GENETIC TESTING IN MYELODYSPLASTIC SYNDROMES – CONTRIBUTION IN DIAGNOSIS, PROGNOSTIC AND CLINICAL MANAGEMENT

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Abstract: Myelodysplastic syndromes (MDS) represent a group of clonal hematological malignancies characterized by ineffective hematopoiesis. Other hematological disorders associating dysplastic features are grouped under the myelodysplastic/ myeloproliferative neoplasms (MDS/MPN) category. The great diversity of the acquired chromosomal abnormalities described in MDS highlights the molecular heterogeneity of these diseases. We report on 12 MDS and 3 MDS/MPN patients investigated by cytogenetic and molecular techniques (FISH). The most frequent chromosomal anomalies were 5q deletion and trisomy 8. Other trisomies, deletions and new translocations were also detected. MDS and MDS/MPN stand as challenging entities in hemato-oncology due to their heterogeneity. Thus, genetic testing provides important means for diagnosis confirmation and offers further insight into the prognosis and management of these patients.

INTRODUCTION

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders originating in pluripotent hematopoietic stem or progenitor cells. They are characterized by ineffective hematopoiesis with peripheral blood cytopenias, and a tendency to evolve towards acute myelogenous leukemia (AML) (Panani and Roussos, 2006) There is a profound heterogeneity in MDS from clinical, morphological and genetic point of view (Haase *et al*. 2007). In this context, the understanding of pathogenetic mechanisms causing MDS is still incomplete.

MDS diagnosis is presently done according to the World Health Organization (WHO) classification criteria. The 1982 French-American-British Cooperative Group (FAB) classification (Bennett *et al.* 1982) was in use until 2001, and still applied in certain circumstances (*e.g.* clinical trials). WHO classifications of MDS succeeded the FAB system, and presently the 4th edition is the current standard (Vardiman *et al*. 2009).

Cytogenetic abnormalities are frequent in MDS, detected in 20-70% of patients at diagnosis (Pozdnyakova *et al.* 2008). The cytogenetic profile of MDS is strongly dominated by unbalanced abnormalities. Most frequently, a loss of genetic material in the form of deletions and monosomies can be observed. Gain of genetic material is also identified. The most frequent single cytogenetic abnormalities reported are 5q deletion, monosomy 7 or 7q deletion, trisomy 8, and 20q deletion (Tefferi and Vardiman, 2009). Complex karyotype changes defined as at least three independent abnormalities identified in the same clone are found in 15% of MDS patients (Haase *et al.* 2007). Several recurring chromosomal abnormalities are considered presumptive evidence of MDS even in the absence of definitive morphological features, as recognized by the 4th edition of WHO classification. These abnormalities include -7 or del(7q) , -5 or del(5q), i(17q) or t(17p), -13 or del(13q).

Cytogenetic abberrations in MDS are strong independent prognostic determinants. Various systems have been proposed for the prognostic stratification of MDS patients, such as International Prognostic Scoring System (IPSS) (Greenberg *et al*. 1997) and WHO classification based Prognostic Scoring System (WPSS) (Malcovati *et al*. 2007). Generally, three cytogenetic prognostic categories are recognized - good: normal karyotype, –Y, del(5q), del(20q) as isolated anomalies; poor: complex (≥three abnormalities), chromosome 7 anomalies; and intermediate: other abnormalities.

WHO overlapping category, myelodysplastic/ myeloproliferative (MDS/MPN) diseases, exhibits both myelodysplasia and myeloproliferative features suggestive of MPN. Besides chronic myelomonocytic leukemia and juvenile myelomonocytic leukemia, this category comprises several other less understood entities, such as atypical chronic myeloid leukemia.

We report on cytogenetic findings from a retrospective study of 15 patients with primary MDS (12 patients) and MDS/MPN (3 patients), with the aim of emphasizing the role of cytogenetic analysis in diagnosis, prognosis assessment, and patients management. Furthermore, by presenting some rare/novel cytogenetic anomalies in MDS we add supplementary genetic data that may offer further insights into disease biology.

MATERIALS AND METHODS

The patients were admitted in Hematology Clinics and Departments from hospitals in Bucharest area for diagnosis and treatment. May-Grünwald-Giemsa panoptic stained peripheral blood smears and bone marrow aspirate smears were examined. Morphological examination of BM biopsy specimens completed the hematologic diagnosis algorithm whenever necessary. Perls stain on bone marrow smears was used for demonstration of ring sideroblasts.

Cytogenetic investigation was performed by classical chromosome studies (GTG banding) and standard fluorescent in situ hybridization (FISH) techniques. Bone marrow samples were cultured for 24h, 48h and 72h respectively, and harvested according to standard protocols. A motorized Axio Imager Z1 Zeiss Microscope equipped with Zeiss CCD camera, Metapher MSearch and Ikaros MetaSystem softwares were used for GTG-banded metaphases analysis. Karyotyping was done following the International System for Human Cytogenetic Nomenclature 2009 guidelines (Shaffer *et al*, 2009). Centromeric, locus specific and painting probes were used for FISH studies following manufacturer recommendations. FISH was applied in selected patients to verify or supplement conventional cytogenetics. For example, all three patients with a MDS/MPN diagnosis were BCR/ABL negative as demonstrated using LSI BCR/ABL Dual Color, Dual Fusion Translocation Probe (*Vysis, Abbott Molecular*). FISH analysis was performed on Axio Imager Z1 Zeiss Microscope equipped with appropriate single band fluorescence filters and using Isis MetaSystem software.

RESULTS AND DISCUSSION

Clinico-hematological features and cytogenetic results of our patients are centralized in table 1. The median age was 62 years and the male-to-female ratio was 1.14 (M:F = 8:7).

9 patients were diagnosed using FAB criteria. WHO classification was available for 6 patients.

Genetic testing revealed abnormalities in 8 out of 15 patients, ranging from complex karyotype to single numerical or structural anomalies. The frequency of cytogenetic abnormalities in our patient group, 53.3%, is in agreement with published literature (Pozdnyakova, 2008).

5q deletion as unique genetic aberration was identified in two patients (no. 5 and 10, table 1), with clinical diagnosis of refractory anemia (RA) and MDS/MPN respectively (Fig. 1 A). Both patients have deletions that encompass 5q31-q33 region, with different proximal breakpoints (q13 in RA patient, and q15 in MDS/MPN patient). The RA diagnosis was refined to MDS with isolated del(5q) according to WHO criteria. The other patient stands as one of the rare reported cases with isolated del(5q) and myeloproliferative characteristics. Cryptic deletion of 5q31-33 was investigated by FISH using ON MDS 5q- Kreatech probe (EGR1 - 5q31; CSF1R - 5q33)(*Kreatech Diagnostics*) in other three patients (no. 4, 14, 15), with no evidence of deletions. Deletion of the long arm of chromosome 5 is the most frequent cytogenetic anomaly in MDS (Haase *et al*. 2007, Greenberg *et al*. 1997). Although the deletions can have variable breakpoints, a common deleted region spanning band 5q31 was delineated, as specifically associated with 5q- syndrome. Haploinsufficiency of gene/genes within the deleted region was suspected as the pathogenetic mechanism in 5q deletion MDS. The relevant gene seems to be RPS41 (Ebert *et al.* 2008), although other genes (CTNNA1, EGR1) are likely to be involved in generating MDS phenotype. The prognostic impact of 5q deletions is favorable, when del(5q) is not part of a complex karyotype (Haase *et al.* 2007, Malcovati *et al.* 2007). The therapeutical management of del(5q) MDS has recently changed following the demonstration of high efficacy of Lenalidomide (List *et al.* 2006, Jädersten 2010) .

Monosomy 7 as isolated anomaly was identified in a patient with refractory anemia with excess of blasts (RAEB) (no. 2, table 1), in all analysed cells. Cytogenetic investigation was supplemented by FISH with chromosome 7 painting probe (*Qbiogene*) (Fig.1 B). Monosomy 7 is the second most frequent isolated chromosome anomaly in MDS. The frequency of monosomy 7 in MDS is approximately 15% (Heim 1992). It may be observed as total or partial monosomy. In the latter case, deletions of various sizes are detected, with clustering

breakpoints at 7q11 and 7q36. Two common minimally deleted regions have been delineated: 7q22 and 7q32-34. The same pathomechanism as in deletion 5q was proposed, haploinsufficiency of gene/genes with tumor-suppressive features within the deleted regions. Monosomy 7 even as an isolated abnormality confers a significantly poor prognosis (Malcovati *et al.* 2007, Haase 2008)

Trisomy 8 as sole cytogenetic anomaly was identified in two patients (no. 9 and 13, table 1), one diagnosed with RA (FAB criteria) and the other with refractory cytopenia with multilineage dysplasia (RCMD, WHO criteria). Trisomy 8 is a common chromosomal abnormality detected in MDS patients (Haase 2008). It is not specific for MDS and can be encountered in a wide variety of myeloid neoplasms. Trisomy 8 as sole anomaly places the patients in the intermediate risk group.

Trisomy 19 was revealed in a MDS/MPN patient (no. 11, table 1) (Arghir A., personal communication). Trisomy 19 as a sole chromosomal abnormality has been described by various authors in myeloid malignancies, particularly in MDS and AML. As a part of complex karyotypes, trisomy 19 has been encountered in chronic myeloid neoplasms such as idiopathic myelofibrosis and polycythemia vera (http://AtlasGeneticsOncology.org). Trisomy 19 as sole anomaly places the MDS patients in the intermediate risk group.

Complex karyotype changes were found in a RAEB patient (no. 1, table 1). Multiple numerical and structural anomalies were detected in a high percentage of bone marrow metaphases (73%). The malignant clone was hyperdiploid (51 chromosomes), showing trisomy 8, 14, 21 and the presence of three structurally abnormal chromosomes: two chromosomes 1 with del(1p) and a supernumerary marker chromosome similar in size to the C group (Fig. 1 C). FISH with chromosome 14 painting probe (*Vysis, Abbott Molecular*), and centromeric probe for chromosome 8 (D8Z1) confirmed the classical cytogenetic findings (Arghir A, personal communication). Quantitative alterations of chromosome 8 (either total or partial trisomy 8) are commonly found in MDS, as well as trisomy 14 Trisomy 21 is the second most frequent acquired trisomy in adult MDS (http://AtlasGeneticsOncology.org). In our case, frequently described trisomies associate with structural aberrations (marker chromosome). The impact of these complex genomic alterations on disease evolution was unfavorable. The patient progressed towards AML and died of infectious complication.

Uncommon cytogenetic findings were detected in one patient (no. 12, table 1). He was diagnosed within the overlap category of MDS/MPN. Bone marrow cytogenetic investigation identified several structural abnormalities (translocation t(6;14), del(11)(p15) and del(1)(q21)) and a genomic instability with the presence of multiple chromosome breaks and double minutes (Arghir A, personal communication). Among the structural anomalies, the translocation t(6;14)(q21;q32) was of particular interest (Fig. 1 D). To our knowledge this rearrangement was not reported by date. The patient had an unfavorable evolution. New cytogenetic findings with unknown prognostic significance are frequently detected in small groups of MDS patients. Large patient cohorts and multicentric studies are needed in order to define the biological behavior of such MDS subgroups.

Interestingly, among the 8 patients with cytogenetics anomalies, all but one (patient no 2, table 1 with monosomy 7) had a mixture of abnormal and normal metaphases, with the malignant clone accounting for only a fraction of metaphases. The all-abnormal pattern correlates with higher risk MDS (Pierre *et al.* 1989, Steensma and List 2005), an assumption also verified in our patient. The abnormal-normal pattern patients have a better overall survival when compared with all-abnormal pattern patients.

CONCLUSIONS

MDS are highly heterogeneous disorders, thus, diagnosis and prognosis assessment is often challenging, and requires integration of clinical, hematological, and genetic data.
Our evaluation of the genetic characteristics of MDS and MDS/MPN patients reveals a cytogenetic profile comparable to data previously published, showing common as well as new/rare cytogenetic findings. Our study proves that cytogenetics is still the gold standard of MDS genetic testing, providing important clues for diagnosis and prognostic impact (*e.g*. deletion 5q, monosomy 7, complex karyotype changes). Cytogenetic findings are also shown to emerge as an important factor in treatment selection. Ultimately, by better understanding the genetic complexity of MDS, genetic testing may offer important insights into pathogenic mechanisms.

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Figure legend
Fig. 1. Cytogenetic features of the investigated patients: A. Isolated deletion 5q in a MDS/MPN patient (patient no. 10). B. Isolated monosomy 7 in patient no 2. confirmed by FISH (inverted DAPI) with painting probe for chromosome 7. C. Complex karyotype abnormalities (patient no. 1). D. Uncomon cytogenetic findings: t(6;14) and del(11)(p15) in a MDS/MPN patient (patient no 12).

Table 1. Patient characteristics

No.	Diagnosis		Sex	Age (years)	Karyotype
1.	RAEB	FAB CLASSIFICATION	M	54	51,XY,+1, del(1)(p3?),del(1)(p3?),+8,+14,+21,+mar, [44]/46,XY[16]
2.	RAEB		F	62	45,XX,-7[30]
3.	RAEB		F	66	46,XX[30]
4.	RAEB		F	37	46,XX[30]
5.	RA		F	75	46,XX,del(5)(q13q33)[9]/46,XX[28]
6.	RA		M	66	46,XY[30]
7.	RA		F	72	46,XX[30]
8.	RARS		M	61	46,XY[30]
9.	RA		F	54	47,XX,+8[7]/46,XX[53]
10.	MDS/MPN	WHO CLASSIFICATION	F	74	46,XX,del(5)(q15q33)[10]/46,XX[20]
11.	MDS/MPN		M	52	46,XY,+19[27]/46,XY[3]
12.	MDS/MPN		M	59	46,XY,t(6;14),del(11)(p15)[2]/ 46,XY,del(1)(q21)[2]/46,XY[26]
13.	RCMD		M	71	47,XY,+8[12]/46,XY[8]
14.	RAEB -II		M	54	46,XY[23]
15.	RAEB II		M	82	46,XY[30]

MDS = myelodysplastic syndrome; RA = refractory anemia; RAEB = refractory anemia with excess of blasts; RARS = refractory anemia with ring sideroblasts; MDS/MPN = myelodysplastic syndrome/myeoproliferative neoplasm; RCMD = Refractory cytopenia with multilineage dysplasia