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 myelodysplasia and, in general, cases with unicytopenia are the most difficult to make the diagnosis. In principle the recent characterization of 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% 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, TP53, 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: Fifty three of the 137 patients (39%) had a mutation in one or more genes. Of the 137 patients, three had tricytopenia, 14 had bicytopenia and 120 had unicytopenia. Two of the three with tricytopenia (66%) had mutations and nine of 14 with bicytopenia (64%) had mutations. In contrast 42 of the 120 patients with unicytopenia (35%) had mutation in one or more genes. Thirty of the 53 patients with mutation (57%) had one gene mutated and only 4 (13%) of these patients had bi- or tricytopenia. Of the remaining 23 patients with mutations in two or more genes a higher percentage (30%) of patients had bi- or tricytopenia. Compared to patients without mutations in the tested genes, those with mutation had significantly lower number of neutrophils (P=0.006), but higher percentage of monocytes (P=0.0002) and slightly higher percentage of lymphocytes (P=0.06).

Twelve of 14 (86%) patients with cytogenetic abnormalities showed mutation in one or more genes and only three patients of the 14 (21%) had bi- or tricytopenia.

Conclusions: Diagnosis of MDS at early stage of disease (blasts <5%) can be significantly enhanced by adding molecular profiling to cytogenetics studies. Molecular profiling using limited number of genes (14) in patients with cytopenia and suspected of having MDS, but no cytogenetic abnormalities, can confirm the diagnosis of MDS in 39% of cases. Compared to MDS with unicytopenia, MDS with bi- or tricytopenia without increase in blasts, is more likely to be confirmed by molecular testing.

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 helps 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. There are numerous reactive processes that cause cytopenia including drug reaction, nutritional or hormonal deficiencies, autoimmune diseases, or chronic infection and all these should be ruled out prior to MDS diagnosis. Molecular studies can be very help in providing objective means for the demonstration of abnormal mutant clone that can confirm the diagnosis of MDS.

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 from community practice, suspected of MDS, with cytopenia and without cytogenetic abnormalities based on conventional cytogenetics and FISH. (Cytopenia was defined as having platelets <100,000 /µl, neutrophils <1,800/µl, or hemoglobin <10g/dL).

A control group of 14 patients with a confirmed diagnosis of refractory anemia (RA) with cytogenetic abnormalities.

DNA extraction and Sequencing

DNA extracted from bone marrow or peripheral blood specimens using QIACube as recommended by the manufacturer.

Direct Sanger sequencing or next generation sequencing (NGS)

Genes investigated

DNA methylation genes (TET2 and IDH1/IDH2), RNA splicing genes (SF3B1, SRSF2, U2AF1 and ZRSR2), chromatin modification genes (ASXL1 and EZH2), transcription gene (RUNX1), DNA repair control gene (TP53), RAS pathway genes (NRAS, CBL) and transcription factor gene (ETV6).

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%) of patients with RA and cytogenetic abnormalities using the same gene panel. This observation supports the assumption that the majority of the patients without both 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- 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 28% 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 reported 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.

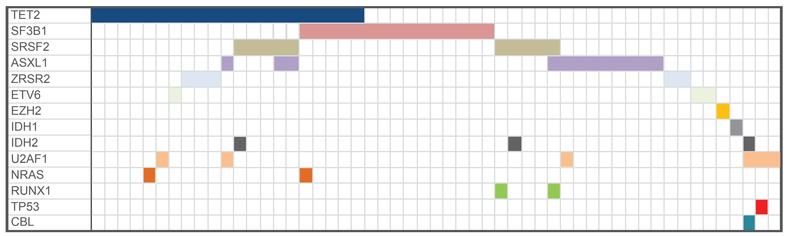
Table 1. Hematologic findings in patients with mutation and without mutation

	Mutation (N=53)			No Mutation (N=84)			
	Median	Minimum	Maximum	Median	Minimum	Maximum	P-Value
WBC	4.8	1.1	25.0	5.0	1.1	16.0	0.46
Hgb	10.4	7.2	15.0	10.7	8.2	17.5	0.41
MCV	100.0	81.2	119.0	97.7	66.9	116.1	0.03
Platelet	132.0	37.0	565.0	180.0	16.0	562.0	0.07
Lymph	27.8	7.2	57.1	23.8	3.7	63.8	0.05
Mono	9.7	3.3	31.0	7.5	0.2	34.4	0.006
Neutro	61.0	16.2	89.3	66.6	32.8	89.2	0.008
Blasts	1.7	0.0	6.0	1.6	0.1	4.3	0.44

Table 2. Association between mutation rate and the lineages involved in the cytopenia

	Mutation (%)	Unmutation (%)
Unicytopenia	42 (79)	78 (93)
Bi-and tricytopenia	11 (21)	6 (7)

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



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:

- More likely to be in TET2 [21 of 53 (40%)].
- More likely to be in SF3B1 gene (28%).
- 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.