Initial Diagnostic Workup of Acute Leukemia
A Pocket Guide for the Clinician

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The recommendations in this guide are based on the Initial Diagnostic Workup of Acute Leukemia, a guideline from the College of American Pathologists (CAP) and the American Society of Hematology (ASH).
Context

A complete diagnosis of acute leukemia requires knowledge of clinical information combined with morphologic evaluation, immunophenotyping and karyotype analysis, and often, molecular genetic testing. Cooperation between clinicians and pathologists is key to ensuring an accurate diagnosis. This pocket guide provides a framework for the multiple steps, including laboratory testing, in the evaluation of acute leukemia samples.

Note: All laboratory testing performed for the initial workup and diagnosis of a patient with acute leukemia and all related specimen handling and storage must be performed in a laboratory that is in compliance with regulatory and/or accreditation requirements.

Comprehensive Sharing and Reporting of Data

The treating clinician should provide relevant clinical data or ensure that this is readily accessible by the pathologist. Similarly, the pathologist needs to be proactive when reporting results and their interpretation with attending clinicians and referral institutions.

Relevant Clinical Data

Data that should be provided or be made readily accessible to the pathologist includes, but is not limited to:

- **Age**
- **Sex**
- **Ethnicity**
- **History of any hematologic disorder or known predisposing conditions or syndromes**
- **Prior malignancy**
- **Exposure to cytotoxic therapy, immunotherapy, radiotherapy, or other possibly toxic substances**
- **Any history of possibly confounding factors, such as recent growth factor therapy, transfusions, or other medications that might obscure or mimic the features of acute leukemia**
- **Family history of any hematologic disorder or other malignancies**
- **Relevant physical examination findings, including neurologic exam findings**
- **Relevant imaging findings**
- **Presence of tumor masses (e.g., mediastinal), other tissue lesions (e.g., cutaneous), and/or organomegaly**
- **Any additional clinical findings deemed to have diagnostic or prognostic importance by the attending physician**

Reporting and Record-Keeping

In the initial report, the pathologist should include laboratory, morphologic, immunophenotypic, and, if performed, cytochemical data, on which the diagnosis is based, along with a list of any pending tests. The pathologist should issue addenda/amended reports when the results of additional tests become available.

The pathologist and treating clinician should coordinate and ensure that all tests performed for classification, management, predicting prognosis and disease monitoring are entered into the patient’s medical records. This information should include the sample source, adequacy, and collection information as applicable.

Note: All records should adhere to the current World Health Organization (WHO) terminology for the final diagnosis and classification of acute leukemia.

Testing and Reporting in the Context of Referral

If after examination of a peripheral blood smear, it is determined that the patient will require immediate referral to another institution with expertise in the management of acute leukemia for treatment, the initial institution should, whenever possible, defer invasive procedures including bone marrow aspiration and biopsies to the treatment center to avoid duplicate procedures, associated patient discomfort, and additional costs.

Note: This recommendation does not preclude evaluation when a rapid diagnosis, such as in cases of suspected APL, is necessary in order to initiate therapy prior to transfer.

Upon referral to another institution, the primary institution should provide the treatment center with all laboratory results, pathology slides, flow cytometry data, cytogentic information, and a list of pending tests at the time of the referral. Pending test results should be forwarded as they become available.

Collection and Evaluation of Samples/Specimens

<table>
<thead>
<tr>
<th>Actions which should be performed for suspected or confirmed leukemia</th>
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<tbody>
<tr>
<td><strong>Review recent or concurrent CBCs and leukocyte differentials</strong></td>
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<tr>
<td><strong>Evaluate peripheral blood smear</strong></td>
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<tr>
<td><strong>Obtain fresh samples for morphologic evaluation and ancillary studies:</strong></td>
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<tr>
<td><strong>Bone marrow (BM) aspirate and/or touch imprints</strong></td>
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<tr>
<td><strong>BM core biopsy and/or marrow clot</strong> may be obtained and should be reviewed concurrently with the BM aspirate and/or touch imprints</td>
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<tr>
<td><strong>BM core biopsies</strong> may be used for ancillary studies when adequate aspirate cannot be obtained</td>
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<tr>
<td><strong>Peripheral blood (PB) may be used for diagnosis and ancillary studies if sufficient blasts are present and the BM aspirate is inadequate or there is compelling clinical reason to avoid BM evaluation</strong></td>
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<tr>
<td><strong>Tissue biopsy</strong> for extramedullary disease without apparent BM or PB involvement</td>
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<tr>
<td><strong>Submit sufficient samples to perform conventional cytogenetic analysis (i.e., karyotype), appropriate molecular genetic and/or fluorescent in-situ hybridization (FISH) testing, and flow cytometric immunophenotyping (FCI)</strong></td>
</tr>
<tr>
<td><strong>Ensure that the flow cytometry panel is sufficiently comprehensive to distinguish AML, B-ALL, T-ALL including early T-cell precursor (ETP), and acute leukemias of ambiguous lineage</strong></td>
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<tr>
<td><strong>Ensure that flow cytometry analysis or molecular characterization is comprehensive enough to allow subsequent detection of minimal residual disease</strong></td>
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<tr>
<td><strong>Use immunohistochemical stains for limited immunophenotyping in the diagnosis and classification of acute leukemia when bone marrow aspirate or peripheral blood material is not available for flow cytometry study</strong></td>
</tr>
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</table>

Actions which may be performed

| **Request and evaluate cytochemical studies to assist in the diagnosis and classification of AML** |
| **Use flow cytometry in the evaluation of CSF** |
| **Use cryopreserved cells or nucleic acid, formalin fixed paraffin-embedded tissue, or unstained marrow aspirate or peripheral blood smears or other involved tissues for molecular or genetic studies in which the use of such material has been validated** |

An example of initial workup for acute leukemia. Bone marrow aspirate smear shows large blasts with azurophilic granules with maturation (upper left); the blasts are CD34+, CD117+, with MPO and CD19 coexpression (upper right); metaphase FISH reveals RUNX1/RUNX1T1 fusion as a result of t(8;21)(q22;q22) (lower left); KIT D816V (Asp to Val, GAC to GTG) mutation is detected (lower right), providing prognostic information in adult patients with RUNX1/RUNX1T1-AML.
**Initial Diagnostic Workup of Acute Leukemia**

- **Patient Suspected of Acute Leukemia**
  - Relevant clinical data
  - Complete blood count and leukocyte differential
  - Peripheral blood smear
  - Bone marrow aspiration with core biopsy
  - Extramedullary tissue biopsy (if applicable)

- **Not Acute Leukemia**
  - Morphologic evaluation
  - Flow cytometry immunophenotyping
  - Conventional cytogenetic analysis
  - Appropriate molecular genetic and/or FISH studies

**If a CSF sample is collected** at the time of intrathecal therapy or for other clinical indications, **should perform**:
- CSF cell count
- Pathologist review of a cytocentrifuge preparation with enumeration of blasts
- May perform: CSF flow cytometry

**Should use** the current WHO terminology for the final diagnosis and classification of acute leukemia.

**Lymphoblastic Leukemia/Lymphoma (ALL)**

**B-Lymphoblastic Leukemia/Lymphoma (B-ALL)**

**T-Lymphoblastic Leukemia/Lymphoma (T-ALL)**

**Acute Leukemia**

- **Should perform** testing for:
  - t(9;22)(q34;q11.2); BCR-ABL1
  - KMT2A (MLL) rearrangement
- May perform:
  - Mutational analysis that includes (but is not limited to) PAX5, JAK1, JAK2, and/or IKZF1
  - CRLF2 overexpression by flow cytometry

**Flow cytometry panel should include** sufficient markers to identify early T-cell precursor (ETP)

**Should perform testing for**:
- t(12;21)(p13;q22); ETV6-RUNX1
- t(9;22)(q34;q11.2); BCR-ABL1
- KMT2A (MLL) rearrangement
- iAMP21
- Trisomy 4 and 10

**May perform**:
- Mutational analysis for NOTCH1 and/or FBXW7
- CRLF2 overexpression by flow cytometry

**Strong Recommendation**

**Recommendation**
### Initial Diagnostic Workup of Acute Myeloid Leukemia

**Acute Myeloid Leukemia (AML)**

- **If acute promyelocytic leukemia (APL) is suspected, should perform:**
  - Rapid detection of PML-RARA
  - Coagulation studies to evaluate for DIC

**Should perform:**
- FLT3-ITD mutation

**May perform:**
- Mutational analysis that includes (but is not limited to): IDH1, IDH2, TET2, WT1, DNMT3A, and/or TP53

**Core Binding Factor (CBF) AML**
- t(8;21)(q22;q22.1); RUNX1-RUNX1T1 or inv16(p13.1q22)/t(16;16)(p13.1;q22); CBFB-MYH11

**AML other than CBF, APL or AML with myelodysplasia-related cytogenetic abnormalities**

**Should perform:**
- Mutational analysis for NPM1, CEBPA and RUNX1

**Should perform:**
- KIT mutation

**May perform:**
- KIT mutation

### Strength of Recommendations and Quality of Evidence


#### Strength of Recommendation

- **Strong recommendation**—Supported by convincing (high) or adequate (intermediate) quality of evidence and/or clear benefit that outweighs any harms.
- **Recommendation**—Indicates there is some limitation in the quality of the evidence, balance of benefits and harms, values, or costs, but the panel concludes that there is sufficient evidence and/or benefit to inform a recommendation.
- **Expert consensus opinion**—Indicates there are serious limitations in the quality of evidence, balance of benefits and harms, values, or costs, but the panel consensus is that the statement is necessary.

#### Quality of Evidence

- **Convincing**—High confidence that available evidence reflects true effect. Further research is very unlikely to change the confidence in the estimate of effect.
- **Adequate**—Moderate confidence that available evidence reflects true effect. Further research is likely to have an important impact on the confidence in estimate of effect and may change the estimate.
- **Inadequate**—Little confidence that available evidence reflects true effect. Further research is very likely to have an important impact on the confidence in the estimate of effect and is likely to change the estimate.
- **Insufficient**—Evidence is insufficient to discern net effect. Any estimate of effect is very uncertain.

### How to Use This Pocket Guide

ASH pocket guides are primarily intended to help clinicians make decisions about diagnostic and treatment alternatives. The recommendations are not intended to serve or be construed as a standard of care. Clinicians must make decisions on the basis of the unique clinical presentation of an individual patient, ideally through a shared process that considers the patient’s values and preferences with respect to the anticipated outcomes of the chosen option. Decisions may be constrained by realities of a specific clinical setting, including, but not limited to, institutional policies, time limitations, or unavailability of treatments. ASH pocket guides may not include all appropriate methods of care for the clinical scenarios described. As science advances and new evidence becomes available, recommendations may become outdated. Although this pocket guide is intended to improve patient care, following the recommendations cannot guarantee successful outcomes. ASH does not warrant or guarantee any products described in this pocket guide.

Drs. Cessna and Wang declare no competing financial interests.

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