

WHO 2008 CLASSIFICATION OF MYELOID NEOPLASMS-WHAT IS NEW AND WHY?

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WHY CLASSIFY

- “Language of medicine: diseases must be described, defined and named before they can be diagnosed, treated and studied”
- Disease should be clearly defined, clinically distinctive and non-overlapping

BASIS OF CLASSIFICATION

- Morphology
- Immunophenotype
- Genetics
- Clinical features

PRE-REQUISITES

- Peripheral blood
 - Good quality May-Grunwald-Giemsa or Wright-Giemsa stain is necessary
 - RBC indices from automated analyzer
 - Manual 200 cell diff recommended in myeloid neoplasms

PRE-REQUISITES

- Bone marrow aspirate
 - Good quality aspirate with adequate particles
 - Very thick smear is inadequate for evaluation
 - High quality staining is essential
 - Do not over diagnose on aspirate
 - Cellularity difficult to assess
 - 500 nucleated cell diff in area close to particles

PRE-REQUISITES

- Bone marrow trephine biopsy
 - “the contribution of adequate bone marrow biopsy sections in the diagnosis of myeloid neoplasms cannot be over stated”
 - Provides information on overall cellularity, topography, proportion and maturation of hematopoietic cells and marrow stroma
 - A 1.5 cm trephine at right angle to cortical bone is considered adequate

PRE-REQUISITES

- BM trephine
 - Well fixed (formalin, Bouin's, B-5)
 - 3-4 micron thick sections
 - H&E stain
 - Immunohistochemical and histochemical stains

MYELOPROLIFERATIVE NEOPLASMS

- CML, BCR-ABL1 positive
- Chronic neutrophilic leukemia
- Polycythemia vera, *
- Primary myelofibrosis, *
- Essential thrombocythemia, *
- Chronic eosinophilic leukemia, NOS
- Mastocytosis
- Myeloproliferative neoplasm, unclassifiable

NEW GENES

- JAK2 V617F- PV, PMF, ET
- MPL W151L/K- PMF, ET
- JAK exon 12- PV
- KIT D816V- Mastocytosis
- PDGFRA, PDGFRB, FGFR1

POLYCYTHEMIA VERA

- Either both major and one minor or first major and two minor
- MAJOR CRITERIA
 - Hb > 18.5 in men, 16.5 in women or other evidence of increased red cell volume
 - Presence of **JAK2 V617F** or other functionally similar mutation such as JAK2 exon 12 mutation

POLYCYTHEMIA VERA

- MINOR CRITERIA

- BM biopsy with hypercellularity for age with trilineage growth with prominent erythroid, granulocytic and megakaryocytic proliferation
- Serum erythropoietin level below reference range for normal
- Endogenous erythroid colony formation in vitro

PRIMARY MYELOFIBROSIS

- Major criteria
 - Presence of megakaryocytic proliferation and atypia, usually accompanied by either reticulin and/or collagen fibrosis or in the absence of fibrosis, meg changes must be accompanied by increased cellularity with granulocytic proliferation and often decreased erythropoiesis
 - Not meeting criteria for PV/CML/MDS
 - Demonstration of **JAK2** or other clonal marker or in absence of clonal marker, exclusion of fibrosis due to other causes

PRIMARY MYELOFIBROSIS

- Minor criteria
 - Leukoerythroblastosis
 - Increase in serum LDH level
 - Anemia
 - Splenomegaly
- 3 major and 2 minor

ESSENTIAL THROMBOCYTHEMIA

- Sustained platelet count $>450 \times 10^9/L$
- BM biopsy showing proliferation mainly of megs with increased numbers of enlarged, mature megs. No significant increase or left shift of neutrophil granulopoiesis or erythropoiesis
- Not PV/PMF/CML/MDS
- Demonstration of **JAK2** or other clonal marker, or in absence of JAK2, no evidence of reactive thrombocytosis

CEL, NOS

- Eosinophilia $>1.5 \times 10^9$
- No BCR/ABL1 or PV/ET/PMF or MDS/MPN
- No t(5;12)(q31-35;p13) or other rearrangement of PDGRFB
- No FIP1L1-PDGRFA fusion gene or other rearrangement of PDGRFA
- No rearrangement of FGFR1
- $<20\%$ blasts & no inv(16) or t(16;16)
- Clonal cytogenetic or molecular genetic abnormality or $>2\%$ blasts PB or $>5\%$ blasts BM

EOSINOPHILIA

- Idiopathic hypereosinophilic syndrome
 - $>1.5 \times 10^9$ -6 months
 - Reactive excluded
 - AML/MPN/MDS and mastocytosis excluded
 - Cytokine producing aberrant T cells excluded
 - Tissue damage
- Idiopathic hypereosinophilia
 - Meets other criteria but no tissue damage

MASTOCYTOSIS

- Clonal neoplastic proliferation of mast cells that accumulate in 1 or more organ systems
- Associated with activating point mutations of KIT (common D816V)
- Express CD9, CD33, CD45, CD68, CD117 and tryptase
- CD2 and CD25 are aberrant markers

MASTOCYTOSIS

- Cutaneous mastocytosis
- Indolent systemic mastocytosis
- Systemic mastocytosis with associated clonal hematopoietic non-mast cell lineage disease(SM-AHNMD)
- Aggressive systemic mastocytosis
- Mast cell leukemia
- Mast cell sarcoma
- Extracutaneous mastocytoma

MPN, UNCLASSIFIABLE

- Not waste-basket category for cases with incomplete clinical data or poor quality PB, BM aspirate or trephine
- Early stages of PV, PMF or ET with lack of diagnostic features
- Advanced stage MPN with myelofibrosis, osteosclerosis or transformation to more aggressive stage
- Co-existing neoplastic or inflammatory disorder obscures some diagnostic features in otherwise convincing MPN

**MYELOID AND LYMPHOID NEOPLASMS
WITH EOSINOPHILIA AND
ABNORMALITIES OF PDGFRA, PDGFRB or
FGFR1**

PDGFRA REARRANGEMENTS

- FIP1L1-PDGFR A most common, other variant translocations
- Detected by nested RT-PCR or FISH
- Cryptic by cytogenetics
- Commonly MPN (CEL), but maybe AML/ALL
- $>1.5 \times 10^9$ eos with some abnormalities
- Organ damage by eos
- Responsive to imatinib

PDGFRB REARRANGEMENTS

- Commonly t(5;12) with formation of ETV6-PDGFRB fusion gene but many variants
- Hematologic features of CMML common, but atypical CML, CEL and AML may occur
- Leukocytosis with variable increase in neutro, eos and monos and precursors
- Hypercellular BM with increased mast cells and some fibrosis
- Responsive to imatinib

FGFR1 ABNORMALITIES

- Heterogeneous- MPN, AML, ALL or mixed phenotype acute leukemia (MPAL)
- 90% with PB, BM eosinophilia
- 8p11 breakpoint translocations with many partners-t(8;13) ZNF198-FGFR1 most common
- Poor prognosis with no response to tyrosine kinase inhibitor therapy

MDS/MPN NEOPLASMS

- CMML (CMML with eosinophilia)
- Atypical CML, BCR-ABL1 NEGATIVE
- JMML
- MDS/MPN, unclassifiable
- Provisional entity- Refractory anemia with ring sideroblasts and thrombocytosis (RARS-T)

MDS/MPN

- Exclude PDGFRA/PDGFRB rearrangements in cases with eosinophilia
- RARS-T is associated with clinical and morphologic features of MDS with marked thrombocytosis and atypical megs
- May be MPN- JAK2 or MPL mutation in majority cases

MDS

- Refractory cytopenia with unilineage dysplasia
- RARS
- RCMD
- RAEB
- MDS with isolated del(5q)
- MDS, unclassifiable
- Childhood MDS

MDS

- Well stained PB and BM essential
- Dysplasia in >10% of cells in lineage
- Blast % very important
- Genetics also very important in diagnosis and prognosis
- Always look for ring sideroblasts under oil
- Detailed history is mandatory and drug/toxin exposure and B12/folate deficiency must be excluded

RCUD

- Includes refractory anemia, refractory neutropenia and refractory thrombocytopenia
- Dysplasia in only 1 lineage
- RA Hb <10g/dl normocytic normochromic with dyserythropoiesis, <5 % blasts and <15% ring sideroblasts
- RN ANC<1.8X10⁹/L, >10% dysplastic neutrophils in PB/BM
- RT plts <100x10⁹, > 10% dysplastic megs with at least 30 megs evaluated

CHILHOOD MDS

- Very uncommon <5% of hematopoietic neoplasms
- De-novo MDS must be distinguished from secondary MDS due to marrow failure
- Neutropenia and thrombocytopenia more common and isolated anemia is rare
- RARS and 5q- are rare
- Hypocellular marrow is more common

REFRACTORY CYTOPENIA OF CHILDHOOD (RCC)

- Provisional entity
- Persistent cytopenia with <5% blasts BM and <2% blasts PB
- Dysplasia required
- BM trephine normally hypocellular
- PB- dysplastic changes in at least 10% neutrophils
- BM aspirate- dysplasia in 10% of erythroid and granulocytic precursors and unequivocal micromegs
- No ring sideroblasts

RCC

- Differential diagnosis
 - Infections
 - Vitamin deficiency
 - Metabolic disorders
 - Rheumatic disease
 - ALPS
 - Mitochondrial deletions
 - Inherited BM failure disorders
 - PNH

AML and related precursor neoplasms

- AML with recurrent genetic abnormalities
- AML with MDS related changes
- Therapy related myeloid neoplasms
- AML, NOS
- Myeloid sarcoma
- Myeloid proliferation related to Down syndrome
- Blastic plasmacytoid dendritic cell neoplasm

AML

- Cytogenetic and molecular abnormalities take precedence over traditional morphologic categories (AML, NOS)
- As more prognostically significant genetic findings are found NOS category will continue to shrink
- Cytogenetics/molecular testing has great prognostic and, sometimes therapeutic, importance

AML with recurrent genetic abnormalities

- AML with balanced translocations/inversions
 - AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
 - Mostly FAB M2- <20% blasts is AML
 - Large blasts with granules and Auer rods
 - Immunophenotype-typical myeloid phenotype with co-expression of CD19, CD79a and PAX5 in some cases
 - Genes-RUNX1(AML1 or CBFA)&RUNX1T1 (ETO)-CBF components
 - Good response to therapy and long term disease free survival

AML with inv(16)(p13.1;q22) or
t(16;16)(p13.1;q22); CBFB-MYH11

- Usually FAB M4 with abnormal marrow **eosinophils**-<20% blasts is AML
- Eos have large purple-violet granules
- Immunophenotype-multiple blast populations with CD34/CD117+ and differentiation to granulocytic and monocytic lineages
- Genes- CBFB-beta subunit of CBF; MYH11-smooth muscle myosin heavy chain
- May be cryptic on karyotype-FISH/RT-PCR
- **Long complete remission when treated with high dose cytarabine in consolidation**

APL with t(15;17)(q22;q12); PML-RARA

- Typical morphology with hypergranular and microgranular variants
- <20% blasts AML
- Immunophenotype-**CD34/HLA-DR/CD11a/CD11b-**, CD33+++**, CD117+/-**
- **ATRA sensitive- favorable**
- **Variant translocations- t(11;17)-ATRA resistant; t(5;17) ATRA sensitive**

AML with t(9;11)(p22;q23); MLLT3-MLL

- **More common in children (9-12%)**
- Strong correlation with FAB M4/5
- Immunophenotype- strong CD33/CD65/CD4/HLA-DR, CD34/CD14/CD13 usually low
- MLL-histone methyltransferase that regulates gene transcription
- Intermediate survival, superior to other MLL translocations
- Over 80 different translocations involving MLL in adult and peds AML and ALL

AML with t(6;9)(p23;q34); DEK-NUP214

- Usually presents with anemia, thrombocytopenia with low TLC
- May be any FAB subtype, except M3 & M7
- >2% PB & BM baso in 44-62%
- Granulocytic and erythroid dysplasia
- Nonspecific immunophenotype
- DEK-NUP214 nucleoporin fusion protein acts as aberrant transcription factor
- Poor prognosis

AML with $\text{inv}(3)(\text{q}21;\text{q}26.2)$ or $\text{t}(3;3)(\text{q}21;\text{q}26.2)$;RPN1-EVI1

- De novo or arising from MDS
- Normal or elevated plts
- Any FAB subtype except M3
- Dysplastic megs
- CD13,CD33, HLA-DR,CD34, CD38+
- Aberrant CD7, some cases with CD41 & CD61
- Fusion gene causes increased cell proliferation and decreased differentiation
- Aggressive disease with short survival

AML with t(1;22)(p13;q13); RBM15-MLK1

- Infants and young children < 3 yrs
- Commonly with Down syndrome
- FAB M7-megakaryoblasts and undifferentiated blasts
- Micromegs and marrow fibrosis
- CD41/CD61+ CD34/CD45/HLA-DR-
- Karyotype or molecular-RNA binding motif protein 15 and megakaryocytic leukemia 1 fusion
- Respond well to intensive therapy with long disease free survival

AML with myelodysplasia related changes

- AML with 20% or more blasts
 - previous history of MDS, MDS/MPN
 - MDS related cytogenetic abnormality
 - AML with multilineage dysplasia
 - Exclude previous cytotoxic or radiation therapy
 - Exclude AML with recurrent genetic abnormalities
 - Usually severe pancytopenia and dysplasia >50% cells in 2 lineages
 - TdT expression with chromo 5&7 abnormalities

AML with myelodysplasia related changes

- Karyotype
- Unbalanced abnormalities -7/del(7q), -5/del(5q), i(17q)/t(17p), -13/del(13q), del(11q), del(12p)/t(12p), del(9q) & idic(X)(q13)
- Balanced abnormalities- t(11;16), t(3;21), t(1;3), t(2;11), t(5;12), t(5;7), t(5;17), t(5;10) & t(3;5)
- D/D RAEB,AML M6/M7
- Poor prognosis with low rate of achieving

Therapy related myeloid neoplasms

- ▣ Mutational events induced by cytotoxic therapy
- ▣ Therapy related AML, MDS, MDS/MPN
- ▣ 5-10 yrs after alkylating agents and/or ionizing radiation
- ▣ 1-5 yrs after DNA topoisomerase II inhibitors
- ▣ Multilineage dysplasia and abnormal karyotype
- ▣ No specific immunophenotype
- ▣ Poor prognosis, especially with abnormalities of chromosomes 5 & 7

AML, NOS

- Cases that do not fulfil criteria for inclusion in one of the previously described groups
- Clinical relevance of some groups is of questionable significance
- Mutation analysis and cytogenetic studies are recommended to offer more prognostic information

Myeloid sarcoma

- Tumor mass composed of myeloid blasts with or without maturation occurring at an anatomical site other than BM
- Skin, LN, GI, bone, soft tissue, testis
- IHC CD68, MPO, CD117, CD99, CD34, TdT, CD56
- D/D Malignant lymphoma

Myeloid proliferations related to Down syndrome

- ▣ Marked increase in leukemia, both ALL and AML
- ▣ 150 fold increase in AML <5 yrs with 70% AML M7
- ▣ **Transient abnormal myelopoiesis (TAM)**
 - Unique disorder in DS with clinical and morphologic findings indistinguishable from AML
 - 10% DS newborns, thrombocytopenia, leukocytosis with blasts, morphology and immunophenotype of meg blasts is common
 - Resolves spontaneously within 3 months

AML in Down syndrome

- Usually under 5 yrs, account for 20% of peds AML/MDS
- May be preceded by RCC like picture
- Meg blasts, core with reticulin fibrosis and dysplastic megs
- CD117, CD13, CD33, CD7, CD4, CD42, CD41, CD61, CD71 are positive (same in TAM)
- Negative for MPO, CD15, CD14 & glycoporphin A
- Somatic mutations of gene encoding transcription factor GATA is pathognomonic of both TAM & AML
- **Good response to therapy and favorable outcome**

Blastic plasmacytoid dendritic cell neoplasm

- Aggressive tumor derived from precursors of plasmacytoid dendritic cells (**plasmacytoid monocytes**)
- Usually present with asymptomatic solitary or multiple skin lesions +/- lymphadenopathy, PB & BM involvement may be minimal at presentation but invariably develops
- **10-20% cases associated with or develop AML M4**
- Medium sized blasts with irregular nuclei, fine chromatin and several nucleoli
- CD4, CD43, CD45RA, CD56, CD123, CD303 & TCL1
- CD68+ in 50%, TdT + in one third, CD34 & CD117 –
- Abnormal karyotype in 2/3rd but not specific
- **Aggressive with median survival of 12-14 months**