# Updates in the Disease Classification of Acute Leukemia

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**Abstract**

The upcoming 5th edition of the World Health Organization Classification of Hematolymphoid Tumors adopts hierarchical reorganization of entities based on data generated from improved diagnostic techniques including Next-Generation Sequencing (NGS) and gene expression. This review explores the update in disease classification of acute leukemia and highlights the changes made in the current edition. The changes include modification in the nomenclature of some entities, addition as well as deletion of some entities, and revision in diagnostic criteria.

**Keywords:** Acute myeloid leukemia, Acute lymphoid leukemia, Classification, Next-Generation Sequencing

## Introduction

Acute leukemia is a malignant disorder of the hematopoietic progenitor cells characterized by maturation arrest in differentiation and aberrant proliferation of blasts. It is of two types, viz, acute myeloid leukemia (AML) involving the myeloid series and acute lymphoblastic leukemia (ALL) involving the lymphoid series. It accounts for 3.1% of all cancer-related deaths, with an incidence of 2.5% of all cancers diagnosed yearly.[1]

For decades, the mainstay of treatment for leukemia has been traditional chemotherapy and radiotherapy. However, with the advent of next-generation sequencing (NGS), several new molecular targets have been identified for classification and prognostication of these, with a paradigm shift to targeted therapies as a mainstay of treatment.

## Updates in the Disease Classification of Acute Myeloid Leukemia[2]

As per the World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th revised edition (WHO-HAEM4R, 2017) , AML was classified into six subcategories: (*i*) AML with recurrent genetic abnormalities, (*ii*) AML with myelodysplasia-related changes, (*iii*) Therapy-related myeloid neoplasms, (*iv*) AML-NOS (not otherwise specified), (*v*) Myeloid sarcoma, (*vi*) Myeloid proliferations associated with Down syndrome; with a separate chapter on myeloid neoplasms with germline predisposition.

In WHO 5th edition (WHO-HAEM5, 2022), AML classification focuses on forming a structural framework that groups AML based on known genetic information and provides scope for new emerging entities.

### Change in nomenclature

1. The significant change is the restructuring of the entire classification, that now, AML is subcategorized into only three, i.e., (*i*) AML with defining genetic abnormalities, (*ii*) AML defined by differentiation, and (*iii*) Myeloid sarcoma. In addition, a separate chapter on secondary myeloid neoplasm is added that includes three sections, i.e., (*i*) Myeloid neoplasm-post cytotoxic therapy, (*ii*) myeloid neoplasm associated with germline predisposition, and (*iii*) myeloid proliferations associated with Down syndrome. This was done to separate a new category that arises in the setting of known predisposition factors, either exposure to cytotoxic therapy or a germline predisposition.
2. AML with recurrent genetic abnormalities is now renamed to **AML with defining genetic abnormalities.** Under this category, entities are now recognized by their fusion partners instead of characteristic cytogenetic abnormality because it was found that identi-fication of fusion partner impacts prognosis and is required for

disease monitoring (**Table 1**).

3. AML-NOS term has been eliminated and replaced by **AML defined by differentiation** to avoid confusing terminology. It encompasses AMLs that currently do not have defining genetic abnormalities and are classified according to the myeloid differentiation seen on blood or bone marrow examination. However, discovering new molecular targets is anticipated to eliminate this category.

4. Within the category of AML defined by differentiation, WHO-HAEM5 renames pure erythroid leukemia to **Acute Erythroid Leukemia (AEL)**. It is an aggressive neoplasm characterized by neoplastic proliferation of erythroid constituting more than 80% of bone marrow elements with increased (>30%) immature erythroid cells (erythroblasts and pronormoblasts) along with the presence of biallelic TP53 mutation. TP53 mutation analysis and cytogenetic studies are important to rule out reactive conditions of erythroid hyperplasia which shows no TP53 mutation and a normal karyotype.[3,4]

5. **AML**, **myelodysplasia-related (AML-MR)** replaces AML with myelodysplasia-related changes. This entity is now included under the heading of AML with defining genetic abnormalities because a history of myelodysplastic syndrome (MDS) or Myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN) or detection of one or more characteristics in molecular and cytogenetic abnormalities is an essential criterion to diagnose AML-MR.[5,6]

6. Therapy-related myeloid neoplasm is included in a separate chapter under the heading of myeloid neoplasm-post cytotoxic therapy. In addition, there is a refining of the definition of this category. The essential criteria for diagnosing this disease entity requires a myeloid neoplasm to meet the diagnostic criteria of any myelodysplastic neoplasms, myelodysplastic/myelo-proliferative neoplasm, or AML with a history of prior exposure to cytotoxic therapy &/or large-field radia-tion therapy for an unrelated disorder.[7,8]

**Addition of new categories**

1. A new AML with characteristic rearrangement with NUP98 is recognized under AML with defined genetic abnormalities along with AML involving rearrange-ments of KMT2A and MECOM as several fusion partners have been discovered, like KMT2A with MLL10, MLLT3, ELL, and AFDN.[9-11] These fusion partners are essential to be recognized by molecular studies because they impact prognosis during disease progression.[12-15]

2. A new section on AML with other defined genetic alterations has been included under AML with defining genetic abnormalities to provide a place for new emerging AML subtypes that might later become defined. Under this category, WHO-HAEM5 includes AML with rare fusions, i.e., RUNX1T3::GLIS2, KAT6A::CREBBP, FUS::ERG, MNX1::ETV6, and NPM1::MLF1.[16-19]

3. There is an emphasis on eliminating ≥20% blast criteria wherever possible, with integrating clinicopathologic evaluation to direct therapeutic decisions. In addition, relaxation of the 20% cut-off will pave a pathway for quantitating clones carrying the genetic alteration estimated by variant allele fusion transcription to

**Table 1. Updates in WHO Classification of AML with defining genetic abnormalities (2022)**

| *WHO-HAEM4R, 2017* | *WHO-HAEM5, 2022* |
|---|---|
| AML with recurrent genetic abnormalities | AML with defining genetic abnormalities |
| AML with t(15;17)(q22:q11-12) | Acute promyelocytic leukemia with PML::RARA fusion |
| AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11 | AML with CBFB::MYH11 fusion |
| AML with t(8;21)(q22;q22.1); RUNX1-RUNX1T1 | AML with RUNX1::RUNX1T1 fusion |
| AML with t(6;9)(p23;q34.1); DEK-NUP214 | AML with DEK::NUP214 fusion |
| AML with t(1;22)(p13.3;q13.1); RBM1-MKL1 | AML with RBM15::MRTFA fusion |
| AML with BCR-ABL1 | AML with BCR::ABL1 fusion |
| AML with t(9;11)(p21.3:q23.3); KMT2A-MLLT3 | AML with KMT2A rearrangement |
| AML with inv(3)(q21.3q26.2) or t(3:3) (q21.3;q26.2) | AML with MECOM rearrangement |
| (New entity) | AML with NUP98 rearrangement |
| AML with mutated NPM1 | AML with NPM1 mutation |
| AML with biallelic mutation of CEBPA | AML with CEBPA mutation |
| AML with mutated RUNX1 | (Removed) |

establish lower limit criteria for diagnosis of AML. So, whenever there is high clinical suspicion along with presence of blast in blood /bone marrow, even if less than 20%, should be followed by molecular studies for defined genetic abnormalities for diagnostic categorisation.

*Only AML with BCR:* ABL1 and AML with CEBPA mutation have retained the blast percentage criteria of 20% for diagnosis to avoid overlap with chronic myeloid leukemia with the former, while there is a lack of adequate data for change in cut-off criteria in the latter.

4. PARP-1 inhibitors are now recognized drugs associated with increased risk for the development of AML, while methotrexate has been removed under myeloid neoplasm post-cytotoxic therapy.[20,21]

**Laboratory Perspective**

The clinically suspected patient should be evaluated with detailed clinical history including drug history, and family history (including history for germline disorders, down syndrome, etc). A peripheral smear examination along with bone marrow examination is required for quantification & morphological assessment of blasts. All cases irrespective of blast percentage should undergo molecular studies for identification of defining genetic abnormalities, except for AML with BCR-ABL and AML with CEBPA mutation, for diagnostic categorization. Conventional cytochemistry holds little importance now, except for those cases which do not show any defining genetic abnormalities & requires classification based on differentiation. Immunophenotyping is essential as part of baseline investigation for prognostication, evaluation of minimal residual disease & disease monitoring.

**Clinical Implications**

Any change in the disease nomenclature is likely to have therapeutic implications. First and foremost, the importance of cytogenetics has decreased and been replaced by gene fusion detection. Nowadays, customized **next generation sequencing** (NGS) panels are available that screen for almost all common mutations as well as for the above gene fusions. Plus, these fusion genes are likely to be available for PCR based minimal residual disease (MRD) monitoring in future.

Simultaneous prognostic classification of these genetic alterations guides for appropriate risk stratification and consolidation therapy with chemotherapy or transplant. Secondly, with 20% blast criteria being diluted for diagnosis of AML, stress is more on disease biology, aggressive behaviour and detection of genetic alterations, paving way for directed therapies for AML in these patients, earlier without any need for MDS like treatment. Next, stress on AML with germline predisposition, as a separate category emphasizes the need for appropriate genetic counselling in these patients, as well as considerations while choosing family members as stem cell donors. Removal of methotrexate as a potential cause of secondary myeloid neoplasm decreases the fear of usage of this common medication from the minds of hematologists, oncologists, rheumatologists and internists.

**Updates in Disease Classification of Acute Lymphoid Leukemia (ALL)**[22]

B-/T-cell ALL was discussed under one roof of precursor lymphoid neoplasm in WHO 4$^{th}$ revised edition. However, the WHO 5$^{th}$ revised classification discusses B and T precursor lymphoid neoplasm separately.

**Change in Nomenclature**

1. With major restructuring, B-lymphoblastic leukemia/lymphoma is now discussed under B-cell lymphoid proliferations and lymphomas, while T-lymphoblastic leukemia/lymphoma is discussed under T-cell lymphoid proliferations and lymphomas.
2. Like AML, precursor B-cell ALL is classified according to molecular alteration over cytogenetic abnormalities for the feasibility of their detection by different techniques (**Table 2**).
3. B-ALL NOS (not otherwise specified) is only used for cases that cannot be classified despite comprehensive testing, while those diagnosed based on morphology and immunophenotype alone are to be reported as **B-ALL NFC (not further classified)**.
4. The term NOS has been removed from T-lymphoblastic leukemia/lymphoma, while Early T-cell precursor lymphoblastic leukemia has been renamed to Early T-precursor lymphoblastic leukemia/lymphoma, Further classification into relevant genetically defined categories has not yet been achieved till date.
5. NK-lymphoblastic leukemia/lymphoma is not a recognized entity now and has been removed from disease classification, because there is a lack of litera-ture on reliable diagnostic criteria and overlap with other disease entities such as CD56+ AML, CD56+ acute undifferentiated leukemia, CD56+ T-ALL and blastic plasmacytoid dendritic cell neoplasm.

**Addition of New Entities**

1. Two new entities have been identified based on molecular signature: B-lymphoblastic leukemia/lymphoma with ETV6::RUNX1-like features and B-lymphoblastic leukemia/lymphoma with TCF::HLF fusion.[23,24]
2. Addition of a new category of B-lymphoblastic leukemia/

**Table 2. Updates in WHO Classification of B-cell lymphoid proliferations (2022)**

| *WHO-HAEM4R, 2017* | *WHO-HAEM5, 2022* |
|---|---|
| B-lymphoblastic leukemia/lymphoma with hyper diploidy | B-lymphoblastic leukemia/lymphoma with high hyper diploidy |
| B-lymphoblastic leukemia/lymphoma with t(9;22)(q34:q11.2); BCR-ABL1 | B-lymphoblastic leukemia/lymphoma with BCR::ABL1 fusion |
| B-lymphoblastic leukemia/lymphoma, BCR-ABL1-like | B-lymphoblastic leukemia/lymphoma with BCR::ABL-like features |
| B-lymphoblastic leukemia/lymphoma with t(v;11q23.3); KMT2A rearranged | B-lymphoblastic leukemia/lymphoma with KMT2A rearrangements |
| B-lymphoblastic leukemia/lymphoma with t(12;21) (q13.2;q22.1); ETV6-RUNX1 | B-lymphoblastic leukemia/lymphoma with ETV6::RUNX1-like features |
| B-lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3); TCF3-PBX1 | B-lymphoblastic leukemia/lymphoma with TCF3::PBX1 fusion |
| B-lymphoblastic leukemia/lymphoma with t(5;14)(q31.1;q32.1); IGH/IL3 | B-lymphoblastic leukemia/lymphoma with IGH::1l3 fusion |
| (same) | B-lymphoblastic leukemia/lymphoma with hypodiploidy |
| (same) | B-lymphoblastic leukemia/lymphoma with iAMP21 |
| (same) | B-lymphoblastic leukemia/lymphoma with other defined genetic abnormalities |
| (New entity) | B-lymphoblastic leukemia/lymphoma with ETV6::RUNX1-like features |
| (New entity) | B-lymphoblastic leukemia/lymphoma with TCF::HLF fusion |

lymphoma with other defined genetic abnormalities to include emerging entities like B-ALL with DUX4, MEF2D, ZNF384 or NUTM1 rearrange-ments.[24–26]

**Laboratory Perspective**

Like AML, a detailed clinical history & examination should be followed by peripheral smear examination & bone marrow examination for quantification & morphological assessment of blasts. Thereafter, molecular studies are to be done for the identification of known genetic abnor-malities. ALL-NOS should be labelled after comprehensive molecular testing. Immunophenotyping has a role in disease monitoring for risk assessment & minimal residual disease status.

**Clinical Implications**

The new nomenclature has certain therapeutic impli-cations for ALL as well. Like AML, importance of cyto-genetics has decreased to detecting hyper or hypo-diploidy. Here, hyperdiploidy (>48 chromosomes) has been replaced with high hyperdiploidy (51-64 chromo-somes), as had been prognostically recognized for several years. Here also, next generation sequencing panels are available that screen for almost all common mutations as well as for the above gene fusions in ALL patients and are likely to play more important role as diagnostic specimen plus PCR based MRD monitoring in future. Addition of new entities further strengthens the existing literature available for these.

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