The Bone Marrow Comprehensive Report: Advances in Molecular and Cytogenetic Risk Stratification in AML and MDS

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Because ancillary testing is necessary to supplement morphologic features in the diagnosis of most hematologic neoplasms, hematopathologists have traditionally consolidated a variety of information into diagnostic pathology reports. Initially, this information included clinical features of the patient, morphologic features and tests that represented cytochemical and immunophenotyping studies. Cytogenetic and molecular genetic studies, however, are increasingly important and necessary for complete diagnosis and for predicting prognosis in most hematologic neoplasms. The logistics of reporting all of this information, which includes tests that may be resulted at different times, is a challenge for all pathology specialties. The complexities of the information produced by next generation sequencing methods significantly increases this challenge for pathologists.

The Current State

Bone marrow studies are particularly complex because they typically include evaluation of peripheral blood, bone marrow aspirate and bone marrow trephine biopsy specimens. All of these samples generate key information and may be tested by a variety of methods. The peripheral blood is typically accompanied by complete blood count data that should be included with the morphologic description. The bone marrow aspirate evaluation typically includes a differential cell count. Both the blood and marrow aspirate material may be subjected to cytochemical stains and flow cytometry immunophenotyping and may be submitted for karyotype analysis. The bone marrow core biopsy may be studied by additional histochemical and immunohistochemical methods. All sample types may undergo additional genetic and molecular genetic testing. Some of these tests are requested at the time of the bone marrow aspiration and biopsy procedure and others are added by the reviewing pathologist based on the morphologic features of the case. All of these tests may generate a separate report and without a consolidated or comprehensive report, the ordering physician is left to sort through a huge amount of potentially contradictory data that may be created by sampling differences and accepted false negative finding based on the methodology. Without an expert reviewing all of the testing performed, the individual reports may suggest incorrect diagnoses and a firm diagnosis may never be documented in the medical record.

Reporting guidelines related to hematopathology samples are published from a variety of organizations. Recognizing the pathologists’ role in this process and the value of
consolidated reporting, the College of American Pathologists recommends the following
elements of a bone marrow report:

- Clinical information
- Aspirate and biopsy sites
- Peripheral blood
- Marrow aspirate/touch preps
- Marrow biopsy/clot
- Immunophenotyping
- Cytogenetics
- Molecular genetics
- Other ancillary tests
- Diagnosis

Genetic Features of Acute Myeloid Leukemia (AML) and
Myelodysplastic Syndrome (MDS)

The complete diagnosis and reporting of samples involved by AML or MDS are highly
dependent on incorporating genetic testing. In the past, using older classification
systems, cytogenetic risk groups were recognized and treated independently of the
pathologic interpretation. Using current classification systems for AML, the correct
diagnosis can only be made by knowing the cytogenetic or molecular genetic testing
results. The specific disease groups within the 2008 WHO classification of hematologic
neoplasms category of acute myeloid leukemias with recurring genetic abnormalities (Table 1) require this information and a significant subset of cases diagnosed as acute
myeloid leukemia with myelodysplasia-related changes (Table 2) are identified only by the
detection of a clonal, myelodysplasia-related cytogenetic abnormality. Because
of these diagnostic requirements in the 2008 WHO classification, cytogenetic studies
must be performed on all cases of suspected acute leukemia.

Mutation analysis is also important in the diagnosis and prognosis of AML; particularly
in the many AML cases with normal karyotypes. The WHO classification currently
includes two provisional entities based on the detection of gene mutations (NPM1 and
CEBPA), but also recommends testing for FLT3, especially in normal karyotype AML,
and KIT in core binding factor AML [AML with t(8;21)(q22;q22); RUNX1-RUNX1I and
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11]. In these
settings, detection of FLT3 or KIT provides prognostic information which may impact
future therapy. While these four relatively common gene mutations are specifically
mentioned in the WHO classification, many other mutations are now recognized in AML
as having prognostic significance (Table 3) and many of them occur in combination
with the different combinations imparting different prognostic significance. Epigenetic
changes, including gene methylation, also have prognostic implications in AML and
will further complicate the interpretation of genetic data.
Karyotype information is also important in the myelodysplastic syndromes with one specific category of the WHO classification defined by the presence of a solitary abnormality [MDS with isolated del(5q)]. The detection of some cytogenetic abnormalities now allows for a diagnosis of MDS, unclassifiable, even in the absence of features of a more specific MDS category (Table 4). For all categories of MDS, prognostic scoring systems are used that rely on knowledge of the karyotypic features of the sample. Detection of gene mutations is also important in MDS and provides additional prognostic information (Table 5). While many of the mutations in MDS are only recently discovered, the demand for this testing is increasing and will quickly become a standard of care.

The large number of genetic changes that may occur in AML and MDS with prognostic significance creates new challenges in testing and diagnosis. In the past, with only a few mutations recognized to be significant, standard polymerase chain reaction (PCR)-based tests could be performed. This approach, however, usually only targets a few “hot spots” for mutation for a given gene and will miss mutations that occur outside of the common hot spots. Now, with many more mutations, often involving multiple exons on a gene, and the knowledge that multiple mutations may occur in a single sample, a targeted PCR approach becomes extremely labor intensive and expensive. Next generation molecular assays, which include but are not restricted to whole genome sequencing, allow broader coverage of multiple genes. The price of this technology is dropping dramatically and is becoming more cost-effective than older methods. This approach can cover very large gene panels for diseases and panels of this type are already available from some laboratories. While this approach will become cost-effective from the technical side, the interpretation of such complex data requires new approaches. Pathologists will need to play an active role in determining what information is reported and how it is incorporated into diagnostic reports.

Obstacles to Comprehensive Reporting

Although new technology is often daunting, pathologists have traditionally embraced advances in technology and must do the same with next generation sequencing. While these methods will probably not become part of the routine testing of most laboratories, they will be tests that are integral to our diagnosis and pathologists must learn the basics of how to order and use these methods for the care of their patients. This would include learning about the specific markers and their diagnostic and prognostic significance. Again, pathologists are usually leaders in the integration of new markers into the diagnosis of disease with their continuous acceptance of new immunohistochemical markers used in a wide variety of disorders.

Unfortunately, technology is a major obstacle to comprehensive reporting in pathology. While methods to quickly analyze large data sets in a way that is suitable for clinical use will be certainly occur in the near future, pathology information systems are generally not developed with complex reporting in mind. These systems are typically developed with each area of testing separated and manual methods are often
necessary to pull data from the different areas into a single report. The testing that is performed is sometimes unnecessary due to a variety of different providers ordering testing without knowledge of results in other areas, making the comprehensive reporting of results not only labor intensive, but awkward. In some centers, such as the Mayo Clinic, pathologists have taken the lead to ensure that the proper tests are performed and reported. In these centers, the pathologist negotiates a testing algorithm with the treating service and only the pathologist involved the diagnosis orders the testing necessary for the case. This approach has been found to result in not only a better disease evaluation, but a cost-savings for the involved institution. Others, such as Vanderbilt University, have taken this approach even further and have developed their own electronic system to pull data from different reports in different information system to create a comprehensive report in the patient’s medical record.

The above efforts suggest that most technical obstacles to comprehensive reporting can be overcome. That would leave apathy as possibly the biggest obstacle to comprehensive reporting. Some pathologists are content with letting the treating physician accumulate and interpret reports. Others feel they just do not have time to put all of the data together. This approach results in excessive testing, which make our areas cost-centers in the eyes of hospital leadership, and diminishes our standing as physicians if we give the impression that we are not interested in provided comprehensive care to the patient that is represented by the sample we receive.
Table 1. AML with recurrent genetic abnormalities in the 2008 WHO classification

- AML with t(8;21)(q22;q22); RUNX1-RUNX1
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11
- Acute promyelocytic leukemia with t(15;17)(q22;q12); PML-RARA
- AML with t(9;11)(p22;q23); MLLT3-MLL
- AML with t(6;9)(p23;q24); DEK-NUP214
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1
- AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1
- Provisional entities
  - AML with mutated NPM1
  - AML with mutated CEBPA

Table 2. Karyotype abnormalities sufficient to diagnose a non-therapy-related AML as being acute myeloid leukemia with myelodysplasia related changes in the 2008 WHO classification.

- Complex karyotype (3 or more clonal abnormalities)
- Unbalanced abnormalities
  - -7/del(7q)
  - -5/del(5q)
  - i(17q)/t(17p)
  - -13/del(13q)
  - del(11q)
  - del(12p)/t(12p)
  - del(9q)
  - idic(X)(q13)
- Balanced abnormalities
  - t(11;16)(q23;p13.3)
  - t(3;21)(q26.2;q22.1)
  - t(1;3)(p36.3;q21.1)
  - t(2;11)(p21;q23)
  - t(5;12)(q33;p12)
  - t(5;7)(q33;q11.2)
  - t(5;17)(q33;p13)
  - t(5;10)(q33;q21)
  - t(3;5)(q25;q34)
Table 3. Gene-specific abnormalities in acute myeloid leukemia. Adapted from Ofran and Rowe.11

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency in AML</th>
<th>Reported prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPM1</td>
<td>30-35%</td>
<td>Favorable</td>
</tr>
<tr>
<td>FLT3 ITD</td>
<td>25%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>15-25%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>NRAS/KRAS</td>
<td>15-20%</td>
<td>Neutral</td>
</tr>
<tr>
<td>WT1</td>
<td>10-15%</td>
<td>Neutral to unfavorable</td>
</tr>
<tr>
<td>RUNX1</td>
<td>10-15%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>IDH2 R132</td>
<td>7-16%</td>
<td>Variable</td>
</tr>
<tr>
<td>IDH2 R140 and R172</td>
<td>8-15%</td>
<td>Variable</td>
</tr>
<tr>
<td>TET2</td>
<td>8-12%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>MLL</td>
<td>5-10%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>ASXL1</td>
<td>3-19%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>FLT3 TKD</td>
<td>7%</td>
<td>Neutral</td>
</tr>
<tr>
<td>CEBPA</td>
<td>6%</td>
<td>Favorable</td>
</tr>
<tr>
<td>PTPN11</td>
<td>3%</td>
<td>Unknown</td>
</tr>
<tr>
<td>PHF6</td>
<td>2.4%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>TP53</td>
<td>2-5%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>KIT</td>
<td>2-3%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>CBL</td>
<td>1-3%</td>
<td>Unknown</td>
</tr>
<tr>
<td>EZH2</td>
<td>1-3%</td>
<td>Unknown</td>
</tr>
<tr>
<td>JAK2</td>
<td>1%</td>
<td>Unfavorable</td>
</tr>
</tbody>
</table>

Table 4. Clonal cytogenetic abnormalities that may be considered as evidence of MDS, unclassifiable in the absence of definite dysplastic morphologic features, but in the setting of persistent cytopenias.

- **Unbalanced abnormalities**
  - -7/del(7q)
  - -5/del(5q)
  - i(17q)/t(17p)
  - -13/del(13q)
  - del(11q)
  - del(12p)/t(12p)
  - del(9q)
  - idic(X)(q13)
- **Balanced abnormalities**
  - t(11;16)(q23;p13.3)
  - t(3;21)(q26.2;q22.1)
  - t(1;3)(p36.3;q21.1)
  - t(2;11)(p21;q23)
  - inv(3)(q21q26.2)
  - t(6;9)(p23;q34)
Table 5. Gene-specific abnormalities in myelodysplastic syndrome. Adapted from Bejar et al.\textsuperscript{21}

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency in MDS</th>
<th>Reported prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>TET2</td>
<td>20.5%</td>
<td>Neutral</td>
</tr>
<tr>
<td>ASXL1</td>
<td>14.4%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>RUNX1</td>
<td>8.7%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>TP53</td>
<td>7.5%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>EZH2</td>
<td>6.4%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>NRAS</td>
<td>3.6%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>JAK2</td>
<td>3.0%</td>
<td>Neutral</td>
</tr>
<tr>
<td>ETV6</td>
<td>2.7%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>CBL</td>
<td>2.3%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>IDH2</td>
<td>2.1%</td>
<td>Unfavorable</td>
</tr>
<tr>
<td>NPM1</td>
<td>1.8%</td>
<td>Neutral</td>
</tr>
<tr>
<td>IDH1</td>
<td>1.4%</td>
<td>Neutral</td>
</tr>
<tr>
<td>KRAS</td>
<td>0.9%</td>
<td>Neutral</td>
</tr>
<tr>
<td>GNAS</td>
<td>0.7%</td>
<td>Unknown</td>
</tr>
<tr>
<td>PTPN11</td>
<td>0.7%</td>
<td>Unknown</td>
</tr>
<tr>
<td>BRAF</td>
<td>0.5%</td>
<td>Unknown</td>
</tr>
<tr>
<td>PTEN</td>
<td>0.2%</td>
<td>Unknown</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>0.2%</td>
<td>Unknown</td>
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References:


