

Laboratorinė medicina.
2018, t. 20, Nr. 3(79), p. 183–188.

Applicability of Flow Cytometry for the Diagnostics of Myelodysplastic Syndromes

Miglė Janeliūnienė¹
Aušra Janiulionienė¹
Agnė Barzinskienė¹
Daiva Taškūnaitė¹
Mantas Radzevičius^{1,2}
Miglė Montrimaitė^{1,2}
Rėda Matuzevičienė^{1,2}
Dovilė Karčiauskaitė^{1,2}

Summary

Background and objectives. Diagnosis of myelodysplastic syndromes (MDS) is based on evaluation of blood cell counts, as well as on morphological features of hematopoietic cells and distinct cytogenetic abnormalities. As about half of cases are cytogenetically “silent” and the results of morphological analysis are sometimes indefinite there is a need for additional objective method to establish the diagnosis of MDS. Flow cytometry can be used to define the percentage of blasts and their phenotype, as well as the regularity of granulocyte, monocyte and erythrokaryocyte maturation patterns and may serve as a powerful tool for the clarification of diagnosis.

Materials and methods. 56 adult patients with MDS in suspicion treated at Vilnius University Hospital Santaros Klinikos were included in the study. Diagnostic bone marrow samples were analyzed on FACS Canto flow cytometer applying 6 color marker panel.

Results. Four deviations from normal phenotype or maturation patterns in blast, granulocyte, monocyte or erythrokaryocyte populations were proven to sufficiently discriminate MDS positive and negative cases. In this setting the specificity of the assay was 73.3% and the sensitivity was 80.0%. These findings were: reduced B-cell precursors count ($p=0.03$), reduced side scatter of granulocyte population ($p=0.10$), changes of CD10/CD64 and CD11b/CD13/CD16 expression pattern on granulocyte population ($p=0.03$ and $p<0.01$ respectively), and changes of CD71/CD235a expression pattern on erythrokaryocyte population ($p=0.05$).

Conclusions. Flow cytometry can be applied to differentiate cytopenias and to identify patients with higher risk of MDS as a part of integral diagnosis.

Keywords: myelodysplastic syndrome, flow cytometry, blasts.

INTRODUCTION

Myelodysplastic syndromes (MDS) are clonal haematopoietic stem cell diseases, characterized by cytopenias, dysplasia in one or more of the major myeloid cell lines, ineffective haematopoiesis, and increased risk of development of acute myeloid leukemia [1]. The diagnosis of myelodysplastic syndromes is based on evaluation of patient's clinical history, peripheral blood and bone marrow (BM)

cell counts and morphological features, cytogenetic information and exclusion of other diseases [2]. However, cytogenetic abnormalities are identified in only 30–40% of MDS cases and morphological evaluation, except in cases with objective changes (excess of blasts, ringed sideroblasts), is often investigator dependent, and that leaves a large proportion of cytopenic patients with an unclassified diagnosis [3]. To address the situation, multiparameter flow cytometry

¹Center of Laboratory Medicine,
Vilnius University Hospital Santaros
Klinikos, Vilnius, Lithuania

Vilniaus universiteto ligoninės
Santaros klinikų Laboratorinės
medicinos centras

E-mail: migle.janeliuniene@santa.lt

²Department of Physiology,
Biochemistry, Microbiology
and Laboratory Medicine,
Faculty of Medicine, Vilnius University,
Vilnius, Lithuania

Vilniaus universiteto Medicinos
fakulteto Fiziologijos, biochemijos,
mikrobiologijos ir laboratorinės
medicinos katedra

was recommended as a co-criterion in the diagnostics of MDS [4]. The recommendations of World Health Organization (2008) indicate that multiple (more than 3) aberrancies in blood cell maturation patterns may be used as a diagnostic co-criterion of MDS. When no cytogenetic alterations are detected and morphological examination is borderline, flow cytometry may assist to identify patients who need a more thorough follow up [5]. A number of studies have reported flow cytometric abnormalities in bone marrow of MDS patients. Most of them have focused on aberrant antigen expression patterns of maturing myelomonocytic cells. This approach was based on the fact that normal haematopoiesis is characterized by sustainable antigen expression patterns. Other studies imply that evaluation of antigen expression just on CD34+ myeloblasts gives more consistent results in the diagnosis and prognosis of MDS, as changes in maturing myelomonocytic cells might be found in non-MDS conditions as well [6, 7]. No single flow cytometric abnormality has been proven to be a specific diagnostic marker of MDS, but good sensitivity and specificity results have been reached using a combination of immunophenotypic parameters [8]. European LeukemiaNet Group proposed standardized flow cytometric assay for categorization of MDS, which included evaluation of aberrations in progenitor cell segment as well as detection of dysgranulopoietic, dysmonopoietic, and dyserythropoietic features [9]. In our study we sought to apply these recommendations constructing a standardized 6 color marker panel and using it to test *de novo* and post-treatment patients with cytopenia.

MATERIALS AND METHODS

Patients

56 adult patients with cytopenia in at least one haematopoietic cell lineage and suspicion of MDS admitted to the Vilnius University Hospital Santaros Klinikos during the period from 2012 until 2014 were included in the study. There were 29 male and 27 female patients. The average age of patients was 59.37±11.59 years. All patients gave an informed consent to participate in this study.

All assays were performed on BM samples obtained by standard BM puncture procedure and anticoagu-

Table. List of monoclonal antibodies used for MDS diagnostics

FITC	PE	PerCP-Cy5.5	APC	PE-Cy7	APC-H7
CD38	CD14	CD34	CD33	CD10	CD45
CD64	CD5	CD34	CD2	CD10	CD45
CD15	CD117	CD34	CD7	CD10	CD45
CD16	CD13	CD34	CD11b	CD4	CD45
CD36	CD123	CD34	CD56	CD19	CD45
CD71	HLA-DR	CD34	CD235a	CD10	CD45

lated with K₃-EDTA. Data on peripheral blood complete blood counts (CBC's) and cytogenetic abnormalities were taken from patient's electronic record. CBC's were performed on Sysmex XE-5000 hematology analyzer. Cytogenetic abnormalities were identified using FISH karyotyping.

Sample preparation

Samples were prepared by lysed whole blood technique. To stain cell surface antigens 100 L of anticoagulated bone marrow were incubated with 20 L of pre-titrated antibodies for 20 minutes in the dark at room temperature. Then 100 L of lysing medium (*BD FACS Lysing Solution*) were added and samples were incubated for 15 minutes in order to fix leukocytes and to lyse red cells. Afterwards cells were washed in 3 ml of wash buffer (*BD CellWash* with 0.5% fetal bovine serum) at 350 g for 5 minutes. After the aspiration of the supernatant, cells were re-suspended in 500 L of PBS (phosphate buffered saline) with 0.5% fetal bovine serum. Samples were analyzed within 24 hours.

Monoclonal antibodies

A marker panel consisting of six-color monoclonal antibodies labeled with FITC, PE, PerCP-Cy5.5, APC, PE-Cy7 and APC-H7 was applied. As anchor markers CD34 (PerCP-Cy5.5) and CD45 (APC-H7) were selected. Most of the tubes also contained CD10 marker for separation and enumeration of B-cell precursors. Full list of monoclonal antibodies is presented in Table. All monoclonal antibodies were manufactured by BD Biosciences (San Jose, US).

Data analysis

Samples were analyzed on *BD FACSCanto* flow cytometer using *FACS Diva v.6.1.3* software. 500 000 cells were acquired from each tube. Parameters for the analysis were cho-

sen according to European LeukemiaNet Group recommendations [9]. Blasts were identified as cells with low to intermediate side scatter (SSC) expressing CD34. In case of negative CD34 expression, cells with low to intermediate side scatter and dim CD45 expression were considered as blasts. Markers were evaluated as positive when they were expressed on more than 20% of cells, except mature myelomonocytic antigens (CD11b, CD15, CD4) where 30% cut-off was set. The percentage of B-cell precursors relative to all CD34 positive events was established. Increase in myeloid blasts over 3%, decrease in haematogones less than 5%, alterations in the expression of early (CD34, CD117, HLA-DR) or myeloid (CD13, CD33) markers, co-expression of lymphoid markers or CD56 and asynchronous mature marker expression (CD15, CD11b and CD4 together with CD34) were considered as blast aberrations associated with MDS.

Granulocytes, monocytes and erythrokaryocytes were defined in each SSC/CD45 scattergram. Granulocytes were examined for reduced SSC value, CD10 expression, regularity of CD11b/CD13/CD16, CD15/CD10 and CD64/CD10 patterns, CD56 co-expression over 30% and asynchronous expression of early markers (CD34, CD117, HLA-DR) over 30%.

In the population of monocytes the proportion of CD14 and CD36 negative immature cells was evaluated (considered as normal up to 20%) as well as CD56 co-expression over 30% and expression of lymphoid antigens (CD2, CD5, CD7, CD19) over 20%. Also HLA-DR and CD11b markers were examined for the reduced intensity.

Erythrokaryocytes were checked for the regularity of CD71 and CD235a expression, reduced CD36 expression or increased proportion of CD117 positive progenitors over 10%.

One point was assigned for each of the mentioned characteristics and the sum of all points was used as Flow cytometric MDS score.

Groups of patients and statistical analysis

Patients were grouped to MDS positive and negative cases according to specific histologic and cytogenetic findings. Erythroid hyperplasia with ringed sideroblasts, internuclear bridging, nuclear budding, karyorrhexis, and evidence of Howell-Jolly bodies were considered to be morphologic features of dyserythropoiesis. Micromegakaryocytes, large mononuclear megakaryocytes, megakaryocytes with multiple dispersed nuclei, hypogranular megakaryocytes and naked nuclei were regarded abnormal. Hyposegmentation, hypo or hypergranulation of neutrophils or abnormal localization of immature granulocytic precursors in central marrow spaces was associated with dysgranulopoiesis [10]. Chromosomal abnormalities -7/del(7q), -5/del(5q), del(13q), del(11q), del(12p) or t(12p), del(9q), idic(X)(q13), t(17p) (unbalanced translocations) or i(17q) (ie, loss of 17p), t(11;16)(q23;p13.3), t(3;21)(q26.2;q22.1), t(1;3)(p36.3;q21), t(2;11)(p21;q23), inv(3)(q21q26.2), t(6;9)(p23;q34) were considered as evidence of MDS in cytopenic patients even without specific morphologic findings [11]. Eventually 32 patients were classified as MDS patients and 23 as non-MDS patients. In MDS group 18 patients did not have any cytogenetic abnormality, three patients had monosomy 5, one had del 7q, one monosomy 7 and del 3q, one trisomy 8, two had del 1p, one del 4q, one del 20q, two – a complex karyotype and for two patients cytogenetic evaluation was not performed. In MDS negative group one patient had t(9;22) and one had a complex karyotype.

Hemoglobin value, leukocyte count and platelet count in peripheral blood, amount of blasts in bone marrow and flow cytometric features of dysplasia

were compared in MDS positive and MDS negative groups. Flow cytometric MDS score, was established in each group and the sensitivity and specificity of Flow cytometric MDS score was calculated.

Statistical analysis was carried out on SPSS 10.0 software. For determination of hemoglobin, leukocyte and platelet count as well as blast count differences in MDS and non-MDS groups Student's t-test was used and Spearman correlation coefficient was applied to study similarities. To determine flow cytometric features that discriminate MDS and non-MDS cases best, logistic regression analysis was performed. For differences in ROC (Receiver Operating Characteristics) curve was used to determine the sensitivity and specificity of Flow cytometric MDS score.

RESULTS

Mean hemoglobin concentration in peripheral blood differed significantly between MDS and non-MDS groups: in MDS group it was 94.8 ± 17.6 g/L and in non-MDS group it was 107.83 ± 17.33 g/L ($p=0.04$), while there was no statistical difference between groups in white blood cell counts ($(3.92 \pm 2.72) \times 10^9/L$ and $(4.69 \pm 2.42) \times 10^9/L$ respectively ($p>0.1$)) and platelet counts ($(104.00 \pm 69.31) \times 10^9/L$ and $(171.26 \pm 86.51) \times 10^9/L$ ($p>0.1$)). Most common warnings ("flags") by hematology analyzer in both groups were "immature granulocytes" (50% in MDS and 43.48% in non-MDS group), "poikilocytosis" (37.5% in MDS and 21.73% in non-MDS group) and "giant platelets" (28.13% and 34.78% respectively).

Significantly larger amount of blasts was found in bone marrow of MDS patients ($10.27 \pm 8.48\%$) comparing to the non-MDS group

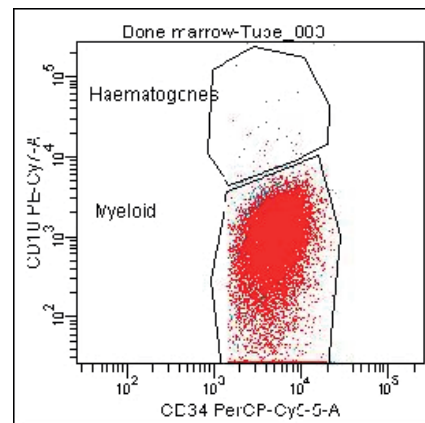


Fig. 1. Decreased amount of B-cell precursors (haematogones) in bone marrow of MDS patient

($2.11 \pm 1.92\%$) ($p<0.01$). Proportion of B-cell precursors was $0.5 \pm 0.91\%$ and $3.5 \pm 2.12\%$ respectively. Decrease in proportion of early (CD34+) B-cell precursors served as a good separation criterion between MDS and non-MDS groups ($p=0.03$) (Fig. 1). Most common phenotypic aberrations in blast population of MDS patients were: hypo-expression of myeloid markers CD13 and CD33 (19.0% of each marker) and cross lineage expression of CD7 (28.1%) and CD56 (15.6%). In non-MDS group these findings were detected in 4.3%, 17.4%, 8.7% and 0.0% of patients respectively. However, no single flow cytometric parameter in blast population differentiated MDS positive and MDS negative groups with statistical significance.

In the granulocyte population reduced SSC value, abnormal CD64/CD10 and CD11b/CD13/CD16 expression patterns were the most frequent alterations (31.3%, 25.0% and 81.25%) in MDS patients (Fig. 2). In non-MDS group these values were lower: 17.4%, 4.35% and 26.09%. All these three parameters were useful to predict MDS (respectively, $p=0.10$, $p=0.05$ and $p<0.01$).

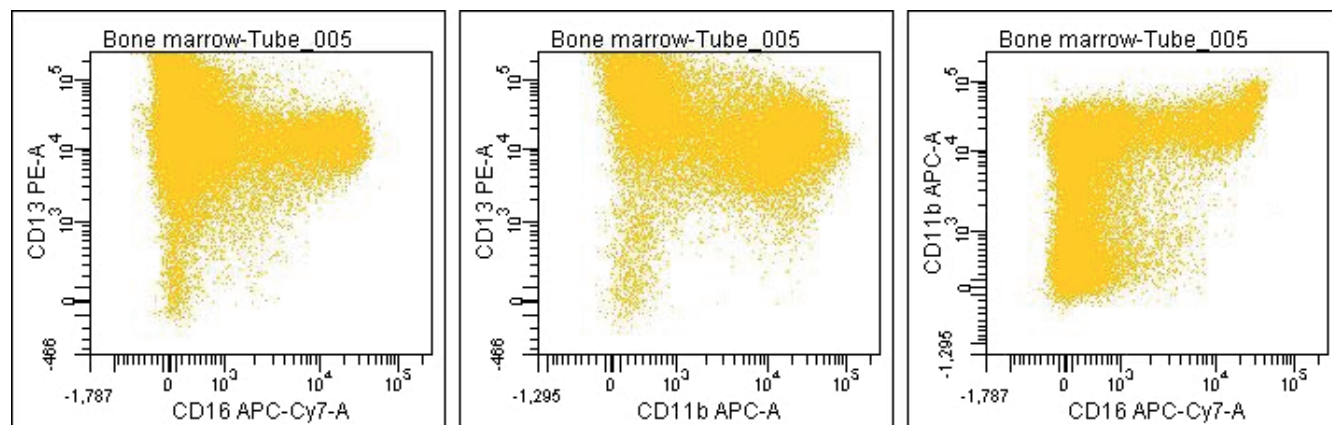


Fig. 2. Dysmorphic pattern of granulocyte CD11b/CD13/CD16 expression in MDS

Elevated amount of monocytes (above 5%) was found in 40.63% of MDS and 39.13% of non-MDS bone marrow samples. The most common phenotypic aberration in MDS patients was co-expression of CD56 (21.88%). In non-MDS cases CD56 was not expressed in a single case. However, this finding did not predict MDS with statistical significance.

Increased amount of erythrokaryocytes (above 25%) was detected in 21.88% of MDS and 13.04% of non-MDS patients. Changes in CD71/CD235a expression pattern showed the ability to predict MDS ($p=0.05$).

Flow cytometric MDS score of four (four phenotypic aberrations or changes in maturation patterns) was chosen as best for discrimination between MDS and non-MDS groups. In this setting specificity of flow cytometric test for diagnostics of MDS was 73.3% and sensitivity was 80.0%. In MDS group median Flow cytometric MDS score was 6.47 ± 1.94 while in non-MDS group it was 2.77 ± 2.29 .

DISCUSSION

Flow cytometry has been proven to be a reliable method in characterizing hematopoietic cells for diagnostics of acute leukemia and chronic lymphocytic proliferations. It has also been evaluated as a potential diagnostic tool for MDS [12].

Several flow cytometric scoring systems have been proposed to assist in diagnostics of MDS. Wells et al. created a multiplex scheme which evaluated 12 parameters of CD34+ progenitors and maturing myeloid cells and attributed different number of points depending on abnormalities found. Authors reported 55% of sensitivity and 100% of specificity in diagnosing MDS at flow score of 3 [13]. Ogata et al. designed more simplified score to diagnose low-grade MDS and evaluated its diagnostic utility in Japanese and Italian patients with low-risk myelodysplastic syndromes. The principal parameters were percentage of blasts, percentage of B-cell progenitors, myeloblast CD45 expression, and side scatter value of granulocytes. This protocol discriminated between low-grade MDS and non-clonal cytopenias with good analytic characteristics: sensitivity was 65% and 89% in Japanese and Italian populations, and specificities were 98% and 90% respectively [14]. Our MDS assessment scheme demonstrated better sensitiv-

ity (73.3%) at Flow cytometry MDS score of four than the Wells et al. study, but specificity was lower (80%) than in mentioned score systems.

The fact that we and other researchers did not reach very good sensitivity and specificity while testing multiple parameters raises a discussion, whether only the most specific aberrations should be selected. However, as a heterogeneous group of diseases, MDS do not have a specific marker abnormality or specific abnormal marker profile that discriminates MDS from other pathologies [15, 16]. European LeukemiaNet guidelines propose to test a broad spectrum of parameters, including immature myeloid and monocytic progenitors, maturing neutrophils, monocytes, progenitor B cells and erythroid compartment [17]. MDS management schemes include flow cytometric evaluation as a recommended, but not a mandatory assay, provided that it is performed according to European LeukemiaNet guidelines [18].

In our study the proportion of blasts in MDS patients significantly differed from non-MDS cases. Reduced amount of B-precursors was one of the parameters useful for discriminating MDS. These features are widely described in other studies as well. Malcovati et al. found that amount of CD45low/SSC low blasts in MDS patients ranged between 0.19 and 20% while in controls it was 2% [19]. Matarraz states that in high proportion of patients even with low-grade MDS (60%) B-cell precursors disappear at all [20]. Maftoun, Banankhah also refers to this trait in his work reporting mean haematogone value of 0% vs. 0.38% in control group [21].

Not only increase in number, but also deviations from normal phenotype are recognized as an attribute of MDS. Alterations of CD45, HLA-DR, CD13, CD33 marker expression are described most often, as well as asynchronous expression of mature antigens (CD11b, CD15) and cross lineage expression of lymphoid markers (CD5, CD7, CD19, CD56) [18, 23–25]. We did find the reduced expression of myeloid markers CD13 and CD33 and cross lineage expression of CD7 and CD56 in a substantial proportion of cases (19% for each myeloid marker; 28% and 16% for lymphoid markers respectively), but asynchronous expression of mature markers was not significant. The reported CD7 expression in MDS is slight and varies from 3.5% to 9% of cases [22–24]. It is known that CD7 can be expressed in a small sub-

set of earliest myeloid precursors especially in recovering bone marrow [25]. Regarding CD56 expression, Mann et al. found that this marker was expressed in 20% of patients with increased myeloblast count not only in MDS, but also in chronic and acute myeloid leukemia [26]. Ogata MDS study did not show that evaluation of CD11b, CD15 and CD56 improves diagnostic power of MDS scheme [14, 27]. These studies would suggest, that there is a little or no need to include CD5, CD11b, CD15, CD56 or CD7 marker evaluation in the diagnostics of MDS [28] and that would correlate with the results of our study. On the other hand, Kussick carried out a large study of more than 800 samples and found that in more than 50% MDS cases abnormal antigen expression of HLA-DR, CD13, CD33, CD38, and CD117 was detected [29]. These findings indicate that lymphoid or mature antigen expression should not be overestimated and alterations of usual markers of myeloid blasts should be taken into account.

Analysis of maturing neutrophils is another important section of MDS examination. It reveals the ability of progenitors to differentiate to the final functional state. Reduced granulocyte SSC value is a marker most often assigned to MDS. For the evaluation of this characteristic Ogata and colleagues suggest using the ratio of granulocytes and lymphocytes SSC channel peak values [14]. In our study we did not measure the ratio, but assumed that SSC was altered when the lower bound of population was overlapping lymphocytes. Decreased SSC parameter was found to be useful in separating MDS from non-MDS ($p=0.1$).

Another widely presented feature of MDS is decreased CD10 expression on neutrophil population [9, 15, 28]. In different studies altered CD10 expression ranges from 11% to 74% [30–32]. In our study not only did we monitor CD10 expression, but also evaluated the synchronization between CD64/CD10 and CD15/CD10 expression. Changes in CD64/CD10 pattern were found to be specific for MDS. Chung et al. notes that another marker combination CD15/CD10 is more useful for MDS diagnostics [33], but in our study it was only as helpful as CD10 decrease alone and did not discriminate MDS patients from non-MDS patients ($p>0.1$).

The analysis of CD13 versus CD16, CD13 versus CD11b and CD11b versus CD16 expression allows assessing the regularity of neutrophil maturation.

tion and detection of maturational blockades [1, 33]. In normal bone marrow these patterns look like “check mark”, “sickle” or “inverted seven”. In our study at least one of these patterns was dysmorphic in 81.25% of MDS patients ($p < 0.01$). It comes in line with the findings of Stetler-Stevenson (70% CD11b/CD16 and 78% for CD13/CD16), but is higher than frequencies reported in other studies [30–32]. However, interpretation of the loss of CD16 may be complicated, since a lack of CD16 is also observed in pathological non-MDS conditions, such as paroxysmal nocturnal hemoglobinuria [15, 28].

According to European LeukemiaNet guidelines in monocytic compartment, the proportion of monocytes, abnormal distribution of their maturational stages, altered expression of monocytic markers, and aberrant expression of CD2 and CD56 have to be evaluated [18]. In our study we found abnormal expression of CD56 in 28% of MDS cases, which is slightly more than, e.g. in Wells study (17%)

[13]. However it is reported that expression of CD56 can be often seen in activated monocytes, chronic myelomonocytic leukemia or in recovering bone marrow after chemotherapy or transplantation [15]. Xu study states that CD56 together with downregulation of HLA-DR is seen in reactive monocytosis, while CD56 expression together with downregulation of myeloid markers is specific for CMML [33]. In our hands CD56 expression did not predict MDS with statistical significance.

First MDS studies did not include evaluation of erythropoiesis in MDS flow cytometric diagnostics. Later abnormalities in CD36, CD71 and CD235a expression have been described [34–37]. Reduced expression of CD71 is mentioned among the most specific MDS related features [34–36]. Mathis et al. introduced the RED-score which is the evaluation of CD36, CD71 and hemoglobin value [38]. RED-score 3 correctly classified MDS in 80% of cases which is a promising finding. We did not measure the

score, but hemoglobin values differed significantly between MDS and non-MDS patients ($p = 0.01$). Also combination of CD71 and CD235a was found to be specific, so combining these features to one score for evaluation of dyserythropoiesis seems very reasonable.

CONCLUSIONS

Flow cytometry can be applied to differentiate cytopenias and to identify patients with higher risk of MDS as a part of integral diagnosis. Yet consistent flow cytometric techniques still have to be developed and antigen expression patterns of normal and regenerating bone marrow established.

STATEMENT OF CONFLICT OF INTEREST

The authors state no conflict of interest. ♦

Gauta: 2018 09 12
Priimta spaudai: 2018 09 28

Santrauka

TĖKMĖS CITOMETRIJOS PRITAIKOMUMAS MIELODISPLAZINIŲ SINDROMŲ DIAGNOSTIKAI

Miglė Janeliūnienė, Aušra Janiulionienė, Agnė Barzinskienė, Daiva Taškūnaitė, Mantas Radzevičius, Miglė Montrimaitė, Rėda Matuzevičienė, Dovilė Karčiauskaitė

Įvadas. Mielodisplazinių sindromų (MDS) diagnostika yra pagrįsta kraujo ląstelių skaičiaus, jų morfologinių savybių vertinimu kraujyje ir kaulų čiulpuose, taip pat citogenetinių pokyčių nustatymu. Visgi daugiau nei pusei ligonių citogenetinių pokyčių neaptinkama, o morfologinių savybių vertinimas, išskyrus labai aiškius atvejus (radus sideroblastų, labai padidėjus blastų skaičiui), gali būti subjektyvus, todėl reikalingi papildomi objektyvūs tyrimo metodai. Tėkmės citometrija gali būti taiko-

ma siekiant tiksliau nustatyti blastų skaičių, vertinti jų fenotipo adekvatumą, taip pat granulocitų, monocitų ir brandulėtųjų eritrocitų brendimo taisyklumą.

Tiriamieji ir metodai. Tirti Vilniaus universiteto ligoninės Santaros klinikose gydyti 56 suaugę ligoniai, kuriems dėl vienos ar daugiau kraujodaros linijų citopenijos įtarta MDS. Kaulų čiulpų mėginiai buvo analizuojami „FACS Canto“ tėkmės citometru taikant standartizuotą šešių spalvų žymenų derinį.

Rezultatai. Nustatėme, kad keturi nukrypimai nuo įprasto fenotipo ar brendimo modelio blastų, granulocitų, monocitų ar brandulėtųjų eritrocitų populiacijose leidžia geriausiai atskirti MDS sergančius pacientus nuo nesergančių. Tyrimo specifiškumas buvo 73,3 %, jautrumas – 80,0 %. Specifiškiausi rodikliai buvo sumažėjęs B ląstelių pirmtakų skaičius ($p = 0,03$), sumažėjusi granulocitų šoninė spindulio sklaida ($p = 0,10$), pakitusi granulocitų CD10 / CD64 ir CD11b / CD13 / CD16 raiška (atitinkamai $p = 0,03$ ir $p < 0,01$) ir brandulėtųjų eritrocitų CD71 / CD235a raiškos pokyčiai ($p = 0,05$).

Išvada. Tėkmės citometrija gali būti naudojama siekiant identifikuoti didesnę mielodisplazinio sindromo riziką ligoniams, kuriems nustatyta citopenija.

Reikšminiai žodžiai: mielodisplazinis sindromas, tėkmės citometrija, blastai.

REFERENCES

- Yoshida Y, Stephenson J, Mufti GJ. Myelodysplastic syndromes: from morphology to molecular biology. Part I. Classification, natural history and cell biology of myelodysplasia. *Int J Hematol* 1993; 57(2): 87–97.
- Bennett JM. A comparative review of classification systems in myelodysplastic syndromes (MDS). *Semin Oncol* 2005; 32(4 Suppl 5): S3–10.
- Ramos F, Fernández-Ferrero S, Suárez D, Barbón M, Rodríguez JA, Gil S, et al. Myelodysplastic syndrome: a search for minimal diagnostic criteria. *Leuk Res* 1999; 23(3): 283–90.
- Valent P, Horny H-P, Bennett JM, Fonatsch C, Germing U, Greenberg P, et al. Definitions and standards in the diagnosis and treatment of the myelodysplastic syndromes: Consensus statements and report from a working conference. *Leuk Res* 2007; 31(6): 727–36.
- Brunning R, Orazi A, Germing U, LeBeau MM, Porwitt A, Baumann I, et al. Myelodysplastic syndromes/neoplasms. In: Swerdlow SH, et al, eds. WHO classification of tumours and haematopoietic and lymphoid tissues. Lyon: IARC, 2008; p. 88–93.
- Xu F, Guo J, Wu L-Y, He Q, Zhang Z, Chang C-K, et al. Diagnostic application and clinical significance of FCM progress scoring system based on immunophenotyping in CD34+ blasts in myelodysplastic syndromes. *Cytometry B Clin Cytom* 2013; 84(4): 267–78.
- Tang G, Jorgensen LJ, Zhou Y, Hu Y, Kersh M, Garcia-Manero G, et al. Multi-color CD34+ progenitor-focused flow cytometric assay in evaluation of myelodysplastic syndromes in patients with post cancer therapy cytopenia. *Leuk Res* 2012; 36(8): 974–81.

8. Della Porta MG, Picone C. Diagnostic utility of flow cytometry in myelodysplastic syndromes. *Mediterr J Hematol Infect Dis* 2017; 9(1): e2017017.
9. Westers TM, Ireland R, Kern W, Alhan C, Balleisen JS, Bettelheim P, et al. Standardization of flow cytometry in myelodysplastic syndromes: a report from an international consortium and the European LeukemiaNet Working Group. *Leukemia* 2012; 26(7): 1730–41.
10. Parmentier S, Schetelig J, Lorenz K, Kramer M, Ireland R, Schuler U, et al. Assessment of dysplastic hematopoiesis: lessons from healthy bone marrow donors. *Haematologica* 2012; 97(5): 723–30.
11. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia* 2014; 28(2): 241–7.
12. Cazzola M. Flow cytometry immunophenotyping for diagnosis of myelodysplastic syndrome. *Haematologica* 2009; 94(8): 1041–3.
13. Wells DA, Benesch M, Loken MR, Vallejo C, Myerson D, Leisenring WM, et al. Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation. *Blood* 2003; 102(1): 394–403.
14. Ogata K, Della Porta MG, Malcovati L, Picone C, Yokose N, Matsuda A, et al. Diagnostic utility of flow cytometry in low-grade myelodysplastic syndromes: a prospective validation study. *Haematologica* 2009; 94(8): 1066–74.
15. Bento LC, Correia RP, Pitangueiras Manguiera CL, De Souza Barroso R, Rocha FA, Bacal NS, et al. The use of flow cytometry in myelodysplastic syndromes: a review. *Front Oncol* 2017; 7: 270.
16. Valent P, Orazi A, Steensma DP, Ebert BL, Haase D, Malcovati L, et al. Proposed minimal diagnostic criteria for myelodysplastic syndromes (MDS) and potential pre-MDS conditions. *Oncotarget* 2017; 8(43): 73483–500.
17. Loosdrecht AA van de, Alhan C, Béné MC, Porta MG, Dräger AM, Feuillard J, et al. Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes. *Haematologica* 2009; 94(8): 1124–34.
18. Malcovati L, Hellström-Lindberg E, Bowen D, Adès L, Cermak J, Del Cañizo C, et al. Diagnosis and treatment of primary myelodysplastic syndromes in adults: recommendations from the European LeukemiaNet. *Blood* 2013; 122(17): 2943–64.
19. Malcovati L, Della Porta MG, Lunghi M, Pascutto C, Vanelli L, Travaglino E, et al. Flow cytometry evaluation of erythroid and myeloid dysplasia in patients with myelodysplastic syndrome. *Leukemia* 2005; 19(5): 776–83.
20. Matarraz S, López A, Barrena S, Fernandez C, Jensen E, Flores J, et al. The immunophenotype of different immature, myeloid and B-cell lineage-committed CD34+ hematopoietic cells allows discrimination between normal/reactive and myelodysplastic syndrome precursors. *Leukemia* 2008; 22(6): 1175–83.
21. Maftoun-Banankhah S, Maleki A, Karandikar NJ, Arbin AA, Fuda FS, Wang H-Y, et al. Multiparameter flow cytometric analysis reveals low percentage of bone marrow hematogones in myelodysplastic syndromes. *Am J Clin Pathol* 2008; 129(2): 300–8.
22. Bardet V, Wagner-Ballon O, Guy J, Morvan C, Debord C, Trimoreau F, et al. Multicentric study underlining the interest of adding CD5, CD7 and CD56 expression assessment to the flow cytometric Ogata score in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. *Haematologica* 2015; 100(4): 472–8.
23. Font P, Subirá D, Mtnes-Chamorro C, Castañón S, Arranz E, Ramiro S, et al. Evaluation of CD7 and terminal deoxynucleotidyl transferase (TdT) expression in CD34+ myeloblasts from patients with myelodysplastic syndrome. *Leuk Res* 2006; 30(8): 957–63.
24. Kern W, Haferlach C, Schnittger S, Haferlach T. Clinical utility of multiparameter flow cytometry in the diagnosis of 1013 patients with suspected myelodysplastic syndrome: correlation to cytomorphology, cytogenetics, and clinical data. *Cancer* 2010; 116(19): 4549–63.
25. Wood BL. Myeloid malignancies: myelodysplastic syndromes, myeloproliferative disorders, and acute myeloid leukemia. *Clin Lab Med* 2007; 27(3): 551–75, vii.
26. Mann KP, DeCastro CM, Liu J, Moore JO, Bigner SH, Traweek ST. Neural cell adhesion molecule (CD56)-positive acute myelogenous leukemia and myelodysplastic and myeloproliferative syndromes. *Am J Clin Pathol* 1997; 107(6): 653–60.
27. Ogata K, Kishikawa Y, Satoh C, Tamura H, Dan K, Hayashi A. Diagnostic application of flow cytometric characteristics of CD34+ cells in low-grade myelodysplastic syndromes. *Blood* 2006; 108(3): 1037–44.
28. Aanei CM, Picot T, Tavernier E, Guyotat D, Campos Catafal L. Diagnostic utility of flow cytometry in myelodysplastic syndromes. *Front Oncol* 2016; 6: 161.
29. Kussick SJ, Fromm JR, Rossini A, Li Y, Chang A, Norwood TH, et al. Four-color flow cytometry shows strong concordance with bone marrow morphology and cytogenetics in the evaluation for myelodysplasia. *Am J Clin Pathol* 2005; 124(2): 170–81.
30. Stetler-Stevenson M, Arthur DC, Jabbour N, Xie XY, Molldrem J, Barrett AJ, et al. Diagnostic utility of flow cytometric immunophenotyping in myelodysplastic syndrome. *Blood* 2001; 98(4): 979–87.
31. Loken MR, van de Loosdrecht A, Ogata K, Orfao A, Wells DA. Flow cytometry in myelodysplastic syndromes: report from a working conference. *Leuk Res* 2008; 32(1): 5–17.
32. Chung J-W, Park C-J, Cha C-H, Cho Y-U, Jang S, Chi H-S, et al. A combination of CD15/CD10, CD64/CD33, CD16/CD13 or CD11b flow cytometric granulocyte panels is sensitive and specific for diagnosis of myelodysplastic syndrome. *Ann Clin Lab Sci* 2012; 42(3): 271–80.
33. Xu Y, McKenna RW, Karandikar NJ, Pildain AJ, Kroft SH. Flow cytometric analysis of monocytes as a tool for distinguishing chronic myelomonocytic leukemia from reactive monocytosis. *Am J Clin Pathol* 2005; 124(5): 799–806.
34. Della Porta MG, Malcovati L, Invernizzi R, Travaglino E, Pascutto C, Maffioli M, et al. Flow cytometry evaluation of erythroid dysplasia in patients with myelodysplastic syndrome. *Leukemia* 2006; 20(4): 549–55.
35. Xu F, Wu L, He Q, Zhang Z, Chang C, Li X. Immunophenotypic analysis of erythroid dysplasia and its diagnostic application in myelodysplastic syndromes. *Intern Med J* 2012; 42(4): 401–11.
36. Chopra A, Pati H, Mahapatra M, Mishra P, Seth T, Kumar S, et al. Flow cytometry in myelodysplastic syndrome: analysis of diagnostic utility using maturation pattern-based and quantitative approaches. *Ann Hematol* 2012; 91(9): 1351–62.
37. Westers TM, Cremers EMP, Oelschlaegel U, Johansson U, Bettelheim P, Matarraz S, et al. Immunophenotypic analysis of erythroid dysplasia in myelodysplastic syndromes. A report from the IMDSFlow working group. *Haematologica* 2017; 102(2): 308–19.
38. Mathis S, Chapuis N, Debord C, Rouquette A, Radford-Weiss I, Park S, et al. Flow cytometric detection of dyserythropoiesis: a sensitive and powerful diagnostic tool for myelodysplastic syndromes. *Leukemia* 2013; 27(10): 1981–7.