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

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ABSTRACT

Although numerous recent publications have demonstrated interest in multiparameter flow cytometry in the investigation of myelodysplastic disorders, it is perceived by many laboratory hematologists as difficult and expensive, requiring a high level of expertise. We report a multicentric open real-life study aimed at evaluating the added value of the technically simple flow cytometry score described by the Ogata group for the diagnosis of myelodysplastic syndromes. A total of 652 patients were recruited prospectively in four different centers: 346 myelodysplastic syndromes, 53 myelodysplastic/myeloproliferative neoplasms, and 253 controls. The Ogata score was assessed using CD45 and CD34 staining, with the addition of CD10 and CD19. Moreover, labeling of CD5, CD7 and CD56 for the evaluation of myeloid progenitors and monocytes was tested on a subset of 294 patients. On the whole series, the specificity of Ogata score reached 89%. Respective sensitivities were 54% for low-risk myelodysplastic syndromes, 68% and 84% for type 1 and type 2 refractory anemia with excess of blasts, and 72% for myelodysplastic/myeloproliferative neoplasms. CD5 expression was poorly informative. When adding CD56 or CD7 labeling to the Ogata score, sensitivity rose to 66% for low-risk myelodysplastic syndromes, to 89% for myelodysplastic/myeloproliferative neoplasms and to 97% for refractory anemia with excess of blasts. This large multicenter study confirms the feasibility of Ogata scoring in routine flow cytometry diagnosis but highlights its poor sensitivity in low-risk myelodysplastic syndromes. The addition of CD7 and CD56 in flow cytometry panels improves the sensitivity but more sophisticated panels would be more informative.

Introduction

Myelodysplastic syndromes (MDS) are heterogeneous myeloid neoplasms characterized by peripheral cytopenia, aberrant differentiation and increased intramedullary abortion of clonal immature myeloid cells. According to the WHO 2008 classification, the gold standard for MDS diagnosis relies on bone marrow (BM) cytomorphology and cytogenetics.[1] The minimal criterion for a morphological diagnosis of MDS is the evidence of at least 10% of cytomorphological abnormal myeloid, erythroid or megakaryoblastic precursors with chronic peripheral cytopenia, after ruling out any other causes. Cytomorphological analysis of BM smears in MDS is, however, challenging, mainly in cases of unilineage MDS with significant persistent normal residual myelopoiesis. Another criterion is required for the diagnosis of MDS, i.e. presence of ring sideroblasts for refractory anemia with ring sideroblasts, (RARS), over 5% BM blasts or 5%-19% circulating blast cells, defining refractory anemia with excess blasts type 1 and type 2, respectively (RAEB 1 and 2), and/or clonal cytogenetic or molecular abnormalities. These anomalies are, however, found in less than half the suspected cases because of blast cell counts lower than 5%, normal karyotype or absence of ring sideroblasts, stressing the need for other diagnostic criteria. The WHO 2008 classification of MDS also recognizes useful features issued from histopathology, molecular biology and multiparameter flow cytometry (MFC). Histopathology is important to recognize BM fibrosis[2] and useful to diagnose border-line cases with myeloproliferative features. Several mutations can be found in MDS.[3] Recently, high throughput sequencing has revealed numerous new mutations involving spliceosome genes (*SF3B1, SRSF2, U2AF1, ZRSR2*), DNA methylation (*TET2, DNMT3A, IDH1/2*), chromatin modification (*ASXL1, EZH2*), transcription regulation (*RUNX1*), DNA repair (*TP53*), signal transduction (*CBL, NRAS, KRAS*) or the cohesin complex (*STAG2*).[4] These mutations are, however, not specific and their place in the diagnosis of MDS has not yet been established. The value of MFC, identifying characteristic expression patterns of cell surface markers in MDS was first reported independently

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Table 1. Patient's characteristics.

MDS (n=346)	Number (M/F)	Age Q2[Q1 ;Q3]	Hb <10g/dL	Platelets <100^9/L	Granulocytes <1.8 x 10^9/L	Proven	Abnormal karyotype	Increase in ringed sideroblasts	IPSS low/ intermediate1/ intermediate2/ high	IPPS-r Very low/ low/ intermediate/ high/ very high
Low-risk (n=218)										
RA	50 (25/25)	79[71 ;87]	13 (30%)	15 (34%)	15 (34%)	12 (24%)	12 (32%)	0 (0%)	18/12/2/0	13/15/2/1/1
RCMD	126 (71/55)	79[73 ;85]	33 (24%)	28 (25%)	38 (33%)	53 (42%)	35 (38%)	28 (37%)	55/25/2/0	23/44/10/5/0
RARS	31 (18/13)	75[66 ;83]	14 (50%)	6 (21%)	4 (13%)	31 (100%)	6 (25%)	31 (100%)	15/7/0/0	8/11/3/0/0
5q-	11 (3/8 ?)	78[63 ;81]	2 (25%)	0 (0%)	4 (50%)	11 (100%)	11 (100%)	0 (0%)	8/0/0/0	0/2/6/0/0
High-risk (n=128)										
RAEB 1	90 (54/36)	66[74 ;79]	39 (44%)	29 (33%)	47 (53%)	39 (43%)	3 (41%)	10 (15%)	0/67/12/0	0/17/47/13/2
RAEB 2	38 (29/9)	72 [65 ;80]	20 (77%)	22 (88%)	20 (77%)	15 (40%)	14 (40%)	4 (24%)	0/0/21/10	0/0/9/13/9
MDS/MPN										
CMML (n=53)	43 (26/17)	67 [77 ;83]	9 (21%)	11 (26%)	12 (29%)	4 (9%)	4 (10%)	2 (5%)	14/14/5/0	7/17/9/0/0
Other	10 (3/7)	74 [71;83]	4 (57%)	1 (14%)	1 (14%)	6 (60%)	3 (30%)	5 (71%)	4/3/0/0	1/2/4/0/0
Controls (n=253)										
Anemia associated with iron and/or B12/folate deficiency	45 (20/25 ?)	78[66 ;83]								
Idiopathic thrombocytopenia	41 (24/17)	64[56;73]								
Idiopathic or iatrogenic anemia	38 (16/22)	64[56 ;73]								
Anemia associated with renal failure	24 (12/12)	81[64 ;84]								
Autoimmune cytopenia	21 (6/15)	82[74; 86]								
Unconfirmed/transient cytopenia	17 (6/11)	72[53;80]								
Spleen enlargement	10 (6/4)	69[43;80]								
Infection	8 (3/5)	70[57;75]								
Excessive alcohol intake	8 (5/3)	63[57;79]								
Hemorragia	8 (3/5)	78[68;91]								
Idiopathic hypoplasia/ aplasia	6 (1/5)	76[67;83]								
Normal	17 (8/9)	68[44;77]								

4 cancers; 3 congenital cytopenia; 3 other peripheral thrombopenia; 2 hemolytic anemia; 2 anemia of thyroid dysfunction; 2 anorexia nervosa; 2 denutrition; 1 anemia associated with liver disease; 1 hypogammaglobulinemia.

between 1997 and 2002 by Bowen and Davis,[5] Elghetany *et al.*,[6] Stetler-Stevenson *et al.*,[7] and Maynadié *et al.*[8] Since then, numerous studies have been published, confirming that the immunophenotype of MDS clonal myeloid precursors is abnormal in almost all cases and could have an important prognostic value. International studies focusing on the harmonization of MFC studies in MDS indicate the importance of targeting the progenitor compartment and of searching for abnormal differentiation patterns. MFC studies in MDS, however, require high expertise in order to recognize normal myeloid immunophenotypic differentiation through the use of appropriate combinations of antibodies.[9-13] This makes MFC in MDS highly technical and difficult to interpret. As an alternative, a simple 3-color MFC protocol based on CD45, CD10 and CD34 labeling of surface antigens was proposed by Ogata *et al.*[13,14] This MFC protocol calculates a 4-point score based on the quantification of myeloid and B-cell progenitors, side scatter appreciation of granulocyte cytoplasmic heterogeneity, and CD45 expression levels on immature myeloid precursors.

Here, an extended version of the Ogata score was evaluated in a series of 346 MDS cases in four different centers. This cohort also included 53 cases of myelodysplastic/myeloproliferative neoplasms (MDS/MPN) and 253 control cases unrelated to any hematologic neoplasm. The Ogata score was first improved by the additional labeling of CD19 to better target B-cell progenitors, and secondly, was enriched by evaluating CD5, CD7 and CD56 expression on blast cells and monocytes.

Methods

Patients

This study was approved by the ethics committee of Limoges University Hospital (*Online Supplementary Appendix*). All patients with a final diagnosis of MDS fulfilled the criteria of the WHO 2008 classification. As reported by Kern *et al.*,[12] patients were classified as proven MDS when a second criterion was present, while suspected MDS was reported on the basis of morphology only with blast counts less than 5%. The eligible cohort of patients

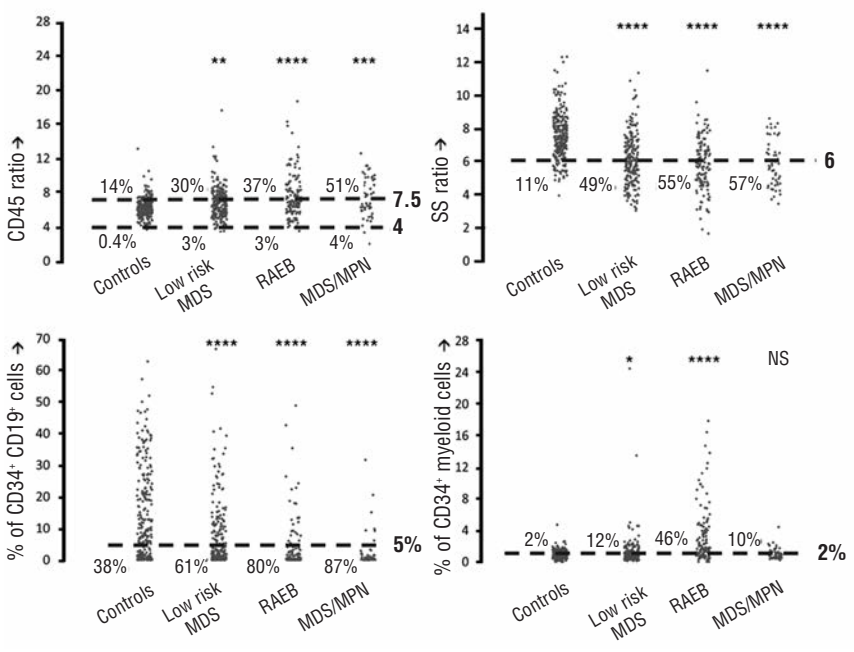

Figure 1. Distribution of Ogata score individual parameter values. Ogata score thresholds are indicated by dashed lines. The percentages of cases outside normal ranges are given for each category. Non-significant (NS) and significant differences are indicated on the top of each graph (*t*-test pvalues: **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001).

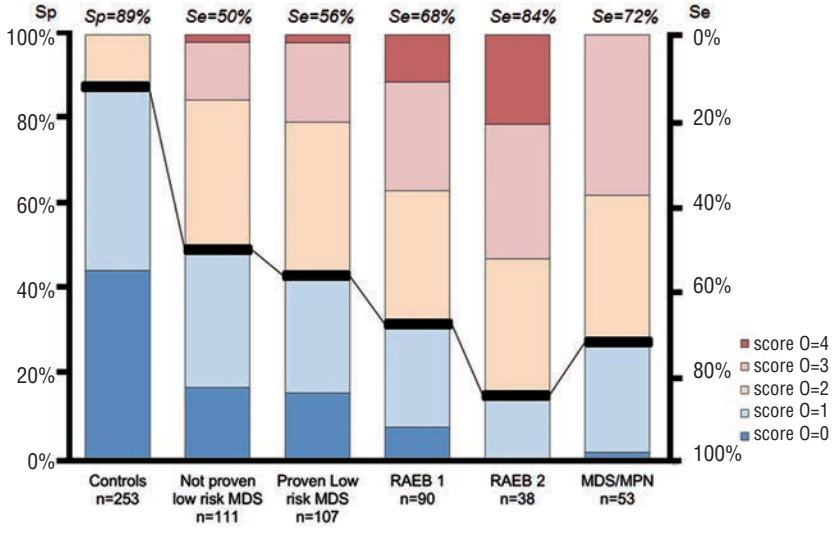

Figure 2. Distribution of Ogata score values. Bold lines indicate the threshold of 2. The left and right ordinates, respectively, indicate specificity (sp) and sensitivity (se).

(n=652) was stratified into four groups (Table 1). The first three groups were made up of patients with proven [(n=235, including 90 RAEB1, 38 RAEB2, 12 RA, 53 refractory cytopenia with multilineage dysplasia, (RCMD), 31 RARS and 11 5q- syndrome) or suspected (n=111 with normal cytochemistry and cytogenetics, morphologically classified as 38 RA and 73 RCMD) MDS or MDS/MPN [chronic myelonocytic leukemia (CMML) n=43, others n=10]. The last group (n=253) included patients with non-hematologic causes of cytopenia (n=236), including transient cytopenia (see Table 1) or with normal BM and normal white blood cell count at the time of analysis (unconfirmed/transient cytopenia n=17). The most frequent non-hematologic causes of cytopenias were, respectively, vitamin deficiency (n=45), idiopathic thrombocytopenia (n=41), idiopathic or iatrogenic cytopenia (n=38), and renal failure (n=24).

Flow cytometry

All centers used Navios instruments (Beckman Coulter, Miami, FL, USA). Before each series, the settings of photomultipliers were checked with fluorescent calibration beads (*Online Supplementary Table S1*). Antibodies used in this study were from Beckman Coulter (Miami, FL, USA) and are listed in *Online Supplementary Table S2*. Direct immunolabeling was performed on 50 µL of whole BM. After 20 min incubation, red blood cells were lysed with VersalyseTM (Beckman Coulter) according to the manufacturer's instructions and the samples washed once in PBS. At least 50,000 events were acquired. The gating strategy is presented in *Online Supplementary Figures S1* and *S2*.

Hemodilution of the BM aspirates by peripheral blood (PB) was calculated according to the method described by Holdrinet *et al.*[15] and Brooimans RA *et al.*[16] Samples with BM purity equal to or higher than 80% were considered as non-hemodiluted. Concordance between centers was checked on 20 randomly chosen electronic files, 10 controls and 10 MDS or MDS/MPN. As described by Della Porta *et al.*,[13] four parameters (1 point each when outside the normal ranges) were analyzed: 1) the lymphocyte/myeloid progenitor CD45 ratio (normal range 4-7.5); 2) the granulocyte/lymphocyte SSC peak channel ratio (threshold for normal 6 or more); 3) the percentage of B-cell progenitors, defined as $CD34^+CD19^+CD10^+$cells, among all $CD34^+$ cells (threshold for

normal 5% or more); 4) the percentage of CD34$^+$ myeloid progenitors among all acquired cells (threshold for normal less than 2%). An extended Ogata score was established by adding 1 point when CD5 or CD7 was expressed on myeloid progenitors or CD56 on monocytes with a threshold of 30% positive cells. A score of 2 or more was considered positive for both the classical and extended Ogata score.

Statistical analysis

Specificity and sensitivity of the various parameters, Student *t*-test and scores according to MDS subtypes were calculated using Excel software by comparison with controls.

Results

Data acquisition and analysis were carried out by each center. Preliminary comparison of low-risk MDS with controls by two investigators (VB: 118 MDS or MDS/MPN patients and 60 controls; EG: 104 MDS or MDS/MPN patients and 75 controls) independently on patients from their own center resulted in the same thresholds as those reported by Della Porta *et al.*[13] Verification of the absence of discrepancies between centers was performed using the same 20 MFC electronic files in each center. Concordance was excellent; only one control case that had been scored 1 by one center was scored 0 by the three others, and one case scored 3 by one center was scored 4 by the three others. In other words, considering a threshold of 2, all centers correctly classified the patients within Ogata's scoring system. The influence of hemodilution on the Ogata score was also verified as described above. Ogata scoring was similar whether BM samples were hemodiluted or not (*Online Supplementary Figure S3*).

Each parameter of the Ogata score was compared between controls, patients with low-risk MDS (proven or suspected) RAEB and MDS/MPN patients (Figure 1). Specificity of the lymphocyte/myeloid progenitor CD45 ratio was 83.6%; sensitivity was 33% for low-risk MDS, 40% for RAEB and 55% for MDS/MPN. Specificity of the granulocyte/lymphocyte SSC ratio was 89%, with sensitivities of 49%, 55% and 57% for low-risk MDS, RAEB and MDS/MPN, respectively. Thus, individual specificities of the CD45 and SSC ratios were good yet showed poor sensitivity, comparable between low-risk MDS and RAEB. The specificity of decreased CD19$^+$ progenitors was poor (62%) with a sensitivity of 61%, 80% and 87% for low-risk MDS, RAEB and MSDS/MPN, respectively. Conversely, the specificity of increased myeloid progenitors was very good (98%) yet with very poor sensitivity in low-risk MDS and MDS/MPN (12% and 10%, respectively), and slightly better sensitivity in RAEB (46%). Altogether, no single Ogata score parameter was satisfactory for the diagnosis of MDS in MFC. CD45 and SSC ratios were independent of MDS stage. Both the decrease in CD19$^+$CD10$^+$ progenitors and increase in myeloid progenitors were associated with advanced disease, and the higher Ogata score of RAEB1 and 2 was mainly due to increased myeloid progenitors and decreased B-cell progenitors, yet without any strong relationship with the Revised International Prognostic Scoring System (IPSS-R) (*data not shown*).

The distribution of Ogata score values was then analyzed according to the different categories of patients (Table 1 and Figure 2). The specificity of a positive Ogata score (≥2) was 89% (29 of 253 controls with a positive Ogata score). Here, controls were real-life patients with cytopenia related to the various causes reported in Table 1. The first causes for such controls to have an Ogata score of 2 or over were transient cytopenia, iron or vitamin deficiency, and autoimmune cytopenia (Table 1). The sensitivity of the Ogata score ranged between 37% and 84% according to the MDS and MDS/MPN categories (Table 1 and Figure 2). Sensitivity increased with the severity of the disease. The additional value of the CD19 marker was estimated on 62 controls and 68 MDS patients chosen randomly, by comparing results when B-cell progenitors were gated on CD19$^+$ and CD34$^+$ events or on CD10$^+$ events followed by backgating on CD45^{low} SSC^{low} events. When compared to the original Ogata score, addition of the CD19 marker allowed the B-cell compartment to be more easily targeted, but only improved the specificity from 80.6% to 85.4%, and did not change the sensitivity.

In parallel to the Ogata score, the contribution of CD5, CD7 and CD56 positivity was evaluated on a subset of 111 controls and 183 MDS or MDS/MPN patients (68 non-proven MDS, 55 low-risk proven MDS, 27 RAEB1, 16 RAEB2, and 17 MDS/MPN). Expression of either CD5 or CD7 on myeloid progenitors or CD56 on monocytes was observed in 6 controls (5.4%), 16 suspected MDS (24%), 19 proven low-risk MDS (35%), 9 RAEB1 (33%), 3 RAEB2 (19%) and 10 MDS/MPN (59%). It is worthy of note that CD56 positivity accounted for 45 of 63 (71%) cases with abnormal expression of one of these three markers. Interestingly, expression of CD56 on granulocytes was

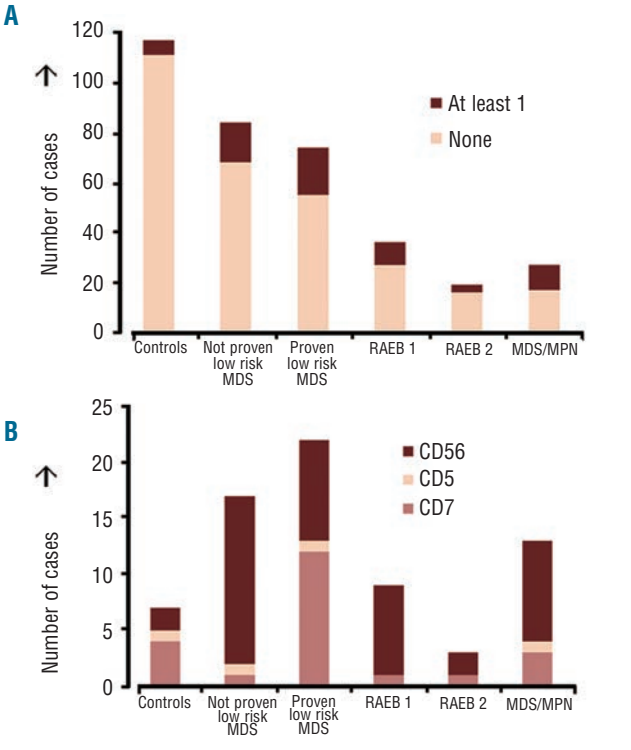

Figure 3. Number of cases with abnormal expression of CD5 or CD7 on myeloid progenitors or CD56 on monocytes. (A) shows patients with at least one abnormality. (B) shows the number of cases with abnormal expression of CD5 or CD7 on blast cells or CD56 on monocytes.

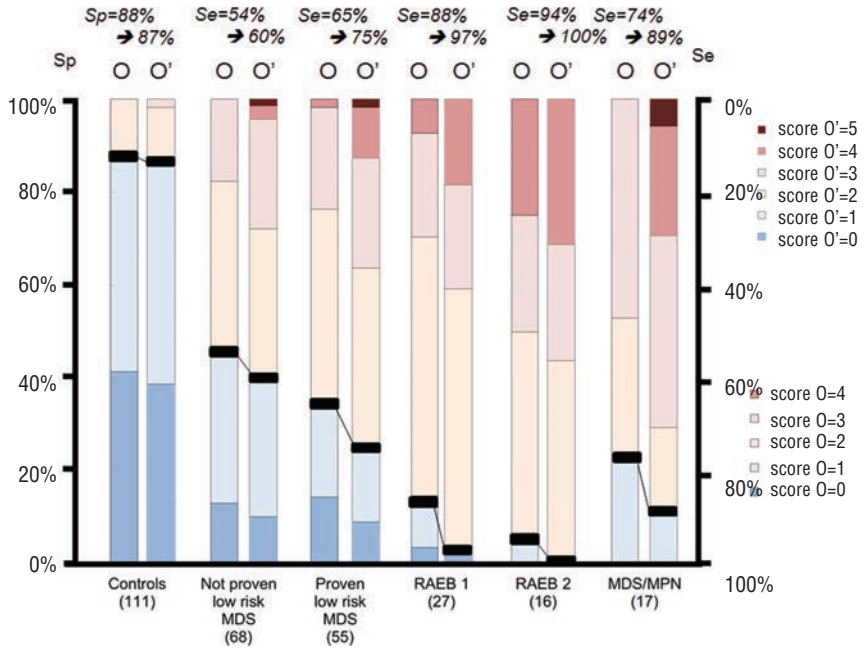

Figure 4. Comparison between classical (left) and extended (right) Ogata scores. Bold lines indicate the threshold of 2. Left and right ordinates, respectively, indicate specificity (sp) and sensitivity (se).

found only in 6 of 183 (3%) MDS/MPN cases; all these cases also had CD56 positive monocytes. Ectopic expression of CD5 on myeloid progenitors was found only in 3 cases, one suspected and one proven RCMD, and one control. CD7 expression on myeloid progenitors was observed in 4 of 111 (3.6%) controls and 15 of 166 (9%) MDS; these were mainly with proven low-risk MDS (12 of 55, 22%). CD56 expression on monocytes was found in 2 of 111 (1.8%) controls and 34 of 166 (21%) MDS patients; incidence of CD56 expression on monocytes was 50% or more in CMML and other MDS/MPN.

The value of the extended Ogata score, built by adding 1 point in case of abnormal expression of either CD5 or CD7 on myeloid progenitors or CD56 on monocytes, was then evaluated. Comparison of the classical and expanded Ogata scores is shown in Figure 4. The specificity was unchanged from 88% to 87%, however, the sensitivity of the extended Ogata score was increased for all categories of patients: 67% for low-risk MDS (60% if suspected and 75% if proven), 97% for RAEB1, 100% for RAEB2, and 89% for MDS/MPN. An extended Ogata score including only CD56 (CD56-Ogata score) was found to have a specificity of 88%, and sensitivities of 64% for low-risk MDS (60% if suspected and 69% if proven), 97% for RAEB and 82% for MDS/MPN. This CD56-Ogata score is, therefore, almost as informative as the extended Ogata score, except for proven MDS.

Discussion

In this study, we report the results of an investigation into the significance of the MFC Ogata score on a series of MDS patients recruited prospectively without any selection criteria, as would be the case in real-life routine diagnosis. We further evaluated the interest of CD5 and CD7 labeling in myeloid progenitors and CD56 on the monocytic compartment.

Several MFC protocols have been developed and published either to diagnose MDS or to predict prognosis. However, these protocols used a wide diversity of techniques and antibody panels.[9,11,17-21] With a view to standardizing MFC in MDS, the European LeukemiaNet (ELN) working group on MDS has defined minimal prerequisites for MFC diagnosis of MDS.[21,22] These include markers of myeloid progenitors allowing detection of an abnormal expression of CD45, CD34, CD117, HLA-DR, CD13 or CD33, asynchronous expression of CD11b or CD15 and ectopic expression of CD5, CD7, CD56 or CD19.21,22 Some intracellular markers have also been assessed in MDS, such as myeloperoxidase activity[23] or, more recently, ferritin content[24] or expression of the myeloid nuclear differentiation antigen (MNDA).[25] Other intracellular markers, such as TREM-1, that have been proven to be important in myelomonocytic differentiation and are altered in other pathological situations, could also be suggested (see review by Arts *et al.*[26]).

To date, MFC in MDS still requires numerous antibodies and a high level of expertise to interpret results in terms of abnormal differentiation patterns and/or abnormal expression of a given marker. The Ogata MFC score was created with a view to simplifying investigation into MDS. Setting up the Ogata MFC score in 4 different routine French laboratories was extremely easy and quite time- and cost-efficient in terms of workload and consumption of reagents. Here, we added CD19 to the Ogata panel to better target the B-cell progenitor compartment. But comparative analysis of MFC data with or without CD19 gating of B-cell progenitors hardly changed the results because of the very particular $CD45^{low}/SSC^{very\ low}$ characteristics of hematogones. Nevertheless, targeting B-cell progenitors with CD19 allowed a better definition and easier quantification of this cell compartment.

One interesting feature of the Ogata score is its relative independence from hemodilution of BM samples.[13,14] We confirmed that the impact of blood dilution was marginal, especially for the quantification of $CD19^+$ B-cell progenitors normalized to the number of $CD34^+$ cells. Only quan-

tification of the latter (CD34+ myeloid progenitors) would be sensitive to hemodilution, which is probably the reason for the poor sensitivity of this parameter, even in RAEB. Interestingly, although BM cells were examined here, it is worth mentioning that some immunophenotypic abnormalities can be found, notably on circulating granulocytes from patients with MDS, such as a decrease in CD10 expression in RAEBs.[27] It has, however, been reported that, except for CD56, these abnormalities tend to be lost between the BM and PB because the disease resides in the BM, and for the fact that cells from the MDS clone are counter-selected to reach the PB compartment.[28]

The same sensitivities and specificities as those already published[13,14] were obtained in this series of patients. Of note, Ogata score tended to be increased in RAEB1 and 2, demonstrating that, as reported by others, BM dysplasia detected in MFC increases with progression of the disease.[9,11,19,29] It was confirmed that none of the four parameters was informative enough on its own, while the CD45 and SSC ratios appeared to be independent of the MDS stage. Thus, the higher Ogata score of RAEB1 and 2 was mainly due to increased myeloid progenitors and decreased B-cell progenitors. Altogether, the sensitivity of the Ogata score was poor, mainly in low-risk MDS, yet with good specificity. The Ogata score was also increased in the majority of MDS/MPN cases, with a rather good sensitivity, suggesting that it could be proposed as a routine diagnostic tool in MDS/MPN, mainly CMML.

The extended Ogata score explored by adding CD5, CD7 and CD56 labeling was shown to be of poor value for CD5. CD7 expression on blast cells was, however, found to be informative mainly in low-risk MDS. CD56 expression on monocytes was mainly found in MDS/MPN, but also in a significant proportion of MDS, as well as in a few controls. These results mean that none of these three markers on its own has any value in diagnosing MDS. The extended Ogata score was found to carry an increased sensitivity of nearly 100% in RAEB1 and 2 and 82% in MDS/MPN. Altogether, this study shows that the Ogata score is reproducible and is technically robust, yet with a poor sensitivity, mainly in low-risk MDS where additional efficient diagnostic tools are mostly needed. The Ogata score can also be positive in patients with no evidence for MDS, such as iron or vitamin deficiency, transient cytopenia and autoimmune disease, indicating the importance of not interpreting the MFC Ogata score on its own, but to use it in conjunction with the clinical context and cytological examination of BM smears. Increased expression of CD56 has been previously reported in MDS and CMML.[28] Taking into account the expression of CD56 on monocytes improved the Ogata score, giving an efficient diagnosis of MDS/MPN. The expression of CD7 on myeloid progenitors was shown to be also informative in MDS, and this was later proven by other methods. Thus, integrated into the global clinical and biological context of the patient, Ogata or extended Ogata MFC scores appear to be helpful to stratify patients with genuine MDS, but are still of variable sensitivity (rather poor in low-grade MDS). Since MFC provides rapid results, it can usefully complement cytomorphological results in an integrated report. However, according to the recommendations of the ELN working group,[21,22] a more sophisticated panel is still needed to fully exploit the value of MFC in identifying early changes associated with emerging MDS.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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