



## THE IMPORTANCE OF MOLECULAR AND CYTOGENETIC MARKERS IN THE PROGNOSTIC OF MYELODYSPLASTIC SYNDROMES

Nicorescu Iuliana-Maria<sup>1</sup>

<sup>1</sup> Hematology, Neolife Medical Center, Bucharest, Romania

**Abstract.** Myelodysplastic syndromes represent clonal diseases of hematopoietic stem cells that occur predominantly in elderly people, but myelodysplastic syndromes can affect younger patients as well. Myelodysplastic syndromes are characterized by ineffective hematopoiesis, causing different forms of cytopenia in peripheral blood, contrasting normal or hypercellular bone marrow. Although less known, due to its absence from current clinical practice or in the prognostic score, molecular analysis could bring a significant contribution in the early diagnosis of disease. In this respect, the latest studies (2011) show that this type of analysis is of great importance in terms of input added to the pathogenic mechanisms of the Myelodysplastic syndromes subtypes establishment, with high conversion. Furthermore, the analysis serves elect the optimal timing for the initiation of specific therapy.

**Keywords:** myelodysplastic syndromes, acute leukemias, molecular analysis

### Introduction

“Myelodysplastic syndromes are clinically heterogeneous disorders characterized by byclonal hematopoiesis, impaired differentiation, peripheral-blood cytopenias, and a risk of progression to acute myeloid leukaemia. Somatic mutations may influence the clinical phenotype but are not included in current prognostic scoring systems.”[1]

Myelodysplastic syndromes represent a heterogeneous group of hematologic disorders difficult to diagnose, which is more often underdiagnosed or identified in a stage of transformation into acute leukaemia.

Myeloid malignancies are clonal diseases of hematopoietic stem cells or cell progenitors. These diseases also contain chronic disease which includes myelodysplastic syndromes, chronic myeloproliferative diseases, chronic myelomonocytic leukaemia and acute disorders represented by acute leukemia.

Being a heterogeneous disease category, both in terms of clinical, biological, morphological as well as cytogenetic and molecular manifestation, during the recent years research on cytogenetic and molecular-level changes has expanded. The purpose of this research is the establishment of a loyal group identifier score of increased risk of transformation of the myelodysplastic syndromes into acute leukaemia, linking all mentioned prognostic factors.

At this point, molecular analysis in MDS is less known

and has not yet been introduced into prognostic score, but according to the latest studies (since 2011) it has a great importance in terms of obtaining information on pathogenic mechanisms.

### Definitions and classification

MDS - represent clonal diseases of hematopoietic stem cells, which predominantly occur in elderly people, characterized by ineffective hematopoiesis causing different forms of cytopenia in peripheral blood, contrasting normal or hypercellular bone marrow.

Acute leukaemia is a heterogeneous group of malignant proliferation of pluripotent or unipotent stem cells, characterized by clonal expansion of immature cells that lost the ability to differentiate.

### MDS classification – WHO classification - 2008 World Health Organization MDS

#### Classification and Criteria

- Refractory cytopenia with unilineage dysplasia (RCUD);
- Refractory anaemia (RA), refractory neutropenia (RN), refractory thrombocytopenia (RT). Unicytopenia or bicytopenia, No or rare blasts (<1%), Unilineage dysplasia: 10% of the cells in one myeloid lineage;
- Refractory anaemia with ring sideroblasts (RARS): Anaemia, No blasts, 15% of erythroid precursors are ring sideroblasts Erythroid dysplasia only <5% blasts;
- Refractory cytopenia with multilineage dysplasia (RCMD): Cytopenia(s), No or rare blasts (<1%), No Auer rods <1% monocytes Dysplasia in 10% of the cells in two or more myeloid lineages, <5% blasts in marrow No Auer rods 15% ring sideroblasts;

**Iuliana-Maria Nicorescu**

40 Ficusului Blvd, 1st District, 013975,

Bucharest, Romania

e-mail: nicorescuiuliana@yahoo.com

- Refractory anaemia with excess blasts-1(RAEB-1): Cytopenia(s) <5% blasts No Auer rods <1% monocytes Unilineage or multilineage dysplasia,5%-9% blasts No Auer rods;
- Refractory anaemia with excess blasts-2 (RAEB-2): Cytopenia(s)5%-19% blasts Auer rods,<1 % monocytes Unilineage or multilineage dysplasia 10%-19% blasts Auer rods.

Myelodysplastic syndrome unclassified (MDS-U): Cytopenias 1% blasts Unequivocal dysplasia in <10% of cells in one or more myeloid cell lines when accompanied by a cytogenetic abnormality considered as presumptive evidence for a diagnosis of MDS <5% blasts.

MDS associated with isolated del(5q) Anaemia Usually normal or increased platelet count No or rare blasts (<1%), Normal to increased megakaryocytes with hypolobated nuclei <5% blasts, Isolated del(5q) cytogenetic abnormality No Auer rods.

*“The IPSS and the WHO classification system incorporate only the most common chromosomal abnormalities. An international effort is under way to develop a comprehensive cytogenetic scoring system for the myelodysplastic syndromes that incorporates rare cytogenetic subgroups, which will inform an ongoing revision of the IPSS. With the advent of more sensitive techniques already available in the research setting, including next-generation genome and transcriptome sequencing and arrays of single-nucleotide polymorphisms for the detection of copy-number alterations, the rate of discovery will accelerate, and the compendium of genetic alterations in the myelodysplastic syndromes will undoubtedly expand. Detailed analyses of bone marrow samples from a larger number of patients with myelodysplastic syndromes are needed to establish the spectrum and frequency of mutations, the degree of genotypic overlap, and their clinical significance, particularly in predicting outcomes for available therapies”.[2]*

At this moment, according to the studies it is estimated that approximately 50% of patients with MDS show karyotype changes (Fig.1.), most common are the partial deletions – del 5q, del 20q; del 7q; del 11q; del 12p; Losses chromosome monosomy 7; y ;17; Extra chromosome – trisomy 8,11,21; translocation;

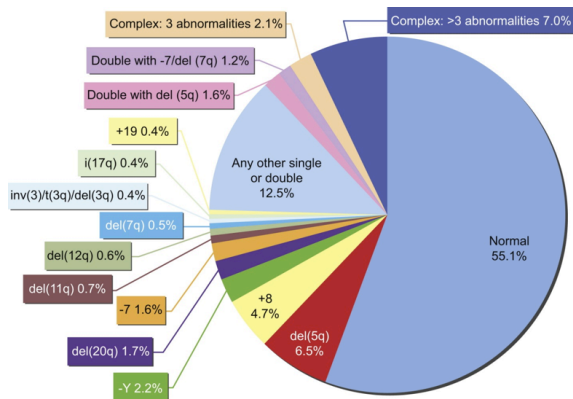


Fig.1.Distribution of cytogenetic findings in MDS [3]

complex abnormalities.

According to cytogenetic analysis we could obtain:

- normal karyotype
- balanced chromosomal abnormalities
- unbalanced chromosomal abnormalities → → → common by MDS with transformation risk
- complex karyotype → → → common by MDS with transformation risk

5q deletion – the most common chromosomal abnormality identified in half of the isolated cases, often associated with refractory anaemia { AR } in terms of FAB classification, most common in women- in general good prognosis . It is characterized by increased transfusion requirements and associated hemochromatosis. (fig 2)

It can occur in association with other abnormalities, being characterized by recurrent and severe anaemia, accompanied by leucopenia and thrombocytopenia. In terms of FAB most cases are AREB1 and AREB2 with

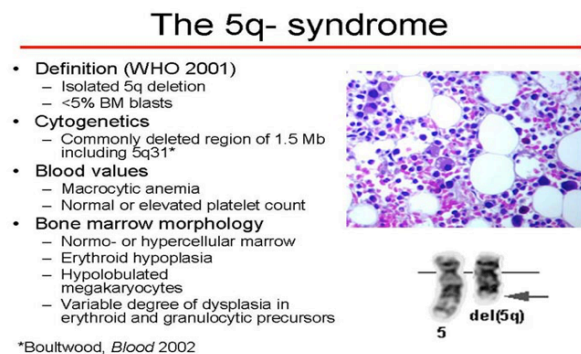


Fig.2.Deletion 5q [4]

increased risk of transformation into acute leukaemia and poor prognosis.[1].

**Del 17p:** (fig.3.) the rearrangement of chromosome 17 includes del. 17p or translocations t(5;17)(p11;p11), t(7;17)(p11;p11) or i(17p)(q10). In general del17p is associated with other chromosomal mutations. The patient frequently presents TP53 mutation with low or no response to chemotherapy. Primary 17p deletion (de novo) occurs in patients who have undergone chemotherapy or radiotherapy for other neoplasia.

Patients are showing a reserved prognosis and 4 months median survival rate after evolution into acute myeloid leukemia. It was reported in AML and MDS a strong correlation between 17p deletion (a clonal cytogenetic anomaly consisting of a deletion of the short arm of chromosome 17), and a particular form of morphological dysgranulopoiesis, we also found in such cases a strong correlation between 17p deletion and p53 mutation; these correlations suggest that AML and MDS with 17p deletion constitute a new morphological-cytogenetic- molecular entity, the 17p syndrome.” [5]

MDS and AML de novo or secondary to an exposure to chemical mutagens or to chemotherapy with alkylating agents may probably also be secondary to immunosuppressive therapy for severe aplastic anaemia. “Chromosome 7 anomalies are not rare in acute

lymphocytic leukemia – ALL. They occur in balanced translocations involving 7p15 or 7q34 in T lineage and 7q22 or 7q32 in B proliferations; monosomy 7 is present in 5 to 6% of ALL, most often as a secondary anomaly of



Fig.3.Deletion 17p [7]

the t(9,22)”[8].

**Deletion 12p** (fig. 4): 12 p abnormalities are common in a broad spectrum of haematological malignancies – ALL; AML, MDS, or chronic myeloproliferative syndromes, nonHodgkin’s lymphoma.

5% of AML secondary MDS after prior mutagenic exposure associated with a poor prognosis (karyotypes

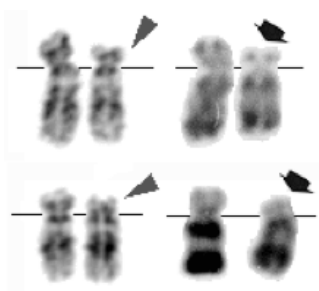


Fig.4.Deletion 12p [7]

mostly complex). Duplication 12 (p1,2p13) described in one MDS case after benzole agent exposure.

**Monosomy 7:** In adults second, at children first as frequency, is associated with monocytosis, splenomegaly and poor prognosis.

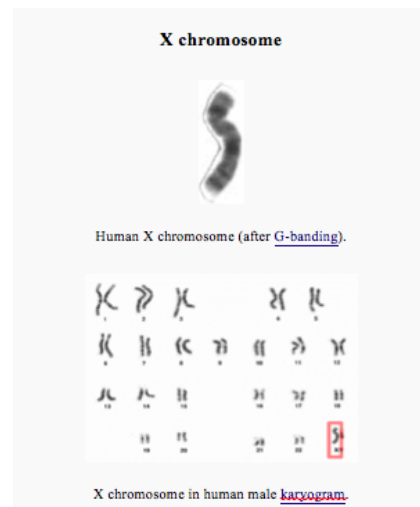
“MDS is rare in childhood and may have a rapidly progressive course with an extremely poor prognosis without hematopoietic stem cell transplantation (HSCT). The disease can arise in a previously healthy child; in this case, it is referred to as de novo or primary MDS. MDS may develop in a child with a known predisposition (egg, previous cytotoxic chemotherapy); this is referred to as secondary MDS (see Etiology). The disease is most common in adults, especially elderly people, and the course varies, ranging from an acute, rapidly fatal illness to a chronic, indolent illness. When a child presents with cytopenias associated with MDS, physicians should administer supportive care until the diagnosis is established. Many patients present with profound cytopenia and a notable risk for infection. Transfusions and broad-spectrum antibiotics may be required to

treat life-threatening anaemia, thrombocytopenia, and infection until definitive therapy can be started. In MDS paediatric patients with refractory cytopenia, hematopoietic stem cell transplantation (HSCT) from a matched related or unrelated donor early in the course of the disease is the treatment of choice.”[6,9,10]

**Trisomy 8** is the only amplified chromosomal disorders associated with MDS and other haematological malignancies and solid tumours. Studies that compare normal cells CD34+ with those in MDS associate with trisomy 8, highlight the presence and increasing imbalance between pro and anti-apoptotic genes. Tested in culture the CD34+ cells combined with trisomy 8 proved to be radiation resistant and gained strength, being able to form hematopoietic colonies, despite the apoptotic protein presence, normally associated with rapid aging and cell death. A subset of patients with MDS and trisomy 8 distinguished a clinical response to immunosuppressive therapy. These patients are young, associate refractory anaemia and show HLA -DR15.

**Y - :** loss of Y chromosome in case of patients with MDS compared to those with normal karyotype, show favourable prognosis according to the IPSS.

**X chromosome abnormalities** (fig.5) – loss of an X chromosome in female patients with MDS has been with FISS to affect blast cells as well as myeloid elements of the marrow. A typical structural rearrangement has been proposed for some case RARS. Isolated deletion of Xq as



a sole cytogenetic abnormality in MDS is relatively rare  
Fig.5.X chromosome abnormalities [7]

with REAB and unfavourable prognostic.

### Evolution and prognosis

Prognostic factors and framing into acute leukaemia transformation and / or death risk group represent continuous changing criteria.

MDS were often called "preleukemic states", which highlights increased risk (10=40% depending on the subtype of the WHO classification) of progression to acute leukaemia. Median survival rate varies depending on the subtype: 20-65 months in AR, 21-76 months ARSI t 5-12 months RAEB.

Based on morphological and biological factors different scores were created. The most used prognostic

The prognostic value of the risk factors	0	0.5-1.0	1.5-2.0	≥2.5
Marrow blasts %	<5	5-10	11-20	21-30
Caryotype (meaning)x	normal	good	intermediate	unfavourable
cytopenia	0	1	2	3

**Table I. IPSS prognostic scoring system**

scoring system is IPSS (table I).

xCaryotype: normal - 46XY; god - Y- / 5q- / 20q-isolate; unfavourable - complex Caryotype (>3 abnormality) or abnormalities of chromosome 7; intermediate - all other abnormalities

xxCytopenia:Hb<10 g/dl, PMN <1.500/mm<sup>3</sup>, Tr<100.000/mm<sup>3</sup>

By identifying the high-risk genetic mutations and the correlation of subgroups with molecular changes in subgroups with cytogenetic changes, associated to already established markers in the diagnosis and tracking patients with MDS, the assay is attempting to establish a more accurate diagnose and monitoring protocol for these patients.

MDS remains one of the most challenging adult malignancies with varied and complex features at presentation. Immunophenotyping, cytogenetic-molecular studies and, more recently, high-resolution genome-wide screening, characterize MDS as a heterogeneous disease with distinct manifestations and prognostic and therapeutic implications.

**ACKNOWLEDGEMENT:** *This presentation has been elaborated and written by Iuliana-Maria Nicorescu, MD and third year PhD student since 2012 at UMF Carol Davila under coordination of Prof. Dr. Ana-Maria Vlădăreanu, MD, PhD. This paper is supported by European Project code POSDRU/159/1.5/S/141531, entitled "Human Resource Development - PhD students and postdocs - for research excellence in health and biotechnology".*

## References

1. **Rafael Bejar, Benjamin L Ebert:** "The genetic basis of Myelodysplastic syndromes" *Elsevier* 2010;

2. **Rafael Bejar, M.D., Ph.D., Kristen Stevenson, M.S., Omar Abdel-Wahab, M.D et al** – „Clinical Effect of Point Mutations in Myelodysplastic Syndromes" *TheNew England journal of medicine* – jun 2011 - 2946- 2948;

3. **Schantz J, Tuchler H, Sole F, Mallo M, Luno E et al** „New comprehensive cytogenetic scoring system for primary MDS and oligoblastic acute myeloid leukemia after MDS derived from an international database merge" *J ClinOncol* 2012, 30: 820-829;

4. *Boultwood Blood* 2002

5. *Wintrobe's Clinical Hematology – Twelfth edition 1956-1976.*

6. **Anne Murati, Mandy Brecqueville, RaynierDevillier, et al:** „Myeloid malignancies: mutations, models and management" *BioMed Central* – 2012;

7. *Atlas of genetics and cytogenetics in oncology and Hematology;*

8. **Grant E, Nybakken and Adam Bagg** "The genetic basis and expanding role of molecular analysis in the diagnosis, prognosis and therapeutic design for MDS" *The Journal of Molecular Diagnostics – Elsevier* –march 2014;

9. **Patnaik MM, Hanson CA, Hodnefield JM, et al:** „Monosomal karyotype in MDS, with or without monosomy 7 or 5 is prognostically worse than an otherwise complex karyotype" *Leukemia* 25: 266-270, 2011;

10. **Prasad Mathew, MBBS, DCH, FAAP; Chief Editor: Robert J Arceci, MD, PhD** „Pediatric Myelodysplastic Syndrome - Monosomy 7" *Medscape;*