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Correlation Between Bone Marrow Blasts Counts With Flow Cytometry and Morphological Analysis in Myelodysplastic Syndromes

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Dear Editor,

Determination of the percentage of blasts in the bone marrow (BM) is one of the critical factors in diagnosing MDS. Flow cytometry (FCM) of BM cells has been introduced as an important co-criterion for diagnosing MDS [1-3]. However, FCM has not been well accepted because of the lack of consensus of the criteria to define a phenotypic myeloblast and the appropriate denominator for calculation. Moreover, BM samples contain variable amounts of peripheral blood mixed with immature cells, thereby complicating interpretation. The present study was designed to choose reagent combinations to identify blasts by FCM in MDS patients, to determine which cell mass as the denominator in the process of counting the percentages of blasts, and to reveal whether the aspirates with high proportions of mature neutrophils should be normalized based on the proportion of dim CD16 maturing myeloid cells.

Sixty-nine patients with primary MDS were enrolled; BM samples were obtained from 20 control subjects without any known hematological disease and with normal blood cell counts. This study was approved by our institutional review board, and writ-ten informed consent was obtained from all subjects. Cell morphology was analyzed by conventional light microscopy, and the same BM samples were analyzed by FCM within 6 hr. The panel included four-color combinations of markers: HLA-DR-fluorescein isothiocyanate (FITC)/CD117-phycoerythrin (PE)/CD34-allophycocyanin (APC)/CD45-peridinin-chlorophyll protein (PerCP), CD16-FITC/CD13-PE/CD11bAPC/CD45-PerCP, anti-ckappa-FITC/anti-lambda-PE/CD138APC/CD45-PerCP, and IgG1-FITC/IgG1-PE/IgG1-APC/IgG1-PerCP. For each criterion, the percentage of blasts was calculated by using both the total number of nucleated BM cells (A) and the number of non-erythroid nucleated cells (N) as the denominator. For the A1 and N1 methods, blasts were defined as events showing a CD34^{hi} and CD45$^{lo/int}$ phenotype. The A2 and N2 strategies identified CD34^{hi} and/or CD117$^+$ cells showing CD45$^{lo/int}$ expression as corresponding to blasts. For the A3 and N3 methods, blasts were defined as events showing a CD34^{hi} and/or CD117$^+$ HLA-DR$^+$ and CD45$^{lo/int}$ phenotype. The ratio of the percentage of dim CD16 neutrophils to the average percentage from BM biopsies (80%) was calculated as a dilution factor of excess mature segmented neutrophils in the sample. We used

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Table 1. Correlation between the percentages of blasts in the bone marrow obtained by flow cytometry and morphology.

Method of analysis	Control subjects (N=20)				MDS patients (N=69)			
	Percentage (%) of blasts (mean±SD)	Correlation with morphology (r^2)	Degree of agreement with morphology		Percentage (%) of blasts (mean±SD)	Correlation with morphology (r^2)	Degree of agreement with morphology	
			Mean difference ±1.96 SD (%)	Limit of agreement (N)			Mean difference ±1.96 SD (%)	Limit of agreement (N)
Morphology	1.40±1.08				7.16±4.47			
A1	0.67±0.44	0.555	0.73±1.59	18	4.85±3.83	0.774	2.3±4.2	65
A2	2.80±1.02	0.584	−1.40±1.43	18	9.72±5.58	0.808	−2.6±5.0	67
A3	1.56±0.90	0.869	−0.16±0.8	19	7.20±4.72	0.897	0±2.9	67
N1	0.86±0.59	0.704	0.54±1.31	19	5.46±4.08	0.780	1.7±4.1	64
N2	3.16±1.16	0.527	−1.76±1.63	19	10.90±5.80	0.766	−3.7±5.6	65
N3	1.97±0.86	0.857	−0.44±0.84	19	8.02±5.12	0.870	−0.9±3.7	67
CA1	0.92±0.70	0.526	0.48±1.52	19	6.47±5.21	0.656	0.7±6	64
CA2	3.66±1.70	0.667	−2.25±1.99	19	13.31±8.89	0.500	−6.2±12.9	66
CA3	2.15±1.60	0.707	−0.80±1.70	19	9.63±6.64	0.723	−2.5±7.2	64
CN1	1.21±1.10	0.588	0.19±1.46	19	7.35±5.84	0.582	0.2±7.4	67
CN2	4.14±1.98	0.621	−2.70±2.50	19	14.89±9.39	0.462	−7.7±14.0	66
CN3	2.50±1.69	0.717	−1.10±1.90	19	10.80±7.40	0.654	−3.6±9	64

the following formula to normalize a BM aspirate to the levels expected for an "average" biopsy: 80%/(% dim CD16×blast count). After normalization, the A1, A2, and A3 strategies were converted to the CA1, CA2, and CA3 strategies, respectively. Similarly, the N1, N2, and N3 methods were converted to the CN1, CN2, and CN3 methods, respectively. Mean values and their SDs along with the median and range values were calculated for all data, and the Pearson correlation (r^2) and Bland-Altman tests were used for analysis. The limits of agreement of the Bland-Altman test were set as the mean difference between the morphological and FCM blast counts±1.96 SD.

The A3 strategy showed the highest degree of correlation and agreement with morphological blasts counts ($P>0.05$). The A1, N1, and CA1 strategies underestimated the numbers of blasts compared with morphological counts. The A2, N2, N3, CA2, CA3, CN2, and CN3 strategies overestimated blasts compared with morphological counts. The CN1 strategy underestimated the blasts in control subjects and overestimated the blasts in patients with MDS ($P<0.05$) (Table 1 and Fig. 1).

The blasts counts were underestimated via a CD34^{hi} and CD45$^{lo/int}$ phenotype strategy, suggesting that use of these markers alone will underestimate blast counts. Multiple experiments have shown that some blasts may not express CD34 [4, 5], and neoplastic cells may lack CD34 expression in MDS [6, 7]. The CD34^{hi} and/or CD117$^+$ and CD45$^{lo/int}$ phenotype strategy overestimated blast

numbers. CD117 is lost during early monocyte development and in the promyelocyte during neutrophil development. This antigen is expressed at high levels on mast cells and mast cell precursors, and occasionally on other neoplastic cells. The present study revealed that ratio of CD34^{hi} and/or CD117$^+$HLA$^-$DR$^+$ cells to the number of total events showed the highest degree of correlation and agreement with morphological blast counts. This could reflect the fact that this criterion includes measurement of CD34$^+$ blasts, CD34$^-$CD117$^+$HLA$^-$DR$^+$ neutrophils, and erythroid-committed precursors as well as early CD117^{lo}HLA-DR^{hi} monocytic and dendritic cell precursors [8-10]. After normalizing by dim CD16 neutrophils, the blast counts were found to be overestimated.

In conclusion, quantification of blasts by FCM based on the ratio of the CD34^{hi} and/or CD117$^+$HLA-DR$^+$ phenotype to the number of total events showed the highest correlation with morphology.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.
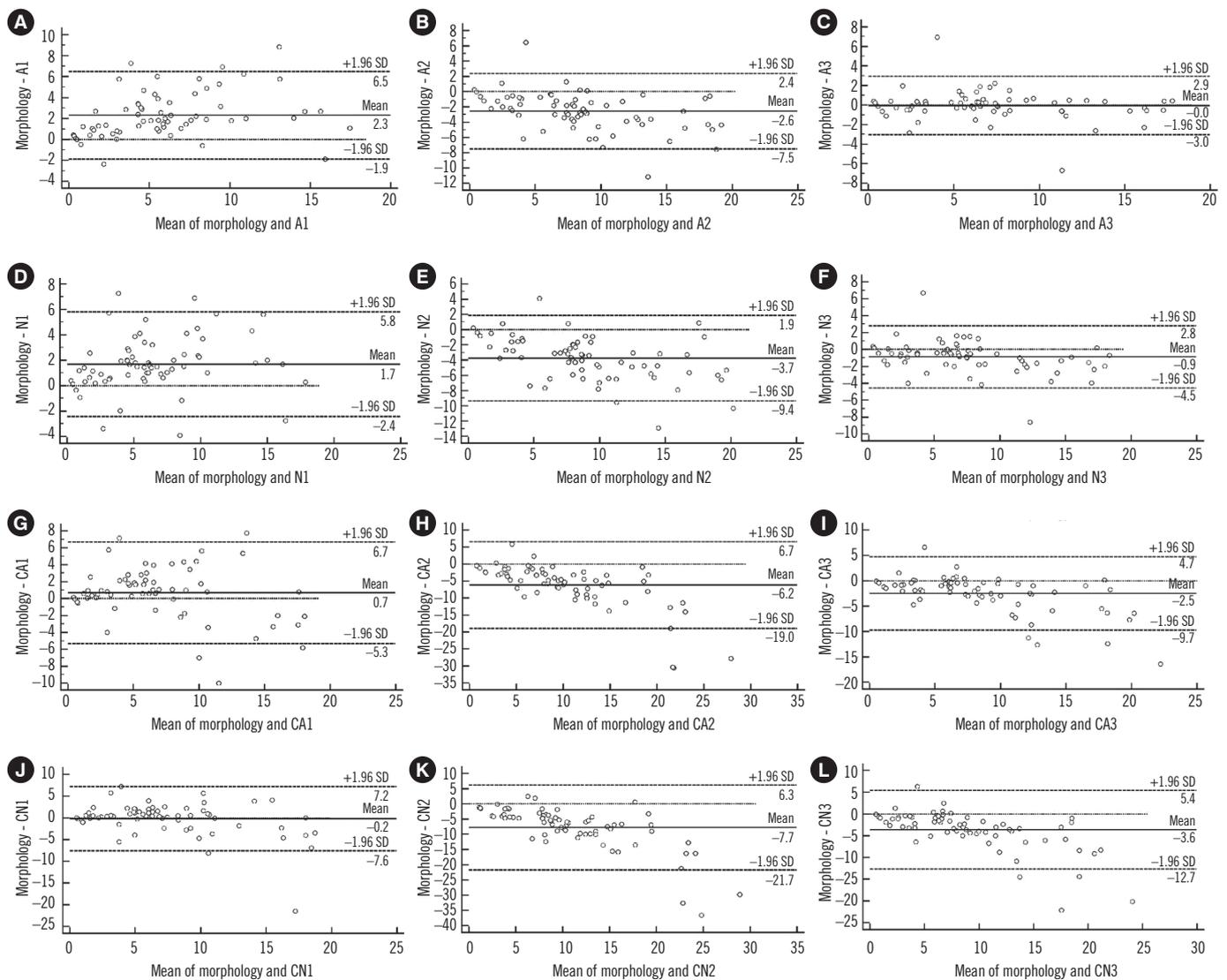
Fig. 1. Degree of agreement between bone marrow blast counts by flow cytometry and conventional morphology in MDS. The abscissae represent the mean percentage of bone marrow blasts obtained by morphology and flow cytometry for each patient, and the ordinates represent the difference in the percentages of bone marrow blasts obtained by morphology and flow cytometry for each patient. Panels A, B, and C show flow cytometry counts obtained with strategies A1, A2, and A3, respectively, and panels D, E, and F show flow cytometry blast percentages with the N1, N2, and N3 strategies, respectively. Panels G, H, and I show flow cytometry counts obtained with strategies CA1, CA2, and CA3, respectively, and panels J, K, and L show the CN1, CN2, and CN3 strategies, respectively. The percentages in the Bland-Altman plots indicate the number of cases within the 95% limits of agreement.

REFERENCES

1. Cazzola M, Della Porta MG, Travaglino E, Malcovati L. Classification and prognostic evaluation of myelodysplastic syndromes. Semin Oncol 2011; 38:627-34.
2. van de Loosdrecht AA and Westers TM. Cutting edge: flow cytometry in myelodysplastic syndromes. J Natl Compr Canc Netw 2013;11:892-902.
3. Porwit A, van de Loosdrecht AA, Bettelheim P, Brodersen LE, Burbury K, Cremers E, et al. Revisiting guidelines for integration of flow cytometry results in the WHO classification of myelodysplastic syndromes-proposal from the International/European LeukemiaNet Working Group for Flow Cytometry in MDS. Leukemia 2014;9:1793-8.
4. van Lochem EG, van der Velden VH, Wind HK, te Marvelde JG, Westerdaal NA, van Dongen JJ. Immunophenotypic differentiation patterns of normal hematopoiesis in human bone marrow: reference patterns for age-related changes and disease-induced shifts. Cytometry B Clin Cytom 2004;60:1-13.
5. 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;98:979-87.
6. Pirruccello SJ, Young KH, Aoun P. Myeloblast phenotypic changes in myelodysplasia. CD34 and CD117 expression abnormalities are common. Am J Clin Pathol 2006;125:884-94.

7. Bellos F and Kern W. Flow cytometry in the diagnosis of myelodysplastic syndromes and the value of myeloid nuclear differentiation antigen. Cytometry B Clin Cytom 2017;92:200-6.

8. 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;22:1175-83.

9. Orfao A, Ortuño F, de Santiago M, Lopez A, San Miguel J. Immunophenotyping of acute leukemias and myelodysplastic syndromes. Cytometry A 2004;58:62-71.

10. Matarraz S, López A, Barrena S, Fernandez C, Jensen E, Flores-Montero J, et al. Bone marrow cells from myelodysplastic syndromes show altered immunophenotypic profiles that may contribute to the diagnosis and prognostic stratification of the disease: a pilot study on a series of 56 patients. Cytometry B Clin Cytom 2010;78:154-68.