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Ned Tijdschr Klin Chem Labgeneesk 2009; 34: 232-234

Diagnostic utility of multiparameter flow cytometry analysis in myelodysplastic syndromes

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Introduction

The myelodysplastic syndromes (MDS) are a heterogeneous group of bone marrow (BM) disorders characterized by ineffective and dysplastic hematopoiesis in one or more blood cell lineages, increased apoptosis and hyper-proliferative BM. Furthermore, MDS has a high rate of transformation to acute myeloid leukemia (sAML). The WHO classification of MDS takes into account both morphologic and cytogenetic features and divides MDS into 8 subgroups. Currently, classification relies on combining clinical information with morphologic and cytogenetic features of peripheral blood and BM. However, cytogenetic abnormalities are found in only ~40% of the cases, while morphologic evaluation is subjective. It has been shown that flow cytometry (FC) can reveal immunophenotypic aberrations in MDS compared to normal BM (1-4). Furthermore, expansion of the knowledge regarding apoptosis and proliferation could lead to new insights in treatment, and may serve as an additional marker for the diagnosis of MDS. The aim of the present study was to improve the diagnosis and classification of MDS by quantitative assessment of hematopoietic differentiation pathways by multiparameter FC.

Methods

Twenty-five normal BM, obtained from patients who were subject to hip -or open heart surgery and 31 BM from patients with a suspicion for MDS were analyzed. Eleven of the 31 MDS suspicious BM were confirmed as MDS by morphologic and cytogenetic features. A panel of eight quadruple immunostainings was developed to characterize erythroid, granulocytic, monocytic and mono/myeloblasts differentiation pathways, according to the protocol of the Dutch workinggroup on MDS (chair Dr. Arjan A. van de Loosdrecht). Shortly, the following 4-colour panel was used; Erythroid differentiation: CD71/CD235a/CD34/CD117; Mono/myeloid blasts: CD34/CD117/CD45/CD13.33 and CD15/HLA-DR/CD45/CD11b; Monocytic differentiation: CD36/CD33/CD45/CD14; Myeloid blasts:

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CD7/CD56/CD45/CD34 and CD5/CD19/CD45/CD34; Basophils: CD123/HLA-DR/CD45/CD34. Apoptosis and proliferation were measured by using AnnexinV/PI and Ki67, respectively. The degree of apoptosis and proliferation was determined in MDS suspected BM (n=30, n=17) and normal BM (n=20, n=12).

The different cell compartments were identified using CD45 expression, SSC characteristics and specific antibodies. Normal BM served as a framework of reference for absolute percentages and normal pattern recognition.[1-4] However, individual immunophenotypic deviations do not have to be specific for MDS. This requires an FC scoring system, which quantifies abnormalities in the erythroid, granulocytic, monocytic and mono/myeloblasts differentiation pathways. With the scoring system used a BM was classified as MDS if the flow score was ≥ 2.0, using the following criteria[1]; an aberrant immunophenotype or percentage >3% or <0.2% of myeloid blasts, 2 points; an aberrant immunophenotype in other lineage, 1 point.

A scoring system for apoptosis measurements was developed using described characteristics of apoptosis in MDS. We defined a BM as increased suspicion for MDS when the flow score was ≥ 3, using the following criteria: an increased/decreased apoptosis overall, 3 points; an increased apoptosis $CD3^{+/-}$ lymphocytes (B- T- & NK-cells) or increased/decreased apoptosis in $CD34^{+}$ cells, 2 points and finally, an increased/decreased apoptosis in other subpopulation, 1 point.

Results

Aberrant expression of membrane markers using FC analysis were detected in MDS BM as shown in the figure 1. The first figure (1A) shows a complete absence of the erythroid lineage. An increased percentage of mono/myeloid blasts is shown in figure 1B, indicating MDS. The maturation of granulocytes and monocytes, revealed a low expression of CD11b on monocytes. Moreover, the entire pattern is collapsed (figure 1C). Regarding the monocytic differentiation a low percentage promonocytes and a high percentage blasts was observed (figure 1D). The myeloid/granulocytic differentiation demonstrated two distinct patterns, 'nike' and 'u-shape', respectively (figures 1E/F). An aberrant relationship between CD11b and CD13 and an a-synchronic shift to the left was observed, indicating an increase of immature granulocytic precursor cells and a reduction in maturing granulocytes. Myeloid blasts were screened for the presence of lineage infidelity markers CD56, CD7, CD19 and/or CD5 or the absence of CD34 expression (figure 1G). In this figure 39% of the blasts are $CD7^{+}$ (p=0.01), compared to healthy BM, suggesting MDS. Furthermore, a MDS specific significant increase (p<.001) in proerythroblasts, median of 1.6% compared to 0.4% in healthy control BM and median of 0.5% to non-MDS diagnosed BM (p<.01). Overall, 82% (9/11) MDS patients showed abnormalities in granulocytic differentiation, like abnormal CD45,

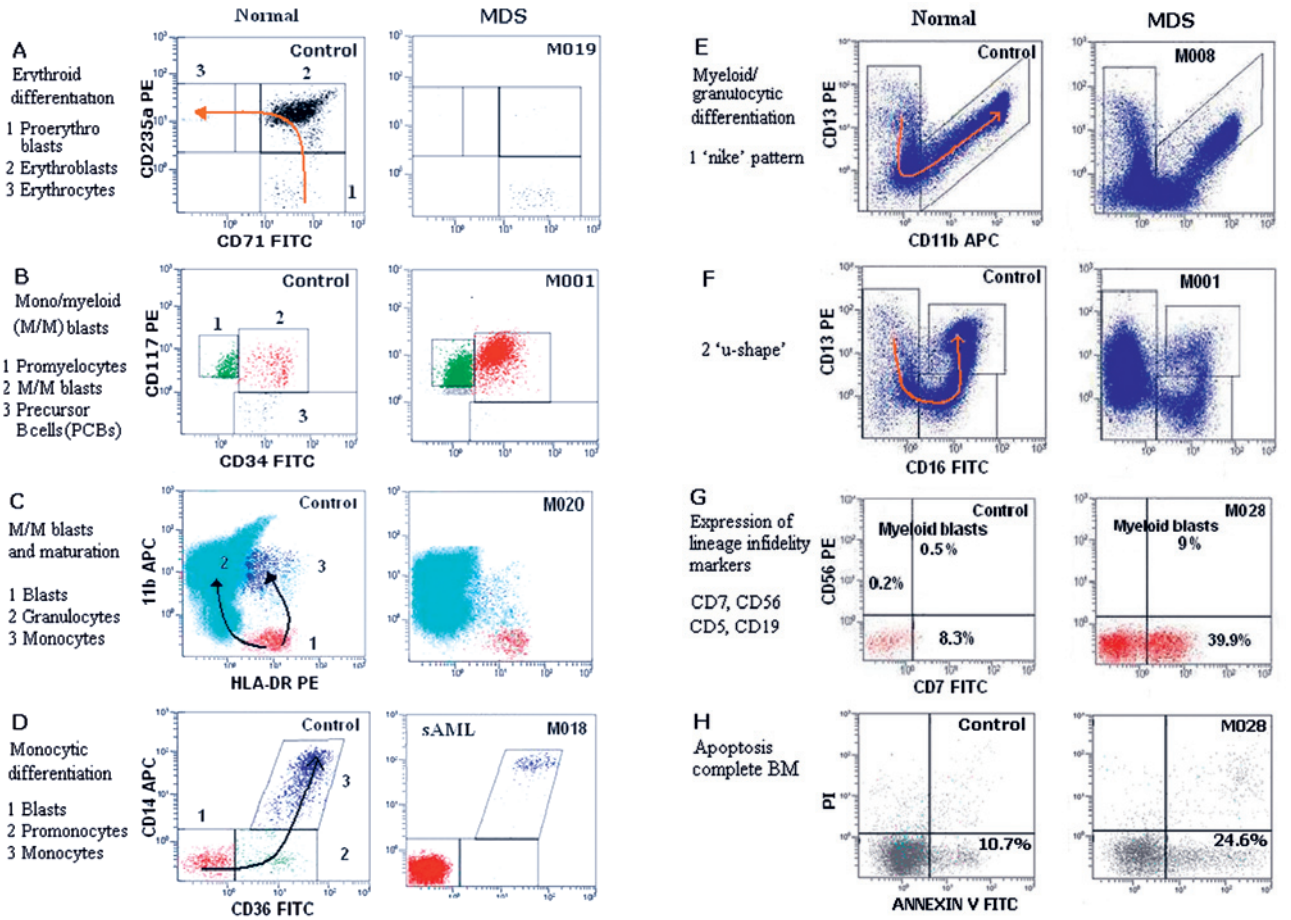

Figure 1. An overview of aberrations found in analyzed MDS BM, regarding each staining.

Table 1. FC score system for panel and apoptosis applied to MDS BM. Erythroid, erythroid lineage; Blasts, myeloid blasts; Granulo, granulocytic lineage; Mono, monocytic lineage; PN, patient number; Ph, phenotype; ND, not determined; A: P ratio, apoptosis:proliferation ratio * not conclusive, treatment observation

PN	Erythroid		Blasts		Granulo		Mono		Score	Flow	Clinic	Apoptosis	A: P ratio
	Ph	%	Ph	%	Ph	%	Ph	%					
M001		x		x	x	x	x	x	4	MDS	RAEB I	2	ND
M007	x	x				x	x		2	MDS	RA	0	ND
M008					x		x	x	2	MDS	MDSU	2	ND
M017	x		x		x		x		5	MDS	RA	6	Aberrant ↑
M019	x			x	x	x		x	3	MDS	RAEB I	5	Aberrant ↓
M020							x		1	Normal	RARS	0	Normal
M025		x	x			x	x		3	MDS	RA*	3	Aberrant ↑
M027	x	x		x	x	x		x	3	MDS	MDS	5	Normal
M028			x	x	x	x		x	5	MDS	RAEB II	9	Aberrant ↑
M030			x				x		3	MDS	RARS	6	Normal
M031				x	x				3	MDS	RA	ND	ND

CD15 and/or CD33 expression, and monocytic lineage, for example abnormal expression of CD45, CD14, CD13 and CD33. When applying the FC score system this resulted in the detection of 91% (10/11) of the morphological and cytogenetically identified MDS patients, with a specificity of 100%. The unrecognized MDS was morphologically distinct through the presence of ringsideroblasts (RARS). FC analysis showed aberrant CD7 expression on myeloid progenitors ($p<.001$) compared to healthy BM, in MDS suspicious BM, amongst one BM were morphology and cytogenetics was inconclusive, underlining the additional value of FC analysis. Since, recent studies suggest this might identify patients with MDS clinically at risk[3]. Detectable change in apoptotic rates in different cell compartments regarding MDS patients was observed. When the apoptosis score system was applied, 60% was marked as suspicious for MDS; however the first three MDS BMs were not analyzed using CD3 antibody. The apoptosis:proliferation ratio was sensitive (4/7) regarding MDS identification compared to normal BM. The final graph (figure 1H), shows increased apoptosis in MDS.

Conclusion

The quantitative assessment of hematopoietic differentiation pathways can improve the diagnosis and classification of MDS, especially when morphology and cytogenetics are inconclusive. Apoptosis and proliferation measurements of BM may further aid in the diagnosis, classification and subsequent treatment of MDS patients.

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