The Role of Molecular Profiling of Bone Marrow Samples in Confirming the Diagnosis of Myelodysplastic Syndrome in Patients Presenting with Cytopenia

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ABSTRACT

Introduction: Diagnosis of myelodysplastic syndrome (MDS) can be very difficult when blast count in bone marrow is <5%. The demonstration of a mutation in one or more of the MDS-related genes is usually considered an objective confirmation of MDS. However, recent reports suggest that normal individuals may have circulating clonal hematopoietic cells carrying MDS-related mutations. We studied the relevance the mutated allele frequency and number of mutated genes in confirming the diagnosis of MDS in patients with cytopenia as determined using bone marrow samples.

Methodology: We analyzed Next Generation Sequencing (NGS) data from 294 consecutive bone marrow samples referred to rule out MDS and reported to be positive for mutation in one or more MDS-related genes. All samples were tested for mutations in the following genes: TET2, SF3B1, ASXL1, DNMT3A, SRSF2, RUNX1, NRAS, ZRSR2, EZH2, ETV6, TP53, CBL, NPM1, JAK2, U2AF1, IDH1, KRAS, IDH2, FLT3, PTPN11, SETBP1, and BCOR. The average depth of NGS testing in this targeted sequencing was approximately 10,000X.

Results: Of the 294 MDS samples with mutations, 103 (35%) had blasts <5%. Of the 103 samples, 33 (32%) showed mutations in one gene; the remaining (65%) had mutations in more than one gene. The frequency of the mutant allele was <20% in only 11 of 103 cases (11%). The remaining 92 patients had either mutations in two genes or in one gene, but the mutant allele frequency was >20%. Four of the 11 patients (36%) with one gene mutation and <20% allele frequency had cytogenetic abnormalities confirming the diagnosis of MDS [der(1;7)(q10;p10), del(5q), trisomy 8. and del(11)(q23)]. Of the remaining 7 patients with allele frequency <20%, 3 had mutations in DNMT3A, 1 in U2AF1 gene, 1 in TET2 gene, 1 in TP53 and 1 in SF3B1 gene. Of these 7 cases, only two cases had an allele frequency <10%, one in TP53 gene and one in SF3B1 gene.

Of the 92 cases with mutations in two genes or in one gene with allele frequency >20%, 26 patients (28%) had cytogenetic abnormalities confirming the diagnosis of MDS. In fact in this group of 26 patients with cytogenetic abnormalities, only one patient had mutations at <20% in all mutated genes (TET2, DNMT3A and TP53), but also had del(17p). Of the remaining patients 65 cases without cytogenetic abnormalities, with more than one gene mutation, at least one gene had mutant allele at >20%.

There was no statistically significant difference in the degree of cytopenia between patients with <20% one mutation and no cytogenetic abnormalities (N=7) and the 96 cases with mutations in two genes or in one gene with allele frequency >20%. There was no significant difference in the degree of cytopenia between the 36 patients with one gene mutation and 67 patients with more than one gene mutation.

Conclusion: This data suggests that bone marrow samples from patients with peripheral cytopenia should be tested by cytogenetic and molecular profiling using NGS and the analysis of MDS-related genes. Our data suggests that when proper criteria are used, molecular profiling of bone marrow in the proper clinical presentation can help in confirming the diagnosis of MDS. Our data suggests that the presence of mutations in more than one gene and the detection of mutant allele frequency >20% may comprise reliable criteria for the diagnosis of MDS. The presence of mutation in 20% of DNA usually reflects mutation in 40% of the bone marrow cells. Patients with mutant allele frequency between 10% and 20% in the bone marrow and cytopenia most likely have MDS, but further studies are needed. Mutant allele frequency in bone marrow of <10% is extremely rare when testing is performed in patients presenting with cytopenia.

INTRODUCTION

The myelodysplastic syndromes (MDS) are a clonal process that involves the transformation of hematopoietic progenitor cells and leads to apoptosis and the clinical manifestation of cytopenia. This ineffective hematopoiesis is caused by genomic abnormalities that accumulate with time in most patients. The clinical syndrome of MDS may vary dependent on the type of genomic abnormalities and the genes involved in the neoplastic process. Also the abnormal neoplastic clone may not cause significant clinical problem if it is not dominant and if it does not suppress the normal hematopoiesis adequately. Recent studies suggests that 5-10% of individuals above the age of 65 may have low level mutant clone without clinical manifestation of MDS, but they may develop a clinically manifested MDS at later time. This suggests that sequential mutations are necessary for the clinical syndrome of MDS to develop, in a fashion similar to multiple hit model described for solid tumors. Of course driver mutations may differ dependent on the gene involved in the process and it is possible that specific mutations in specific genes will develop into full-blown MDS.

The diagnosis of MDS depends on the presence of cytopenia. The presence of cytogenetic or molecular abnormalities and the demonstration of the presence of a dominant abnormal (mutant) clone driving hematopoiesis is very important for the confirmation of diagnosis of MDS. However, it is not clear how to define "dominance" at this time. In the case of solid tumors, breaking through the basement membrane by proliferating abnormal cells is considered adequate for classification of cancer. In MGUS, serum M protein >30 g/L or plasma cells in bone marrow>10% are considered indication of myeloma diagnosis. In this study, we attempted to define the criteria that are adequate to consider that the molecular abnormalities diagnostic for MDS in patients presenting with cytopenia.

METHODS

Patients and samples

Bone marrow samples from 294 consecutive patients with MDS were used for testing by NGS, of which 103 had early MDS (Blasts<5%) in bone marrow. All work was performed with Institutional Review Board (IRB) approval.

DNA isolation

Total nucleic acid was isolated from plasma using QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

Gene sequencing

NGS was performed using an Illumina MiSeq system (San Diego, CA); NGS, amplification, and indexing were performed as recommended by the manufacturer. Amplicons were confirmed for each sample by running an agarose gel. Samples were pooled and the experiment sheet was generated using Illumina Experiment Manager. MiSeq Reporter was used for analysis and Variant Studio was used for calling. For confirmation of variant calling, NextGene software (SoftGenetics, State College, PA) was used. Average sequencing coverage across the entire coding regions was 10,000 in 90% of the sequenced amplicons.

RESULTS

1. Early and late MDS show similar mutation patterns when analyzing bone marrow samples

- Of the 294 MDS samples with mutations, 103 (35%) had blasts <5%.

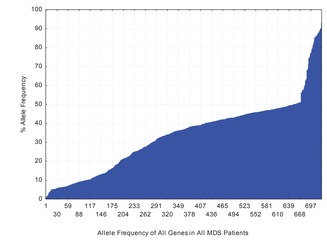

Figure 1a. *Profile of percentage of mutant allele frequency in all genes in all tested MDS patients.*

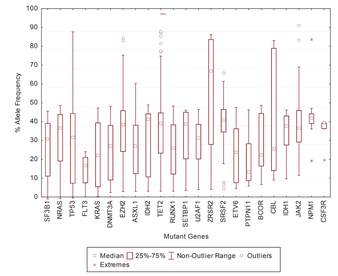

Figure 1b. *Levels of mutant allele in various genes as detected in all MDS patients*

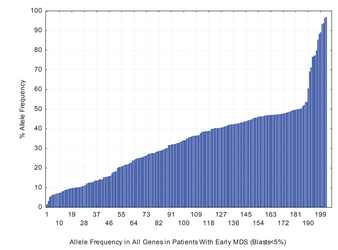

Figure 1c. *Profile of percentage of mutant allele frequency in all genes in early MDS patients*

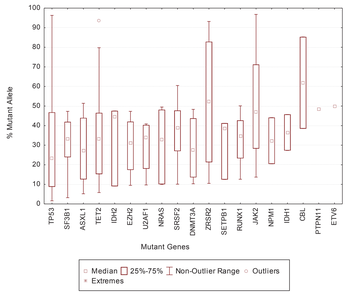

Figure 1d. *Levels of mutant allele in various genes as detected in early MDS patients*

2. Multigene mutations using bone marrow samples

- Most (68%) of the early MDS cases showed mutations in multiple genes. A similar ratio was detected when all MDS patients were considered.

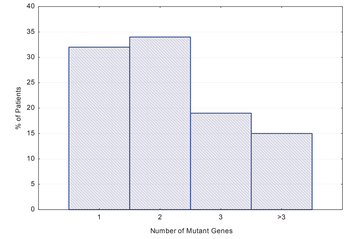

Figure 2a. *Number of mutant genes in early MDS (blasts <5%), n=103.*

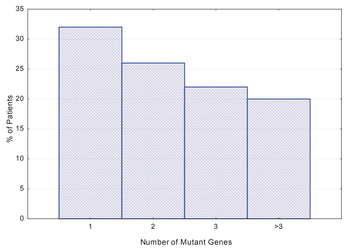

Figure 2b. *Number of mutant genes in all MDS, n=294.*

3. Complementary cytogenetic abnormalities and the type of mutant gene as indication of clinical significance

When considering patients with one gene mutated, the frequency of the mutant allele was <20% in only 11 of 103 cases (11%). The remaining 92 patients had either mutations in two genes or in one gene, but the mutant allele frequency was >20%.

However, four of these 11 patients (36%) had cytogenetic abnormalities confirming the diagnosis of MDS [der(1;7)(q10;p10), del(5q), trisomy 8. and del(11)(q23)]. Of the remaining 7 patients with allele frequency <20%, 3 had mutations in DNMT3A, 1 in U2AF1 gene, 1 in TET2 gene, 1 in TP53 and 1 in SF3B1 gene. Of these 7 cases, only two cases had an allele frequency <10%, one in TP53 gene & one in SF3B1 gene (Table 1).

	Mutated Gene	% Allele Frequency	Cytogenetics
Pat. # 1	ZRSR2	10.5	der(1;7)(q10;p10)
Pat. # 2	DNMT3A	14.2	del(5q)
Pat. # 3	ZRSR2	12.7	trisomy 8
Pat. # 4	SF3B1	15.6	del(11)(q23)
Pat. # 5	TP53	1.6	Diploid
Pat. # 6	DNMT3A	13.6	Diploid
Pat. # 7	DNMT3A	15.5	Diploid
Pat. # 8	DNMT3A	10.3	Diploid
Pat. # 9	SF3B1	3.2	Diploid
Pat. # 10	U2AF1	18	Diploid
Pat. # 11	TET2	11.4	Diploid

Table 1. *Correlation of mutated gene with cytogenetics*

Of the 92 cases with mutations in two genes or in one gene with allele frequency >20%, 26 patients (28%) had cytogenetic abnormalities confirming the diagnosis of MDS. In this group of 26 patients with cytogenetic abnormalities, only one patient had mutations at <20% in all mutated genes (TET2, DNMT3A and TP53), but also had del(17p). Of the remaining patients 65 cases without cytogenetic abnormalities, with more than one gene mutation, at least one gene had mutant allele at >20%.

4. No correlation between depth of cytopenia and molecular abnormalities in early MDS

There was no statistically significant difference in the degree of cytopenia between patients with <20% one mutation and no cytogenetic abnormalities (N=7) and the 96 cases with mutations in two genes or in one gene with allele frequency >20%. There was no significant difference in the degree of cytopenia between the 36 patients with one gene mutation and 67 patients with more than one gene mutation.

SUMMARY

- **Confirmation of MDS diagnosis using NGS is reliable when based on percent of mutant allele in bone marrow as well as the type of driver genes.**
- **Mutation analysis to confirm MDS diagnosis should be based on analyzing bone marrow samples.**
- **Cytogenetic and molecular profiling using NGS should be considered together for the confirmation of the diagnosis of MDS.**
- **The presence of mutation in more than one gene and the detection of mutant allele frequency >20% in bone marrow samples is adequate for the diagnosis of MDS.**
- **Patients with mutant allele frequency between 10% and 20% in the bone marrow in one gene and cytopenia most likely have MDS, but further studies are needed.**
- **Mutant allele frequency in bone marrow of <10% is extremely rare when testing is performed in patients presenting with cytopenia.**

Authors are employees of a diagnostic company offering NGS testing.