Classification of Acute Leukemias – Past, Present, and Future

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The perspective of the classification of any disease is to treat them according to their biologic behavior. Standard criteria to distinguish between myeloid and lymphoid acute leukemias were laid down as the first of its kind, by the French-American-British (FAB) working group. The FAB classification had a cursory correlation with clinical outcome, poor concordance owing to inter-observer variation, and failure to incorporate cytogenetic data. Hence, the World Health Organization (WHO) classification of leukemias evolved in 1997 with the goal of improving the objectivity and reproducibility, which had incorporated cytogenetic abnormalities and immunology as principal designating criteria, other than the morphology. Major changes were made in the subsequent editions of WHO classification, incorporating newer genetic abnormalities such as mutations of nucleophosmin member 1, CCAAT/enhancer-binding protein alpha; renaming of the existing classes, etc. The role of the genes encoding guanine nucleotide-binding protein gamma 11, amphiregulin, and ceruloplasmin; the biomarkers platelet factor 4 and connective tissue-activating peptide III, complement fragment C3a; the mRNA coding for plexin C1, leukotriene B4 receptor 1, and Immunoglobulin superfamily member 2; mixed lineage leukemia gene rearrangement in the prognosis of leukemias is proven. Thus, the approach of diagnostics using cytogenetics and immunophenotyping may further be modified.

Keywords: Acute myeloid leukemia, Classification, Lymphoid leukemia

INTRODUCTION

The perspective of the classification of any disease is to treat them according to their biologic behavior. As acute leukemias are a heterogeneous group of neoplasms with differences in clinical course, prognosis and treatment between the groups, with the invent and application of target-based approach to therapy, their classification needs to be precise, facilitating non-overlapping identification of the differing entities, incorporating all the essential and new information.

EMERGENCE OF FRENCH-AMERICAN-BRITISH (FAB) CLASSIFICATION

The attempt to classify leukemias was initiated by Nikolaus Friedreich in 1857 who categorized leukemias as acute and chronic. In 1868, Neumann used the term "myelogenous" to imply that leukemias arise from the bone marrow.[1]

Though the morphological approach to classify acute leukemias has always been in progress, standard criteria to distinguish between myeloid and lymphoid acute leukemias and to subtype them further, based on morphology and cytochemistry were laid down as the first of its kind, in 1976, by the FAB working group.[2,3] Subsequently, with the recognition of new morphological subsets, the original FAB classification was modified further viz. addition of acute myeloid leukemia – minimally differentiated disease (AML-M0) with expression of myeloid antigen, acute megakaryoblastic leukemia (AML-M7).[4-6] Acute lymphoblastic leukemia (ALL) had been classified into L1, L2, and L3 (Table 1).[7] In the FAB system, the cut off blast percentage for making a diagnosis of acute leukemia was 30%.[8]

PITFALLS OF FAB CLASSIFICATION AND INTRODUCTION OF WORLD HEALTH ORGANIZATION (WHO) CLASSIFICATION

But still, the FAB classification had such major disadvantages as cursory correlation with clinical outcome, poor concordance owing to inter-observer variation, and failure to incorporate cytogenetic data. Furthermore, many cytogenetic abnormalities were identified in the subtypes of leukemias in the latter half of twentieth century.[9] Genetic abnormalities are present in more than 80% of ALLs and

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more than 90% of AMLs and most of them are recurrent.[10] The lymphoblasts of B- and T-ALL may be morphologically indistinguishable.[4] The heterogeneity of acute leukemias is not determined just by their biology and clinical course, but also because of the fact that, patients belonging to the same group show marked variation in their response to therapy e.g. patients with acute promyelocytic leukemia with t(11;17)(q23;q21) are resistant to treatment with pharmacologic doses of all trans-retinoic acid (ATRA), whereas patients with t(15;17)(q22;q21), t(5;17)(q35;q21), and t(11;17)(q13;q21) are responsive to ATRA. By and large, the WHO classification of leukemias evolved in 1997 with the goal of improving the objectivity and reproducibility.[11] It was framed by the European Association for Hematopathology and the society for Hematopathology.[9] Indeed, it was a trendsetter in the approach to classification of hematopoietic neoplasms.

Thus, the WHO classification had incorporated cytogenetic abnormalities and immunology as principal designating criteria, despite retaining morphology as the mainstay of the diagnosis.[9,12] Immunophenotype and genetic features have now become an essential integral part of the definition of hematopoietic neoplasms; with these, making a consensus diagnosis is easier, than that with morphology alone.[2,12] The recognition of genetic abnormalities and immunophenotypic features not just furnish defining criteria for the disease entities but also facilitate targeting the therapy towards specific antigens, genes or pathways.[2,9,13]

In the WHO system, the cut off blast percentage for making a diagnosis of acute leukemia was lowered to 20%. The AML classification includes five groups, the fourth group being a modification of the FAB AML classification. The acute promyelocytic leukemia is no longer classified in terms of morphology, but has been placed in the category of AML with recurrent genetic abnormalities. The introduction of genetic abnormalities as defining criteria in the classification system has changed the requisite blast percentage for a diagnosis of

AML, so that it can even be less than 20%, provided there is an associated t(8;21)(q22;q22) or inv(16)(p13q22) or t(16;16)(p13;q22) or t(15;17)(q22;q12). The WHO classification divides ALL into 3 categories: Precursor B-cell, mature B-cell (Burkitt Leukemia), and precursor T-cell (Table 2).[9]

In 1995, the European Group for Immunological Characterizing of Acute Leukemia (EGIL) formulated guidelines for classification of acute leukemia with biphenotypic marker expression.[14] These criteria had been incorporated in the WHO 2001 guidelines for classifying acute leukemia of ambiguous lineage.[15]

The first three categories in the WHO AML classification are based on the pathogenesis of disease. The fourth category is based on morphology.[9] Thus, the individual categories are not in accordance with each other.

MAJOR DIFFERENCES BETWEEN 2001 AND 2008 WHO CLASSIFICATIONS

The genetic abnormality t(8;21)(q22;q22) mentioned in WHO classification 2001 as AML 1/Eight twenty-one has

Table 1: FAB classification of acute leukemias

Myeloid

  M0: Minimally differentiated leukemia

  M1: Myeloblastic leukemia without maturation

  M2: Myeloblastic leukemia with maturation

  M3: Promyelocytic leukemia

  M4: Myelomonocytic leukemia

  M5: Monocytic leukemia

  M6: Erythroleukemia

  M7: Megakaryoblastic leukemia

Lymphoid

  L1: Small, homogenous cells with inconspicuous/1-2 nucleoli

  L2: Large cells with variable size with 1-2 nucleoli

  L3: Large cells, homogenous, finely stippled chromatin with basophilic vacuolated cytoplasm

FAB: French-American-British

Table 2: WHO classification of acute leukemias 2001

Myeloid

  AML with recurrent cytogenetic abnormalities

    AML with t (8;21) (q22;q22), (AML 1/ETO)

    AML with inv (16) (p13q22) or t (16;16) (p13;q22), (CBFβ/MYH11)

  Acute promyelocytic leukemia with t (15;17) (q22;q12), (PML/RARα) and variants

    AML with 11q23 (MLL) abnormalities

  AML with multilineage dysplasia

    With prior myelodysplastic syndrome

    Without prior myelodysplastic syndrome

  AML and myelodysplastic syndrome, therapy related

    Alkylating agent-related

    Topoisomerase II inhibitor-related

  AML not otherwise categorized

    AML, minimally differentiated

    AML without maturation

    AML with maturation

    Acute myelomonocytic leukemia

    Acute monoblastic and monocytic leukemia

    Acute erythroid leukemia

    Acute megakaryoblastic leukemia

    Acute basophilic leukemia

    Acute panmyelosis with myelofibrosis

    Myeloid sarcoma

  Acute leukemia of ambiguous lineage

    Undifferentiated acute leukemia

    Bilineal acute leukemia

    Biphenotypic acute leukemia

Lymphoid

  Precursor B-cell neoplasm

    Precursor B-lymphoblastic leukemia

  Mature B-cell neoplasm

    Burkitt leukemia

  Precursor T-cell neoplasm

    Precursor T-lymphoblastic leukemia

WHO: World Health Organization, AML: Acute myeloid leukemia, AML1/ETO: Acute myeloid leukemia 1/Eight twenty-one, CBFβ: Core-binding factor, subunit beta, RARα: Retinoic acid receptor α, MLL: Mixed lineage leukemia

been renamed in WHO classification 2008 as Runt-related transcription factor 1; translocated to, 1 (cyclin D-related) (RUNX1-RUNX1T1).[9,16] The genes RUNX1, core-binding factor, subunit beta or retinoic acid receptor α encode transcription factors; rearrangements of these genes affect the differentiation of myeloid cells. Nevertheless, studies have shown that not only rearrangements of these genes but also a second genetic abnormality viz. mutations of genes such as fms-like tyrosine kinase 3 (FLT3) or KIT (v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog) which encode proteins activating signal transduction pathways is necessary to promote proliferation/survival of the neoplastic clone.[16,17]

Furthermore, genetic mutations have been identified in the so-called "cytogenetically normal" AML in the recent past. These include mutations of enhancer-binding protein alpha (CEBPA) (encoding the CCAAT/enhancer binding protein-α), nucleophosmin member 1 (NPM1), FLT3, neuroblastoma RAS viral (v-ras) oncogene homolog/Kirsten rat sarcoma viral oncogene homolog and meningioma 1 gene (MN1). These mutations have been found to be significant prognostic factors and are likely targets of new approach therapy.[16-19] Gene over- and under-expression, loss of heterozygosity, and copy number variants are increasingly gaining importance in having an influence over the diagnosis and prognosis of leukemias and these are detected by array-based approaches. Consequently, in the WHO classification 2008 (Table 3), six new additions have taken part in the group of AML with recurrent genetic abnormalities.[16,17]

Myeloid sarcoma is now considered as a distinct entity and has been separated from the category of AML-not otherwise specified (NOS). Myeloid proliferations related to Down syndrome and Blastic plasmacytoid dendritic cell neoplasm, have been added newly. Acute leukemias of ambiguous lineage have further been subtyped with the inclusion of natural killer cell lymphoblastic leukemia/lymphoma, which was earlier grouped under precursor lymphoid neoplasms. B lymphoblastic leukemia/lymphoma also has been subtyped in this updated classification.[9,16]

Acute megakaryoblastic leukemia, previously included in the category of AML-NOS should be categorized according to the specific genetic abnormality if they are associated with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); ribophorin 1 gene-ecotropic virus integration 1 gene or with t(1;22) (p13;q13); RNA binding motif protein 15-megakaryoblastic leukemia 1.[16]

The subgroup termed in 2001 classification as "AML with multilineage dysplasia" has been renamed as "AML with myelodysplasia-related changes". This category includes

patients having previously documented myelodysplastic syndrome, those having specific cytogenetic abnormalities related to myelodysplasia and patients who have a normal karyotype but exhibiting morphological

Table 3: WHO classification of acute leukemias 2008

Myeloid
  AML with recurrent cytogenetic abnormalities
    AML with t (8;21) (q22;q22); RUNX1-RUNX1T1
    AML with inv (16) (p13.1q22) or t (16;16) (p13.1;q22); CBFβ–MYH11
    Acute promyelocytic leukemia with t (15;17)(q22;q12); PML-RARα
    AML with t (9;11) (p22;q23); MLLT3-MLL
    AML with t (6;9) (p23;q34); DEK-NUP214
    AML with inv (3) (q21q26.2) or t (3;3) (q21;q26.2); RPN1-EVI1
    AML (megakaryoblastic) with t (1;22) (p13;q13); RBM15-MKL1
    AML with mutated NPM1*
    AML with mutated CEBPA*
  AML with myelodysplasia-related changes
  Therapy-related myeloid neoplasms
  AML NOS
    AML with minimal differentiation
    AML without maturation
    AML with maturation
    Acute myelomonocytic leukemia
    Acute monoblastic and monocytic leukemia
    Acute erythroid leukemia
    Acute megakaryoblastic leukemia
    Acute basophilic leukemia
    Acute panmyelosis with myelofibrosis
  Myeloid sarcoma
  Myeloid proliferations related to Down syndrome
    Transient abnormal myelopoiesis
    Myeloid leukemia associated with Down syndrome
  Blastic plasmacytoid dendritic cell neoplasms
Acute leukemias of ambiguous lineage
  Acute undifferentiated leukemia
  Mixed phenotype acute leukemia with t (9;22) (q34;q11.2); BCR-ABL1
  Mixed phenotype acute leukemia with t (v; 11q23); MLL rearranged
  Mixed phenotype acute leukemia, B/myeloid, NOS
  Mixed phenotype acute leukemia, T/myeloid, NOS
  NK cell lymphoblastic leukemia/lymphoma
Lymphoid
  Precursor B-cell neoplasm
    B lymphoblastic leukemia/lymphoma, NOS
    B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
    B lymphoblastic leukemia/lymphoma with t (9;22)(q34;q11.2); BCR-ABL1
    B lymphoblastic leukemia/lymphoma with t (v; 11q23); MLL rearranged
    B lymphoblastic leukemia/lymphoma with t (12;21)(p13;q22); TEL-AML1 (ETV6-RUNX1)
    B lymphoblastic leukemia/lymphoma with hyperdiploidy
    B lymphoblastic leukemia/lymphoma with hypodiploidy (hypodiploid ALL)
    B lymphoblastic leukemia/lymphoma with t (5;14)(q31;q32); IL3-IGH
    B lymphoblastic leukemia/lymphoma with t (1;19)(q23;p13.3); E2A-PBX1 (TCF3-PBX1)
  Precursor T-cell neoplasm
    T lymphoblastic leukemia/lymphoma

*These are provisional entities. WHO: World Health Organization, AML: Acute myeloid leukemia, RUNX1-RUNX1T1: Runt-related transcription factor 1; translocated to, 1 (cyclin D-related), CBFβ: Core-binding factor, subunit beta, RARα: Retinoic acid receptor α, MLL: Mixed lineage leukemia, MLLT3: Mixed lineage leukemia gene T3, RPN1-EVI1: Ribophorin1 gene-ecotropic virus integration 1 gene, RBM15-MKL1: RNA binding motif protein 15-megakaryoblastic leukemia 1, NPM1: Nucleophosmin member 1, CEBPA: CCAAT/enhancer-binding protein alpha, NOS: Not otherwise specified, NK: Natural killer, TEL: Translocation-ETS-leukemia, ALL: Acute lymphoblastic leukemia

multilineage dysplasia. These patients with apparently normal cytogenetics are found to harbor FLT3, NPM1, CEBPA, additional sex comb-like 1 (ASXL1), and MN1-translocation-ETS-leukemia mutations.[8,16-20] Patients who have worse survival are frequently found to have FLT3 mutations.[17,20] The features which are more frequently seen to be associated with NPM1 mutations are significant and they are FLT3-internal tandem duplication (FLT3-ITD) and FLT3-tyrosine kinase domain mutations, myelomonocytic or monocytic morphology, extramedullary involvement with lymphadenopathy, female predilection, higher leucocyte count, higher platelet counts, higher bone marrow blast counts, higher lactate dehydrogenase, lower CD34 expression.[8,18] Signatures for NPM1 provides a more accurate subtyping than does that of FLT3-ITD.[20] In the future, perhaps, this group would be classified as a separate entity rather than being designated as provisional entity.

Therapy-related myeloid neoplasms are no longer subdivided in 2008 classification based on the drug given, as in the 2001 classification, because most of the patients receive both alkylating agents and topoisomerase II inhibitors.[16]

In WHO 2008 classification, both bilineal and biphenotypic acute leukemias are grouped as "Mixed phenotype Acute Leukemia;" these two were different entities in the EGIL (European Group of Immunological Markers for Leukemias) and WHO 2001 classification systems. A single expression of myeloperoxidase (MPO) (cytoplasmic) or CD3 (surface/intracellular) is now considered sufficient to label the blasts as myeloid or T-lymphoid lineage respectively. Acute leukemias that express both MPO (cytoplasmic) and CD19 are now diagnosed as "mixed phenotype acute leukemia." But unlike the EGIL classification, the WHO 2008 classification excludes acute leukemias with certain cytogenetic abnormalities from the group of "mixed phenotype acute leukemia," e.g. acute leukemia with t(8;21), t(15;17) or inv(16) are classified as AML with recurrent cytogenetic abnormalities, though they possess typical phenotypic expression.[15]

Specific chromosomal aberrations, their molecular counterparts and ploidy pattern have been included as important parameters in WHO classification of ALL. Hence, cases especially those with ambiguous morphology should be evaluated using flow cytometry for a more precise classification.[2,16,21,22]

The term Burkitt leukemia is no longer used to denote the morphological subtype of ALL.[16]

THE FUTURE ERA OF CLASSIFICATION

The impact of cytogenetic diagnosis in the management of hematological malignancies has improved dramatically over the past decade with the aid of molecular techniques such as fluorescent *in situ* hybridization, Southern blot, and polymerase chain reaction-based assays.[2,17] Microarray profiling studies, though potentially important in the research setting for the molecular classification of leukemias, have not yet been tested in clinical practice.[17] Apparently uniform chromosomal abnormalities such as t(1;19), t(9;22), t(8;14) or t(15;17) may differ at the molecular level.[23] Furthermore, patients with AML and a normal karyotype may have cryptic/submicroscopic genetic abnormalities and some of these have significance in prognostication also.[2,17] These include: FLT3-ITD, mutations in the NPM1, CEBPA, E26 transforming sequence related gene, ASXL1, IDH1 (isocitrate dehydrogenase 1), IDH2 genes, partial tandem duplication of the mixed lineage leukemia (MLL) gene, and high expression of the brain and acute leukemia, cytoplasmic gene.[8,17] Of these, mutations in the NPM1 and CEBPA genes have been assigned to be important in determining prognosis and thus in subtyping; and so, these two have been incorporated in the 2008 WHO classification.[16,20]

The genes encoding guanine nucleotide-binding protein gamma 11 and amphiregulin are found to be down-regulated in AML, B- and T-ALL. However, the gene encoding ceruloplasmin is up-regulated in AML, but not in B- and T-ALL.[24] The queries "whether these 3 genes will have any role in the distinction of acute leukemias in the future?" and "whether these will become a part of the upcoming classification?" have to be answered in the future. The prognosis of MLL rearrangement in AML depends on the specific partner gene. MLLT3 (MLL gene T3) [t(9;11)] and MLLT1 [t(11;19)] rearrangements have good prognosis, whereas, MLLT10 [t(10;11)] and MLLT4 [t(6;11)] have a poor prognosis.[8] However, the distinction is not made out in the WHO 2008 classification.

Studies have shown that, gene expression profiling can explore the specific expression signatures of leukemias and non-leukemic conditions and can divide leukemias into prognostic subgroups. Microarray-based gene expression profiling can be employed to classify leukemias in cases where cytogenetic analysis is not feasible. Gene expression microarray along with flow cytometry should be adjuncts to the usual diagnostic procedures.[25] This revolutionary evolution will, by all means, have a great impact in the approach to therapy in the future. Studies to evaluate the role of biomarkers also are in progress.[26] Proteomic analysis of peripheral blood plasma and quantification of selected proteins using mass spectroscopy is an emerging trend in subtyping leukemias and in assigning targeted therapy for the same. It is proven to predict the recurrence of ALL in adult patients and thus its clinical behavior.[27] The protein biomarkers platelet factor 4 and connective tissue active

peptide III (fragment of pro-platelet basic protein precursor) are down-regulated in ALL whereas two fragments of C3a are up-regulated.[28,29] The mRNA coding for plexin C1 is decreased in the blasts of AML. The levels of mRNA coding for leukotriene B4 receptor 1 gene and immunoglobulin superfamily member 2 gene are decreased in CML in blast crisis.[27] Hope the next generation or the present generation technophilic hematologists and hematopathologists would be routinely applying these advanced procedures, if feasible.

Though, clinical history, for instance leukemogenic therapy, has been given importance in the WHO 2008 classification of acute leukemia, further details such as pre-leukemic myeloid neoplasm, unrelated to Down's syndrome; history of myelodysplastic syndrome, recent therapy with growth factors may well be incorporated in the classification.[8,16]

CONCLUSION

However, the advanced, if not sophisticated, approach of diagnostics using cytogenetics and immunophenotyping makes it difficult for countries like India to implement WHO classification in routine use.[11]

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