FONDAZIONE ITALIANA SINDROMI MIELODISPLASTICHE

L'attuale approccio clinico al paziente con **Sindrome Mielodisplastica**

La nuova classificazione WHO delle Sindromi Mielodisplastiche

Gemelli
Fondazione Policlinico Universitario A. Gemelli
Università Cattolica del Sacro Cuore

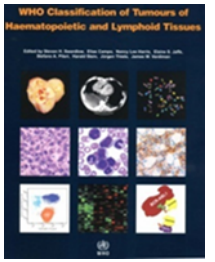
Gina Zini, MD. PhD. Hematology Prof.

FPG - Università Cattolica, Roma

Learning objectives

2016 WHO Updated Classification
of
Myelodysplastic Syndromes (MDS)

Principles of the WHO classification



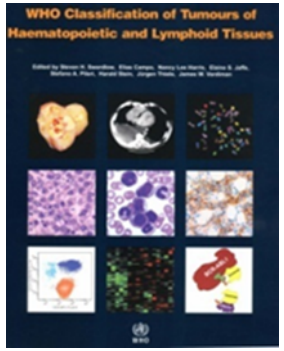
- Cytochemistry, immunophenotype, genetics and clinical features to define clinically significant disease entities.
- A classification that can be used in daily clinical practice.
- A classification that can serve as a common language for clinical trials and laboratory investigation.
- The term myeloid includes all cells belonging to the granulocytic, monocytic/macrophage, erythroid, megakaryocytic and mast cell lineages.
- Blast percentage ≥ 20% on PB and BM remains fundamental for categorizing and for evaluating disease progression.

Myelodysplastic syndromes

Clonal disorders characterized by

- Simultaneous proliferation and apoptosis of hematopoietic cells ▸ ineffective hematopoiesis
- Cytopenia(s) ▸ Hb< 10g/L, ANC < 1.8x109/L, Plt <100x109/L according to the IPPS (values are not exclusionary)
- Dysplasia in one or more of the three myeloid lineages
- Increased risk of development of AML

WHO 2008 MDS Classification



Disease	Blood findings	Bone marrow findings
Refractory cytopenias with unilineage dysplasia (RCUD) Refractory anaemia (RA); Refractory neutropenia (RN); Refractory thrombocytopenia (RT)	Unicytopenia or bicytopenia[1] No or rare blasts (<1%)[2]	Unilineage dysplasia: ≥10% of the cells in one myeloid lineage <5% blasts <15% of erythroid precursors are ring sideroblasts
Refractory anaemia with ring sideroblasts (RARS)	Anaemia No blasts	≥15% of erythroid precursors are ring sideroblasts Erythroid dysplasia only <5% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenia(s) No or rare blasts (<1%)[2] No Auer rods <1x10^9/L monocytes	Dysplasia in ≥10% of the cells in ≥ two myeloid lineages (neutrophil and/or erythroid precursors and/or megakaryocytes) <5% blasts in marrow No Auer rods ±15% ring sideroblasts
Refractory anaemia with excess blasts-1 (RAEB-1)	Cytopenia(s) <5% blasts[2] * No Auer rods <1x10^9/L monocytes	Unilineage or multilineage dysplasia 5-9% blasts[2] No Auer rods
Refractory anaemia with excess blasts-2 (RAEB-2)	Cytopenia(s) 5–19% blasts Auer rods ±[3] <1x10^9/L monocytes	Unilineage or multilineage dysplasia 10–19% blasts Auer rods ±[3]
Myelodysplastic syndrome – unclassified (MDS-U)	Cytopenias ≤1% blasts [2]	Unequivocal dysplasia in less than 10% of cells in one or more myeloid cell lines when accompanied by a cytogenetic abnormality considered as presumptive evidence for a diagnosis of MDS (See Table 5.04) <5% blasts
MDS associated with isolated del(5q)	Anaemia Usually normal or increased platelet count No or rare blasts (<1%)	Normal to increased megakaryocytes with hypolobated nuclei <5% blasts Isolated del(5q) cytogenetic abnormality No Auer rods

[1] Bicytopenia may occasionally be observed. Cases with pancytopenia should be classified as MDS-U.
[2] If the marrow myeloblast percentage is <5% but there are 2-4% myeloblasts in the blood, the diagnostic classification is RAEB 1. Cases of RCUD and RCMD with 1% myeloblasts in the blood should be classified as MDS, U.
[3] Cases with Auer rods and <5% myeloblasts in the blood and <10% in the marrow should be classified as RAEB 2.

WHO 2016: Proposed changes

- Nomenclature
- Morphology
- Immunophenotyping
- Genetics and molecular genetics

WHO 2016: MDS Revised Nomenclature

- WHO scheme classifies based on dysplasia and blast count, not cytopenia
- Type of dysplasia often does not fit with the cytopenic lineage in RCUD
- Subgroups of Refractory Anemia, Refractory Neutropenia and Refractory Thrombocytopenia are eliminated

WHO 2016: MDS Revised Nomenclature

Current or prior WHO categories

- Refractory cytopenia with unilineage dysplasia
  - Refractory anemia
  - Refractory neutropenia
  - Refractory thrombocytopenia
- RA with ring sideroblasts
- Refractory cytopenia with multilineage dysplasia
- Refractory anemia with excess of blasts
  - RAEB-1
  - RAEB-2
- MDS with isolated del(5q)
- MDS unclassifiable
- Childhood MDS
  - Refractory cytopenia of childhood

2016: Proposed changes

- MDS with single lineage dysplasia (MDS-SLD)
- MDS with ring sideroblasts with single lineage dysplasia (MDS-RSSLD)
- MDS with ring sideroblasts with multilineage dysplasia (MDS-RDMLD)
- MDS with multilineage dysplasia (MDS-MLD)
- MDS with excess of blasts (MDS-EB)
  - MDS-EB-1
  - MDS-EB-2
- MDS with isolated del(5q)
- MDS unclassifiable
- Childhood MDS
  - Refractory cytopenia of childhood

MDS Morphology Issues

- Cut-off of 10% to detect lineage dysplasia is maintained

- Cut-off of 2% of blasts introduced by the IPSS-R: difficult, poorly reproducible distinction between categories 0-2% vs >2% vs <5%

  Recommendation to report the exact blast count, rather than <5%

- Diagnosis of AML in cases with less than 20% of blasts

  - detection of t(8;21)(q22;q22); *RUNX1-RUNX1T1*; inv(16)(p13.1;q22) or (16;16)(p13.1;q22); *CBFB-MYH11* or *PML-RARA* is still considered diagnostic for AML regardless of blast count

  - detection of other genetics event such as t(9;11)(p21.3;q23.3); *KMT2A-MLLT3*, t(6;9)(p23;q34.1), *DEK-NUP214* and *NPM1* mutation remain controversial

- Similarities between myeloid neoplasms with inv3(q21;q26.2) or t(3;3)(q21.3;q26.2) regardless of blast count

Morphological dysplastic features

Dyserythropoiesis

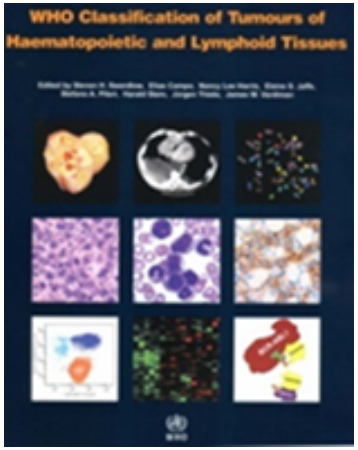
- Nuclear
  - Nuclear budding
  - Internuclear bridging
  - Karyorrhexis
  - Multinuclearity
  - Nuclear hyperlobation
  - Megaloblastic changes
- Cytoplasmic
  - Ring sideroblasts
  - Vacuolization
  - Periodic acid-Schiff positivity

Dysgranulopoiesis

- Small or unusually large size
- Nuclear hypolobation (pseudo Pelger-Huët; pelgeroid)
- Irregular hypersegmentation
- Decreased granules; agranularity
- Pseudo Chediak-Higashi granules
- Auer rods

Dysmegakaryocytopoiesis

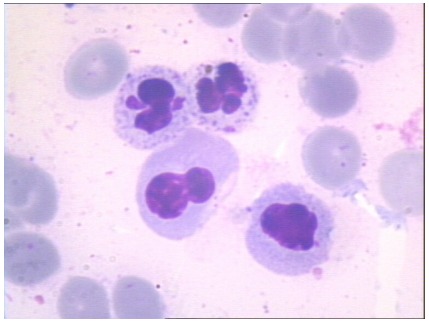
- Micromegakaryocytes
- Nuclear hypolobation
- Multinucleation (normal megakaryocytes are uninucleate with lobulated nuclei)



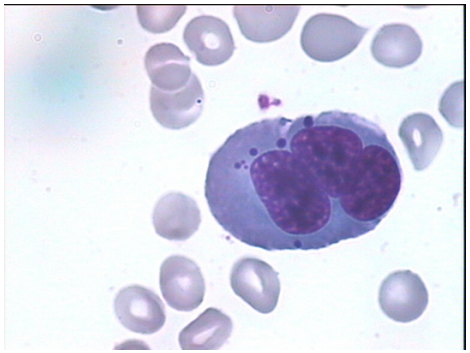
Morphology: WHO Qualitative recommendations

Dyserythropoiesis

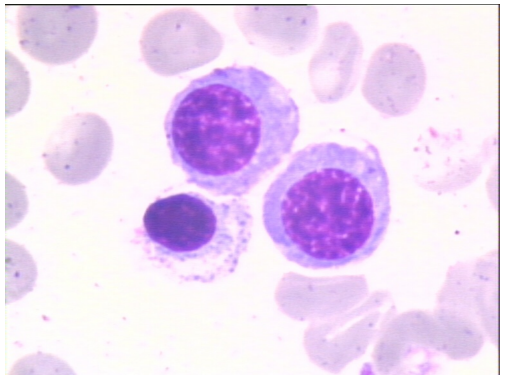
Nuclear budding



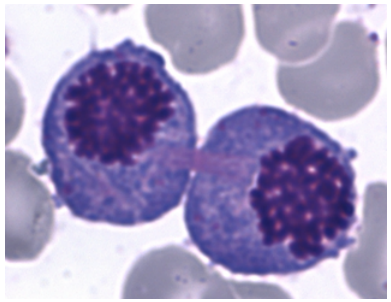
Multinuclearity



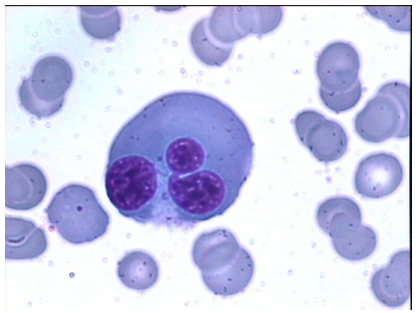
Vacuolization



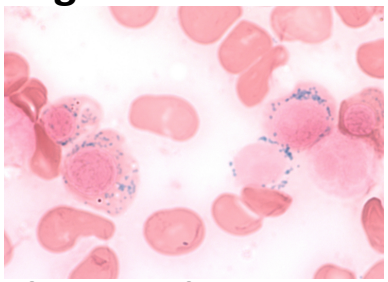
Internuclear bridging



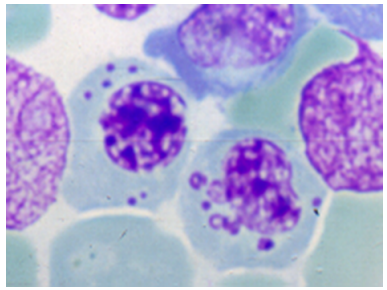
Nuclear hyperlobulation



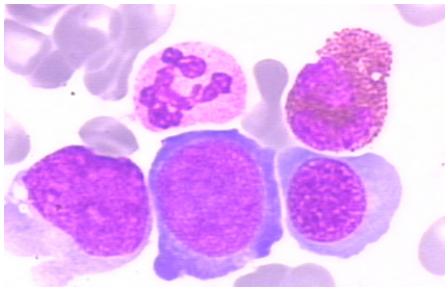
Ring sideroblasts



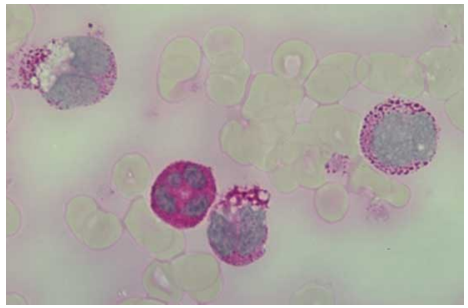
Karyorrexis



Megaloblastic changes



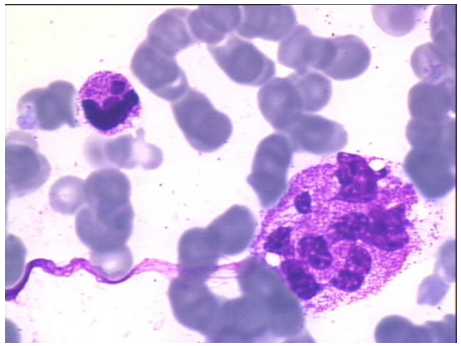
Abnormal PAS positivity

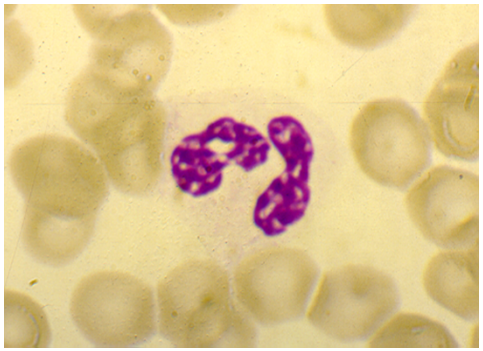


Morphology: WHO Qualitative recommendations

Dysgranulopoiesis

Small or unusually large size

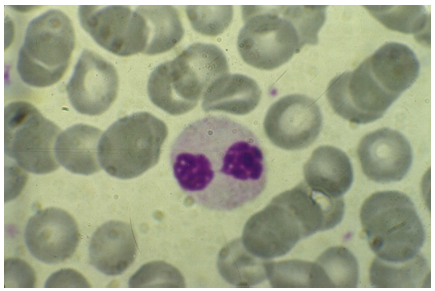




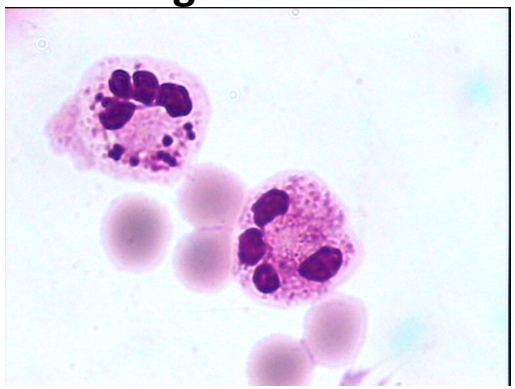
Decreased granules (with at least 2/3 reduction of the content of granules**, agranularity**

Leuk Res. 2014 , Goasguen JE, Bennett JM, Zini G et al. International Working Group on Morphology of MDS (IWGM-MDS).

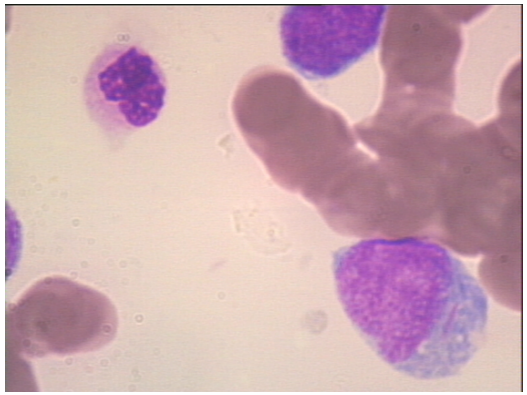
Nuclear hypolobulation (pelgeroid)



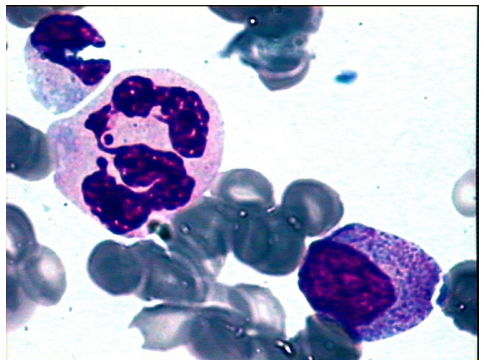
Pseudo Chediak-Higashi granules



Auer rods*



Irregular hypersegmentation

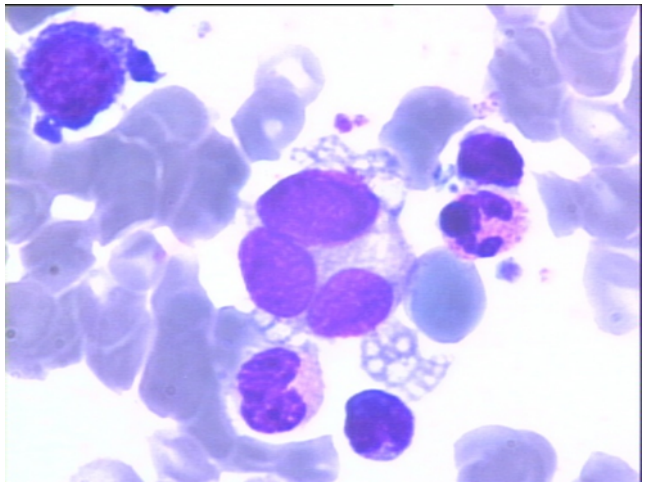


*Cases with Auer rods should be classified as RAEB-2 irrespective of blast count

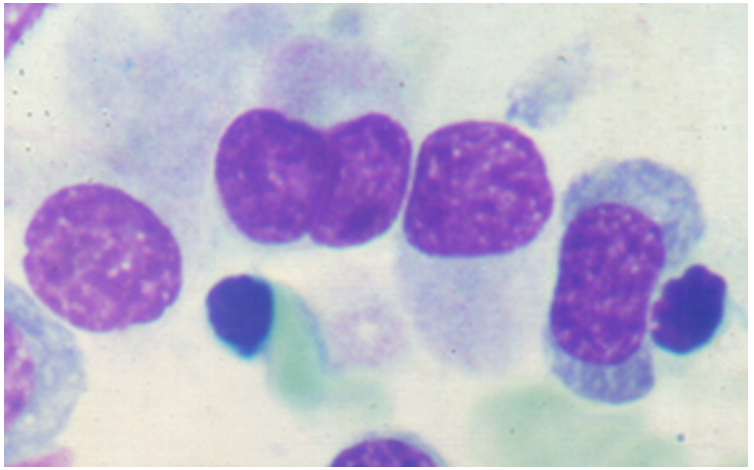
Morphology: WHO: Qualitative recommendations

Dysmegakaryocytopoiesis

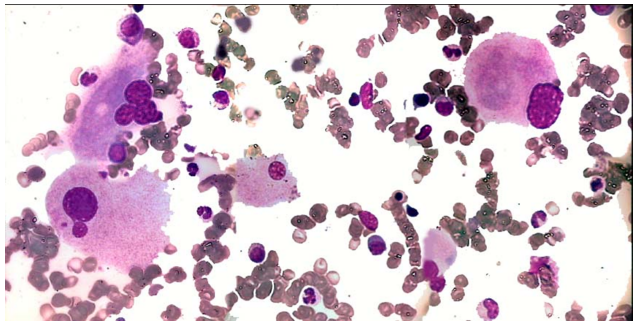
Multinuclearity



Micromegakaryocytes



Nuclear hypolobulation



Quality control initiative on the evaluation of the dysmegakaryopoiesis in myeloid neoplasms: Difficulties in the assessment of dysplasia.
Goasguen JE, Bennett J,Zini G et al., International Working Group on Morphology of MDS IWGM-MDS. Leuk Res. 2016

WHO 2016: MDS Unclassifiable

- MDS with single lineage dysplasia or multilineage dysplasia with <5% of blasts in the BM but 1% of blasts in PB:

  Recommendation: 1% of blasts in PB must be measured on at least two separate occasions

- MDS with single lineage dysplasia but pancytopenia:

  Recommendation: cytopenia is below IPSS level: ANC $<1.8x10^9/L$, HGB<10g/dL, $PLT<100x10^9/L$

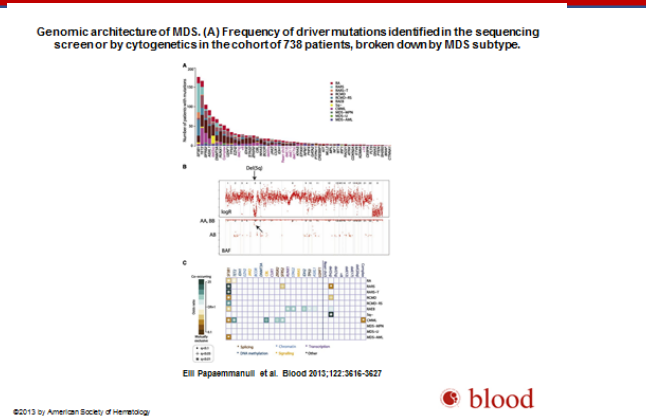
- MDS-associated cytogenetic abnormality in association with cytopenias, <1% PB and <10% BM blasts, but <10% dysplasia in any cell line

Immunophenotyping in MDS

- Abnormal flow cytometry patterns do predict MDS with good sensitivity and specificity
- Specific antibody panels should be carefully chosen and validated according to published guidelines
- Flow cytometry results should be integrated with the BM morphology report
- Flow cytometry immunophenotyping:
  Is not required but will be considered as "***supportive***" of MDS
  Will ***not alone be sufficient*** for making diagnosis of MDS

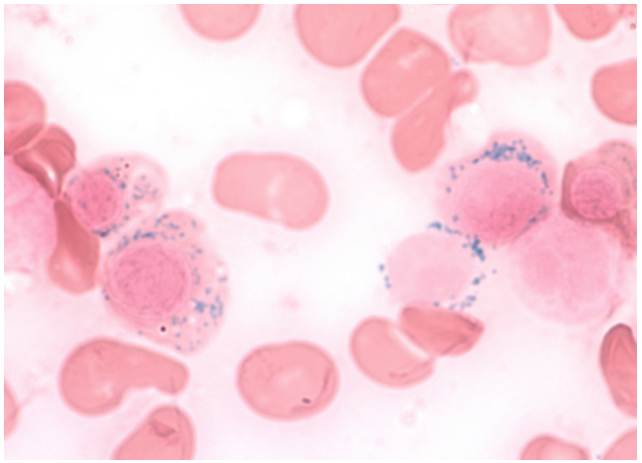
Genetics in MDS

- ✓ Somatic mutations in MDS
  - Prognostic significance of mutations of *TP53, EZH2, ETV6, RUNX1, ASXL1* and others.

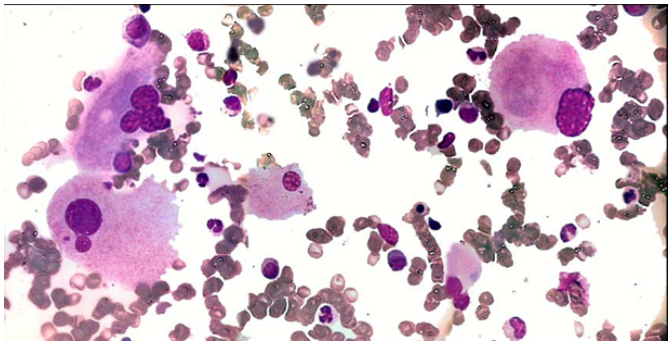


- ✓ Mutation of the spliceosoma gene *SF3B1* in MDS with ring sideroblasts (MDS-RSSLD & MDS-RSMLD)
  - ≥15% ring sideroblasts (among erythroid precursors)

  or
  - ≥5% ring sideroblasts in presence of an *SF3B1* mutation
  - Blasts cell increase exclude this diagnosis
  - If multilineage dysplasia without a blast cell increase is present, a case is classified as MDS with ring sideroblasts and multilineage dysplasia.



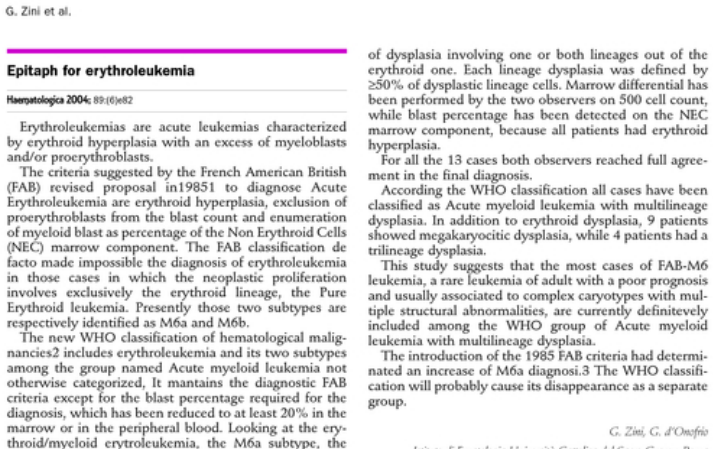
- ✓ MDS with isolated del(5q)
  - Del(5q) as the only abnormality
  - Except for presence of monosomy 7, WHO 2016 does not allow a second cytogenetic abnormality for this category
  - Recommendation to assess *TP53* mutation or p53 staining.



Acute erythroid leukemia (erythroid/myeloid type) proposed to become MDS with excess of blasts

WHO 2001 & 2008 diagnostic criteria:

- AML NOS ≥50% BM erythroid precursors & ≥20% blasts NEC



G. Zini et al.

Epitaph for erythroleukemia

Haematologica 2004; 89:(6)e82

Erythroleukemias are acute leukemias characterized by erythroid hyperplasia with an excess of myeloblasts and/or proerythroblasts.

The criteria suggested by the French American British (FAB) revised proposal in19851 to diagnose Acute Erythroleukemia are erythroid hyperplasia, exclusion of proerythroblasts from the blast count and enumeration of myeloid blast as percentage of the Non Erythroid Cells (NEC) marrow component. The FAB classification de facto made impossible the diagnosis of erythroleukemia in those cases in which the neoplastic proliferation involves exclusively the erythroid lineage, the Pure Erythroid leukemia. Presently those two subtypes are respectively identified as M6a and M6b.

The new WHO classification of hematological malignancies2 includes erythroleukemia and its two subtypes among the group named Acute myeloid leukemia not otherwise categorized, It mantains the diagnostic FAB criteria except for the blast percentage required for the diagnosis, which has been reduced to at least 20% in the marrow or in the peripheral blood. Looking at the erythroid/myeloid erytroleukemia, the M6a subtype, the of dysplasia involving one or both lineages out of the erythroid one. Each lineage dysplasia was defined by ≥50% of dysplastic lineage cells. Marrow differential has been performed by the two observers on 500 cell count, while blast percentage has been detected on the NEC marrow component, because all patients had erythroid hyperplasia.

For all the 13 cases both observers reached full agreement in the final diagnosis.

According the WHO classification all cases have been classified as Acute myeloid leukemia with multilineage dysplasia. In addition to erythroid dysplasia, 9 patients showed megakaryocitic dysplasia, while 4 patients had a trilineage dysplasia.

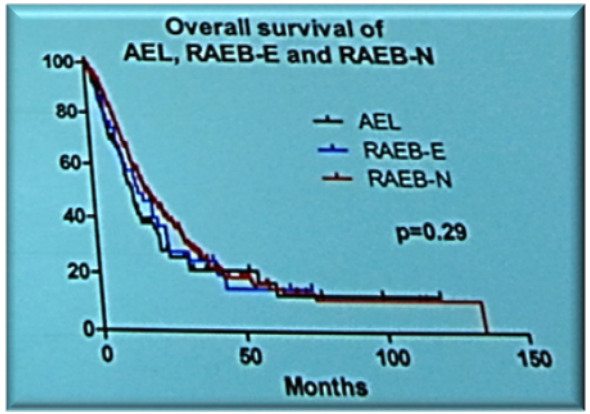
This study suggests that the most cases of FAB-M6 leukemia, a rare leukemia of adult with a poor prognosis and usually associated to complex caryotypes with multiple structural abnormalities, are currently definitevely included among the WHO group of Acute myeloid leukemia with multilineage dysplasia.

The introduction of the 1985 FAB criteria had determinated an increase of M6a diagnosi.3 The WHO classification will probably cause its disappearance as a separate group.

G. Zini, G. d'Onofrio

WHO 2016

- These cases will now be classified as MDS based on the blasts ANC count.



- The different AML and MDS subtypes with predominant erythropoiesis may be combined into one category.
- Pure erythroid leukemia remains a subtype of AML.

Summary
WHO 2016 & MDS

- Limitations of current criteria support the introduction of ICUS

- Somatic mutations in hematopoietic cells leading to clonal expansion are commonly acquired during human aging

- Clonally restricted hematopoiesis is associated with an increased risk of subsequent diagnosis of myeloid or lymphoid neoplasia and increased all-cause mortality

Screening of somatic mutations on DNA from PB cells might be of value in the diagnostic work-up of patients with unexplained anemia or cytopenia.

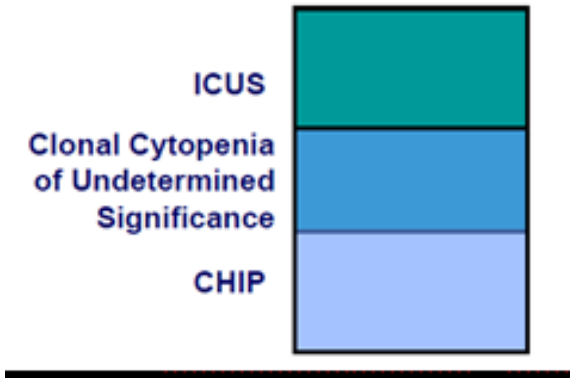
Blurred borders of MDS, MPN and/or AL:
- Idiopathic Cytopenias of Undetermined Significance (ICUS)
- Clonal Cytopenia of Undetermined Significance (CCUS)
- Clonal Hematopiesis of Indeterminated Potential (CHIP)

ICUS:
- persistent cytopenia
- no significant dysplasia
- no specific cytogenetic abnormalities considered as presumptive evidence of MDS
- no potentially related hematologic or non-hematologic disease

CCUS:
- persistent cytopenia (one or more lineage) not explained by any other disease
- no diagnostic criteria for hematological neoplasm
- presence of a somatic mutations associated with hematological neoplasia

CHIP
- presence of somatic mutations associated with hematological neoplasia at variant allele frequency of at least 2%
- absence of definitive morphological evidence of hematological neoplasm, no diagnostic criteria for PNH, MGUS or MBL
- CHIP may have normal blood counts, have cytopenias unrelated to MDS, or cytopenias that do not meet the criteria for MDS
- Broad list of involved genes (eg. *DNMT3A, TET2, JAK2, SF3B1, ASCL1, TP53, CBL, GNB1, BCOR, U2AF1, CREBBP, CUX1, SRSF2, MLL2, SETD2, SETDB1, GNAS, PPM1D, BCORL1*)

- 35% percent of ICUS carry MDS-associated somatic mutations and can be identified as CCUS.
- CCUS and MDS patients share similar mutations may have diagnostic relevance.



Kwok et al. 2015 Blood,126:2355-61.
Steensma et al. 2015 Blood,126:9-16

FONDAZIONE ITALIANA SINDROMI MIELODISPLASTICHE

L'attuale approccio clinico al paziente con **Sindrome Mielodisplastica**

Napoli

Thank you for listening

Gemelli

Fondazione Policlinico Universitario A. Gemelli
Università Cattolica del Sacro Cuore

Gina Zini, MD. PhD. Hematology Prof.

FPG - Università Cattolica, Roma