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**Acute Leukemia**

Rapid advances in understanding the molecular biology of acute leukemia are transforming the approach to diagnosis, prognosis, and treatment of these diseases. This article will briefly review some of the basics of contemporary leukemia diagnosis.

The 2008 WHO classification of acute leukemia is based on clinical features, morphology, immunophenotyping, cytogenetics and molecular genetics, representing a navigation of the leukemia's biology and clinically significant disease subtypes. Genetic changes in AML and ALL are probably the most important feature for prognosis.

The FAB classification which was based on morphology and cytochemical studies did not represent clinically significant subtypes.

2008 **WHO Classification of Acute Leukemia**

- Acute myeloid leukemia with recurrent genetic abnormalities
  - AML with t(15;17)(q22;q12): (RUNX1-RUNX1T1)
  - AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22): (CBFB-MYH11)
  - AML with t(15;17)(q22;q21): (PML-RARA)
  - AML with t(8;21)(q22;q22): (MLLT3-MLL)
  - AML with t(6;9)(p23;q34): (DEK-NUP214)
  - AML with inv(3)(p14p23.3) or t(3;3)(p21q26.2): (RUNX1-EVI1)
  - AML (megakaryoblastic) with t(12;22)(p13;q12): (MLL-AF4)
  - Provisional entity: AML with mutated NPM1
  - Provisional entity: AML with mutated CEBPA

- Acute myeloid leukemia with myelodysplasia-related changes

- Therapy-related myeloid neoplasms

- Acute myeloid leukemia, not otherwise specified (NOS)
  - AML with minimal differentiation
  - AML without maturation
  - AML with maturation

- Acute myelomonocytic leukemia

- Acute monoblastic-monocytic leukemia

- Acute erythroid leukemia
  - Pure erythroid leukemia
  - Erythroleukemia, erythroid/myeloid

- Myeloid Proliferations related to Down Syndrome
  - Transient abnormal myelopoiesis
  - Acute myeloid leukemia associated with Down Syndrome
Acute leukemia of ambiguous lineage

- Acute undifferentiated leukemia
- Mixed phenotype acute leukemia
- Mixed phenotype acute leukemia with t(9;22)(q34.11;22): BCR-ABL1
- Mixed phenotype acute leukemia with t(1;19): MLL rearranged
- Mixed phenotype acute leukemia: B/myeloid: NOS
- Mixed phenotype acute leukemia: T/myeloid: NOS
- Mixed phenotype acute leukemia: NOS: rare types
- Natural killer (NK)-W/O-cell lymphoblastic leukemia

Lymphoblastic leukemia/lymphoma

- Lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
- Lymphoblastic leukemia/lymphoma with t(9;22)(q34.11;22): BCR-ABL1
- Lymphoblastic leukemia/lymphoma with t(1;19): MLL rearranged
- Lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22): TEL-AML1
- Lymphoblastic leukemia/lymphoma with hyperdiploidy
- Lymphoblastic leukemia/lymphoma with hypodiploidy
- Lymphoblastic leukemia/lymphoma with t(1;19)
- Lymphoblastic leukemia/lymphoma with t(11;19)

Diagnostic approach

1. Clinical history & physical examination
2. Complete blood count and peripheral blood smear examination
3. Bone marrow aspiration and trephine biopsy morphology examination
4. Immunophenotyping of leukemic cells by flow cytometry or immunohistochemistry
5. Bone marrow cytomorphology studies
6. Molecular studies in some cases

Clinical features

Clinical findings play an important role in the 2016 WHO classification. Patients with AML, and preceding history of MDS are diagnosed as AML with myeloid lineage dysplasia. If previously treated with chemotherapy, these cases are classified as therapy-related AML. If a specific recurrent abnormality is present these are diagnosed as AML with the specific chromosomal abnormality, e.g., therapy-related AML with t(9;11). Patients with Down syndrome are put in a separate category from the non-Down syndrome cases.

Morphology

Laboratory evaluation of a suspected case of leukemia begins with peripheral blood examination; morphological evaluation of bone marrow aspirate and biopsy is recommended in all cases. Morphology remains the standard for enumeration of blasts which are expressed as a percentage of nucleated cells based on a 200-cell count in peripheral blood and a 500-cell count in bone marrow.

- Acute erythroleukemia the erythroid precursors are excluded from the blast count.
- In cases with t(8;21), t(15;17), or t(10;11), a diagnosis of AML is made even if the blast count is >20%.
- If other genetic abnormalities are present and the blast count is <20%, a diagnosis of MDS is made.

Morphological evaluation is essential for identification of multianeu copy syndromes, for the identification of blasts with features of hypergranular or hypogranular acute promyelocytic leukemia and for detecting the presence of abnormal eosinophils in AML with inv(16)(q22q22).

Immunophenotyping

Flow cytometric immunophenotyping is recommended in all potential cases of acute leukemia. It is essential for the diagnosis of all cases of AML, minimally differentiated AML, and mixed phenotype acute leukemia. Identification of immunophenotypic patterns that correspond to specific disease groups is useful for guiding genetic studies.

- Antigenic pattern: MPO+/CD13+CD33weak/HLA-DR+/CD20+/CD19+

Characterizes AML with t(15;17)(q22q21)

As aberrant expression is frequently encountered, a battery of antibodies is required for definitive characterization of blast lineages.

Cytogenetics

Cytogenetic studies should be performed on all new cases of acute leukemia; recurrent chromosomal abnormalities are found in approximately 40% of cases and define 5 risk groups in AML and B-ALL. Favorable, intermediate, and adverse 60% of cases are cytogenetically normal (CN-AML) are included in the intermediate group, however prognosis depends on specific molecular abnormalities.

In B-ALL, to detect cryptic abnormalities such as t(12;21), molecular studies by FISH or PCR are indicated.

Cytogenetic indicators of prognosis are less clearly defined in T-ALL. Myeloid blasts related to leukemic chromosomal abnormalities are complex karyotype, balanced and unbalanced abnormalities, some of which occur most commonly in therapy-related disease.

Molecular studies

Cytogenetically normal AML (CN-AML) is a heterogeneous group in which gene mutations are highly relevant for prognostic stratification.

Testing for NPM1, CEBPA, and FLT3-ITD mutations is recommended for all cases of AML.

- The class I mutations eg FLT3 confer a proliferation and survival advantage, these occur in clonally associated with disease progression.
- Class II mutations CEBPA and NPM1 lead to impaired differentiation, occur early and are proposed to be the initiating mutations. Class I and II mutations occur together, however, class II mutations are mutually exclusive.
- FLT3-ITD occur in many subtypes of AML including 20% of CN-AML, and are associated with a worse outcome.

NPM1 mutations are the most common genetic abnormality in AML occurring in approximately 30% of adult de novo AML and in 60% of CN-AML and are associated with favorable prognosis and in vitro sensitivity to FLT3-ITD inhibition, hence both mutations should be tested together.

CEBPA is found in 5-10% of AML blasts in AML and in the mature myeloid subsets and in 15% CN-AML; this mutation is considered to be an independent prognostic marker and is associated with a favorable prognosis.

Molecular studies by FISH or PCR are utilized for definitive diagnosis and to target therapy with APML and ASML in AML using specific treatment.

Current trials are exploring other molecular targets for therapy.

Monitoring levels of early treatment response and low level disease persistence provides an opportunity for early intervention.

It is expected that more targeted treatment will increase the demand for more sophisticated molecular testing.

Role of the Clinical Laboratory in Acute Leukemia Diagnosis

1. Peripheral blood examination
2. Bone marrow morphology
3. Flow cytometric immunophenotyping
4. Cytogenetic studies
5. FISH/PCR

Correlation with clinical features is essential for final conclusion

Chronic myeloid leukemia

Clinical features especially splenomegaly, indicate the need for further investigations for diagnosis of CML. Investigations comprise CEC & PBF examination, bone marrow aspirate and trephine biopsy morphology and cytogenetic studies for Ph chromosome. FISH or molecular studies are indicated to detect the presence of the BCR-ABL fusion gene and for monitoring therapy.

Flow cytometric immunophenotyping is not necessary for the diagnosis of CML in chronic phase, however in blast crisis it can provide important information regarding blast lineage and can also assist in disease acceleration.

Polychromatophilia Vera

Major criteria

- Hb >15.5 g/dL in men or 16.5 g/dL in women, or Hb >17 g/dL in men or >21 g/dL in women if associated with 3rd degree increase from baseline that is not attributed to correction of iron deficiency anaemia. Hb or hematocrit increase of reference range for age sex/alcohol & drugs OR Hb >75% above median predicted value
- Presence of JAK2 V617F or CALR exon 12 mutation

Diagnosis of PV requires both major criteria and one minor criterion or the presence of first major criterion and two minor criteria.

Minor criteria

- Bone marrow showing polychromatophilia
- Serum EPO level below the reference range for normal
- Endogenous erythroid colony formation

References


FROM THE EDITOR’S DESK

Rapid advances in understanding the molecular biology of acute myeloid leukemia are transforming the approach to diagnosis, prognosis, and treatment of these cases. Molecular diagnostics is in addition paving the way for targeted therapy and personalized medicine.

In this issue, Dr. Arvind Kalsi details the current AML classification with a particular emphasis on the need for a complete approach to the diagnosis and its impact on the management of acute myeloid leukemia and other malignancies.

Cytogenetics, which played a central role in older leukemia classification schemes, is no longer required, although in rare cases it can be helpful in the identification of monogenic differentiation. The current standard of care is to perform immunophenotyping by multicolor flow cytometry to further subcategorize (e.g., myeloid leukemia, B-cell leukemia, and T-cell leukemia) and to supplement the findings with other studies as discussed in this review.

It is indeed encouraging to have received a tremendous response to the previous issue of INSIGHT and we continue to look forward to your valuable feedback and suggestions to guide us in addressing the various issues in need of diagnostic service.

We would welcome your valuable suggestions, queries and ideas to help us serve you better.

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