Test Utilization in Newly Diagnosed Myelodysplastic Syndrome and Acute Myeloid Leukemia: An Algorithmic Approach

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DISCLOSURES:

Relevant Financial Relationship(s)
None

Off Label Usage
None
Learning Objectives

• Identify standard components in the bone marrow evaluation for a new diagnosis/ suspicion of MDS and AML

• Compare the benefits and limitations of conventional cytogenetic analysis and FISH in these disorders

• Discuss an algorithmic approach to the diagnostic work-up for MDS and AML
MDS

- Clonal hematopoietic stem cell disorder
- Heterogeneous disorder; varying risk for transformation to AML
- Ineffective hematopoiesis
- Diagnosis: cytopenia(s), dysplasia in 1 or more cell lineages, blast % in PB and BM/presence of Auer rods, hypercellular bone marrow, genetics
<table>
<thead>
<tr>
<th>Category</th>
<th>Blood</th>
<th>BM</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCUD</td>
<td>Uni- or bicytopenia, &lt;1% blasts</td>
<td>Unilineage dysplasia; &lt;5% blasts; &lt;15% ring sideroblasts</td>
</tr>
<tr>
<td>RARS</td>
<td>Anemia, no blasts</td>
<td>Erythroid dysplasia; ≥15% ringed sideroblasts; &lt;5% blasts</td>
</tr>
<tr>
<td>RCMD (RS)</td>
<td>Cytopenia(s), no monocytosis, &lt;1% blasts</td>
<td>Dysplasia ≥10% of cells in ≥2 lineages, &lt;5% blasts, no Auer rods, (≥15% ringed sideroblasts)</td>
</tr>
<tr>
<td>RAEB (1, 2)</td>
<td>Cytopenia(s), no monocytosis</td>
<td>1; uni- or multilineage dysplasia, 5-9% blasts, no Auer rods 2; uni- or multilineage dysplasia, 10-19% blasts, and/or Auer rods</td>
</tr>
<tr>
<td>MDS-U</td>
<td>Cytopenias, ≤1% blasts</td>
<td>Unilineage dysplasia, no Auer rods Unequivocal dysplasia in &lt;10% but with cytogenetic abnormality = presumptive</td>
</tr>
<tr>
<td>Isolated del5q</td>
<td>Anemia, normal or increased platelets, &lt;1% blasts</td>
<td>NI to ↑megakaryocytes with hypolobated nuclei, &lt;5% blasts, isolated del5q, no Auer rods</td>
</tr>
</tbody>
</table>
IPSS/IPSS-R and Prognosis

- Blast percent (bone marrow)
  - ≤2%
  - >2 - <5%
- Cytogenetic risk group
  - 5 groups
- Hemoglobin
- ANC
- Platelet count

Impact on Survival

Greenberg PL et al. Blood 2012;120:2454-2465
Components in the Pathologic Evaluation of Newly-Diagnosed MDS

- CBC with differential count and peripheral blood smear review
- Bone marrow aspiration and biopsy
- Iron stain
- Conventional cytogenetics
- Fluorescence in situ hybridization
Recurring Cytogenetic Abnormalities in MDS

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Incidence (%)</th>
<th>Risk group</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDS</td>
<td>t-MDS</td>
<td></td>
</tr>
<tr>
<td>5q-</td>
<td>10-15</td>
<td>Good (isolated or +1)</td>
</tr>
<tr>
<td>-7 / 7q-</td>
<td>10</td>
<td>Poor</td>
</tr>
<tr>
<td>+8</td>
<td>10</td>
<td>Intermediate</td>
</tr>
<tr>
<td>20q-</td>
<td>5-8</td>
<td>Good</td>
</tr>
<tr>
<td>-Y</td>
<td>5</td>
<td>Very good</td>
</tr>
<tr>
<td>i(17q)/t(17p)</td>
<td>3</td>
<td>Intermediate</td>
</tr>
<tr>
<td>12p-</td>
<td>3</td>
<td>Good</td>
</tr>
<tr>
<td>Complex &gt;3</td>
<td>&lt;5</td>
<td>Very poor</td>
</tr>
</tbody>
</table>

Conventional cytogenetics and vs FISH in MDS

Key Questions

Q1) How does FISH compare to routine cytogenetics in providing genetic information in MDS?

Q2) How does FISH compare to routine cytogenetic analysis for detection of del(5q)?
Study Methods

• Specimen requirements
  • Bone marrow specimen with morphology
  • Cytogenetics performed
  • FISH performed for -5/5q, -7/7q, +8, 11q-, 13q-, 20q-, t(3;21), inv(3)/t(3;3)

• Cases from January 2007-October 2011

• Compared conventional cytogenetic and FISH findings
Results
In the setting of 20 metaphases

458 total cases

- Concordance (91.5%)
  - 310 Normal karyotype and Normal FISH (68%)
  - 96 Abnormal karyotype and Abnormal FISH (21%)
  - 12 Non-clonal cytogenetics and Normal FISH (2.5%)

- Discordance (8.5%)
  - 11 Normal cytogenetics and Abnormal FISH (2.5%)
  - 27 Abnormal cytogenetics and Normal FISH (6%)
  - 2 Non-clonal cytogenetics and Abnormal FISH (<1%)
Normal Cytogenetics and Abnormal FISH (11 cases)

- **MDS (6):** 7q- (18%), +3/+8/+13 (3%), +8 and 20q- (~7.5%), +3q26 (MECOM) (25%), 21q- (RUNX1) (65%)/ amp 3q26 (MECOM) (14%), +3/+5 (6%)

- **MDS/MPN (1):** 7q-(15%)

- **AML (2):** +8 (2%); +RUNX1x2 and +8 (~5%)

- **No myeloid malignancy (myeloma) (2):** +3 (1.8%); 11q23-/13q- (35%)
Abnormal Cytogenetics and Normal FISH (27 cases)

- 19 cases (70%): FISH probes not targeted to abnormal regions
- 8 cases: MDS (2), MDS/MPN (2), no myeloid malignancy (4)
  - 7 cases: low numbers of clonal metaphases (<3)
  - 1 case (del(11)(q13q23) [20]): Probe may not cover the region. Metaphase FISH not done.
Results

In the setting of 1-19 metaphases

• 34 total cases
  • Concordant (28 cases) (82%)
    • 21 Normal cytogenetics and Normal FISH
    • 7 Abnormal cytogenetics and Abnormal FISH (match)
  • Discordant (6 cases) (18%)
    • 4 Normal cytogenetics and Abnormal FISH
    • 2 Abnormal cytogenetics and Normal FISH
Discordant Cases with <20 Metaphases

- Normal Cytogenetics and Abnormal FISH (n = 4) (12%)
  - MDS (3): 5q- and 7q-; 7q-; 20q –
  - AML (1): +8

- Abnormal Cytogenetics and Normal FISH (n = 2)
Cytogenetics versus FISH to detect del(5q) in MDS

- Detection of del(5q) predicts for response to lenalidomide therapy

- 302 total cases
  - Concordance (92%)
    - Absence of del(5q) in 256 cases
    - Presence of del(5q) in 22 cases
Cytogenetics versus FISH to detect del(5q) in MDS

- Discordance (8%)
  - (3%) Non-clonal (1 metaphase) showing del(5q)/ FISH negative (n = 8)
  - (5%) Absence of classic del(5q) by cytogenetics/ FISH positive (n = 16)
    - 14 with add(5q) (13 w/ complex karyotype)
    - 2 with <20 metaphases

→ No cases with normal karyotype showed del(5q) by FISH
Conclusions

• Conventional cytogenetics
  • Should be performed in all newly diagnosed MDS cases

• FISH
  • Should not routinely be performed with an adequate cytogenetic study (20 metaphases)
  • Excellent correlation of MDS FISH panel and cytogenetics including del(5q)
Conclusions

• FISH (cont’d)
  • Reserve FISH testing in MDS for cases with inadequate karyotype
  • Perform targeted FISH
    • to resolve karyotypic ambiguities
    • to confirm specific genetic alteration as necessary
## Selected Literature on Cytogenetics vs FISH in MDS

<table>
<thead>
<tr>
<th>Author/Year</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jiang et al./2012</td>
<td>FISH has limited value with successful metaphase analysis</td>
</tr>
<tr>
<td>Coleman J et al./2011</td>
<td>FISH was rarely abnormal in cases with 20 normal metaphases</td>
</tr>
<tr>
<td>Yang et al./2010</td>
<td>FISH testing informative in karyotypic failure</td>
</tr>
<tr>
<td>Pitchford et al./2010</td>
<td>FISH is of little additional value in the setting of adequate normal karyotype</td>
</tr>
<tr>
<td>Douet-Gilbert et al./2011</td>
<td>FISH does not improve del(5q) or del(20q) detection with a normal karyotype</td>
</tr>
<tr>
<td>Bernasconi et al/2003</td>
<td>FISH detects occult defects in about 15% of cytogenetically normal MDS patients</td>
</tr>
<tr>
<td>Cherry et al/2003</td>
<td>Interphase FISH studies are nearly as sensitive as cytogenetic analyses</td>
</tr>
<tr>
<td>Ketterling et al/2002</td>
<td>Limited additional utility for FISH in MDS in the setting of an adequate karyotypic analysis</td>
</tr>
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</table>
Practical Algorithm for Effective Test Utilization in MDS

Clinical Suspicion of MDS

CBC, Differential, Peripheral Blood Smear, Iron Stain, Bone Marrow Evaluation
Pathology: MDS/Rule out MDS

Conventional Cytogenetic Analysis

- <20 Metaphases
  - MDS FISH Panel

- 20 Metaphases; normal or clonal
  - STOP
  - or
  - Do targeted FISH to confirm/resolve genetic abnormality
Acute Myeloid Leukemia (AML)

- Clonal hematopoietic stem cell disorder
- Clonal expansion of myeloid blasts
- Heterogeneous disorder
- Requisite blast count is \( \geq 20\% \) unless in select cases there is an associated specific genetic abnormality
WHO 2008 Classification of AML

• AML with recurrent genetic abnormalities
• AML with myelodysplasia-related changes
• AML, therapy-related
• AML, not otherwise specified
• Provisional entities
  • AML with mutated NPM1
  • AML with mutated CEBPA
Potential Components in the Pathologic Evaluation of Newly-Suspected AML

• CBC with differential count and peripheral blood smear review
• Bone marrow aspiration and biopsy
• Flow cytometric immunophenotyping
• Conventional cytogenetics
• Fluorescence in situ hybridization
• Molecular analysis
Impact of cytogenetic entities recognized in 2008 WHO classification

### Recurring Cytogenetic Abnormalities in AML

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Incidence %</th>
<th>Risk group</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(15;17)(q24;q21); <em>PML-RARA</em></td>
<td>12</td>
<td>Favorable</td>
</tr>
<tr>
<td>t(8;21)(q22;q22); <em>RUNX1-RUNX1T1</em></td>
<td>5</td>
<td>Favorable</td>
</tr>
<tr>
<td>inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); <em>CBFB-MYH11</em></td>
<td>5</td>
<td>Favorable</td>
</tr>
<tr>
<td>t(9;11)(p22;q23); <em>MLLT3-MLL</em></td>
<td>2</td>
<td>Intermediate</td>
</tr>
<tr>
<td>t(6;9)(p23;q34); <em>DEK-NUP214</em></td>
<td>1</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>inv(3)(q21q26.2) or t(3;3)(q21;q26.2); <em>RPN-MECOM</em></td>
<td>1</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>t(1;22)(p13;q13); <em>RBM15-MKL1</em></td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Normal karyotype</td>
<td>40-50%</td>
<td>Dependent on molecular alterations</td>
</tr>
<tr>
<td>Complex &gt;3</td>
<td>15-25%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>Monosomatal karyotype</td>
<td>15-18%</td>
<td>Unfavorable</td>
</tr>
</tbody>
</table>

Conventional cytogenetics and vs FISH in AML

Key Question
How does FISH compare to routine cytogenetics in providing cytogenetic information in AML?

t(8;21)(q22;q22); RUNX1-RUNX1T1
Study Methods

• Specimen requirements
  • Bone marrow specimen with AML morphology
  • Cytogenetics performed
  • FISH performed for RUNX1/RUNX1T1, PML/RARA, MLL, MYH11/CBFB, and in some cases DEK/NUP214, BCR/ABL1, -5/5q, -7/7q, +8.

• Cases from 2006-2013

• Compared conventional cytogenetic and FISH findings
Results
In the setting of 20 metaphases

220 total cases

• Concordance (93%)
  • 89 Normal karyotype and Normal FISH
  • 116 Abnormal karyotype and Abnormal FISH

• Discordance (7%)
  • 7 Normal cytogenetics and Abnormal FISH
  • 8 Abnormal cytogenetics and Normal FISH
Normal Cytogenetics and Abnormal FISH (n = 7 cases)

- **Significant /potentially significant (n = 2) (1%)**
  - Cryptic inv(16); *CBFB-MYH11* (68%) (abnormal eos and monocytic morphology)
  - +8 and del 13q (9%) (Non-clonal cytogenetic abnormality showed +8)

- **No clear significance (n = 5)**
  - *AML1x3* (5%)
  - +8 , *AML1x3*, *PMLx3* (5-12%)
  - 13q- (27%) (pt also has CLL)
  - 13q- (26%) (pt also has CLL)
  - Del(9p) (Not part of the AML FISH panel)
Abnormal Cytogenetics and Normal FISH (n = 8 cases)

• 7 cases:
  • FISH probes not targeted to abnormal regions

• 1 case:
  • Trisomy 8 in two (of 30) metaphases (low number)
Discordant Cases with <20 Metaphases

- Normal Cytogenetics and Abnormal FISH (n = 3)
  - Non-clonal abnormalities (2 cases)
    - Intermediate risk group assignment (trisomy 8 in 31%) (Total of 2 metaphases)
    - Potential adverse risk group assignment (del(13q) in 11%)
  - Only 3 metaphases (1 case)
    - No significance, trisomy 4 by FISH (not part of usual panel)

- Abnormal Cytogenetics and Normal FISH (n = 0)
Conclusions

• Conventional cytogenetics
  • Should be performed in all newly diagnosed cases of AML

• FISH
  • Should not be routinely performed with an adequate study (20 metaphases)
    • Excellent correlation of AML FISH panel and cytogenetics
Conclusions

• FISH (cont’d)
  • If monocytic differentiation, FISH for *CBFB-MYH11* [Inv(16)]
  • Reserve FISH testing
    • for cases with inadequate karyotype
    • to confirm utility for potential MRD testing
    • to resolve karyotypic ambiguities
# Selected Literature on Cytogenetics vs FISH in AML

<table>
<thead>
<tr>
<th>Author/Year</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vance GH et al</td>
<td>Concordance rate for cytogenetics and FISH was 98-100%</td>
</tr>
<tr>
<td>/2007</td>
<td></td>
</tr>
<tr>
<td>Frohling S et al</td>
<td>In most AML cases, FISH should not replace cytogenetics. FISH should be used in detection of subtle abnormalities (MLL, inv(16) and suspected t(8;21)var].</td>
</tr>
<tr>
<td>/2002</td>
<td></td>
</tr>
<tr>
<td>Cox MC et al</td>
<td>FISH screening does not add relevant information to cytogenetics, with an adequate study.</td>
</tr>
<tr>
<td>/2003</td>
<td></td>
</tr>
<tr>
<td>Klaus M et al</td>
<td>In most AML cases, FISH should not replace cytogenetics. FISH should be used in detection of subtle abnormalities (MLL), ETV6 (12p13) and chr. 13 abnormalities.</td>
</tr>
<tr>
<td>/2004</td>
<td></td>
</tr>
</tbody>
</table>
Practical Algorithm for Effective Test Utilization in AML

1. **Clinical Suspicion of AML**
2. **CBC, Differential, Peripheral Blood Smear, Bone Marrow Evaluation**
   - Pathology: Acute Leukemia
3. **Flow Cytometric Immunophenotyping**
   - Conventional Cytogenetic Analysis
   - Cytochemistries
   - DNA extract and hold
4. **AML Diagnosis established, not APL**
Practical Algorithm for Effective Test Utilization in AML (cont’d)

Genetic Testing for Additional Prognostication and Potential Therapeutic Purposes

- **NO**
  - STOP

- **YES**
  - Testing done in at least normal karyotype or all cases
    - FLT3, NPM1, CEBPA
  - Testing done based on initial pathologic and genetic findings
    - FISH for MLL (11q23) and CBFB-MYH11[Inv(16)], if monocytic differentiation
    - FISH for RUNX1-RUNX1T1 [t(8;21)], if suspect by morphology and/or immunophenotyping
    - FISH to clarify use of FISH probe to detect an abnormality based on cytogenetics
    - KIT sequencing in core binding factor-AML
Summary

Diagnostic tests for patients with suspected MDS

• Bone marrow aspirate and biopsy
• Iron stain
• Conventional cytogenetic analysis
• FISH testing generally unnecessary
Summary

Diagnostic tests for patients with suspected AML

- Bone marrow aspirate and biopsy
- Flow Cytometric Immunophenotyping
- Cytochemical stains (as available)
- Conventional cytogenetic analysis
- DNA Extract and Hold
- FISH and DNA testing as per algorithm
Summary

- Algorithms reflect clinical, pathology, genetic and laboratory collaboration
- Algorithms are not static
- At least annual review of algorithms and modification as necessary
- Cost savings
Questions & Discussion