Molecular Profiling in Confirming the Diagnosis of Early Myelodysplastic Syndrome

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ABSTRACT

Background: Diagnosis of myelodysplastic syndrome (MDS) based on bone marrow morphology can be very difficult when blasts are not increased. The demonstration of cytogenetic abnormalities in these cases can confirm the diagnosis, providing cytopenia is documented. Cytopenia is usually the major reason for initiating work-up for MDS and, in general, cases with uncytopenia are the most difficult to make the diagnosis. In patients in whom the presence of cytopenia is without cytogenetic abnormalities, the molecular abnormalities underlying the biology of MDS should provide objective biomarkers that can be used to confirm the diagnosis of MDS in the absence of cytogenetic abnormalities.

Toward this goal, we developed a 14-gene panel to detect molecular abnormalities in patients referred to rule out MDS with blast count <5% and no cytogenetic abnormalities. Cytopenia is usually the major reason for initiating work-up for myelodysplasia and, in providing cytopenia is documented. Cytopenia is usually the major reason for initiating work-up for MDS and, in general, cases with uncytopenia are the most difficult to make the diagnosis. In patients in whom the presence of cytopenia is without cytogenetic abnormalities, the molecular abnormalities underlying the biology of MDS should provide objective biomarkers that can be used to confirm the diagnosis of MDS in the absence of cytogenetic abnormalities.

Methods: Cytopenia is defined as having platelets <100,000/µl, neutrophils <1,800/µl, or hemoglobin <10g/dL. Total nucleic acid was extracted from bone marrow or peripheral blood samples and tested for mutations in any of the following genes: ASXL1, ETV6, EZH2, IDH1, IDH2, Nras, CBL, RUNX1, SF3B1, SRSF2, TET2, TF3, U2AF1, and ZRSR2. Direct bidirectional Sanger sequencing, as well as next generation sequencing were used for testing. Samples from 137 patients fulfilling the criteria described above were analyzed. As cytogenetic abnormalities is a marker of MDS, a control group of 14 patients with cytogenetic abnormalities but no increase in blasts were evaluated using the same molecular panel.

RESULTS

1. Molecular testing can define definite MDS patients

39% of patients showed evidence of the presence of an abnormal neoplastic clone, confirming the diagnosis of MDS. While a diagnosis of MDS cannot be completely ruled out in the remaining patients, it is highly unlikely they have MDS. Follow-up of these patients is essential to rule out MDS in these patients.

Patients with mutations had significantly lower number of neutrophils and higher number of monocytes (Table 1).

In contrast, mutations were detected in 12 of 14 (86%) patients with RA and cytogenetic abnormalities using the same gene panel. This observation supports the assumption that the majority of the patients without cytogenetic abnormalities, and mutations can be presumed to have reactive cytopenia rather than MDS.

2. Higher prevalence of mutation in patients with bi- and tri-cytopenia

21% of patients with mutations had bi-cytopenia and tri-cytopenia had mutations compared with only 7% of patients without mutation. This finding suggests that the possibility of confirming a diagnosis of MDS by molecular studies is more likely when more lineages are involved in the cytopenia.

3. Mutations profile associated with good prognosis in patients with early MDS (RA)

Mutations were detected in 53 patients (39%, see Figure). The most common mutation detected in this group of patients was TET2, detected in 21 of 53 (40%) of the patients with mutation. The second most common was SF3B1, detected in 22% of patients. Both mutations have been reported to be either neutral prognosis or associated with good prognosis. Furthermore, majority of patients (55%) had a mutation in one gene, which is also associated to be associated with better outcome compared with mutations in multiple genes. Mutations in two genes were detected in 32% of the patients in this group.

Figure. Mutations detected in the 53 patients with early MDS (RA)

Table 1. Hematologic findings in patients with mutation and without mutation

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<tr>
<th>Parameter</th>
<th>Unmutation (%)</th>
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<tr>
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Table 2. Association between mutation rate and the lineages involved in the cytopenia

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CONCLUSION

1. 39% of patients presenting with cytopenia without cytogenetic abnormality or increase in blast have a mutation in one or more genes.

2. Molecular abnormalities in early MDS (refractory anemia) are more likely to be associated with good prognosis:

   a. More likely to be in TET2 (21 of 53 (40%))
   b. More likely to be in SF3B1 gene (22%)
   c. More likely to be involving a single gene (55%)

3. 86% of patients with RA and cytogenetic abnormalities show a mutation in one or more genes.

4. Patients with bi- and tri-cytopenia have higher rate of mutation.

5. The 14 genes used in this study are likely adequate to screen patients suspected to have MDS for confirming diagnosis. These genes may also provide adequate information on prognosis.

INTRODUCTION

Myelodysplastic syndrome (MDS) is a neoplastic disease characterized by ineffective hematopoiesis manifesting as peripheral blood cytopenias. The cytopenia is usually the early manifestation of the disease. Diagnosis of MDS is currently based on examining bone marrow for the presence of dysplasia, which is subjective and not well-defined. The presence of an increase in blasts in bone marrow or the presence of cytogenetic abnormalities in making the diagnosis, but the diagnosis can be very difficult to confirm in refractory anemia (RA) and no cytogenetic abnormalities. Unfortunately more than 50% of patients with MDS may not have cytogenetic abnormalities. Therefore, diagnosis of MDS, especially RA can be very difficult to confirm.

OBJECTIVES

Evaluate the utility of a 14-gene panel to detect molecular abnormalities in patients referred to rule out MDS with blast count <5% and no cytogenetic abnormalities.

SAMPLES AND METHOD

One hundred and thirty seven (137) consecutive patients referred to rule out MDS with blast count <5% without cytogenetic abnormalities, but with documented cytopenia.

Methods: Cytopenia is defined as having platelets <100,000/µl, neutrophils <1,800/µl, or hemoglobin <10g/dL. Total nucleic acid was extracted from bone marrow or peripheral blood samples and tested for mutations in any of the following genes: ASXL1, ETV6, EZH2, IDH1, IDH2, Nras, CBL, RUNX1, SF3B1, SRSF2, TET2, TF3, U2AF1 and ZRSR2. Direct bidirectional Sanger sequencing, as well as next generation sequencing were used for testing. Samples from 137 patients fulfilling the criteria described above were analyzed. As cytogenetic abnormalities is a marker of MDS, a control group of 14 patients with cytogenetic abnormalities but no increase in blasts were evaluated using the same molecular panel.

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