Article

Molecular profiling in confirming the diagnosis of early myelodysplastic syndrome

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Abstract: Confirming a diagnosis of myelodysplastic syndrome (MDS) in the absence of cytogenetic abnormalities can be very difficult, especially in the early stages of the disease. This study investigated the utility of a 14 gene panel (TET2, IDH1/IDH2, SF3B1, SRSF2, U2AF1, ZRSR2, ASXL1, EZH2, RUNX1, TP53, NRAS, CBL and ETV6) in the diagnosis of suspected MDS. Specimens were analyzed from 137 patients, referred to rule out MDS, who had documented cytopenias with blast count <5% and without cytogenetic abnormalities. Fifty-three of the 137 patients (39%) showed evidence of an abnormal clone, characterized by mutations in one or more genes - including two of the three patients with tricytopenia (66%), nine of 14 with bicytopenia (64%) and 42 of the 120 patients with unilineage cytopenia (35%). Thirty of the 53 patients with mutations (57%) had one mutated gene; of these patients, only 4 (13%) 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 indicating that cases with bi- or tricytopenia are more likely to be confirmed by molecular testing than cases with unicytopenia. Compared to patients without mutations in the tested genes, those with mutations 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). This study demonstrates that a relatively small molecular panel may be a valuable and objective diagnostic means for the diagnosis of MDS patients with <5% blasts and without cytogenetic abnormalities.

Keywords: Early myelodysplastic syndrome, gene mutations, diagnosis

Introduction

Myelodysplastic syndrome (MDS) is a neoplastic disease characterized by ineffective hematopoiesis manifesting as peripheral blood cytopenias. Cytopenia is usually the earliest manifestation of the disease. Diagnosis of MDS is currently based on examination of bone marrow for the presence of dysplasia, which is subjective and not well-defined. Increase in blasts in bone marrow and clonal cytogenetic abnormalities identified by conventional cytogenetics and/or fluorescence in situ hybridization (FISH) are also used for the diagnosis of MDS. However, cytogenetic abnormalities are observed in only approximately 50% of cases. In the absence of cytogenetic abnormalities, confirming a diagnosis of MDS, especially refractory anemia (RA), can be very difficult. Numerous reactive processes can cause cy-

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topenia, including drug reaction, nutritional or hormonal deficiencies, autoimmune diseases, or chronic infection, and should be ruled out prior to making a diagnosis of MDS. Sequencing studies have identified driver mutations in RNA splicing, DNA methylation, chromatin modification, transcription regulation, DNA repair, signal transduction, and cohesin complex genes in MDS [1–4] heightening the interest of utilizing them in the diagnosis of MDS. Studies to date have been retrospective and included cases with and without cytogenetic abnormalities [5–7]. To determine if molecular studies can help provide objective means for the demonstration of abnormal mutant clones and confirm the diagnosis of MDS, in patients referred to rule out MDS with blast count <5% and without cytogenetic abnormalities we developed a 14-gene panel to detect molecular abnormalities.

Materials and Methods

One hundred and thirty-seven (137) consecutive patients referred from community practice, suspected of MDS, with cytopenia and without cytogenetic abnormalities by conventional cytogenetics (and FISH studies for abnormalities involving chromosomes 5, 7, 8 and 20), were included in the study. All samples were collected in compliance with an Institutional Review Board approved protocol. Cytopenia was defined as platelets <100 x 10^9/L, neutrophils <1.8 x 10^9/L, or hemoglobin <10 g/dL. Fourteen patients with RA and cytogenetic abnormalities (and blast count less than 5%) were also studied.

DNA Extraction and Sequencing

Total nucleic acid was extracted from bone marrow or peripheral blood samples using QIACube as recommended by the manufacturer.

Direct bidirectional Sanger sequencing or next generation sequencing (NGS) was used to investigate mutations in: 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*).

In our practice, when one gene is mutated, allele frequency of >20% is considered pathogenic and relevant for confirming diagnosis. When mutations in two genes or more are detected, we consider the sample as positive for a mutation. All the provided data, including clinical information, flow cytometry results, morphology evaluation, cytogenetic and/or FISH data were reviewed.

Statistical Analysis

The correlations were calculated using Spearman rank correlation coefficients. The Wilcoxon rank sum test or Kruskal-Wallis test was used to compare among categorical variables. *P* values less than 0.05 were considered statistically significant.

Results

Molecular testing can define definite MDS patients

Fifty-three of the 137 patients (39%) without cytogenetic abnormalities showed molecular evidence of an abnormal neoplastic clone. Mutation was detected in one gene in 30 cases and in more than one gene in 23 cases, confirming the diagnosis of MDS [Figure 1]. A diagnosis of MDS in the remaining patients cannot be completely ruled out; however, it is highly unlikely that they have MDS and follow up of these patients is needed. Patients with mutations had significantly lower number of neutrophils and higher number of monocytes (Table 1). In contrast, using the same gene panel mutations were detected in 12 of 14 patients (86%) with RA and cytogenetic abnormalities, supporting the assumption that the majority of the patients without mutation can be presumed to have reactive cytopenia rather than MDS.

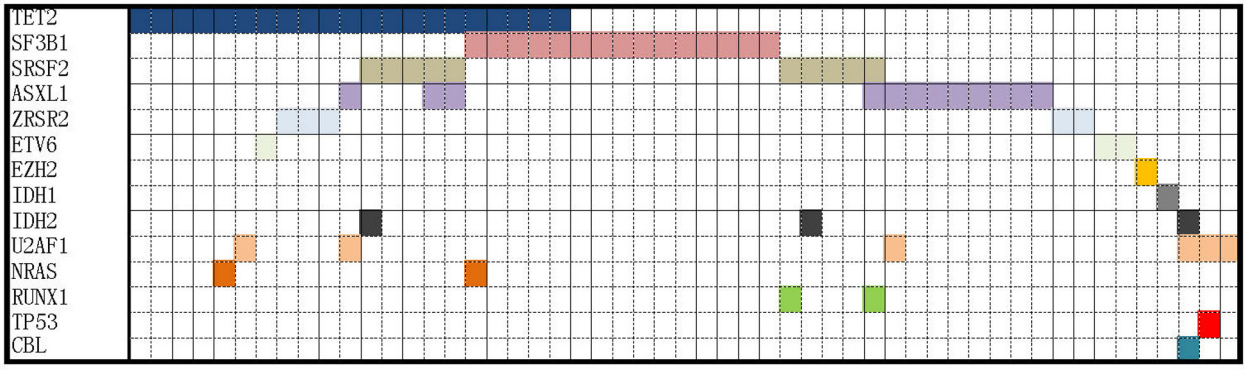

Figure 1: Mutations detected in the 53 patients.

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

Eleven of the 53 patients (21%) with mutations had bi or tricytopenia compared to only 6 of 84 (7%) of the patients without mutation (Table 2), suggesting that the more lineages with cytopenias, the more likely is confirmation of a MDS diagnosis by molecular studies.

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

The mutations detected in the 53 patients with bi- and tri-lineage cytopenias are shown in Figure 1. The most common mutation in this group of patients is *TET2*, which is detected in 21 of 53 (40%) patients with mutations. The second most common is *SF3B1*, detected in 15 of 53 (28%) patients. Both mutations have been reported to be either neutral prognosis or associated with good prognosis [2, 5]. Furthermore, the majority of patients (55%) had a mutation in one gene, which is also reported to be associated with better outcome as compared with patients with mutations in multiple genes [3]. In 17 of 21 (81%) cases with *TET2* mutation and 5 of 15 (33%) cases with *SF3B1* mutation, one or more additional gene was mutated.

Discussion

Results of this study demonstrate the clinical utility of molecular studies in identifying abnormal clones in cases suspected of MDS with <5% blasts and without cytogenetic abnormalities. The identification of mutations could aid in the diagnosis of MDS in 39% of these cases.

A number of features of the data in this cohort are similar to those observed in previously reported cohorts of confirmed MDS cases. The relative fre-

Table 1: Comparing hematologic findings between patients with mutation and those without mutation.

	Mutated (N=53)			Wild type (N=84)			
	Median	Minimum	Maximum	Median	Minimum	Maximum	P-Value
WBC (K/mm^3)	4.8	1.1	25.0	5.0	1.1	16.0	0.46
Hgb (g/dL)	10.4	7.2	15.0	10.7	8.2	17.5	0.41
MCV (fL)	100.0	81.2	119.0	97.7	66.9	116.1	0.03
Platelet K/mm^3)	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

quency of specific gene mutations in this series is comparable to that reported in the literature of confirmed MDS cases (Table 3) [2, 3, 5, 7]. In addition, the positive and negative correlations of mutations (positive correlation of *TET2* and *ZRSR2* mutations and negative correlation of *ASXL1* and *SF3B1* mutations) in the current cohort is similar to those in previous studies [3, 5]. These findings indicate the significance of mutations in the suspected MDS with <5% blasts and therefore the utility of these studies in confirming the diagnosis of MDS.

Mutations in *TET2* and *SF3B1*, reported to be indicators of neutral or good prognosis [2, 5, 8], were most frequent in this cohort of cases. Furthermore, the majority of patients with mutations, 29 of 53 (55%) had a mutation in one gene, which is also reported to be associated with better outcome compared with mutations in multiple genes [3]. These data suggest that the molecular abnormalities and characteristics in early MDS (such as RA) are more likely associated with good prognosis. The molecular characteristics, which indicate good prognosis in these cases, may be valuable as a base line for monitoring disease progression that may be accompanied by additional molecular changes and/or cytogenetic abnormalities. In samples with mutation in one gene, allele frequency >20 is required to be considered pathogenic, whereas mutations detected in two or more genes are considered as acceptable criteria for MDS. These criteria are used to rule out low level mutation detected in pre-MDS cases [9].

Mutations were observed most frequently in *TET2*, *SF3B1*, *ASXL1* and *SRSF2*. Analysis of only these genes would have identified abnormal clones in 44 of 137 (32%) cases compared to 39% with a panel of 14 genes. As the genes listed above showed mutations in cases with <5% blasts, they are likely adequate to initiate MDS but additional genetic changes may be required for clinically overt disease. For example, the majority of cases with *TET2* mutation also have additional gene mutations (17 of 21; 81%). However, *TET2*, *SF3B1*, *ASXL1* and *SRSF2* may not be the only initiating mutations in MDS, since 8 cases lacked mutations in these genes, but had mutations in other genes. This limitation should be considered when designing a molecular panel to aid in the confirmation of a MDS diagnosis.

Table 2: Association between mutation and number of lineages involved in the cytopenia.

	Mutated n(%)	Wild type n(%)
Unicytopenia	42 (79)	78 (93)
Bicytopenia	9 (17)	5 (6)
Tricytopenia	2 (4)	1 (1)

Current knowledge of genotype/phenotype relationships includes association of *SF3B1* mutation with RA with ring sideroblasts [10] and *TET2*/*SRSF2* co-mutation with chronic myelomonocytic leukemia [2]. While mutations in *SF3B1* are associated with good overall survival, mutations in *SRSF2*, *U2AF1*, *IDH1/IDH2*, *ASXL1*, *EZH2*, *RUNX1* and *TP53* have been reported to be unfavorable [6, 11–14]. From the perspective of therapy, TET2 mutations have been reported to identify patients who respond to hypomethylating agents [15]. Therefore, identification of mutations in suspected of MDS is important not only for diagnosis but also for determining prognosis and clinical management.

Driver mutations, identified in MDS, are likely integral to the etiology of the disorder. Cases of MDS with apparently unrelated cytogenetically abnormal clones also suggest that driver mutations may be the primary initiators with chromosomal abnormalities as secondary changes in these cases. The question arises as to how early in the disease process driver mutations can be detected. If these changes are present and detectable in the early stages of the disease process, then this information could be used in early diagnosis and these patients could potentially benefit from early therapeutic intervention based on the genetic characteristics.

The data from this study suggest that an appropriately designed, relatively small molecular panel is a valuable and objective diagnostic means for the

Table 3: Frequency of genes mutated in the current cohort and in published literature.

Mutated Genes	This study (%)	Cazzola *et al.* [2] (%)	Papaemmanuil *et al.* [3] (%)	Malcovati *et al.* [1] (%)	Haferlach *et al.* [5] (%)
TET2	21 (15)	20-30	22	20-25	30-35
SF3B1	15 (11)	15-30	24	25-30	30-35
ASXL1	12 (9)	15-20	12	10-15	25-25
SRSF2	10 (7)	10-20	14	10-15	15-20
U2AF1	6 (4)	<10	<10	5-10	5-10
ZRSR2	4 (3)	<10	<5	5	5-10
ETV6	3 (2)			2	<5
IDH2	3 (2)	5		2-3	0-5
NRAS	2 (1)			5-10	<5
RUNX1	2 (1)	10		10-20	1-15
IDH1	1 (<1)				<5
EZH2	1 (<1)	5		5	10
TP53	1 (<1)	5		5-10	5-10
CBL	1 (<1)			1-2	5

diagnosis of MDS with low levels of blasts and without cytogenetic abnormalities. Additional studies in a larger cohort of patients with these characteristics would be useful in further substantiating the observations in this study. The relatively small number of genes investigated in this study may have limited the ability to detect abnormal clones in a higher proportion of cases with MDS. Next generation sequencing will aid in overcoming this limitation and a higher number of genes may be studied in future investigations to gain greater sensitivity for confirming the diagnosis of MDS in cases with subclinical/subtle features.

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