Therapeutics, Pharmacology and Clinical Toxicology
Vol XXII, Number 1, March 2018
Pages: 9 - 13

THE ROLE OF CITOMETRY AND CITOGENETICS IN THE MANAGEMENT OF MYELODYSPLASTIC SYNDROMES

Nicorescu Iuliana-Maria[1], Vlădăreanu Ana-Maria[2], Georgescu Daniela[3], Mihai Felicia[2]

[1] *NeoLife Clinics, Bucharest*
[2] *Universitary Emengency Hospital, Bucharest*
[3] *Colentina Clinical Hospital*

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.
To achieve the highest success rate of treatment and consequently to increase survival chances, it is necessary to implement ongoing diagnostic and prognostic systems adapted to the specificity of the disease. Cytogenetics, through the approach it proposes, significantly supports this process, becoming in the last few years an integral and indispensable part of it.

Key words: myelodysplastic syndromes, cytogenetics, cytochemistry, flow cytometry

Introduction

Myelodysplastic syndromes (MDS) are clonal diseases of hematopoietic stem cells characterized by ineffective hematopoiesis (variable cytopenias in peripheral blood, in contrast to normal / hypocellular haematopoietic marrow), which associates an increased risk of transformation into acute myeloid leukemia (AML).[1]

Diagnosis of these diseases is suspected based on history and changes in peripheral blood, while cytological and IHC of bone marrow examinations have an essential contribution to the process of confirmation. At the same time, this process of diagnosis can be supported by a series of cytogenetic and molecular analyzes, which help to understand the pathogenic mechanisms and the framing of the disease in the risk groups.

As regards the management of MDS patients, due to their generally advanced age, lack of full response to most standard therapies and variability of prognosis, the treatment of these patients remains problematic.

Myelodysplastic syndromes include a series of haematological conditions characterized by chronic cytopenias (anemia, neutropenia, thrombocytopenia) accompanied by abnormal cell maturation.

Most MDS patients, especially those in the lower risk category, die from the consequences of medullary failure, rather than AML transformation. In fact, the distinction between MDS and AML is arbitrary, because patients with 20-30% blasts are considered to have MDS based on FAB classification criteria, but according to World Health Organization (WHO) classification, they are considered to have AML.[2,3]

The standard of care for MDS remains the support therapy and treatment of symptoms.However, for some categories of MDS patients (depending on age, clinical status and prognostic risk category), other specific forms of therapy are considered.

International consortia, such as NCCN (National Comprehensive Cancer Network), European Leukemia Net (ELN), European Group for Blood and Marrow Transplantation (EBMT) have been trying to provide evidence-based guidance for the development of MDS management guidelines.The uses of the IPSS classification system, age and performance status have been major determinants of therapeutic strategies. For diagnosed patients in the low or intermediate -1 risk category, progressing to the intermediate -2 risk category, the therapeutic options are those in the high-risk categories. The age criterion may be based on the biological or chronological status, according to the institution-specific guidelines, for the indication of intensive chemotherapy or bone marrow transplantation. Performance status is based on institution guidelines, with patient evaluation after correction of remedy causes.[2,3]

Analysis of literature data shows that prognosis

Iuliana Nicorescu
40 Ficusului Str., 1 District, Bucharest
e-mail: nicorescuiuliana@yahoo.com

assessment for MDS patients is based on different risk models, some related to the disease:FAB, WHO, IPSS, WPSS, MDACC, R-IPSS systems, and others related to the patient and comorbidities: MDS-CI.

The FAB and WHO classifications do not take into account cytogenetic changes.

The International Prognostic Score System (IPSS) is most used for newly diagnosed patients, but underestimates the role of transfusion and cytogenetic markers.

The International Prognostic Score System based on World Health Organization classification (WPSS) can also be used during disease progression, but does not take into account chromosome changes.

The system proposed by MD Anderson Cancer Center (MDACC), which includes cytogenetic aspects, can also be applied to patients with secondary MDS.

The MDS Comorbidity Index (MDS-CI) evaluates the role of comorbidities.

It is evident that in MDS patients with low and intermediate risk groups, comorbidities represent an independent risk factor, so revaluation of current prognostic systems is required

In 2012, Peter L. Greenberg and his collaborators public in Blood the revised International Prognostic Score System (R-IPSS), which includes MDS patients in 5 prognostic groups, depending on cytogenetic changes.

Indications for biological evaluation and initiation of treatment.

All patients with MDS should have clinical and laboratory history including documentation of the need for initiation of treatment. It may be supportive (transfusion), symptomatic and / or chemotherapeutic (alkylating agents or hypomethylates).

The paraclinical investigations necessary to support the diagnosis and management of a MDS patient are presented below:

• Laboratory analyzes include blood count, reticulocyte count, serum erythropoietin level, serum vitamin B12 and folic acid, serum ferritin, sideremia, total iron binding capacity, copper serum level, and serology for HIV infection.

• HLA typing (human leukocyte antigen) should be performed in patients who are candidates for haematopoietic stem cell transplantation and for those who require HLA standard platelets.HLA-DR15 testing can identify low-risk or intermediate-1 MDS patients who can respond to immunosuppressive drugs.[2,3]

• Unilateral biopsy bone marrow aspirate is the recommended assay for all patients. This sample should be submitted for pathological examination, specific stains, including iron deposits and FISH testing (fluorescence in in situ hybridization) to detect common cytogenetic abnormalities in MDS and those who may affect therapy, such as del 5q, 7q minus, +8, MLL (11q23) rearrangements, 13q minus, minus 20q, and inv (3).

• Screening of lymphocytotoxic antibodies (anti HLA) for multiparous women and pluralized transfusion patients.

• Patients with LMMC should be evaluated for 5q31-33 translocations and / or PDGFRbeta rearrangements.

• JAK2 mutation analysis should be considered in patients with thrombocytosis.

• Peripheral blood flow cytometry can evaluate a HPN clone in patients with hypoplasia and MDS and differentiate from lymphocyte leukemia with large granular lymphocytes in patients with large granular lymphocytes on the peripheral smear.

• Performance status should be evaluated using either the Eastern Cooperative Oncology Group (ECOG) scale or the Karnofsky Performance Scale.

• Revised IPSS and / or IPSS (R-IPSS) should be used to stratify patients with MDS in risk groups.[2,3]

The role of flow cytometry and cytogenetics in MDS management

Immunophenotyping, cytogenetic and molecular markers under assessment will allow for the definition of cytogenetic and molecular profile for MDS patients, indicating new therapeutic targets, especially for patients with reserved prognosis and refractory to therapy.

Cytochemistry and immunophenotyping

Cytochemical stains and immunophenotyping may indicate a decrease or loss of myeloid maturation or the presence of non-normally expressed antigens.

Useful cytochemical methods include:
- Colors to identify ring sideroblasts;
- PAS coloration for erythroblasts to evaluate diseritropoiesis;
- Peroxidase or black B Sudan: confirms myeloid blasts;
- Esterases, to discern abnormal granulocytes and monocitoid forms.

Immunocytochemistry may be helpful in order to:
- exclude the lymphoid origin of blasts;
- distinguish erithrocyte precursors by the glycophorin A antibody reagent;
- quantify the myeloid progenitor cells and blasts, using antibodies for CD34, CD117, CD13, CD14, CD33;
- detect dysplastic or immature megakaryocytes by antibodies with specificity for von Willebrand factor, factor VIII, CD41 or the HPI-ID monoclonal antibody actor-specific antibodies, factor VIII, CD41 or the HPI-ID monoclonal antibody
- detect line disparity (e.g., myeloid lineages express non-myeloid antigens) and to confirm the presence of bi- or trilinear dysplasia.

Automated flow cytometry systems for MDS have been developed and are in the process of being standardized.These systems seem to have diagnostic and prognostic value in MDS patients.

Flow cytometry for the diagnosis of myelodysplastic syndrome

Flowcitometry was applied in MDS to characterize the blast population, especially in MDS at risk of disease

in transformation.

Although this role remains important, many other abnormalities (within all diagnostic and prognostic groups) have been described and flow cytometry is expected to become an important adjuvant to the diagnosis and prognosis of the disease.

It should be noted that in the literature most of the published materials refer to the medullary aspiration analysis,data on peripheral blood being limited.

The detection and interpretation of anomalies in this group of disorders (in MDS) can be influenced by:

1. Bone marrow cells, especially granulocytes, which remain biologically active after sampling - and therefore sensitive to environmental changes - may lead to alteration of surface antigen expression. Medullary aspiration should preferably be collected in heparin and promptly analyzed (ideally within 24 hours), otherwise prolonged storage in EDTA may modify certain antigens, especially CD10, CD11b and CD16. [4,5]Many of the documented abnormalities are detected as flux thus becoming an important adjuvant in the diagnosis and prognosis of the disease.
2. Abnormalities are detected on myeloblasts, as well as on different types of cell maturation and on different lines within the same patient, who will present various abnormalities.Thus, unlike a typical case of acute lymphoblastic leukemia (ALL) or AML, for example (if there is only one homogeneous neoplastic population),in MDS we have multiple lines that may have different abnormalities, which makes construction of antibody panels difficult.For example, similar abnormalities can be observed under reactive conditions such as CD64 expression on granulocytes from septic processes, CD56 on myeloid precursors as a result of G-CSF administration and other myeloid disorders as expression of CD7 aberrant in myeloblasts in CSB domain. CD16 and CD33 are not always found on dysplastic myeloid cells, but this may be secondary to loss by constitutional genetic polymorphisms, and CD16 is absent in nocturnal paroxysmal hemoglobinuria (PNH).

Thus, the flow data interpretation must be performed with a complete knowledge of the clinical situation. Therefore, great variability in discoveries is possible and reproducibility in laboratories is inevitably difficult.

In this context, it becomes imperative to standardize the methodology and develop reporting and score systems, in order to help diagnose and predict clinical outcome.

Cytogenetics in MDS

Cytogenetic studies have revealed structural and numerical chromosomal abnormalities, and their deepening could clarify the pathogenic mechanism. Cytogenetic analyzes revealed the presence of clonal cellular abnormalities in approximately 50% of patients with de novo MDS, and in 80% of those with secondary MDS.[6,7,8]

The incidence of chromosomal abnormalities is increased in aggressive forms with increased evolutionary risk (AREB, AREB-t) compared to AR, ARS.

Good prognostic anomalies (del (5q)) predominate in RA and those with reserved prognosis (monosomy 7) are more present in cases of AREB, AREB-t.

wIn chemotherapy secondary forms predominate monosomy 7, translocations, complex anomalies.

	MDS de novo	Secondary MDS
Partial deletions		
Del 5q	20	20
Del 20q	3-4	<1
Del 7q	1-2	10
Del 11q	2-3	<1
Del 12p	1-2	3-4
Chromosomal losses		
Monosomy 7	10-15	50
Loss crs Y	3-4	10
Monosomy 17	3	5-7
Translocations		
t(3;3)(q21;q26)	1-2	3
t(1;7)(p11;p11) 3)	<1	4-5
t(5-17)(q11,p11)	1-2	4-5
t(7,17)(q11,p11)	1-2	2
Complex chromosomal abnormalities	(>3)	15-20

Table I. Carotid anomalies in MDS

The karyotype analysis can group patients with MDS in patients with:

- normal karyotype (does not rule out the diagnosis of MDS!) - present in almost 1/2 of the cases, apparently due to the neoplastic clonal disability to divide into culture;

- balanced chromosomal abnormalities - lead to the generation of fusion oncogenes; translocations are more common in secondary forms (e.g., translocations involving PDGFR-β have been identified in 5-10% of cases of chronic myeloid monocytic leukemia (LMMC)[9];

- unbalanced chromosomal abnormalities (loss or gain of genetic material) - the more frequent genetic material increases are trisomy 8, 11 and 21;

- the anomalies predominantly found in MDS are non-random deletions (suggesting a pathogenic mechanism based on loss of tumor suppressor genes or haploinsufficiency of genes required for normal myelopoiesis): loss crs 7 and Y, partial deletions of crs 5q, 20q, 11q, 7q16;at the molecular level, they translate into: - affect of coding genes for GM-CSF, M-CSF, IL-3, PDGF-del (5q), erythropoietin - del (7q) coding gene

involvement, the coding gene involvement for G-CSF-del (17q);

- complex karyotype (presence of > 3 chromosomal abnormalities) - associated with the most unfavorable prognosis among MDS and AML subtypes; MDS cases with complex karyotype have a bigger "chance" to evolve to AML[10]. Cytogenetic abnormalities are both an independent prognostic factor and a predictive factor for the therapeutic response.[1,11]

The identification of a cytogenetic anomaly confirms the presence of a clonal disease,helping to differentiate MDS from secondary, reactive dysplasia,also having prognostic value.Cytogenetic analysis will be performed in all patients where the medullary examination is indicated.

Molecular changes in myelodysplastic syndrome

Anomalies of certain genes have been identified in patients with MDS and AML, with or without the presence of chromosomal anomalies.Although these genetic mutations affect DNA methylation, tumor suppressor genes and oncogenes, the prognostic significance of these mutations is not well characterized.

"2 to 15% of MDS patients found mutations in NRAS, TP53 and RUNX1 (AML1) genes, but these are not specific to MDS patients.More recently, it has been discovered that the abnormalities of the JAK2, FLT3, RSP14 and TET2 genes contribute both to the outline of the clinical picture of the disease and to the prognosis of the disease".[12]

RAS mutations may appear sooner or later in MDS, but the relationship with AML transformation remains uncertain, although some studies indicate that RAS mutation in MDS is associated with disease progression and reduced survival.The acquired gene mutations RAS (especially N-RAS) are found in 20 to 40% of MDS and AML.Although some N-RAS mutations are correlated with cytogenetic changes in MDS (5q-, -7, and -8), there may also be mutations in normal MDS cases with normal karyotype.

The "core binding factor" (CBF) complex is a heterodimeric transcription factor that is composed of two subunits, CBFa (located at 21q22) and CBFß (located at 16q22), which plays an important role in hematopoiesis.Both members are frequently changed by translocations or inversions.[13]

"The JAK2V617F activating mutation is found in an increased percentage of patients with chronic myeloproliferative syndrome (CMS). As a result of this mutation, multiple signaling pathways are abnormally activated, but the exact role in CMS pathogenesis is not well known.WHO 2008 classification provisionally classifies MDS / CMS borderline clinical entities. Among these, ARSI-T (refractory anemia with ringed sideroblasts and marked thrombocytosis) frequently presents the JAK2V617F mutation.Patients with ARSI might acquire the JAK2V617F mutation (possibly in the context of a gene instability) or this mutation in a subset of patients with SMPC might lead to blockage of Fe deposits in mitochondria and the occurrence of diseritropoieza"[12]

"TET2 Mutations - DNA methylation is a prognostic marker and predictor of treatment response in patients with MDS and appears to be a mechanism of disease progression to AML.[14]In addition, hypomethylates inhibiting DNA methyltransferases (DNMTs) have demonstrated activity in MDS and have become a key component of initial therapy for many MDS patients. The TET (ten-eleven translocation) gene family encodes proteins involved in the epigenetic control of DNA expression by demethylation.Somatic mutations at TET2 level occur in about 15% of cases of myeloid neoplasia, including MDS.Functional mutations of TET2 lead to increased methylation and suppression of genes that are expressed normally.The TET2 mutation was initially identified as a deletion at chromosome 4q24. When present in MDS, TET2 mutations were associated with a more favorable prognosis."In myeloproliferative diseases with both mutations present, TET2 and JAK2, it appears that the first mutation occurred in the TET2 gene, followed by the JAK2 mutation.These mutations were found in both multipotent cells and progenitor cells, indicating a role in myelopoiesis".[14]

"RPS14 is the ribosomal protein gene usually located in the deleted region of chromosome 5.Recent studies have shown that haploinsufficiency of the RPS14 gene is the key event involved in the pathogenesis of patients with 5q- syndrome.Patients with 5q syndrome have a distinct morphology and have anemia as the main clinical manifestation.The high proliferative rate of erythroblasts requires a high rate of ribosomal biogenesis, and therefore, RPS14 deficiency greatly affects erythropoiesis and less other cell lines.This mutation is not responsible for the clonal evolution of MDS patients 5q- and, therefore, it is likely that other mutations also play a determining role in the progression of this disease".[15]

RUNX1 ("transcriptional core-binding factor gene") - RUNX1 mutations are observed in 7-15% of de novo MDS cases; are more common in therapy-related MDS cases and have more reserved prognosis.

TP53 tumor suppressor gene - TP53 tumor suppressor gene is located on 17p and mediates cell cycle inhibition,in response to a variety of cellular stressors. In MDS, about 5 to 15% of cases have TP53 mutations at the time of diagnosis.[16]Abnormalities in p53 are more common in patients with MDS associated with previous exposure to alkylating agents or irradiation.The wild-type TP53 mutation is associated with treatment resistance and is a reserved prognostic marker independent of the IPSS risk score.

ASXL1 mutations ("additional sex-comb like-1"): The ASXL1 gene encodes a protein involved in the epigenetic regulation of gene expression and is mutant in about 10% of cases of MDS.The prognostic implications of these mutations are not yet known.[15]

DH Mutations - Isocitrate dehydrogenase oncogenic mutations (IDH1 and IDH2) have been reported in some cases of MDS; determines DNA hypomethylation and gene expression alteration and is considered an unfavorable prognostic factor.

FLT3 mutations - "FLT3 is a kinase receptor tyrosine molecule with an important role in regulating the proliferation and differentiation of hematopoietic cells.The mutations of this gene by FLT3-ITD

tandem internal duplication result in the constitutive activation of multiple cytoplasmic effector molecules in the pathways involved in apoptosis, proliferation and differentiation of hematopoietic cells.FLT3 gene mutations are uncommon in MDS and have been associated with a reserved prognosis."[12;17]

SF3B1 genetic mutations - The SF3B1 gene encodes a portion of the nuclear ribonucleoproteins that, along with other nuclear ribonucleoproteins, form the spliceosome, responsible for splicing the messenger RNA.Somatic punctate recurrent mutations in this gene were identified in 72 of 352 patients (20%) with MDS. [18]Within this group, SF3B1 mutations were common among patients with MDS with ringed sideroblasts (65%) but less common in AR patients (10%), refractory cytopenia and multilinear dysplasia (6%), and AREB (5%).Subsequent studies have confirmed the presence of SF3B1 gene mutations in a subset of MDS patients and have also identified mutations in other genes that affect messenger RNA splicing (e.g., U2AF1, U2AF35, ZRSR2 and SRSF2) in patients with MDS. [15]

Apoptosis (programmed cell death) is an active cellular process that regulates the size of the cell population by decreasing cell survival.Increase of apoptosis may play an important role in the early pathogenesis of MDS, a mechanism that may be responsible for the paradox of medullary hypercellularity versus pancytopenia in peripheral blood in MDS.

* * *

The association of abnormal cell growth and death make MDS one of the most difficult hematological diseases to treat.

It is therefore imperative that the current prognostic and staging systems of this disease be continually evolving, capable of adapting to the unpredictable course of MDS.

Cytogenetics has gained an undeniable place in specific actions aimed at diagnosing and monitoring disease progression, becoming at this time indispensable to this process.

In this way, the early diagnosis and the high accuracy of the disease can be successfully accomplished, so that treatment is initiated as soon as possible, aspect which significantly influences the growth of survival chances.

Aknowledgements

This presentation has been elaborated and written by Iuliana-Maria Nicorescu, MD and PhD student at UMF Carol Davila, under coordination of Prof. Dr. Ana-Maria Vlădăreanu, MD, PhD.

References

1. DeAngelo DJ, Stone RM. Myelodysplastic syndromes: byology and treatment. In: Hoffman R, Benz E, Shattil S, eds. Hematology: basic principles and practice. 4th ed. *New York: Churchill Livingstone,* **2005:1195-1208;**

2. Stanley L Schrier et al. Overview of the treatment of myelodysplastic syndromes *UpToDate* **Oct 2013;**

3. Nordic MDS Group. Guidelines for the diagnosis and treatment of Myelodysplastic Syndromes and Chronic Myelomonocytic Leukemia. *Issue 7 6th update*, **1st of February 2014;**

4. Farrow A, Jacobs A, West RR. Myelodysplasia, chemical exposure and other environmental factors. *Leukemia* **1989; 3:33;**

5. Brandt L. Exposure to organic solvents and risk of hematological malignancies. *Leuk Res* **1992; 16:67;**

6. LeBeau MM, Albain KS, Larson R, et al. Clinical and cytogenetic correlations in 63 patients with therapy-related myelodysplastic syndromes and acute nonlymphocytic leukemia: further evidence for characteristic abnormalities of chromosomes no. 5 and 7. *J Clin Oncol* **1986;4:325;**

7. Nowell P. Chromosome abnormalities in myelodysplastic syndrome. *Semin Oncol* **1992; 19:25;**

8. Anastasi J, Feng J, LeBeau MM, et al. Cytogenetic clonality in myelodysplastic syndromes studied with fluorescence in-situ hybridization: lineage, response to growth factor therapy and clonal expansion. *Blood* **1993; 81:1580;**

9. Golub TR, Barker GF, Lovett M, et al. Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. *Cell* **1994; 77:307;**

10. Rossi G, Pelizzari AM, Bellotti D, et al. Cytogenetic analogy between myelodysplastic syndrome and acute myeloid leukemia of elderly**;**

11. Look T. Molecular pathogenesis of myelodysplastic syndromes. *Hematology ASH* **2005;**

12. Danaila C, Dascalescu A. Hematologie – Patologia neoplazica Elemente de diagnostic si tratament, Iasi: *Junimea*, **2011;**

13. Alan F. List, et al: Myelodysplastic Syndromes. 2009 Lippincott Williams & WilkinsJohn P. Greer, John Foerster, John N. Lukens, George M. Rodgers, Frixos Paraskevas, Bertil Glader, Wintrobe's Clinical Hematology,12th Edm**;**

14. Kosmider O, Gelsi-Boyer V, Cheok M, et al. TET2 mutation is an independent favorable prognostic factor in myelodysplastic syndromes (MDSs). *Blood* **2009; 114:3285;**

15. Yanming Zhang, MD et al. Cytogenetics and molecular genetics of myelodysplastic syndromes; *UpToDate* **21.6. Oct 2013;**

16. Wolfler A, et al. A functional single-nucleotide polymorphism of the G-CSF receptor gene predisposes individuals to high-risk myelodysplastic syndrome. *Blood* **2005;105:3731–3736;**

17. Tehranchi R, et al. Persistent malignant stem cells in del(5q) myelodysplasia in remission. *N Engl J Med* **2010; 363:1025;**

18. Papaemmanuil E, Cazzola M, Boultwood J, et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. *N Engl J Med* **2011; 365:1384.**