Asia's largest and most advanced diagnostic lab

...at National Reference Lab, Sector- 18, Rohini.

- Fully automated high throughput lab with rapid turnaround time (TAT)
- Cutting-edge technology to deliver the most accurate test results from the widest test menu
- Dedicated central sample distribution facility for quick access and distribution of samples
- Conforms to strict international standards of quality and poised to handle the highest workload in Asia
- One stop solution for world-class diagnostics - pathology super specialized tests, radiology and cardiology
- An ergonomically designed and environment-friendly building

Volume 15 June, 2011 News Letter

Dr Lal PathLabs
INSIGHT

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, prognostication, 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 distinct biologic and clinically significant disease subtypes.

Genetic changes in AML and ALL are probably the most important feature for prognostication.

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(8;21)(q22;q22); (RUNX1;RUNX1T1)
  - AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); (CBFB-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)(q21q26.2) or t(3;3)(q21;q26.2); (RNP1-EVI1)
  - AML (megakaryoblastic) with t(1;22)(p13;q13); (RBM15-MKL1)
  - 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 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
  - Mixed phenotype acute leukemia, NOS-rare types
  - Natural killer(NK)-cell lymphoblastic leukemia/lymphoma
- 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
  - B lymphoblastic leukemia/lymphoma with hyperdiploidy
  - B lymphoblastic leukemia/lymphoma with hypodiploidy
  - B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32); IL3-IGH
  - B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.1); E2A-PBX1

Diagnostic approach

1. Clinical history & physical examination
2. Complete blood cell 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 cytogenetic studies
6. Molecular studies in some cases

Clinical Features

Clinical findings play an important role in the 2008 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 eg. 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.

- 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% a diagnosis of MDS is made.

Morphological evaluation is essential for identification of multilineage dysplasia, 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) or t(16;16)

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

eg. Antigenic pattern MPO+/ CD13+/CD33weak/HLA-DR+/ CD34+/CD19+ characterizes AML with t(8;21)(q22;q22)

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

Cytogenetics

Cytogenetics studies should be performed on all new cases of acute leukemia ; recurrent cytogenetic abnormalies are found in approximately 40% of cases and define 3 risk groups in AML and B-ALL: favorable, intermediate and adverse

60% of cases 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 indicators of prognosis are less clearly defined in T-cell ALL

Myelodysplasia-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 mutations is recommended for all cases 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 class II mutations occur together, however class II mutations are mutually exclusive.

Class II mutations are associated with characteristic clinico-pathological features and possibly define distinct biological entities.

- 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 a favorable prognosis in the absence of FLT3-ITD mutation, hence both mutations should be tested together.
- CEBPA is found in 5-9% of AML usually in M1 & M2 morphological subtypes 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 eg. APML with PML-RARA responds to 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

   Flow cytometric immunophenotyping

   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 & PBF examination, bone marrow aspirate and trephine biopsy morphology and cytogenetic studies for Philadelphia 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 suggest disease acceleration.

Polycythemia Vera

Major Criteria	Minor Criteria
1. Hb >18.5 g/dL in men or 16.5 g/dL in women, or Hb > 17 in men or >15 in women if associated with 2g/dL increase from baseline that is not attributed to correction of iron deficiency anemia. Hb or Hct >99 percentile of reference range for age/sex/altitude or RCM >25% above mean predicted value 2. Presence of JAK2 V617F or JAK2 exon 12 mutation	1. Bone marrow showing panmyelosis 2. Serum EPO level below the reference range for normal 3. Endogenous erythroid colony formation

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

Chronic Lymphocytic Leukemia and Other Lymphoid Leukemias

Laboratory investigations recommended are:

a. CBC and PBF examination

b. Bone marrow aspirate and trephine biopsy morphology

c. Immunophenotyping by flow cytometry

d. Cytogenetic analysis

e. Molecular studies by FISh or PCR

References:

1. Betz BL, Hess JL Acute Myeloid Leukemia Diagnosis in the 21st Century. Arch Pathol Lab Med 2010;134(10): 1427-1432
2. Arber DA. Algorithmic approach to the classification of leukemia: United States & Canadian Academy of Pathology (USCAP)
3. Mahaptra M, Rathi S Polycythemia Vera. Hematology Today 2010: 113-117
4. Kjeldsberg CR Pratical Diagnosis of Hematologic Disorders 4th ed.

Dr. Anjali K. Kale MBBS, DCP, MD,FCAP
Diplomate American Board of Pathology
Chief Consultant Pathologist National reference laboratory
Dr. Lal PathLabs, New Delhi

FROM THE EDITOR'S DESK

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

In this issue, Dr Anjali Kale 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.

Cytochemistry, 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 monocytic differentiation. The current standard of care is to perform immunophenotyping by multicolor flow cytometry to further subclassify by lineage (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.

Dr Reena Nakra

Chief Editor,

Head Integration Group, M&A labs

E-mail: reena.nakra@lalpathlabs.com

Dr Lal PathLabs