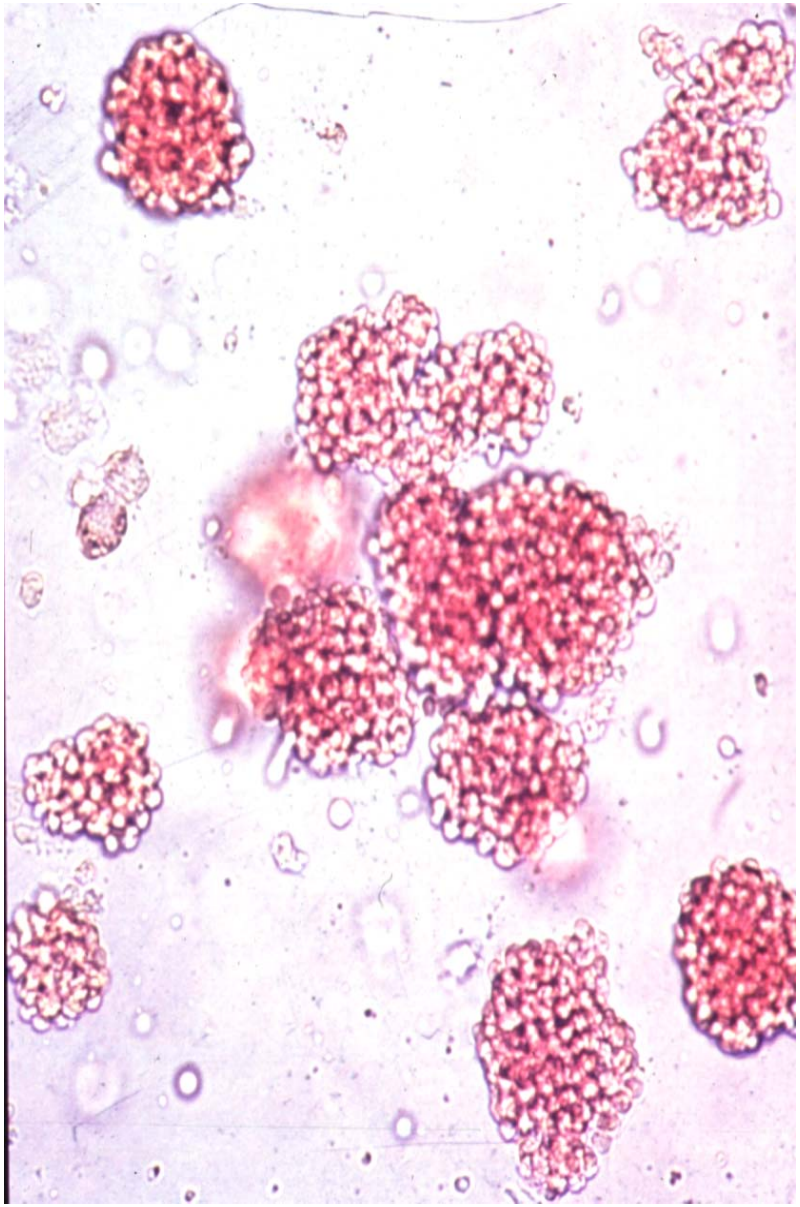
Genes	Chr. Localitation	% (MDS)
TET2	4q24	25
RPS14	5q32	15
CTNNA1	5q31	15
Mir145/146[a]	5q33	15
AXL1	20q11.21	10
N-RAS	1p13.2	10
P53	17p13.1	5-10
RUNX1/AML1	21q22.3	5-10
NPM1	5q35	5
JAK2	9p24	5
FLT3	13q12	2-5
C/EBPalpha	1913.1	1-4
EVI-1	3q26	2
CBL	11q23.3	1-2
EZH2	7q	6

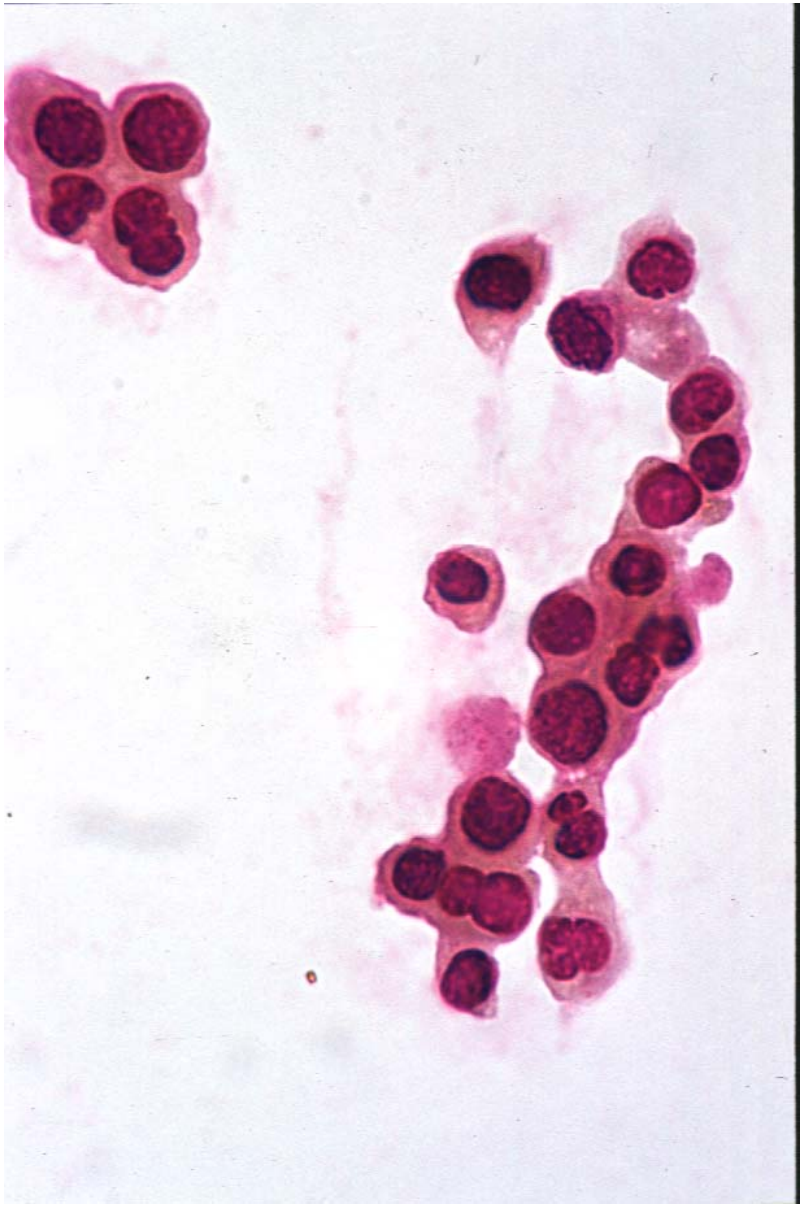
Its presence indicates clonality

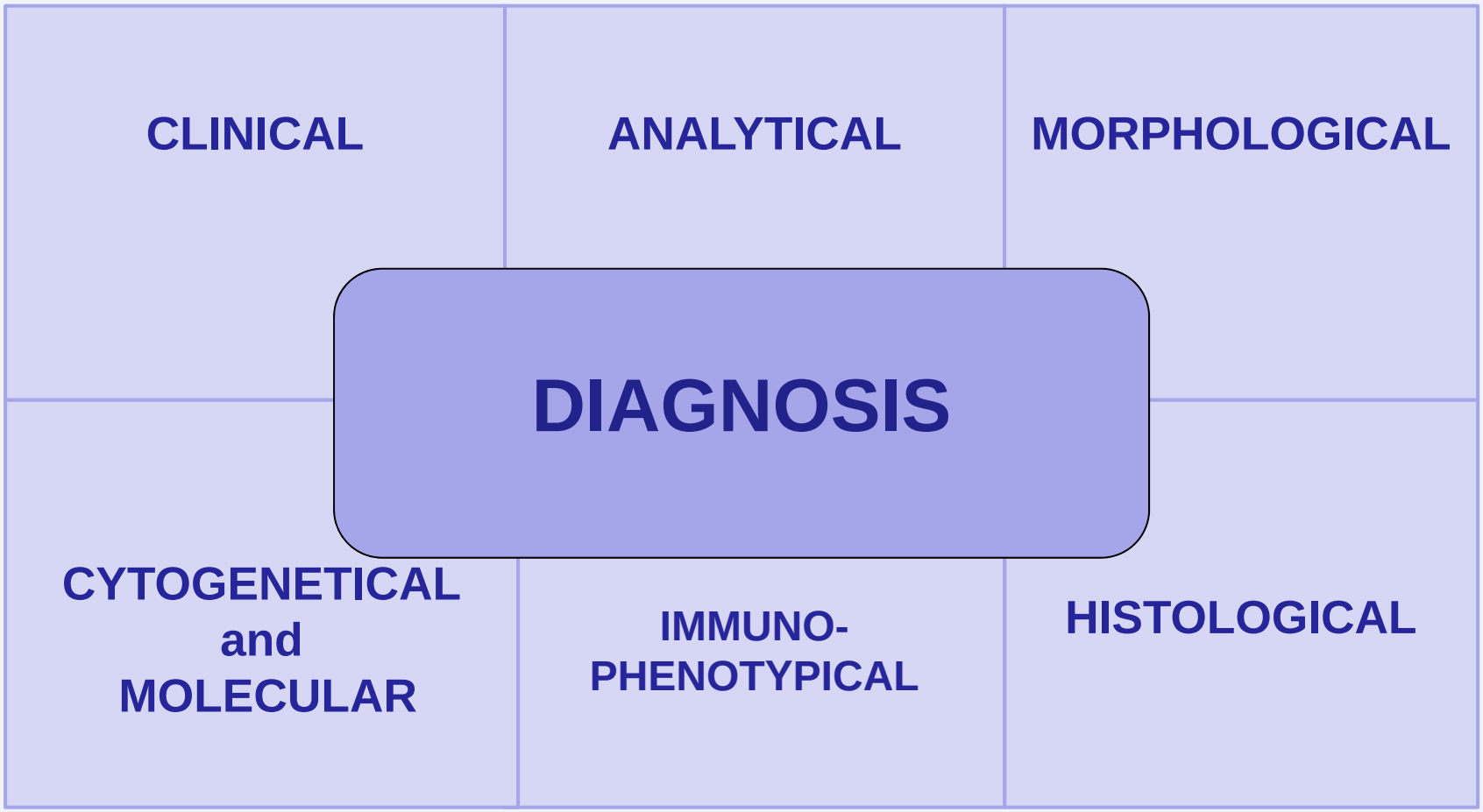
THERE IS NO EXCLUSIVE ALTERATION OF MDS

Jansen JH. EHA 2010

BFU-E



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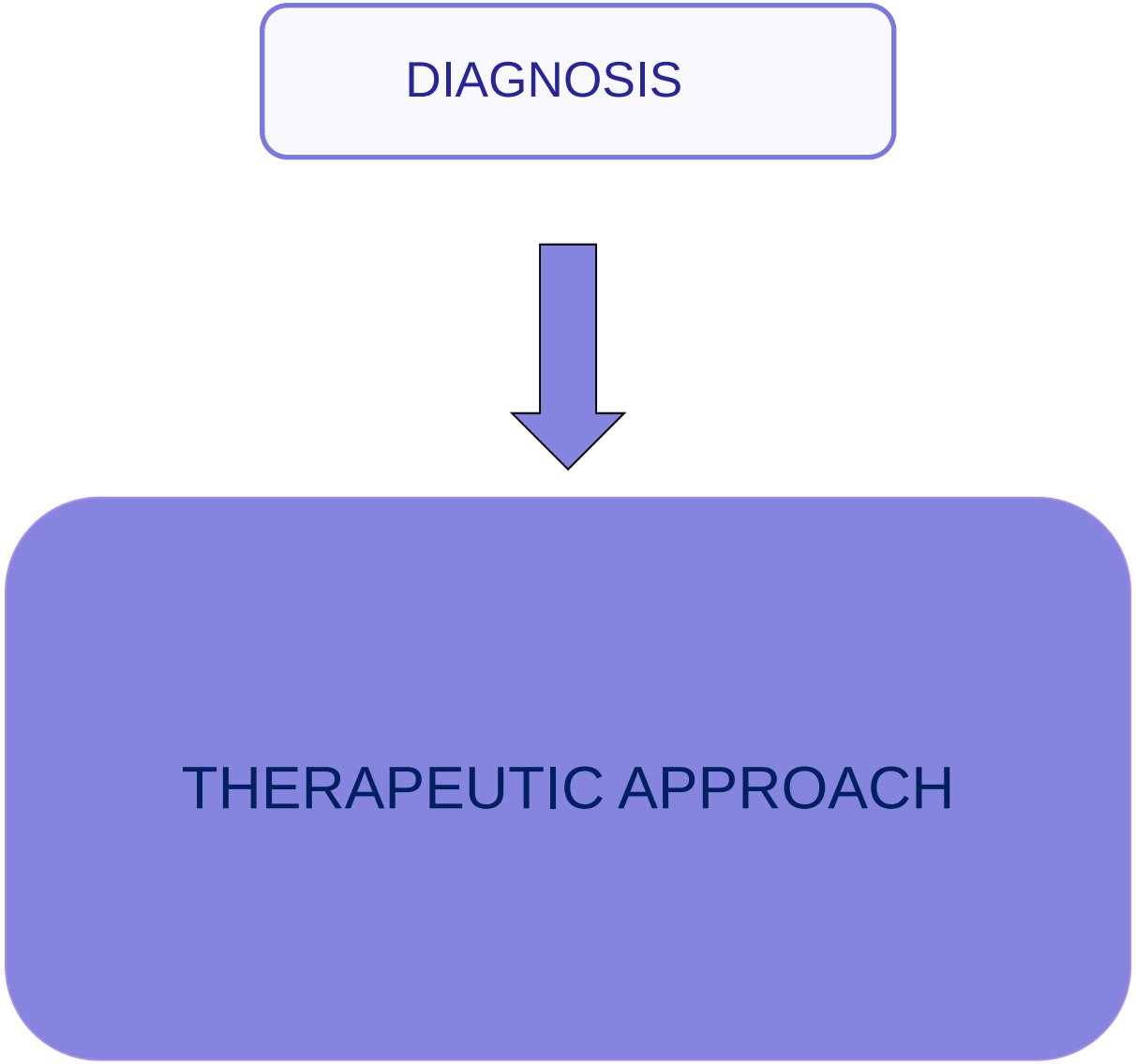
DIAGNOSIS
THERAPEUTIC APPROACH