Work up of Acute Leukemia

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Learning objectives

• Discuss the updated testing guidelines for acute leukemia from College of American Pathologist (CAP)/American Society of Hematology (ASH)
• To know about the samples and tests needed at the time of initial evaluation on all patients
• Discuss the tests needed on a subset of acute leukemia patients
• Understand the prognostic/therapeutic implications of newer molecular tests in acute leukemia
• To be familiar with the newly approved targeted therapies

Agenda

• Introduction and CAP/ASH guidelines for specimen requirement and testing guidelines
• Discuss the broader classification of acute leukemia
• Discuss the specific subtypes of Acute lymphoblastic leukemia (ALL)
• Discuss the specific subtypes of Acute myeloid leukemia (AML)
• Elaborate the molecular genetics gene mutations with prognostic/therapeutic implications in Acute myeloid leukemia (AML)
Introduction

• Definition
  • ≥ 20% blasts (blood or marrow)
    • Select recurrent genetic abnormalities (with or without 20% blasts)
• Two broad categories: Lymphoid and Myeloid

• Complete diagnosis requires knowledge of clinical information, peripheral smear and bone marrow evaluation, immunophenotyping and karyotype analysis

• Molecular studies are often required

Introduction: Statistics

• Acute lymphocytic leukemia (ALL): 5,960 new cases/year
  • 75% cases seen in <6 years
  • 80-85% are precursor B-cell phenotype
  • 5 year survival rate = 85%

• Acute myeloid leukemia 19,520 new cases/year
  • Commonly seen in adults
  • 5 year survival rate = 27%
Key questions asked during initial work up

- What clinical and lab information should be available?
- What specimens and sample types should be evaluated?
- What tests are required for all patients?
- Which tests should be performed on only a subset of patients?
- Where should laboratory testing be performed?
- How should test results and diagnosis be correlated and reported?

1. What clinical and lab information should be available?

- Why do we need clinical information?
  - Down syndrome
  - Myeloid neoplasm with germline predisposition
  - Prior therapy
  - Use of recombinant granulocytic growth factors
  - Vitamin B12 or folic acid deficiency

2. What specimens and sample types should be evaluated on all cases?

- Peripheral blood, bone marrow (BM) aspirate and/or touch imprints
- BM core biopsy and/or marrow clot
- Peripheral blood (PB) may be used for ancillary studies
  - If there are adequate blasts
  - BM is inadequate
  - There is compelling reason to avoid BM
- Tissue biopsy for extramedullary disease without apparent BM or PB involvement
- Flow cytometry - should be comprehensive enough to distinguish between AML, B-ALL, T-ALL, and acute leukemia of ambiguous lineage
  - Essential for lineage assignment
- Conventional cytogenetics
2. What specimens and sample types should be evaluated? - continued

- If sufficient BM aspirate is not available for flow, a second core biopsy can be used for flow and genetic studies
- Should be unfixed (culture media)
- Non-decalcified paraffin-embedded (FFPE) or unstained BM aspirate can be used for nucleic acid extraction
  - Usually the clot sections
  - Depends on the lab and the validation

### Acute Leukemia: broader classification

- **Acute myeloid leukemia**
  - MPO (by flow cytometry, immunohistochemistry, or cytochemistry
  - Monocytic differentiation (≥2 of the following strongly expressed: CD11c, CD14, CD64, lysozyme)

- **Acute lymphoid leukemia**
  - Acute undifferentiated
  - Mixed phenotype

### Utility of cytochemical stains

- Not useful for ALL
- Can be useful sometime for AML
  - MPO and non-specific esterase
  - Speed and low cost
Cytogenetics and FISH studies

• Role of cytogenetics is critical for prognostic implications
• Provides a basis for classification and choice of initial and post remission therapy
• FISH -complimentary to an adequate cytogenetics
  • Many of the abnormalities of ALL are cryptic t(12;21) ETV6-RUNX1 or intrachromosomal amplification of chromosome 21
  • STAT FISH can be very helpful in acute promyelocytic leukemia (APL)
  • In other AMLs?

Molecular studies

• Most of the molecular studies can be performed on EDTA PB (if enough blasts) or bone marrow
• DNA and RNA extract and hold should be done on all the sample
• Molecular studies can be added later
• DNA or RNA extraction can also be performed on cryopreserved cells
Further work up of ALL

- Early T-cell precursor should be identified
- 10-13% of T-ALL
- Limited T-cell differentiation
- Express cytoplasmic CD3
- CD7+, lacks CD8 and CD1a and is positive for one or more myeloid associated markers (CD11b, CD13, CD33)
- Usually negative for CD5 and may express CD2 and/or CD4
- Mutation profile by NGS similar to AML
- NOTCH1 and FBXW7 mutations frequently seen
  - Lack prognostic significance

T-acute lymphoblastic leukemia (T-ALL)

- Early T-cell precursor should be identified
  - 10-13% of T-ALL
  - Limited T-cell differentiation
  - Express cytoplasmic CD3
  - CD7+, lacks CD8 and CD1a and is positive for one or more myeloid associated markers (CD11b, CD13, CD33)
  - Usually negative for CD5 and may express CD2 and/or CD4
  - Mutation profile by NGS similar to AML
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B-acute lymphoblastic leukemia (B-ALL): FISH

<table>
<thead>
<tr>
<th>Pediatric</th>
<th>Adult</th>
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</thead>
<tbody>
<tr>
<td>ETv6/RUNX1 (TEL/AML1) t(12;21) – good prognosis</td>
<td>BCR/ABL1 t(9;22)</td>
</tr>
<tr>
<td>Trisomy 4 and 10 – good prognosis</td>
<td>MLL (11q23)</td>
</tr>
<tr>
<td>BCR/ABL1 t(9;22)</td>
<td>AIMP21</td>
</tr>
</tbody>
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- ETV6/RUNX1 (TEL/AML1) t(12;21) – good prognosis
- Trisomy 4 and 10 – good prognosis
- BCR/ABL1 t(9;22)
- MLL (11q23)
- AIMP21
- BCR/ABL1 t(9;22)
BCR-ABL1 or Ph-like B-ALL: Why is it important to identify these patients?

- 10-15% in children and 25% in adults

- The most common abnormalities include CRLF2 rearrangements, JAK mutations, and erythropoietin receptor (EPOR) rearrangements
  - All three of these categories lead to activation of the JAK/STAT pathway

- Mutations involving ABL-class genes include ABL1, ABL2, CSF1R, PDGFRα, and PDGFRβ. Other mutations and fusions include IKZF1, FGFR1, and RAS
  - Can be treated with tyrosine kinase inhibitors

Ph-like ALL test algorithm

- Positive for one of the major genetic abnormalities by FISH:
  - BCR-ABL1
  - MLL
  - Hyperdiploidy
  - ETV6-RUNX1
  - TCF3-PBX1

- Flow Cytometry + Ped/adult ALL FISH panel

- Further testing if needed

Distribution of Ph-like ALL subgroups among children, adolescents, and young adults.
Acute leukemia of ambiguous lineage

**Broader classification**

Acute Leukemia: Broad Classification

**Acute leukemia of ambiguous lineage**

- Acute undifferentiated
- Mixed phenotype
  - Mixed phenotype acute leukemia with t(9;22) BCR-ABL1
  - Mixed phenotype acute leukemia with t(1;14)(q32.3) KMT2A-rearranged
  - Mixed phenotype acute leukemia, B/myeloid not otherwise specified
  - Mixed phenotype acute leukemia, T/myeloid not otherwise specified

Acute myeloid leukemia (AML)
Acute promyelocytic leukemia (APL)

- Bone marrow packed with highly granular abnormal promyelocytes (no maturation)
- Hypergranular or microgranular
- Unique risk of fatal hemorrhage due to activation of both coagulation and fibrinolytic pathway on top of production defect
- Medical emergency
- Highly curable: Vitamin A (ATRA) / arsenic trioxide and chemotherapy

APL: rapid diagnosis

AML: further testing

- For pediatric or adult patients with suspected or confirmed AML of any type
  - FLT3-ITD should be performed on all AML cases
- Other mutational testing including IDH1, IDH2, TET2, WT1, DNMT3A and or TP53 is recommended
AML: Cytogenetics

- AML without regard to blast count
- Core binding factor (CBF) leukemia and FLT mutation

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>%</th>
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<tbody>
<tr>
<td>t(15;17)</td>
<td>10</td>
</tr>
<tr>
<td>inv(16)/t(16;16)</td>
<td>9</td>
</tr>
<tr>
<td>11q23</td>
<td>7</td>
</tr>
<tr>
<td>Other</td>
<td>32</td>
</tr>
<tr>
<td>t(8;21)</td>
<td>8</td>
</tr>
<tr>
<td>-5/-5q</td>
<td>-</td>
</tr>
<tr>
<td>-7/-7q</td>
<td>-</td>
</tr>
<tr>
<td>+8</td>
<td>-</td>
</tr>
<tr>
<td>+11</td>
<td>-</td>
</tr>
<tr>
<td>del(9q) without t(8;21)</td>
<td>-</td>
</tr>
<tr>
<td>11q23, 17p, 20q or 21q</td>
<td>-</td>
</tr>
<tr>
<td>t(9;22)</td>
<td>-</td>
</tr>
<tr>
<td>t(6;9)</td>
<td>-</td>
</tr>
<tr>
<td>inv(16) AML (n=30)</td>
<td>-</td>
</tr>
<tr>
<td>t(15;17) AML (n=19)</td>
<td>-</td>
</tr>
<tr>
<td>t(8;21) AML (n=15)</td>
<td>-</td>
</tr>
<tr>
<td>11q23 AML (n=11)</td>
<td>-</td>
</tr>
</tbody>
</table>

Recurring Cytogenetic Abnormalities in Adult AML

Cytogenetic Risk Groups

<table>
<thead>
<tr>
<th>Low</th>
<th></th>
<th>Intermediate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(8;21)</td>
<td></td>
<td>Normal karyotype</td>
<td>Complex (≥3)</td>
</tr>
<tr>
<td>inv(16)/i(14;16)</td>
<td></td>
<td>Single abnormally</td>
<td>abnormalities</td>
</tr>
<tr>
<td>t(15;17)</td>
<td></td>
<td>+8</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+11</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12p abnormalities</td>
<td>+</td>
</tr>
</tbody>
</table>

Molecular studies in AML

Mutational complexity of AML

**FLT3 and IDH1/2**

**FLT3 (FMS-like tyrosine kinase 3)**
- Receptor tyrosine kinase involved in hematopoiesis
- Frequency in AML:
  - ITD ~23%
  - TKD ~7%
- Prognostic significance:
  - ITD = negative
  - TKD = unclear
- Therapeutic significance:
  - Midostaurin and other drugs approved for FLT3 mutated AML
- How to measure:
  - RT PCR
  - Next generation sequencing

**IDH1 and IDH2**
- Isocitrate dehydrogenase 1, 2
- Intracellular metabolism and epigenetic regulation, DNA methylation
- Frequency in AML:
  - IDH1 ~ 6-10%
  - IDH2 ~ 8-10%
- Prognostic significance:
  - unclear
- Therapeutic significance:
  - Enasidenib and other drugs approved to treat relapsed/refractory IDH1 or IDH2 mutated AML
- How to measure:
  - RT PCR, Sanger sequencing, NGS
**NPM1 and CEBPA**

**NPM1 (nucleophosmin)**
- Phosphoprotein involved in ribosome biogenesis, cell proliferation, and apoptosis
- Frequency in AML:
  - 27-35%
- Prognostic significance
  - NPM1<sup>mut</sup> and FLT3-ITD<sup>wt</sup> favorable
  - NPM1<sup>mut</sup> better prognosis than normal karyotype AML and NPM1<sup>wt</sup>
- Therapeutic significance
  - May not need allogeneic HCT in first remission

**CEBPA (CCAAT/enhancer- binding protein alpha)**
- Transcription factor involved in neutrophil differentiation
- Frequency in AML
  - Monoallelic: 3-4%
  - Biallelic: 4-6%
- Prognostic significance
  - Monoallelic similar to wild type
  - Biallelic and normal karyotype has favorable prognosis
- Therapeutic significance
  - May not need allogeneic HCT in first remission


**KIT**
- Receptor tyrosine kinase involved in proliferation, differentiation, survival
- Frequency in AML
  - 3<sup>+</sup>
- Prognostic significance
  - Adults: KIT (high >25% VAF)+ CBF AML worse prognosis
  - Pediatric: unclear
- Therapeutic significance
  - Allogeneic HCT in relapsed/refractory KIT+ CBF AML
- How to measure?
  - PCR, NGS


**Molecular Testing Algorithm**

- Strong recommendation
- Recommendation
- Expert consensus
- AML
- BFU
- AML other than CBF, APL, and MDS
  - Complex karyotype, MDS
- MDS, CMML
- APL
- PML-RARA
  - Coagulation studies
- Should test KIT
- May test NPM1, CEBPA

- Should test KIT
- May test AML
PCR and NGS methodologies used for molecular testing in routine practice

<table>
<thead>
<tr>
<th>Method</th>
<th>PCR-based methods</th>
<th>Traditional Advent-Genetic sequencing</th>
<th>NGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol specificity</td>
<td>Rapid, specific with high throughput</td>
<td>High degree of specificity with high throughput</td>
<td>High throughput with high specificity</td>
</tr>
<tr>
<td>Limitation of detection</td>
<td>Limited range of detection, high cost, limited range of information per experiment</td>
<td>Low cost, high throughput, limited range of detection</td>
<td>High throughput with high specificity</td>
</tr>
<tr>
<td>Methodological hurdles</td>
<td>High cost, limited sensitivity, high throughput, limited range of information per experiment</td>
<td>Low cost, high throughput, limited range of detection</td>
<td>High throughput with high specificity</td>
</tr>
</tbody>
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Case #1

- A 37-year-old man presents to the emergency department complaining of fatigue and shortness of breath with a two-week history of worsening exercise tolerance and a rather abrupt onset of shortness of breath over the past several hours. The patient has no major past medical history and works as an architect. Her laboratory results reveal the following:
  - White blood cells - 74.1 × 10^9/L
  - Hemoglobin - 7.3 g/dL
  - Platelet count - 24 × 10^9/L
  - White blood cell (WBC) differential is notable for 39% blasts (don’t look like promyelocytes)
Next Step

• Flow cytometry was performed

• Showed CD34, CD13, CD33, HLADR, CD117 and MPO
  • AML

• What should be our next step?

Testing algorithm

• Two options
  • Targeted PCR/RT or Sanger Sequencing- NPM1, CEBPA, FLT3
  • NGS sequencing- will have all the genes
    • Turn around time is longer
    • Might not work for FLT3 testing
Case #2

• A 37-year-old man presents to the emergency department complaining of fatigue and shortness of breath with two-week history of worsening exercise tolerance and a rather abrupt onset of shortness of breath over the past several hours. The patient has no major past medical history and works as an architect. Her laboratory results reveal the following:
  • White blood cells -74.1 × 10⁹/L
  • Hemoglobin-7.3 g/dL
  • Platelet count-24 × 10⁹/L
  • White blood cell (WBC) differential is notable for 39% blasts.

Next Step

• Flow cytometry was performed as the initial step
  • Showed CD34, CD10, CD19, CD22 and TdT
  • Diagnosis - B-ALL

• What should be our next step?

Testing algorithm
Conclusion

• Laboratory evaluation is critical, though complex
• Morphologic evaluation, immunophenotyping, and karyotype analysis should be performed on all cases
• Molecular genetic testing is evolving with targeted therapies
• Ongoing updates will be needed for the guideline to remain relevant

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Thank you!