

# The WHO classification of acute myeloid leukaemias

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## Introduction

The World Health Organization (WHO) classification of Tumours of the Haemopoietic and lymphoid tissues was published in 2001 and resulted from an extensive collaborative work of haematopathologists and molecular biologists recognized experts in the various fields with the advice of clinical haemato-oncologists<sup>1</sup>. Special attention was made that experts from different parts of the world were represented. The classification aimed at establishing: 1) uniform criteria for the definition of haemopoietic malignancies which could be useful for diagnosis and 2) a common nomenclature which could be adopted worldwide. Such “common language” and diagnostic guidelines have facilitated over the last years the communication among clinicians and scientists allowing them to perform comparative multicenter studies and develop therapeutic approaches with the ultimate benefit to the patients. The previous WHO classification (3<sup>rd</sup> edition) provided an excellent framework to be used in the current practice and, indeed this has been the case, for most pathologists, haematologists and clinicians devoted to classify, diagnose and treat patients with the various types of haemopoietic malignancies<sup>1</sup>. In 2006, the investigators met again as it was apparent that the classification needed to be updated in the light of the major advances in the field of molecular biology and availability of novel treatments. Again various committees integrating members from different disciplines were set up in order to incorporate in the classification the relevant new information available and an updated version of the WHO classification has been published in November 2008 (4<sup>th</sup> edition)<sup>2</sup>.

Because there is no gold standard to classify a haematopoietic tumour within a particular group, major emphasis were made on the following: 1) the characterization and definition of the various disease entities requires a multiparametric analysis which should include morphology, immunophenotype and genotype and recognises that the diagnostic weight of one or another investigation varies among the different diseases and, 2) the stratification or subdivision of the neoplasms should consider not only clinicopathological and genetic features but also prognostic features to ensure that provides relevant information to the clinician for the optimal management of

the patients. In a broad sense, the WHO classification recognises three main disease categories according to the cell origin of the neoplastic cell: 1) lymphoid neoplasms (precursor and mature); 2) myeloid disorders including chronic myeloproliferative disorders, acute myeloid leukaemias and myelodysplastic syndromes (MDS) and, 3) a miscellaneous that comprises, among others, histiocytic and dendritic cell derived diseases.

I shall briefly review the basis and principles of the WHO classification in its present status with a focus on the classification of acute myeloid leukaemia (AML), highlight the changes introduced in the revised WHO classification and briefly outline some distinct disease entities that are included in sections other than AML but that may present as AML or the lineage of the neoplastic cell involved is related to the myeloid line.

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## WHO classification of AML. Diagnostic tools

Classification of the myeloid neoplasms relies on a combination of clinical and laboratory features the latter including morphology, bone marrow histology, immunophenotype and molecular genetics. Morphology allows estimation of the proportion of blasts and evaluation of the presence of dysplastic changes. Further, certain AML with recurrent chromosome abnormalities have a characteristic morphology that points towards the diagnosis of a particular AML subtype. Good examples are cases with hypergranular acute promyelocytic leukaemia (APL) and those with acute myelomonocytic leukaemia with immature eosinophils (AML-M4-Eo) which are associated respectively with the t(15;17) and inv(16) or t(16;16). The proportion of bone marrow infiltrating blasts, unlike in acute lymphoblastic leukaemia (ALL), is very relevant to the diagnosis of AML and to distinguish it from MDS. The blast count should be estimated in all cases by morphological examination of the blood and bone marrow smears and should not be derived from CD34 cell counts by flow cytometry. Megakaryoblasts should be considered in the blast differential but not erythroblasts unless we are dealing with a pure erythroid leukaemia in which there is a neoplastic proliferation of poorly differentiated blasts or proerythroblasts accounting for greater than

80% of the bone marrow cellularity with few, if any, myeloblasts. The bone marrow trephine biopsy is also contributive to this analysis and it is particularly useful in haemodilute specimens and fibrotic bone marrows from megakaryoblastic AML and acute panmyelosis with myelofibrosis; it also allows evaluation of the bone marrow stroma and the more accurate assessment of the cellularity. Problems may arise in distinguishing in the bone marrow smears monoblasts from promonocytes and the latter cells from atypical monocytes. The distinction between monoblasts and promonocytes is not critical for the diagnosis of AML as both are considered blasts; however, distinction between promonocytes and atypical/dysplastic monocytes is relevant to establish a diagnosis of AML and rule out that of chronic myelomonocytic leukaemia (CMML). Cytochemistry is nowadays rarely carried out on the routine practice as it has been largely substituted by immunophenotyping. Nevertheless, stains for myeloperoxidase (MPO) and non-specific esterases may add information to the immunophenotyping and contribute to diagnosis.

Immunophenotyping using a comprehensive panel of monoclonal antibodies that detect antigens in myeloid and lymphoid progenitor cells is a solid stone for the diagnosis of AML by determining the nature or lineage of the blasts. It is critical in distinguishing minimally differentiated AML (M0-AML) from ALL, in confirming or establishing the diagnosis of megakaryoblastic AML and in detecting mixed phenotype acute leukaemia (MPAL) and blastic plasmacytoid dendritic cell leukaemia. In addition, certain immunophenotypic patterns are associated with some recurrent chromosomal abnormalities such it is the case of APL in which the blasts are often CD34 and HLA-Dr negative and have a strong MPO expression. The detection of phenotypes that raise suspicion of the presence of a cytogenetic abnormality may help on setting or planning specific genetic investigations in these cases. Furthermore, atypical or aberrant immunophenotypic profiles are found in greater than 60% of AML and these facilitate investigation and detection of minimal residual disease (MRD) at follow-up after chemotherapy.

Molecular genetic studies of the neoplastic cells should be performed in AML when ever possible. However it has to be acknowledged that this is not always feasible due to the lack of laboratory facilities and/or failure in the sample processing. The WHO classification considers a variety of distinct disease entities on the basis of chromosome translocations/inversions and/or gene mutations. These abnormalities can be detected by standard cytogenetics, fluorescence in situ hybridization (FISH) and/or reverse-transcriptase polymerase chain reaction (RT-PCR). The use of one or another methodology will

depend on the abnormality that is being looked for. In addition, these genetic changes may have a major prognostic impact and might be of great help to the clinician to devise or establish optimal and tailored treatments. Microarray genome-wide expression profiling is still in its infancy in myeloid derived tumours and the information derived from this investigation has not been incorporated in the WHO classification. Although gene expression profiling is relevant for research and provides key information on the biology of the disease, it has not yet found its place on a diagnostic or clinical scenario<sup>3,4</sup>.

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### Revised WHO classification of AML

AML is a disease that results from the clonal expansion and accumulation of an “aberrant” myeloid progenitor cell; it may involve more than one lineage (i.e. myeloid and megakaryocytic) and it is the most common form of leukaemia in adults. It affects predominantly adults without a particularly geographical distribution but exposure to certain agents (ie, benzene) predisposes to the disease.

As in the 3<sup>rd</sup> Edition of the WHO, in the revised version, the threshold of blasts to make the diagnosis of AML is >20%; however, this does not apply to patients presenting with: 1- isolated “myeloid sarcoma”, a tumour of myeloblasts occurring in other sites than bone marrow such as skin or lymph node and that responds well to AML directed therapy<sup>5</sup>, 2-patients in whom blasts harbour the following recurrent genetic abnormalities t(8;21)(q22;q22), t(15;17)(q22;q12), and inv(16)(p13.1q22) or t(16;16)(p13.1q22) and 3-some cases of erythroid leukaemia when erythroid precursors account for greater than 50% of the cellularity.

The five major subgroups of AML are shown in Table 1. As in the previous edition, the WHO has considered the following issues: 1) whether there is a background or a previous clinical history suggesting that the disease is “secondary” or related to chemo/radiotherapy; 2) whether it arises from a silent phase of MDS the latter designated AML with myelodysplasia-related changes and 3) whether the neoplastic cells have certain recurrent genetic abnormalities. Such stratification of AML not only has given insights on the putative origin of the clonogenic cell (i.e. myeloid committed or myeloid stem cell in cases with myelodysplasia-related changes) but also has provided relevant information on the prognosis and outcome of the patients<sup>6</sup>. Globally, the three subcategories of cases with recurrent genetic abnormalities already defined in the previous WHO edition but not the new three subcategories added in the updated version have a favorable prognosis whilst those

**Table 1. Acute myeloid leukaemias (AML). WHO classification**

1. AML with recurrent genetic abnormalities
AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
AML with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); CBFβ-MYH11
APL with t(15;17)(q22;q12); PML-RARA
AML with t(9;11)(p22;q23); MLLT3-MLL
AML* with t(6;9)(p23;q34); DEK-NUP214
AML* with inv(3)(q21;q26) or t(3;3)(q21;q26.2); RPN1-EVI1
AML* (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1
AML with gene mutations: Provisional entity: AML with mutated NPM1 Provisional entity: AML with mutated CEBPA
2. AML with myelodysplasia-related changes
3. Therapy - related myeloid neoplasms
4. AML, not otherwise specified (NOS)
AML with minimal differentiation (M0)
AML without maturation (M1)
AML with maturation (M2)
AML (myelomonocytic) (M4)
AML (monoblastic/ monocytic) (M5)
AML (erythroid): Pure erythroid
Erythroid/myeloid (M6)
AML (megakaryoblastic) (M7)
AML (basophilic)
Acute panmyelosis with myelofibrosis
5. Myeloid sarcoma
6. Myeloid proliferations related to Down syndrome:
Transient abnormal myelopoiesis
AML associated with Down syndrome
7. Blastic plasmacytoid dendritic cell neoplasms

\* New entities incorporated

with myelodysplasia-related changes and secondary to therapy fare worst. Although rearrangement of genes encoding for transcription factors such as the retinoic acid receptor alpha (*RARA*), *CBFB* or *RUNX1* are considered to be early events in leukaemogenesis by impairing cell differentiation, second hits in these cells are required to maintain and promote proliferation, survival and expansion of the neoplastic clone. These second hits may be multiple and comprise deregulation of a variety of genes such as mutations or internal tandem duplications of the *fms*-related tyrosine kinase 3 (*FLT-3*) gene, mutations of nucleophosmin (*NPM1*), *c-KIT*, *CEBPA* (CCAAT/enhancer binding protein alpha gene), Wilms' tumour 1 (*WT1*) and/or *N* and *K-RAS*. The two-hit model will explain the additional abnormalities such as *c-KIT* mutations seen in cases with t(8;21) or inv(16). Investigation of the presence of these secondary events is particularly

important in patients in whom a primary abnormality (normal cytogenetics) is not detected such it is the case of the category of AML Not Otherwise Specified (AML NOS) which represents a substantial proportion of cases, around 30%. The presence of these abnormalities may also help to stratify these patients in several prognostic groups as patients with *c-KIT* and *WT1* mutations and/or internal tandem duplication of *FLT-3* have an unfavorable outcome<sup>7-10</sup>. Incorporation of these genetic changes in the updated WHO classification has been a challenge as by themselves do not define a distinct "clinico-pathologic-genetic" entity since they are present in a range of AML subgroups with or without other abnormalities; in addition, they are not mutually exclusive. Despite of this, some of them have been considered and included in the classification as provisional entities (i.e. AML with mutated *NPM1* or with nucleophosmin cytoplasmic expression and cases with mutated *CEBPA*). Further studies are needed to establish whether these provisional groups constitute separate disease entities. Both these abnormalities of *NPM1* and *CEBPA* are relatively frequent in adult AML but *NPM1* mutations appear to be less common in children<sup>11</sup>. In cases with a normal karyotype and absence of *FLT-3* internal tandem duplications, appear to be associated with a favorable prognosis. Although internal tandem duplications of *FLT-3* have not been considered for disease stratification, its prognostic significance should not be disregarded particularly in cases of AML NOS. In summary, the current classification has incorporated some but not all new information concerning genetic abnormalities and, according to this, few changes have been introduced as outlined below.

### Modifications introduced in the new WHO classification

1. AML with recurrent genetic abnormalities includes now three new genetic defined entities (Table 1) and two provisional entities: cases with mutations of *NPM1* and cases with mutations of *CEBPA*. It is worth to note that whilst in the previous WHO classification, the prognosis was favorable in these patients, this is not the case in the updated version as the new three added definitive entities have an unfavorable prognosis. In addition, cases with acute promyelocytic leukaemia (APL) with variant translocations of *RARA* (17p12) involving partner chromosomes different than 15 such as 11q23, 11q13, 5q35 and 17q11.2, are considered separately. The rationale

behind this is that some of these variant cases do not have typical APL features and may be resistant to all-trans retinoic acid (ATRA). Therefore, these cases should be diagnosed as AML with a variant RARA translocation. AML with 11q23 (*MLL*) abnormalities has been more specifically renamed as AML with t(9;11) (p22;q23)/*MLLT3/MLL* and cases with other *MLL* abnormalities such as a tandem duplication of this gene are not included in this category.

**2.** AML with multilineage dysplasia is renamed AML with myelodysplasia-related changes and this group includes cases with: a) AML arising from a previous MDS or MDS/myeloproliferative disorder; b) AML with specific dysplasia related chromosome abnormalities namely unbalanced abnormalities of chromosomes 7 and 5 but also balanced translocations which are not included in the group of AML with recurrent genetic changes and c) AML with greater than 50% of cells from two myeloid lineages being dysplastic, designated AML with multilineage dysplasia. This category also includes some of the cases previously classified as AML NOS erythroid/myeloid leukaemia (see later). Validation of such criteria to classify these three diseases under the umbrella of AML with myelodysplasia-related changes has been recently documented in a series of a 100 consecutive AML patients<sup>12</sup>.

**3.** Therapy related myeloid neoplasms are not further subclassified in alkylating/radiation and type II topoisomerase related. This group of myeloid neoplasms includes both therapy related AML and therapy related MDS occurring after exposure to chemotherapy and/or radiotherapy. The two subcategories are regarded as a unique clinical disease independently of the proportion of blasts. The rationale for not subdividing into two subgroups according to the type of agent responsible for the development of AML or MDS is that in practice most patients receive combination chemotherapy that include both types of drugs. Still, it is worth to consider as to whether they are related to one or another type since these two subgroups have characteristic clinical, morphological and genetic features.

**4.** AML NOS. This group comprises cases that do not fulfill the criteria to be included in the previous three categories. Subclassification into the various AML NOS subgroups is based on the major lineage involved (i.e. megakaryoblastic or monocytic) and degree of differentiation of the blasts and this can be achieved by morphology, cytochemistry and immunophenotyping. Overall, these subgroups overlap with the previous French-American-British (FAB) classification of AML. Cases of M7-AML with t(1;22)(p13;q23) and AML with inv(3)(q21;q26.2) or t(3;3)(q21;q26.2) are now considered in the group of AMLs with recurrent chromosome abnormalities and

some cases assigned before as AML NOS (erythroid/myeloid leukaemia) are reclassified in AML with myelodysplasia-related changes.

**5.** Myeloid proliferations related to Down syndrome include AML and transient abnormal myelopoiesis (TAM) or transient myeloproliferative disorder; in the latter patients, the disease evolves into AML in around 20-30% of cases. AML in infants and children with Down syndrome are often megakaryoblastic (50% of cases) and their frequency is similar to ALL; this incidence is sharply different than what is seen in the other children in whom ALL is significantly more frequent than AML (ALL/AML: 4/1). In both AML and TAM, in addition to trisomy 21, somatic mutations of the gene encoding the transcription factor *GATA* are frequently present and pathognomonic. The distinct clinical and laboratory features of these diseases in Down syndrome are the rationale to consider as a separate entity from other forms of AML in children.

**6.** Blastic plasmacytoid dendritic cell leukaemia was previously included in the WHO 3<sup>rd</sup> Edition under blastic natural killer cell lymphoma /leukaemia but in the revised version is considered as a separate AML category. This is an aggressive disease thought to derive from plasmacytoid dendritic cells and presents with a high frequency with extramedullary involvement, particularly of the skin. Some cases are associated or develop acute myeloid or monocytic leukaemia. The immunophenotype is characteristic with expression of CD4, CD56, CD123 and TCL-1; in around half of the cases the blasts are CD68<sup>+</sup><sup>13</sup>. Despite of these characteristic phenotypic features, some cases may be problematic and difficult to diagnose as not all the typical dendritic cell markers are expressed whilst cells may be positive for some B and T lymphoid not strictly specific lymphoid markers.

In addition to these AML groups there are cases that may present as AML or ALL and they have been included in other sections. This is the case of mixed phenotype acute leukaemia (MPAL) and that of cases with myeloid and lymphoid neoplasms associated to eosinophilia and harboring abnormalities of the platelet derived growth factor alpha and beta (*PDGFA/PDGFB*) or *FGFR1* mutations. The blasts in MPAL cases do not show a clear evidence of differentiation along a single cell lineage and most likely represent leukaemias of early haemopoietic stem-cells with myeloid and lymphoid features. Criteria for the definition of MPAL has been refined and, in particular, it has been considered as major diagnostic criteria: 1) the expression of highly specific markers for the myeloid (i.e., MPO) and lymphoid (i.e. CD3) lineages regardless of the expression of other less specific markers and 2) exclusion of AML cases with recurrent genetic abnormalities. Myeloid and lym-

phoid neoplasms with eosinophilia and abnormalities of *PDGFA/PDGFB* or *FGFR1* are thought to derive from a pluripotent lymphoid/myeloid progenitor cell and may present as T-lymphoblastic lymphoma, AML, including myeloid sarcoma in the cases with *FGFR1* mutations or the patients develop AML following a phase of chronic eosinophilic leukaemia<sup>14</sup>. Patients with AML will have infiltration by blasts co-existing with eosinophilia (often mature abnormal eosinophils).

## Conclusions

The WHO classification of AML in its updated version (4<sup>th</sup> edition) has provided a useful framework in a clinical and laboratory settings for haemato-pathologists and clinicians devoted to diagnose and treat patients with this haemopoietic tumour. As in the previous edition, major emphasis has been made on the value and need for a multiapproach and comprehensive analysis that includes a range of different investigations to diagnose and subclassify this leukaemia into distinct groups. However, it is also apparent that facilities and resources to carry out all the investigations are not available worldwide. The modifications introduced in this 4<sup>th</sup> edition largely derive from the new information available on gene deregulation. It is recognized that, some AML categories defined by genetic abnormalities and introduced in the updated WHO classification may not represent a “clinical-pathological-genetic” disease entity but have been considered as a provisional category because patients have a different prognosis and outcome.

What is the future of the WHO classification? To this end, it is essential to consider that a classification should not be static but dynamic and therefore subject to further changes and modifications. The incorporation of new findings may well help to define more precisely some disease entities which at present lack a genetic signature and refine the definition of the already well established conditions. In future, if data on gene profiling expression matures and its assessment becomes more simple and accessible, it is possible that a new updated classification may come into light.

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