Classification of acute myeloid leukemias

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Identity

Note
Basis of classification in conformity with WHO recommendations.
The classification of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) includes clinical data (previous history, age) and biologic characteristics (morphology, cytochemistry, immunophenotype, cytogenetic and molecular biology). The separation of homogeneous classes allows us to distinguish prognostic parameters and to identify groups of patients sensitive to drugs or to specific treatment. Recurrent cytogenetic abnormalities are strong prognostic indicators in AML and MDS. Molecular studies of structural chromosomal changes have enabled the cloning of genes located at chromosomal breakpoints and have helped to characterize the proteins involved in leukemogenesis. Morphologic studies remain important because of a strong correlation with cytogenetic and molecular abnormalities.
The clinico-biological classification of acute myeloid leukemia (AML) should include morphological, cytochemical, immunophenotypic, cytogenetic and molecular characterization of the leukemia blasts. The identification of homogeneous categories would allow the development and refinement of treatment strategies.
- Recurrent cytogenetic abnormalities are important as prognostic indicators in AML. The identification of specific abnormalities is used increasingly to decide treatment. Cytogenetic findings have contributed to the understanding of morphological and clinical heterogeneity of AML. Molecular genetic analysis of recurrent translocations and inversions has led to clone genes adjacent to chromosome breakpoint and to characterize their protein products involved in the leukemogenesis process.
- Over the years, leukemia classifications have been mainly descriptive, which was open to regular criticism, revision and reassessment. During the last 20 years, classification according to morphological features of leukemia has been proposed (F.A.B. defined classification). This classification is based on cell morphology on May-Grunwald-Giemsa (MGG) staining of peripheral blood and bone marrow smears with the addition of simple cytochemical techniques.

Rationale for a new classification approach
- The age-incidence of AML is subtly bimodal. Between early childhood and age 45, the annual incidence of acute myeloid leukemia (AML) remains constant at 0.8 cases/10^5 population. The incidence rises exponentially after the age of 45, exceeding 15 cases/10^5 population by age 75. AML has been extensively characterized using cytogenetic since the mid-1970s.
- Available data have suggested an alternative classification in four main groups; a first one for patients identified with specific balanced translocations, the second group for patients with "multilineage" deregulation, a third one for "secondary" AML (after exposure to mutagenic agent or chemo/radiotherapy). Although this is a more rational model of AML classification, some patients cannot be classified into the three first groups and defined a fourth group. At least for the moment, the diagnosis of this last group of patients must rely on the classical cytologic approach (FAB) defining "morphological"-based category.
The first group is characterized by recurring chromosomal abnormalities, mainly balanced reciprocal translocations and affects children and young adults. In this group, it is assumed that there is involvement of committed precursor. This may explain the cellular involvement of a specific subset of myeloid cells for example pure granulocytic cells in t(15;17) AML, granulocytic and eosinophilic cells in t(8;21)
AML, and monocytes and eosinophils in inv(16). These patients often have a high rate of complete remission with cytotoxic chemotherapy.

The second group has similar abnormalities to those which are associated with myelodysplastic syndromes, occur mainly in the elderly population and are rare in childhood. They are characterized by multilineage involvement of bone marrow cells suggesting an early commitment precursor (stem cell). Cytogenetic studies usually show complex chromosome aberrations, mainly loss of genetic material. These diseases are associated with a poor prognosis and a lesser incidence of complete remission after chemotherapy.

The third group concerns "secondary" AML (mainly after treatment for malignant diseases) usually morphologically and cytogenetically related with the second group, or more rarely with the first one, depending of the type of triggering drug used.

**Clinics and pathology**

**Disease**

**First group WHO: AML with "recurrent cytogenetic translocations"**

**Note**

Although the term "de novo" is not fully appropriate (see below "secondary AML"), this category of patients is usually referred as such in the literature since MDS or chemo/radiotherapy does not usually precede them either. The most commonly identified abnormalities are reciprocal translocations: t(8;21), inv(16) or t(16;16), t(15;17), t(11;17), t(9;11), t(6;9), t(1;22) and t(8;16). Molecular studies have shown that these structural chromosome rearrangements create a fusion gene encoding a chimeric protein. Most can be detected by RT-PCR including complex and cryptic cytogenetic variants. The altered expression and/or structure of cellular gene products leads to functional activation that may contribute to the initiation or progression of leukemogenesis.

The most frequent anomalies are: t(8;21)(q22;q22) - inv/del(16)(p13q22)/del(16)(q22)/t(16;16)(p13q22) - t(15;17)(q22;q21) - t(11;17)(q23;q21) - 11q23.

**Cytogenetics**

**t(8;21)(q22;q22)**

DEFINITION: the translocation t(8;21)(q22;q22) is one of the most common structural aberrations in acute myeloid leukemia and is found in 5-12% of AML and in one-third of karyotypically abnormal M2 cases according to the French-American-British (FAB) classification.

MORPHOLOGY AND CYTOCHEMISTRY: among the non-random chromosomal aberrations observed in AML, t(8;21)(q22;q22) is one of the best known and usually correlates with AML M2, with well defined and specific morphological features. AML M2 FAB is the morphological type predominating in correlation with t(8;21), but some AML M1 or AML M4 cases have been also reported. Rare cases with a low bone marrow blast cell count.

IMMUNOLOGICAL MARKERS: M2 AML with t(8;21) show frequent co-expression of the B lymphoid marker CD19 with CD33 and CD34 and less often CD56.

CLINICAL FEATURES: t(8;21) is usually associated with a good response to chemotherapy and a high remission rate with long-term disease-free survival. A large number of patients demonstrate additional chromosome abnormalities: loss of sex chromosome and del(9)(q22); no adverse outcome have been noted for either additional abnormality. Tumoral manifestation such as bony chloromas, may be seen at presentation; in such cases the initial bone marrow aspiration may show a limited and misleadingly low number of blast cells. These should not be confused with MDS. In these particular cases, AML M2 can still be diagnosed even if the morphological features described above are present, although the blasts are below 20% (see below).

MOLECULAR ANALYSIS: both heterodimeric components of the core binding factor complex (CBF), CBFalpha (also known as AML1) and CBFbeta are known to be involved in translocations associated with leukemia. The translocation t(8;21)(q22;q22) involves the AML1 (21q22) and ETO (8q22) genes. The AML/ETO - fusion transcript is consistently detected in patients with t(8;21) AML. Disruption of the AML1 gene is clustered within a single intron. AML1 has similarities to the drosophila segmentation gene RUNT. Some AML M2 patients with the cytological profile described above, demonstrate rearrangement of AML1 and ETO despite being cytogenetically negative for the 8;21 translocation.

**inv(del)(16)(p13q22)/del(16)(q22)/t(16;16)(p13q22)**

DEFINITION: patients with inv(16)(p13q22) usually correspond to the subclass of AML M4, with a specific abnormal eosinophil component and is considered as a distinct entity in correlation with these specific chromosomal abnormalities. These cases of AML M4 are referred as AML M4EO. MORPHOLOGY AND CYTOCHEMISTRY: in addition to the morphological features of AML M4, the bone marrow shows a variable number of eosinophils at all stages of maturation without significant maturation arrest. The most striking abnormalities involve the immature eosinophilic granules. Whils the majority of inv(16)(p13q22) have been identified as AML M4EO, this abnormality may occasionally been seen in other myeloid malignancies, including AML M2, M4 without eosinophilia, M5 and MDS.

IMMUNOPHENOTYPE: although no specific markers for the monocytic cell line have been identified, some positive markers such as CD14, CD15, CD4, CD11b and CD11c in addition to CD13 and CD33 may be a
good indication for monocytic differentiation. In M4
AML with inv(16), co-expression of CD2 with myeloid
markers have been demonstrated.

CLINICAL FEATURES: convergent studies has
revealed that patients with M4 AML with inv(16) and
t(16;16) achieved higher complete remission (CR)
rates. Conversely del(16q) is different and do not have
a better outcome than other M4 AML or MDS. It
remains to be defined whether CBFbeta is involved in
these deletions.

MOLECULAR ANALYSIS: inv(16) and t(16;16) both
result in the fusion of the CBFbeta gene at 16q22 to the
smooth muscle myosin heavy chain (MYH11) at
16p13. CBFbeta codes for Core Binding Factor
(CBFbeta) sub-unit, a heterodimeric transcription factor
known to bind the enhancers of various murine
leukemia viruses and similar motifs in the regulatory
regions of T cell (TCR), myeloperoxidase, neutrophil
elastase and several growth factor receptor gene. The
CBFbeta sub-unit is identical to AML1, one of the gene
involved in the t(8;21) translocation usually associated
with AML M2. Occasionally cytological features of
AML M4EO may be present without karyotypic
evidence of abnormality of chromosome 16. The
CBFbeta/MYH11 is usually demonstrated by molecular
studies. Thus, at diagnosis, the use of FISH and RT-
PCR methods are important when evaluating inv(16).

\( t(15;17)(q22;q21) \)
DEFINITION: \( t(15;17)(q22;q21) \) is associated
consistently with M3 AML. This chromosomal
abnormality first appeared to be confined to the
characteristic or morphologically typical M3 AML or
“hypergranular promyelocytic leukemia”, defined by
bone marrow replacement with highly granulated blast
cells, with occasional pseudo Pelger-Huet cells.
MORPHOLOGY AND CYTOCHEMISTRY. The
nuclear size and shape is irregular and highly variable;
they are often kidney-shaped or bilobed. The cytoplasm
is completely occupied by densely packed or even
coalescent granules, staining bright pink, red or purple
by MGG. In some cells the cytoplasm is filled with fine
dust-like granules. Characteristic cells contain bundle
of Auer rods ("faggot cells"). In M3 AML, MPO is
always strongly positive in all blast cells. Cases with a
similar \( t(15;17) \) but with different morphological
features, have been subsequently reported and have
been called alternatively "M3-variant" AML, or
"microgranular" variant. Distinct morphological
features such as paucity or absence of granules, and a
prominently bilobed nuclear shape characterize them.
IMMUNOLOGICAL MARKERS: M3 AML with
\( t(15;17) \) is usually characterized by the association of
the lymphoid marker, CD2 and CD19, with myeloid
markers and the negativity of HLA-DR and CD34.
CLINICAL FEATURES: M3/M3-variant AML is
frequently associated with disseminated intra-vascular
coagulation (DIC). A particular sensitivity to treatment
with all-trans retinoic acid (ATRA) has been
demonstrated. ATRA act as a differentiation therapy
for acute promyelocytic leukemia. The prognostic
value of M3 AML/t(15;17) is inferior to t(8;21) and
inv(16) and superior to the poor prognostic group
(AML with abnormalities of the chromosomes 5 and
7). AML M3 patients are however increasingly treated
in independent protocols, rendering such comparison
difficult.

MOLECULAR ANALYSIS: the sensitivity of M3 cells
to all-trans retinoic acid led to the discovery that the
retinoic acid receptor alpha (RARalpha) gene on 17q21
fuses with a zinc finger binding transcription factor on
15q22 (promyelocytic leukemia or PML) gene, thus
giving rise to a PML-RARalpha fusion gene product.
Chromosomal variant of \( t(15;17) \). Rare cases lacking
the classical \( t(15;17) \) have been described either having
complex variant translocations involving both
chromosomes 15 and 17 with additional chromosome(s),
expressing in all studied cases, the
PML/RARalpha transcript, or cases where neither
chromosome 15 nor chromosome 17 are apparently
involved, but with submicroscopic insertion of
RARalpha into PML leading to expression of the
PML/RARalpha transcript; these latter cases are
considered as cryptic or masked \( t(15;17) \).
Morphological analysis showed no major difference
between the \( t(15;17) \) positive control group and the
PML/RARalpha positive patients without \( t(15;17) \).

\( t(11;17)(q23;q21) \)
DEFINITION: several AML cases with translocation
\( t(11;17)(q23;q21) \), in which the promyelocytic
leukemia zinc finger (PLZF) gene is translocated to
RARalphagene on 17q21 have been reported. This
finding that the RARalpha gene is involved in both
t(15;17) and \( t(11;17) \) suggests the importance of the
modified RARalpha in AML.
MORPHOLOGY AND CYTOCHEMISTRY: Patients
were initially reported as having M3 morphology.
Interestingly, the \( t(11;17)(q23;q21) \) PLZF/RARalpha
subgroup showed clearly morphological differences
with predominance of cells with regular nuclei, many
granules, usually no Auer rods, increased number of
pseudo Pelger-Huet cells and a strong MPO activity.
These particular characteristics could allow the
definition of a separate morphological entity among
APL.
CLINICAL FEATURES: M3-like patients with
\( t(11;17)(q23;q21) \) are resistant to ATRA, both in vivo
and in vitro.
MOLECULAR ANALYSIS: in patients with
\( t(11;17)(q23;q21) \), where RARalpha is fused to the
PLZF (promyelocytic leukemia zinc finger) gene,
chromosome 17 and RARalpha but not PML are
involved.
11q23
DEFINITION: molecular studies have identified a human homologue of the drosophila trithorax gene (designated HRX or MLL). MLL is a developmental regulator and is structurally altered in leukemia associated translocations that show an abnormality at band 11q23.

MORPHOLOGY AND CYTOCHEMISTRY: there is a strong association between AML M5/M4 and deletion and translocations involving 11q23. Sometimes cases of 11q23 M5B and M4, and occasionally M2 or M1 also show MLL rearrangement. Two clinical subgroups of patients have a high frequency of 11q23 aberration and M5 subtypes: one is AML in infants with MLL rearrangement more frequently than is revealed by translocations with different partner chromosomes. The MLL gene on 11q23 is involved in a number of t(9;11), t(11;19). MOLECULAR ANALYSIS: the same as those occurring in "de novo" leukemia i.e. t(9;11), t(11;19). MOLECULAR ANALYSIS: the MLL gene on 11q23 is involved in a number of translocations with different partner chromosomes. The most common translocations observed in childhood AML are the t(9;11)(p21;q23) and the t(11;19)(q23;p13.1); other translocations of 11q23 involve at least 50 different partners chromosomes. A partial tandem duplication of MLL gene has also been reported in the majority of adult patients whose leukemic blast cells have a +11 and in some with normal karyotype. Molecular studies have shown that MLL is rearranged more frequently than is revealed by conventional cytogenetic studies.

Disease
Second group WHO: mAML: multilinear AML.
Note
DEFINITION: this category is defined by the presence of multilineage dysplasia on cytological analysis. In contrast to the patients with "recurrent translocation", "multilineage AML" by definition involve all myeloid cell lineages. This category of AML occurs mainly in elderly patients and is rare in children. Translocations typical of "de novo AML" in young patients are not found in "multilineage AML". Dysplasia may be analyzed according to standard criteria (presence in >50% of each cell category). Granulocytic dysplasia (DysG) may be defined as polymorphonuclear neutrophils (PMN) with agranular or with hyposegmented nuclei (pseudo Pelger-Huet anomaly). Dysplastic features of erythroblastic precursors define Erythroid dysplasia (DysE): (megablastic or macroblastic aspects, karyorexis, nuclear fragments or multinuclearity). Megakaryocytic dysplasia (DysM) may be diagnosed when micromegakaryocytes, large megakaryocytes with monolobed or with multiple separated nuclei are found. A special mention has to be made of the high frequency of dysmegakaryopoiesis and the utmost importance of clearly separating abnormal megakaryocytic cells with normal ploidy and non lobed ("monolobed") nuclei from hypoploid ("micromegakaryocytes") megakaryocytes and from megakaryocytes with multiple separated nuclei.

Cytogenetics
KARYOTYPIC/MOLECULAR ANALYSIS: in this group of patients chromosomes abnormalities include gain or loss of major segments of chromosomes: -5, -7/del(7q), +8, +9, +11, del(11q), del(12p), del(17p), -18, +19, del(20q), +21 and less often specific translocations t(2;11), t(1;7)(q10;p10) and translocations involving 3q21 and 3q26.

Disease
Third group WHO: "Secondary AML".
Note
DEFINITION: The term "secondary" AML has been utilized to encompass several different situations. A first class of secondary AML include those patients with a longstanding exposure to environmental toxins, including smoking, occupational chemicals such as benzene and related petrochemicals. The importance of detailed occupational history of all patients cannot be overstated.

The second category corresponds to patients who received prolonged administration of chemotherapy and/or radiotherapy for non-MDS/MPS malignancies (epithelial cancer, malignant lymphomas, myelomas, Hodgkin's disease). These AML occur after a latent period of a few years. They may present with myelodysplastic features evolving rapidly to AML. Until recently these were assumed to be exclusively the result of administration of alkylating agents. These AML are frequently associated with acquired chromosomal abnormalities involving 5q, -7/del(7q) and other complex rearrangements, and more rarely with translocations. The morphological presentation and cytogenetic features of these two first types of "secondary" AML (sAML) are somewhat similar to "multilineage AML" (mAML).

Another situation that has been described more recently is AML developing after the administration of agents that bind to DNA-topoisomerase II. In contrast to the loss of chromosomal material after alkylating agent exposure, balanced translocations ("de novo" type AML): 11q23, usually t(9;11), or 21q22, t(8;21) or even t(15;17) have been noted in these leukemias. This category has a morphologic presentation similar to the corresponding "de novo" AML and a much more favorable outcome with chemotherapy.

Disease
Fourth group WHO: morphological and immunophenotyping classification.
Note
DEFINITION: a morphological and immunophenotypic classification remains necessary for
the other situations which do not fit with the two preceding main categories, respectively: "recurrent translocations AML" (so-called "de novo") and "multilineage AML". Morphologically, the diagnosis of AML is based on the cytological aspect of the blast cells and the maturation of the different cell lineages in bone marrow aspirate, in addition to quantitative parameters obtained from the peripheral blood. Blood films, although essential, are not considered sufficient for diagnosis. The major criteria required for sub-classification are based on bone marrow aspirates. This explains the care required in difficult cases, in which the bone marrow aspirate is hypocellular. In these cases, as well as those with myelofibrosis, precise diagnosis needs the additional information of histological examination of a bone marrow biopsy. When the bone marrow is hypercellular or normocellular and easy to aspirate, bone marrow biopsy is usually not essential and cytological examination of smears is sufficient. With some reservations the sub-classification criteria can also be used for the material from patients with relapsing acute leukemia.

MORPHOLOGICAL CATEGORIES: the categories of this fourth group reflect the previous FAB classification with eight main types of AML (from M0 to M7 AML) and one additional category for the so-called "biphenotypic AL". AML M1 and M2 show predominantly granulocytic (neutrophil) differentiation. Very specific hypergranular cells characterize M3 AML. AML M4 and M5 both show monocytic differentiation, predominantly monocytic for M5, and mixed monocytic-granulocytic for M4. Predominantly erythroblastic and megakaryoblastic differentiation are characteristic of AML M6 and M7 AML respectively; the myeloid nature of M0 is defined only on immunological markers (myeloid and no lymphoid markers) in patients lacking morphological or cytochemical criteria for AML. Biphenotypic acute leukemias are defined for patients having both lymphoid and myeloid immunological markers.

References


This article should be referenced as such: