Genetic Testing in Acute Myeloid Leukemia and Myelodysplastic Syndromes

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KEYWORDS

• Myelodysplastic syndrome • Acute myeloid leukemia • Mutations • Translocations • Cytogenetics • Molecular genetic analysis

ABSTRACT

Cytogenetic analysis of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) is essential for disease diagnosis, classification, prognostic stratification, and treatment guidance. Molecular genetic analysis of *CEBPA*, *NPM1*, and *FLT3* is already standard of care in patients with AML, and mutations in several additional genes are assuming increasing importance. Mutational analysis of certain genes, such as *SF3B1*, is also becoming an important tool to distinguish subsets of MDS that have different biologic behaviors. It is still uncertain how to optimally combine karyotype with mutation data in diagnosis and risk-stratification of AML and MDS, particularly in cases with multiple mutations and/or several mutationally distinct subclones.

OVERVIEW

Myelodysplastic syndromes (MDSs) are clonal hematopoietic stem cell neoplasms characterized by morphologic dysplasia, ineffective hematopoiesis resulting in peripheral blood cytopenias, and risk of progression to acute myeloid leukemia (AML). AML is a clonal hematopoietic neoplasm with increased myeloblasts, usually comprising at least 20% of leukocytes in the bone marrow and/or blood. Both MDS and AML are heterogeneous diseases with variable morphologic, immunophenotypic, and genetic features; a range of clinical aggressiveness; and multiple treatment options.

Key Features

- Conventional karyotyping of bone marrow provides critical information regarding risk stratification of both MDS and AML and should be obtained in all cases.
- Mutational analysis of AML routinely includes *FLT3*, *NPM1* and *CEBPA*, but is moving towards including an additional small group of genes (*IDH1*, *IDH2*, *RUNX1*, *MLL*, *DENMT3A*, and others) that have been shown to have prognostic and/or therapeutic relevance in large-scale genomic studies.
- Mutational analysis of a limited set of genes in MDS is also becoming a useful tool for the purposes of prognosis (with *TP53*, *EZH2*, *ASXL1*, and *RUNX1* among genes conferring a poor prognosis independently of other factors); however, the finding of gene mutations alone in a cytopenic patient is currently considered insufficient to establish a primary diagnosis of MDS in the absence of required diagnostic criteria.
- While there is still a role for single gene testing in some contexts, the field is moving towards testing multiple genes (from 10 to over 100) at once in dedicated panels, often using next generation sequencing technology.

In the World Health Organization's (WHO) 4th edition *Classification of Tumors of Haematopoietic and Lymphoid Tissues* published in 2008,[1] several

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recurrent genetic abnormalities were formally incorporated in the diagnostic algorithms of AML and MDS, given their major impact on the prognosis[2,3] and clinical management of these diseases. Regarding the diagnosis of MDS and AML, certain specific cytogenetic abnormalities are considered as presumptive evidence for MDS when they are detected in a patient with unexplained cytopenias.[1] Similarly, a diagnosis of AML can be made with less than 20% myeloblasts when the specific AML-defining chromosomal abnormalities t(8;21)(q22;q22), inv(16)(p13.1q22), t(16;16)(p13.1;q22), or t(15;17)(q22;q12) are detected. With regard to disease classification, genetic abnormalities have been incorporated into the definitions of certain AML and MDS disease categories. These are generally cytogenetic abnormalities, but mutations in *NPM1* and *CEBPA* genes were used to define two new provisional AML subtypes.

Since the publication of the 2008 WHO classification, the advent of high-throughput next generation sequencing (NGS) technologies has revealed the complexity of the genomic landscape of MDS and AML.[4–8] These technologies have led to the discovery of numerous recurrent mutations in genes and cellular pathways not previously implicated in these neoplasms (or previously missed by older, less sensitive methods of analysis), several of which have been shown to have diagnostic, prognostic, and/or therapeutic implications. Although conventional karyotyping to detect numerical chromosomal abnormalities and translocations remains a cornerstone in the diagnosis, classification, and management of AML and MDS, our review focuses mainly on the recently unraveled molecular genetic abnormalities in these diseases. These newly discovered genetic markers are refining existing prognostic schemes for AML and MDS and may help dictate targeted therapies.

CYTOGENETIC TESTING IN MYELODYSPLASTIC SYNDROME AND ACUTE MYELOID LEUKEMIA

Cytogenetic abnormalities are present in approximately 50% to 60% of MDS and AML cases at diagnosis. The cytogenetic findings provide critical diagnostic and prognostic information for both MDS and AML, and a conventional karyotype should always be performed on bone marrow taken at the time of primary diagnosis. Targeted fluorescent in situ hybridization (FISH) studies that interrogate for gains or losses of specific loci or translocations may miss abnormalities that are not included in the panel. FISH analysis for common abnormalities in MDS may be helpful if the karyotype fails or is insufficient (less than 20 metaphases),[9] but probably does not add information if the karyotype is successful.[10,11]

The most common recurring clonal cytogenetic aberrations in MDS are shown in **Table 1**.[12–15] Most of the genetic abnormalities in MDS are chromosomal gains or losses, such as –7, del(5q), –5, and +8. Translocations are less frequent in MDS and, if present, are often unbalanced. According to the 2008 WHO classification, the presence of any of the MDS-defining cytogenetic abnormalities listed in **Table 1** (with the exception of +8, del20q, and –Y), is sufficient to confirm a diagnosis of MDS in a cytopenic patient, even if significant morphologic dysplasia is lacking.[16–18] A recent study suggests that +15, often accompanied by –Y, is another cytogenetic abnormality that does not necessarily indicate MDS.[19] The only genetic abnormality that currently defines a specific MDS subtype is an isolated del(5q), reflecting the strong association of this abnormality with a particular disease phenotype (**Fig. 1**A), response to a specific therapy (lenalidomide), and favorable prognosis. A central role of the del(5q) in the pathogenesis of this MDS subtype has been recently validated by its identification in the most primitive MDS stem cells and its occurrence as an apparent founding event before the acquisition of any other mutations.[20] A number of genes in the commonly deleted region have been hypothesized to contribute to the disease pathogenesis. Haploinsufficiency of the RPS14 ribosomal structural protein,[21] as well as the microRNAs miR-145 and miR-146a in the deleted region, are thought to influence the characteristic megakaryocyte abnormalities and anemia,[22] whereas casein kinase 1A1 (*CSNK1A1*) haploinsufficiency that dysregulates the WNT/beta-catenin pathway has been implicated in proliferation of the del(5q) clone.[23] Beyond the del(5q), it is well established that specific cytogenetic abnormalities strongly influence the prognosis of MDS, and thus the karyotype findings represent a critical aspect of MDS risk-stratification schemes, such as the revised International Prognostic Scoring System (IPSS-R).[3]

Although chromosomal gains and losses are also common in AML, recurring translocations that activate oncogenes are a hallmark of many types of AML. A listing of the common cytogenetic aberrations in AML is shown in **Table 2**. As with MDS, cytogenetics is very important in AML risk stratification. Karyotype abnormalities are strongly correlated with clinical behavior in AML and certain abnormalities define specific AML disease subtypes that often have distinctive morphologies. Examples of these genetic-morphologic correlations in AML