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**Diagnosis of Leukemia Role of the Laboratory**

**Acute Leukemia**

Rapid advances in understanding the molecular biology of acute leukemias 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. It is a representation of disease 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(6;11)(q27;q22): (RUNX1/RUNX1T1)
  - AML with inv(16)(p13.1q22) or del(16)(p13.1q22): (CBFB-MYH11)
  - APL with t(15;17)(q22;q12): (PML-RARA)
  - AML with t(8;11)(q22;q21): (MLLT3-MLL)
  - AML with (16;17)(p13;q22), (CBF-beta-MYH11)
  - AML with inv(3)(p21q26.3) or del(3)(p21q26.3): (RUNX1-EVI1)
  - AML with rearrangement of 11q23 (AML1-ETO)
  - Provisional entity: AML with mutated NPM1
  - Prognostic 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/myelomonocytic leukemia
  - Acute erythroid leukemia
    - Pure erythroid leukemia
    - Erythroleukemia, erythroid/myeloid

- Myeloid Proliferations associated with Down Syndrome
  - Transient abnormal myelopoiesis
  - Acute myeloblastic leukemia associated with Down Syndrome

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**Dr Lal PathLabs**
Acute leukemia of ambiguous lineage
- Acute undifferentiated leukemia
- Mixed phenotype acute leukemia with T(9;22)(q34:11.2): BCR-ABL1
- Mixed phenotype acute leukemia with t(17;22): MLL rearranged
- Mixed phenotype acute leukemia
- T/myeloid, NOS
- Mixed phenotype acute leukemia
- T/lymphoid, NOS
- Mixed phenotype acute leukemia
- NOS, rare types
- Natural killer (NK)/o T-cell lymphoblastic leukemia/symphoma
- B lymphoblastic leukemia/lymphoma
- B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
- B lymphoblastic leukemia/lymphoma with t(12;21): BCR-ABL1
- B lymphoblastic leukemia/lymphoma with t(12;21):MLL rearranged
- B lymphoblastic leukemia/lymphoma with t(12;21):hyperdiploidy
- B lymphoblastic leukemia/lymphoma with hyperdiploidy
- B lymphoblastic leukemia/lymphoma
- E2A- PBX1

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

Clinical Features
Clinical finding play an important role in the 2018 WHO classification. Patients with AML, and preceding history of MDS are diagnosed as AML with multilineage 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(15;17). 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.

- In acute erythroleukemia the erythroid precursors are excluded from the blast count.
- In cases with t(8;21), inv(16), t(16;16) or t(15;17), 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%, the diagnosis of AML is made.
- Morphological evaluation is essential for identification of multi-neuropenia, for the identification of blasts with features of hypergranulocytic or hypogranulocytic acute myeloproliferative leukemia and for detecting the presence of abnormal eosinophils in AML with myeloid or myeloid/lymphoid. NOS.

Immunophenotyping
Flow cytometric immunophenotyping is recommended in all potential cases of acute leukemia. It is essential for the diagnosis of all cases of ALL, 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+/ CD204+/CD19+ characters MLL with t(12;21):MLL2.
- As blastic expression is frequently encountered, a battery of antibodies is required for definitive characterization of blast lineage.

Cytogenetics
Cytogenetic studies should be performed on all new cases of acute leukemia; recurrent cytogenetic abnormalities are found in approximately 40% of cases and define five risk groups in AML and B-ALL: favorable, intermediate and adverse 60% are cytogenetically normal (CN-AML), and 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 abnormalities of prognosis are less clearly defined in T-cell-AML. Myeloid-lineage-related cytogenetic abnormalities comprise 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 significant for prognostic stratification.

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

The class I mutations eg FLT3 confer a proliferation and survival advantage, these occur late and are 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 our class I mutations are mutually exclusive.

FLT3-ITDs occur in many subtypes of AML including 30% of CN-AML and are associated with a poor outcome.

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

- CEBPA is found in 5-10% of AML in t(8;21) and in 15% of CN-AML, this mutation is considered to be an independent prognostic marker and is associated with a favorable prognosis.

Molecular studies by ISH or PCR are utilized for definitive diagnosis and to target therapy. APML with RUNX1-TAL1 responds to specific treatment.

Current trials are exploring other molecular targets for therapy.

Monitoring levels of early treatment response and low level disease persistence provide 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 examination
3. Flow cytometric immunophenotyping
4. Cytogenetic studies

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 CBC & FBC examination, bone marrow aspirate and trephine biopsy morphology and cytogenetic studies for Ph chromosome. FISH or PCR 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 suggest disease acceleration.

Polycythemia Vera

Major Criteria

- HB >18 g/dl in men or >16 g/dl in women, or >17 g/dl in women if associated with 3rd degree increase from baseline that is not attributable to correction of iron deficiency anemia. Hb or Hct that persists despite adequate iron therapy.

Minor Criteria

- Presence of JAK2 V617F or JAK2 exon 12 mutation.
- Bone marrow showing polycytemia.
- Serum EPO level below the reference range for normal.
- Endogenous erythropoietin formation.

Diagnosis of PV requires both major criteria and one minor criterion or the presence of 1 major criterion and 2 minor criteria.

Chronic Lymphocytic Leukemia and Other Lymphoid Leukemias

Laboratory investigations recommended are:

- CBC and FBC examination.
- Bone marrow aspirate and trephine biopsy morphology.
- Hemoglobinopathy, flow cytometry.
- Cytogenetic analysis.
- Molecular studies by FISH or PCR.

References: