Initial Diagnostic Work-Up of Acute Leukemia: ASCO Clinical Practice Guideline Endorsement of the CAP and ASH Guideline

de Haas, et al.
Introduction

- In 2017, an evidence-based guideline for the initial work-up of AL was published by The College of American Pathologists (CAP) and the American Society of Hematology (ASH).

- Since that time, advances in molecular techniques and identification/validation of new molecular markers via large cohorts, have contributed to better risk-stratification of AL patients.

- Secondly, a revision of the WHO-classification of tumors of hematopoietic and lymphoid tissues was described in 2016 and fully published in 2017 also leading to new risk-categories and refined subclassification.

- Therefore, the current ASH/CAP guidelines were reviewed by ASCO Endorsement Expert Panelists, and discussion points are used to summarize issues that were identified from the updated literature.
ASCO Endorsement Methodology

The ASCO Clinical Practice Guidelines Committee endorsement review process includes:

- a methodological review by ASCO guidelines staff
- a content review by an Expert Panel
- final endorsement approval by ASCO CPGC.

The full ASCO Endorsement methodology supplement can be found at:

www.asco.org/hematologic-malignancies-guidelines

CAP & ASH Guideline Methodology can be found at:

Clinical Questions

- The CAP-ASH guideline addressed:
  - What clinical and laboratory information should be available during the initial diagnostic evaluation of a patient with AL?
  - What specimens and sample types should be evaluated during the initial workup of a patient with AL?
  - At the time of diagnosis, what tests are required for all patients for the initial evaluation of an AL?
  - Which tests should be performed on only a subset of patients, including in response to results from initial tests and morphology?
  - Where should laboratory testing be performed?
  - How should test results and the diagnosis be correlated and reported?
Target Population and Audience

Target Population
Children and adults with acute leukemia

Target Audience
Primary care providers, nurses, medical oncologists, pediatric oncologists, hematologists, pathologists, radiation oncologist, other providers
Summary of Recommendations

**Recommendation 1.** The treating clinician should provide relevant clinical data or ensure that this is readily accessible by the pathologist (*Strong recommendation*).

Note.—These data include, but are not limited to, the patient’s age, sex, and ethnicity; history of any hematologic disorder or known predisposing conditions or syndromes; any prior malignancy; exposure to cytotoxic therapy, immunotherapy, radiotherapy, or other possibly toxic substances; and any additional clinical findings of diagnostic or prognostic importance. The treating clinician should also include 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. The treating clinician should also obtain and provide information regarding any family history of any hematologic disorders or other malignancies.
Summary of Recommendations

**Recommendation 2.** The treating clinician should provide relevant physical examination and imaging findings or ensure that those results are readily accessible by the pathologist (Recommendation).

Note.—This includes, but is not limited to, neurologic exam findings and the presence of tumor masses (eg, mediastinal), other tissue lesions (e.g. cutaneous), and/or organomegaly.

**Recommendation 3.** The pathologist should review recent or concurrent complete blood cell (CBC) counts and leukocyte differentials and evaluate a peripheral blood smear (Strong recommendation).
Summary of Recommendations

**Recommendation 4.** The treating clinician or pathologist should obtain a fresh bone marrow aspirate for all patients suspected of acute leukemia, a portion of which should be used to make bone marrow aspirate smears for morphologic evaluation. If performed, the pathologist should evaluate an adequate bone marrow trephine core biopsy, bone marrow trephine touch preparations, and/or marrow clots, in conjunction with the bone marrow aspirates (*Strong recommendation*).

Note.—If bone marrow aspirate material is inadequate or if there is compelling clinical reason to avoid bone marrow examination, peripheral blood may be used for diagnosis and ancillary studies if sufficient numbers of blasts are present. If a bone marrow aspirate is unobtainable, touch imprint preparations of a core biopsy should be prepared and evaluated, and an additional core biopsy may be submitted unfixed in tissue culture medium for disaggregation for flow and genetic studies. Optimally, the same physician should interpret the bone marrow aspirate smears and the core biopsy specimens, or the interpretations of those specimens should be correlated if performed by different physicians.
Recommendation 5. In addition to morphologic assessment (blood and bone marrow), the pathologist or treating clinician should obtain sufficient samples and perform conventional cytogenetic analysis (ie, karyotype), appropriate molecular genetic and/or fluorescent in situ hybridization (FISH) testing, and flow cytometric immunophenotyping (FCI). The flow cytometry panel should be sufficient to distinguish acute myeloid leukemia (including acute promyelocytic leukemia), T-cell acute lymphoblastic leukemia (T-ALL) (including early T-cell precursor leukemias), B-cell precursor ALL (B-ALL), and acute leukemia of ambiguous lineage on all patients diagnosed with acute leukemia. Molecular genetic and/or FISH testing does not, however, replace conventional cytogenetic analysis (Strong recommendation).

Note.—If sufficient bone marrow aspirate or peripheral blood material is not available for FCI, immunohistochemical studies may be used as an alternative method for performing limited immunophenotyping. In addition, a second bone marrow core biopsy can be obtained and submitted, unfixed in tissue culture media, for disaggregation for genetic studies and flow cytometry.
Summary of Recommendations

**Recommendation 6.** For patients with suspected or confirmed acute leukemia, the pathologist may request and evaluate cytochemical studies to assist in the diagnosis and classification of acute myeloid leukemia (AML) (*Expert consensus opinion*).

**Recommendation 7.** The treating clinician or pathologist may use cryopreserved cells or nucleic acid, formalin fixed, nondecalcified paraffin-embedded (FFPE) tissue, or unstained marrow aspirate or peripheral blood smears obtained and prepared from peripheral blood, bone marrow aspirate or other involved tissues for molecular or genetic studies in which the use of such material has been validated. Such specimens must be properly identified and stored under appropriate conditions in a laboratory that is in compliance with regulatory and/or accreditation requirements (*Recommendation*).
Summary of Recommendations

**Recommendation 8.** For patients with acute lymphoblastic leukemia (ALL) receiving intrathecal therapy, the treating clinician should obtain a cerebrospinal fluid (CSF) sample. The treating clinician or pathologist should ensure that a cell count is performed and that examination/enumeration of blasts on a cytocentrifuge preparation is performed and is reviewed by the pathologist *(Strong recommendation).*

**Recommendation 9.** For patients with acute leukemia other than those with ALL who are receiving intrathecal therapy, the treating clinician may, under certain circumstances, obtain a cerebrospinal fluid (CSF) sample when there is no clinical contraindication. The treating clinician or pathologist should ensure that a cell count is performed and that examination/enumeration of blasts on a cytocentrifuge preparation is performed and is reviewed by the pathologist *(Expert consensus opinion).*
Summary of Recommendations

**Recommendation 10.** For patients with suspected or confirmed acute leukemia, the pathologist may use flow cytometry in the evaluation of CSF (*Recommendation*).

**Recommendation 11.** For patients who present with extramedullary disease without bone marrow or blood involvement, the pathologist should evaluate a tissue biopsy and process it for morphologic, immunophenotypic, cytogenetic, and molecular genetic studies, as recommended for the bone marrow (*Strong recommendation*).

Note.—Additional biopsies may be indicated to obtain fresh material for ancillary testing.

**Recommendation 12.** For patients with suspected or confirmed acute leukemia, the pathologist or treating clinician should ensure that flow cytometry analysis or molecular characterization is comprehensive enough to allow subsequent detection of minimal residual disease (MRD) (*Strong recommendation*).
Summary of Recommendations

**Recommendation 13.** For pediatric patients with suspected or confirmed B-ALL, the pathologist or treating clinician should ensure that testing for t(12;21)(p13.2;q22.1); ETV6-RUNX1, t(9;22)(q34.1;q11.2); BCR-ABL1,KMT2A (MLL) translocation, iAMP21, and trisomy 4 and 10 is performed (Strong recommendation).

**Recommendation 14.** For adult patients with suspected or confirmed B-ALL, the pathologist or treating clinician should ensure that testing for t(9;22)(q34.1;q11.2); BCR-ABL1 is performed. In addition, testing for KMT2A (MLL) translocations may be performed. [Strong recommendation for testing for t(9;22)(q34.1;q11.2) and BCR-ABL1; Recommendation for testing for KMT2A (MLL) translocations].

**Recommendation 15.** For patients with suspected or confirmed ALL, the pathologist or treating clinician may order appropriate mutational analysis for selected genes that influence diagnosis, prognosis, and/or therapeutic management, which includes, but is not limited to, PAX5, JAK1, JAK2, and/or IKZF1 for B-ALL and NOTCH1 and/or FBXW7 for T-ALL. Testing for overexpression of CRLF2 may also be performed for B-ALL (Recommendation).
Summary of Recommendations

**Recommendation 16.** For pediatric and adult patients with suspected or confirmed acute myeloid leukemia (AML) of any type, the pathologist or treating clinician should ensure that testing for FLT3-ITD is performed. The pathologist or treating clinician may order mutational analysis that includes, but is not limited to, IDH1, IDH2, TET2, WT1, DNMT3A, and/or TP53 for prognostic and/or therapeutic purposes. [Strong recommendation for testing for FLT3-ITD; Recommendation for testing for other mutational analysis].

**Recommendation 17.** For adult patients with confirmed core-binding factor (CBF) AML (AML with t(8;21)(q22;q22.1); RUNX1-RUNX1T1 or inv(16)(p13.1q22)/t(16;16)(p13.1;q22); CBFB-MYH11), the pathologist or treating clinician should ensure that appropriate mutational analysis for KIT is performed. For pediatric patients with confirmed CBF-AML; RUNX1-RUNX1T1 or inv(16)(p13.1q22)/t(16;16)(p13.1;q22); CBFB-MYH11—the pathologist or treating clinician may ensure that appropriate mutational analysis for KIT is performed. [Strong recommendation for testing for KIT mutation in adult patients with CBF-AML; Expert consensus opinion for testing for KIT mutation in pediatric patients with CBF AML].
Summary of Recommendations

**Recommendation 18.** For patients with suspected acute promyelocytic leukemia (APL), the pathologist or treating physician should also ensure that rapid detection of PML-RARA is performed. The treating physician should also order appropriate coagulation studies to evaluate for disseminated intravascular coagulation (DIC) *(Strong recommendation).*

**Recommendation 19.** For patients other than those with confirmed core binding factor AML, APL, or AML with myelodysplasia-related cytogenetic abnormalities, the pathologist or treating clinician should also ensure that mutational analysis for NPM1, CEBPA, and RUNX1 is also performed *(Strong recommendation).*

**Recommendation 20.** For patients with confirmed acute leukemia, no recommendation is made for or against the use of global/gene-specific methylation, microRNA (miRNA) expression, or gene expression analysis for diagnosis or prognosis *(No recommendation).*
Summary of Recommendations

**Recommendation 21.** For patients with confirmed mixed phenotype acute leukemia (MPAL), the pathologist or treating clinician should ensure that testing for t(9;22)(q34.1;q11.2); BCR-ABL1, and KMT2A (MLL) translocations is performed *(Strong recommendation)*.

**Recommendation 22.** All laboratory testing performed for the initial workup and diagnosis of a patient with acute leukemia must be performed in a laboratory that is in compliance with regulatory and/or accreditation requirements *(Strong recommendation)*.

**Recommendation 23.** 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 *(Strong recommendation)*.
Summary of Recommendations

**Recommendation 24.** If a patient is referred to another institution for treatment, the primary institution should provide the treatment center with all laboratory results, pathology slides, flow cytometry data, cytogenetic information, and a list of pending tests at the time of the referral. Pending test results should be forwarded when they become available *(Strong recommendation).*

**Recommendation 25.** 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 *(Strong recommendation).*
Summary of Recommendations

**Recommendation 26.** 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 (*Strong recommendation*).

Note.—This information should include the sample source, adequacy, and collection information, as applicable.

**Recommendation 27.** Treating physicians and pathologists should use the current World Health Organization (WHO) terminology for the final diagnosis and classification of acute leukemia (*Strong recommendation*).
Discussion

- These guideline recommendations have been reviewed based upon available and mostly updated literature between 2015-2018 and expertise of the Expert Panel.

- The discussion points included mostly addresses issues regarding diagnostics that involve flow cytometry and molecular techniques.

- The Expert Panel strongly advises understanding the distinction between diagnostics that are needed in the first phase to start treatment (by available karyotyping, FISH and PCR techniques, or if possible, NGS) and subsequently treatment stratification, in contrast to the use of the findings in broader research [i.e. whole exome sequencing (WES), whole genome sequencing (WGS), RNA sequencing and epigenome study].

- Finally, complete reporting, including notification of the major risk- and stratification factors should be done in one final report, preferably available within 2 weeks from diagnosis.
Endorsement Statement

ASCO endorses the initial diagnostic workup for acute leukemia guideline from CAP and ASH.
Additional Resources

More information, including a Data Supplement with a reprint of all CAP & ASH recommendations, a Methodology Supplement, slide sets, and clinical tools and resources, is available at

www.asco.org/hematologic-malignancies-guidelines

Link to CAP & ASH guideline:


Patient information is available at www.cancer.net
# ASCO Guideline Panel Members

<table>
<thead>
<tr>
<th>Name (and designation)</th>
<th>Affiliation/Institution</th>
<th>Role/Area of Expertise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valerie de Haas, MD (Co-chair)</td>
<td>Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands</td>
<td>Pediatric Oncologist</td>
</tr>
<tr>
<td>Ling Zhang, MD (Co-chair)</td>
<td>Moffitt Cancer Center, Tampa FL</td>
<td>Hematopathologist</td>
</tr>
<tr>
<td>Rob Pieters, MD</td>
<td>Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands</td>
<td>Pediatric Oncologist</td>
</tr>
<tr>
<td>Dipti Patel Donnelly, MD</td>
<td>Virginia Cancer Specialist, Fairfax VA</td>
<td>Hematology/Medical Oncologist</td>
</tr>
<tr>
<td>Anjali Advani, MD</td>
<td>Cleveland Clinic, Cleveland OH</td>
<td>Hematology/Medical Oncologist</td>
</tr>
<tr>
<td>Kendra Sweet, MD</td>
<td>Moffitt Cancer Center, Tampa FL</td>
<td>Hematology/Medical Oncologist</td>
</tr>
<tr>
<td>Ching-Hon Pui, MD</td>
<td>St. Jude Children's Research Hospital, Memphis, TN</td>
<td>Hematology/Medical Oncologist</td>
</tr>
<tr>
<td>Daniel A Arber MD</td>
<td>University of Chicago Medical Center, Chicago, IL</td>
<td>Hematopathologist</td>
</tr>
<tr>
<td>Raetasha Dabney, MD</td>
<td>Keesler Medical Center, Ocean Springs, MS</td>
<td>PGIN representative</td>
</tr>
<tr>
<td>Elizabeth Kitlas</td>
<td>The Leukemia &amp; Lymphoma Society; Rye Brook, NY</td>
<td>Patient representative</td>
</tr>
<tr>
<td>Nofisat Ismaila, MD</td>
<td>American Society of Clinical Oncology, Alexandria, VA</td>
<td>Staff/health research methodologist</td>
</tr>
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