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Automated leukocyte parameters are useful in the assessment of myelodysplastic syndromes

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Abstract

Background: Study utility of seven automated VCS parameters (V-volume, C-conductivity and S-scatter) in leukocytes as an objective read-out of dysplasia in Myelodysplastic Syndromes (MDS).

Methods: Peripheral blood was analyzed by Beckman-Coulter DxH800 hematology analyzer in 43 patients with low-grade, high-grade MDS and 21 control individuals. The differences in mean (MN) and standard deviation (SD) of each parameter were examined. The optimal sensitivity and specificity to predict MDS were determined by statistical analysis.

Results: In neutrophils, all means of the light scatters were significantly lower in high-grade MDS than in the control group. Mean median angle light scatter (MN-MALS-NE) and mean upper median angle light scatter (MN-UMALS-NE) were significantly different between low-grade MDS and control patients. MN-MALS-NE as a MDS predictor revealed 63% sensitivity and 67% specificity with a cutoff value of ≤133. SDs of each parameter in neutrophils differed significantly among three groups. SD of neutrophil upper median angle light scatter (SD-UMALS-NE) had 77% sensitivity and 82% specificity (cutoff value of ≥11.16) to predict MDS.

Conclusions: MDS patients have a significant decrease with a linear trend in VCS parameters in neutrophils, indicating cell dysplasia. The degree of the heterogeneity measured by SD is the most predictive of MDS.

KEYWORDS

automatic hematology analyzer 2, light scatter parameters 3, myelodysplastic syndromes 1

1 | INTRODUCTION

Myelodysplastic syndromes (MDS) are heterogeneous clonal hematological diseases characterized by chronic cytopenia, dysplasia and increased progression rate to acute myeloid leukemia (Bennett et al., 1982; Foran & Shammo, 2012; Hasserjian, 2017; Montalban-Bravo & Garcia-Manero, 2018; Zini, 2017). MDS occur in 4 individuals per 100,000 people in the U.S. population, affecting males more frequently and its prevalence increases with age (Aul, Gattermann, & Schneider, 1992; Cogle, Craig, Rollison, & List, 2011). Although majority of MDS (80%) are acquired diseases and are age-related, exposure to toxic substance, ionizing radiation, and anti-neoplastic cytotoxic or immunosuppressive therapy (10–20%) are some known risk factors of the MDS (Foran & Shammo, 2012; Mutlu, Akca, Teke, & Ugur, 2011; Zini, 2017). Notably, recent advancements in successful anti-neoplastic therapy result in the increased survival rate and therefore lead to an increased rate of the secondary therapy-associated MDS (Foran & Shammo, 2012). Heterogeneity of the diseases results in a variable degree of cytopenia posing a challenge for clinical suspicion of MDS and a delay in initiating the diagnostic work up for the MDS (Foran & Shammo, 2012; Shammo et al., 2011). Definitive diagnosis of MDS is based on the morphologic dysplasia of the bone marrow cellular elements and complemented by the flow cytometry, karyotype, cytogenetics and molecular genetics studies (Alhan, Westers, Cremers,

et al., 2016; Haase et al., 2007; Haferlach et al., 2014; Hasserjian, 2017; Kern et al., 2013; Montalban-Bravo & Garcia-Manero, 2018). World Health Organization (WHO) classification of tumors of haematopoietic and lymphoid tissues, revised 4th edition, provides diagnostic criteria for six distinct groups of MDS: MDS with single lineage dysplasia, MDS with multilineage dysplasia, MDS with ring sideroblasts with single or multilineage dysplasia, MDS with isolated 5q deletion, MDS with excess blasts and MDS unclassifiable Hasserjian, 2017. Subtypes of MDS can be divided into three risk groups based on survival time and evolution to acute myeloid leukemia – low-, intermediate- and high-risk Hasserjian, 2017. The low-risk group contains MDS with single lineage dysplasia, MDS with ring sideroblasts with single lineage dysplasia, and MDS with isolated 5q deletion. The intermediate-risk group contains MDS with multilineage dysplasia and MDS with ring sideroblasts with multilineage dysplasia. The high-risk group contains MDS with excess blasts (EB1—2–4% blasts in peripheral blood or 5–9% blasts in bone marrow and EB2—5–19% blasts in peripheral blood or 10–19% blasts in bone marrow).

Cytogenetic aberrations, including deletion 5q, although fairly specific for MDS, are present in ~50% of patients (Greenberg, 2012; Schanz, Tuchler, Sole, et al., 2012). Majority of the MDS cases bear associated somatic mutations such as *TET2*, *IDH1*, *IDH2*, *EZH2*, *DNMT3A*, *ASXL1*, *TP53* and *SF3B1* (Bejar, Levine, & Ebert, 2011; Haferlach et al., 2014). These mutations are not specific to MDS and not sufficient to establish a diagnosis of MDS (Itzykson & Fenaux, 2014; Sperling, Gibson, & Ebert, 2017; Steensma et al., 2015). Therefore, morphologic dysplasia remains to be the hallmark and gold standard for the diagnosis of MDS (Goasguen et al., 2016; Goasguen & Bennett, 1992). Subjective interpretation of the bone marrow cellular morphology by pathologists may lead to the uncertainty in the utility of the bone marrow examination, especially in patients with early disease, with the diagnostic discrepancy in up to 20% of patients (Naqvi et al., 2011; Sandhaus, Wald, Sauder, Steele, & Meyerson, 2007). Therefore, there is a need for more objective tool for initial screening of MDS.

Assessment of the morphology of the peripheral blood is a powerful tool to diagnose MDS. Peripheral blood smear might show macrocytic anemia, aniso- and poikilocytosis along with variable dysgranulopoiesis or circulating blasts, features suggestive of MDS. Currently, automated hematology analyzers perform initial assessment of the peripheral blood. In addition to complete blood count (CBC), the hematology analyzers report a range of immature-appearing cells that triggers manual review of the smear. The hematology analyzers are not set to detect subtle features of dysgranulopoiesis, including hypolobated nuclei and hypogranular cytoplasm in granulocytes, and do not "flag" peripheral smear for the manual review. Since it is not feasible for the pathologist to review all blood smears that show cytopenia, an objective measure of the dysplastic cellular morphology is needed to "flag" peripheral blood for the manual review.

The Beckman Coulter DxH800 automated hematology analyzer performs CBC, white blood cell (WBC) differential and nucleated red blood cells count based on a combination of three physical parameters: flow cell volume (V), conductivity (C), and five light scatter measurements (S) by the VCS flow technology (Krause, 1990; Richardson-Jones, 1990; Tang, Jing, Bo, & Xu, 2012). The size of the leukocytes is determined by measuring their volume by the direct current impedance. The nuclear details are analyzed by the radio frequency opacity and reflect conductivity. Three light scatter measurements approximate cytoplasmic granularity and membrane surface: median angle light scatter (MALS), lower median angle light scatter (LMALS), and upper median angle light scatter (UMALS). In addition, the axial light loss (AL2) and the low angle light scatter (LALS) reflect cellular transparency and complexity respectively. Altogether, these seven parameters are displayed as mean (MN) values and standard deviation (SD) values (Jean, Boutet, Lenormand, et al., 2011). Dysgranulopoiesis involving cytoplasmic granularity and nuclear morphology can be reflected in objective VCS parameters that are measured during the routine CBC count.

Although recent advancements have been made in utilizing VCS parameters to "flag" potential patients with MDS, the cutoff values and the sensitivity/specificity of the assays vary (Jean et al., 2011; Miguel et al., 2007; Raess, Njo, et al., 2014). Aim of this study was to ensure identification of patients with subtle dysplasia and low/absent blast count. Therefore, we defined low-grade MDS group as a combination of WHO-defined low- and intermediate-risk groups of patients with MDS. High-grade MDS group was defined as a WHO-defined high-risk group of patients with MDS. This study thrived to establish the cutoff values in VCS parameters that are measured in leukocytes in peripheral blood to screen for the dysplastic cell morphology in MDS patients. Current study attempted to implement an objective "flag" to initiate manual examination of the peripheral blood morphology to identify dysgranulopoiesis and initiate bone marrow biopsy (Invernizzi, Quaglia, & Porta, 2015).

2 | MATERIALS AND METHODS

2.1 | Patient selection

Sixty-four (n = 64) patients with MDS (43 patients) and controls (21 patients) were identified at University of California, Irvine from 2014–2016. Each patient, either control or patient with MDS, had a bone marrow biopsy performed at our institution. Thirteen of them were high-grade (HG) MDS cases (Group A—high-grade MDS; >2% but <20% blast counts in peripheral blood or >5% but <20% in bone marrow) (Bennett, 2016). Thirty cases were low-grade (LG) MDS (Group B—low-grade MDS; <2% blast count in peripheral blood or <5% in bone marrow) (Bennett, 2016). Twenty-one control patients with no significant marrow abnormalities or myeloid neoplasms (CBC and differential counts were within normal limits) were selected randomly during the study as a control group (Group C—normal). All control cases with anemia, leukocytosis and erythrocytosis were diagnosed as non-neoplastic reactive processes (8/21). Rest of the control cases constituted bone marrow biopsies that were performed as part of the staging for lymphoma or multiple myeloma (13/21). Ten control cases did not show any evidence of involvement in the

peripheral blood. Three control cases showed minimal involvement with B-cell lymphoma. The peripheral blood samples were collected on the day of the bone marrow biopsy procedure and analyzed using automated hematology Beckman Coulter DxH800. All peripheral blood smears were reviewed manually. Patients' final pathology bone marrow reports were reviewed for clinicopathologic features. Patients' age, gender, and CBC data are summarized in Table 1. This prospective study was approved by University of California, Irvine Institutional Review Board (HS#2013–9903).

2.2 | Peripheral blood analysis using Beckman Coulter DXH800

Peripheral blood analysis was performed by Beckman Coulter DxH800 (Beckman Coulter, Inc, Brea, CA) in all 64 patients. Mean values of the seven VCS parameters, were collected in addition to the routine CBC and differential counts. Such parameters include: (a) Mean cell volume (MN-V); (b) Mean cell conductivity (MN-C); (c) Mean median angle light scatter (MN-MALS); (d) Mean upper median angle light scatter (MN-UMALS); (e) Mean lower median angle light scatter (MN-LMALS); (f) Mean lower angle light scatter (MN-LALS); and (g) Mean axial light loss (MN-AL2); in neutrophils (NE), lymphocytes (LY), monocytes (MO), eosinophils (EO) and early granulated cells (EGC). In addition, standard deviations (SDs), which reflect the variabilities of the measurements of these mean values of the above VCS parameters in each cell type, were also collected.

2.3 | Statistical analyses

All analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC). The results of VCS parameters were expressed as the mean ± *SD*. Shapiro–Wilk test was utilized to examine the normality of the distribution in each variable. The analysis of variance (ANOVA) was performed to examine the difference in mean of each variable among three groups when distribution followed normal Gaussian distribution. In non-normally distributed variables Kruskal–Wallis test was applied to examine the mean ranks of the variable among the three groups. The Bonferroni–Holm adjusted method was applied to unadjusted *p*-value for multiple testing in seven outcomes within each cell type. The Tukey–Kramer method was applied to adjust the multiple comparisons among groups in each ANOVA model. The *p*-value <.05 was considered to be significant.

A univariate logistic regression model was utilized to predict the presence of MDS with each variable as a predictor. A receiver operating characteristic (ROC) curve was constructed to determine the area

TABLE 1 Clinicopathologic characteristics of patients

	MDS		
	High-grade MDS (*N* = 13)	Low-grade MDS (*N* = 30)	Control (*N* = 21)
	Mean ± *SD*	Mean ± *SD*	Mean ± *SD*
Age (y)	68.4 ± 17.3	71.5 + 16.8	62.2 ± 17.9
Gender (F/M)	6/7	15/15	8/13
WBC ($\times 10^3$/mcl)	3.6 ± 2.4	6.5 ± 5.1	6.4 ± 5.8
HGB (g/dl)***	**7.8 ± 0.8§ ¶**	**9.7 ± 2.3†**	11.8 ± 2.6
HCT (%)***	**23 ± 2.6§ ¶**	**29.0 ± 6.9‡**	35.8 ± 7.9
PLT ($\times 10^3$/mcl)**	**49.5 ± 37.4§**	**105.8 ± 100.0†**	173.8 ± 92.7
RBC (mill/mcl)***	**2.54 ± 0.3§**	**3.1 ± 0.8‡**	4.0 ± 1.1
MCV (fl)	91.2 ± 9.9	**95.2 ± 11.1†**	85.9 ± 19.0
MCH (pg)	31.0 ± 3.5	31.8 ± 3.4	29.9 ± 3.2
MCHC (g/dl)*	**34 ± 0.9†**	33.4 ± 1.1	33.1 ± 1.0
RDW-CV (%)*	**18.9 ± 4.5†**	**17.5 ± 3.8†**	15.2 ± 2.2
MPV (fl)	9.3 ± 1.5	9.5 ± 1.6	8.8 ± 0.6

Note: Significant differences are indicated in bold.

Abbreviations: HCT, hematocrit; HGB, hemoglobin; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; PLT, platelets; RBC, red blood cells; RDW-CV, red cell distribution width–coefficient of variation; WBC, white blood cells.

*p < .05 among all groups.

**p < .01 among all groups.

***p < .001 among all groups.

†p < .05 versus control group.

‡p < .01 versus control group.

§p < .001 versus control group.

¶p < .05 versus low-grade MDS group.

under the curve (AUC) for the best thresholds (cutoff levels) and to calculate the optimal sensitivity and specificity of each variable.

Furthermore, the univariate logic model was used to examine whether the significant relationship between these predictors and the outcome are maintained. The entire dataset was first divided into two equal subsets while the proportion of disease in each subset was the same as in the entire cohort and then further divided into another two subsets based on the 2:1 ratio while the proportion of disease in each subset was the same as in the entire cohort. The stepwise selection procedure was performed onto the two sets separately to predict the outcome, that is, MDS, using previously determined 11 significant predictors in neutrophils, monocytes, and early granulated cells including MN-MALS-NE, MN-LMALS-NE, MN-LALS-NE, MN-UMALS-MO, MN-LALS-MO, MN-C-EGC, SD-V-NE, SD-MALS-NE, SD-UMALS-NE, SD-LMALS-NE, and SD-AL2-NE. The significant value for a variable to enter or to abandon the model was 0.05 or 0.10, respectively.

The results of the age and CBC data were expressed as the mean ± *SD*. The ANOVA was performed to examine the difference in mean of each variable among three groups when distribution followed normal Gaussian distribution. The Tukey–Kramer method was applied to adjust the multiple comparisons among groups in each ANOVA model. In non-normally distributed variables Kruskal–Wallis test was applied to examine the mean ranks of the variable among the three groups. The *p*-values <.05 were considered to be significant.

3 | RESULTS

3.1 | General clinicopathologic features

For the clinical purposes, MDS can be classified as low-grade or high-grade MDS groups. Low-grade MDS poses diagnostic challenges to both clinicians and pathologists. Therefore, splitting patients in two groups, which is done based on the blast count, provides higher statistical power to make conclusions on light scatter parameters. Based on the blast count in peripheral blood and bone marrow we split patients with MDS into low-grade group (<2% blasts in peripheral blood or <5% in bone marrow and high-grade group (2%– < 20% blast in peripheral blood or >5%–<20% in bone marrow).

The clinical information including age, gender and CBC data of the study subjects is summarized in Table 1. The average age was not significantly different among the three groups. It ranged from 62.2 years in the control group, 68.4 years in high-grade MDS group, and 71.5 years in low-grade MDS group. The female to male ratio was 0.86 (6F/7M) in high-grade MDS group, 1 (15F/15M) in low-grade MDS group, and 0.62 (8F/13M) in the control group. There were no significant differences in WBC counts, mean corpuscular hemoglobin and mean platelet volume among the three groups. However, the hemoglobin, hematocrit, RBC count, mean corpuscular volume, red cell distribution width - coefficient of variation, and platelet count were significantly different between low-grade MDS and the control groups. There was a significant difference in, hemoglobin and hematocrit between high-grade MDS and low-grade MDS groups (Table 2).

3.2 | Mean values of the VCS parameters

Means and SDs of seven VCS parameters in neutrophils, lymphocytes, monocytes, eosinophils and early granulated cells (Table 3) were analyzed and the differences were calculated among high-grade MDS (n = 13), low-grade MDS (n = 30) and the control (n = 21) groups (Table 4). The selected data from Table 4 were depicted in box and whiskers plot to emphasize differences between low-grade MDS in comparison to high-grade MDS and control groups (Figure 1).

The mean cell volume in lymphocytes was significantly higher in high-grade MDS than in low-grade MDS (p < .001) and control (p < .05) groups respectively (Figure 1a). However, there was no difference in mean cell volume in leukocytes between low-grade MDS and control groups (Table 4). Similarly, there was no difference in MN-C found in leukocytes in all three groups except for a significantly lower MN-C in high-grade MDS group than in the control group (Table 4). In neutrophils, MN-MALS and MN-UMALS in low-grade MDS group were significantly lower than that in the control group (Figure 1b). In addition, all five mean values of VCS parameters were significantly lower in high-grade MDS group than that in the control group (MN-MALS-NE, MN-LMALS-NE, and MN-LALS-NE; MN-UMALS-NE and MN-AL2-NE). However, there were no differences in these parameters between the two MDS groups (Table 4). MN-LALS-MO was significantly lower in high-grade MDS than in the control

TABLE 2 Selected CBC data significantly different in high-grade MDS, low-grade MDS and control groups

Change in the CBC data	High-grade MDS versus control	Low-grade MDS versus control	High-grade MDS versus low-grade MDS
▼	Red blood cells Hemoglobin Hematocrit Platelets	Red blood cells Hemoglobin Hematocrit Platelets	Hemoglobin Hematocrit
▲	MCHC RDW-CV	MCV RDW-CV	

Note: Significant increase (▲) or decrease (▼) in the CBC parameters.
Abbreviations: MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RDW-CV, red cell distribution width—coefficient of variation.

TABLE 3 Abbreviations of VCS parameters measured in leukocytes

Labels	Neutrophils (NE)	Lymphocytes (LY)	Monocytes (MO)	Eosinophils (EO)	Early granulated cells (EGC)
Mean cell volume SD cell volume	MN-V-NE SD-V-NE	MN-V-LY SD-V-LY	MN-V-MO SD-V-MO	MN-V-EO SD-V-EO	MN-V-EGC SD-V-EGC
Mean cell conductivity SD cell conductivity	MN-C-NE SD-C-NE	MN-C-LY SD-C-LY	MN-C-MO SD-C-MO	MN-C-EO SD-C-EO	MN-C-EGC SD-C-EGC
Mean median angle light scatter SD median angle light scatter	MN-MALS-NE SD-MALS-NE	MN-MALS-LY SD-MALS-LY	MN-MALS-MO SD-MALS-MO	MN-MALS-EO SD-MALS-EO	MN-MALS-EGC SD-MALS-EGC
Mean upper median angle light scatter SD upper median angle light scatter	MN-UMALS-NE SD-UMALS-NE	MN-UMALS-LY SD-UMALS-LY	MN-UMALS-MO SD-UMALS-MO	MN-UMALS-EO SD-UMALS-EO	MN-UMALS-EGC SD-UMALS-EGC
Mean lower median angle light scatter SD lower median angle light scatter	MN-LMALS-NE SD-LMALS-NE	MN-LMALS-LY SD-LMALS-LY	MN-LMALS-MO SD-LMALS-MO	MN-LMALS-EO SD-LMALS-EO	MN-LMALS-EGC SD-LMALS-EGC
Mean lower angle light scatter SD lower angle light scatter	MN-LALS-NE SD-LALS-NE	MN-LALS-LY SD-LALS-LY	MN-LALS-MO SD-LALS-MO	MN-LALS-EO SD-LALS-EO	MN-LALS-EGC SD-LALS-EGC
Mean axial light loss SD axial light loss	MN-AL2-NE SD-AL2-NE	MN-AL2-LY SD-AL2-LY	MN-AL2-MO SD-AL2-MO	MN-AL2-EO SD-AL2-EO	MN-AL2-EGC SD-AL2-EGC

Abbreviations: MN, mean; SD, standard deviation.

group. MN-LALS-LY was significantly higher in high-grade MDS than in low-grade MDS groups (Figure 1c). There was no difference in these mean values between low-grade MDS and the control groups. There was a significant decrease in MN-LALS-EO, MN-MALS-EGC, and MN-UMALS-EGC in high-grade MDS group than in low-grade MDS and the control groups (Figure 1d).

3.3 | Mean standard deviations of each VCS parameter

The means of SD of each VCS parameter in leukocytes were analyzed (Table 5). The data with significant differences between low-grade MDS and the control, and between high-grade MDS and low-grade MDS groups from the Table 5 were depicted in box and whiskers plot (Figure 2). In neutrophils, SD-V-NE, SD-LMALS-NE, SD-SD-AL2-NE, SD-UMALS-NE and SD-MALS-NE were significantly higher in low-grade MDS group than in the control group. All means of SDs except for SD-LMALS-NE were significantly higher in high-grade MDS group than in low-grade MDS group (Figure 2a). All means of SDs of the seven VCS parameters in high-grade MDS group were significantly higher than that in the control group (Table 5). In lymphocytes, there was no difference in the mean SDs of all seven parameters between low-grade MDS and the control groups. However, SD-V-LY, SD-MALS-LY, SD-LMALS-LY and SD-LALS-LY were significantly higher in high-grade MDS than that in low-grade MDS group. In addition, high-grade MDS group revealed significantly higher SD-V-LY and SD-LALS-LY than that of the control group (Figure 2b). In monocytes, SD-MALS-MO, SD-UMALS-MO and SD-LMALS-MO in low-grade MDS group were significantly higher than that in the control group (Figure 2c). In eosinophils, there was no difference in the mean SDs of all seven parameters between low-grade MDS and the control groups. SD-MALS-EO and SD-AL2-EO in high-grade MDS were higher than in low-grade MDS group. SDs of all six VCS parameters were significantly higher in high-grade MDS group than that in the control group (Figure 2d). Similarly, there was no difference in the mean SDs of all seven parameters between low-grade MDS and the control groups in early granulated cells. Only SD-LALS-ECG in high-grade MDS group was significantly higher than in low-grade MDS group (Table 5).

3.4 | Differentiation of MDS from the control patients using VCS parameters

Initially, 20 variables including six mean parameters in leukocytes (MN-MALS-NE, MN-LMALS-NE, MN-LALS-NE, MN-UMALS-MO, MN-LALS-MO, and MN-C-EGC), five SDs of neutrophils (SD-V-NE, SD-MALS-NE, SD-UMALS-NE, SD-LMALS-NE, and SD-AL2-NE), four SDs of monocytes (SD-MALS-MO, SD-UMALS-MO, SD-LMALS-MO, and SD-AL2-MO), and five SDs of eosinophils (SD-C-EO, SD-MALS-EO, SD-UMALS-EO, SD-LMALS-EO, and SD-AL2-EO) have been identified by the univariate logistic models to have a statistically significant association between the outcome and the predictor of MDS. ROC curve was established and AUC with 95% confidence interval was calculated for these 20 variables. Ten of the 20 variables including two means in neutrophils and early granulated cells (MN-MALS-NE, MN-C-ECG) and eight SDs in neutrophils, eosinophils and monocytes (SD-MALS-NE, SD-UMALS-NE, SD-LMALS-NE, SD-AL2-NE, SD-V-NE, SD-MALS-EO, SD-MALS-MO and SD-UMALS-MO) had AUCs with fair (0.70–0.79) or good (0.80–0.89) accuracy (Table 6).

MN-MALS-NE and MN-C-EGC demonstrated a fair accuracy (AUC 0.64–0.68) in predicting MDS. The value of MN-MALS-NE as a screening parameter in evaluating dysplasia revealed a sensitivity of

TABLE 4 Mean values of VCS parameters in neutrophils, lymphocytes, monocytes, eosinophils and early granulated cells

	V	C	MALS	UMALS	LMALS	LALS	AL2
Neutrophils-NE (mean ± SD)							
High-grade MDS	161.46 ± 27.86	141.62 ± 6.41	**123.54 ± 18.92‡**	**127.23 ± 22.89†**	**114.92 ± 16.49‡**	**138.08 ± 22.17‡**	**131.54 ± 16.97†**
Low-grade MDS	155.23 ± 20.70	144.10 ± 6.29	**129.73 ± 11.30†**	**132.63 ± 11.50†**	122.13 ± 11.90	151.90 ± 23.59	134.30 ± 13.37
Control	152.29 ± 11.50	146.71 ± 6.33	136.81 ± 9.91	138.62 ± 6.58	129.71 ± 12.80	165.10 ± 24.41	135.24 ± 5.57
Lymphocyte (mean ± SD)							
High-grade MDS	**103.46 ± 16.10**** †	121.15 ± 7.63	75.62 ± 14.59	76.85 ± 17.37	67.23 ± 13.09	**52.08 ± 20.26***	75.54 ± 16.20
Low-grade MDS	90.96 ± 8.99	118.90 ± 5.22	72.37 ± 8.51	73.40 ± 12.26	64.07 ± 7.18	39.30 ± 4.02	66.70 ± 8.17
Control	91.71 ± 9.55	118.86 ± 6.58	76.19 ± 6.81	78.67 ± 9.44	66.43 ± 6.12	39.81 ± 5.01	68.24 ± 8.19
Monocyte (mean ± SD)							
High-grade MDS	**195.83 ± 27.11†**	120.42 ± 11.96	85.17 ± 13.78	**92.33 ± 13.61* †**	74.42 ± 15.57	**85.25 ± 21.15‡**	121.50 ± 20.77
Low-grade MDS	178.60 ± 17.22	124.03 ± 9.29	88.83 ± 10.95	97.37 ± 12.04	76.37 ± 10.68	91.37 ± 15.64	118.13 ± 16.29
Control	178.10 ± 6.96	127.67 ± 6.38	92.95 ± 4.62	102.48 ± 5.85	78.67 ± 5.60	98.38 ± 11.68	121.67 ± 8.57
Eosinophils (mean ± SD)							
High-grade MDS	155.00 ± 27.23	155.54 ± 30.26	191.39 ± 22.34	201.90 ± 22.22	175.85 ± 23.74	**137.54 ± 22.27** ‡**	125.08 ± 15.12
Low-grade MDS	159.07 ± 15.64	151.82 ± 17.62	192.14 ± 8.39	200.64 ± 12.74	179.00 ± 6.93	164.14 ± 19.06	121.79 ± 8.68
Control	164.80 ± 13.00	147.85 ± 6.29	194.40 ± 9.46	205.15 ± 10.54	179.65 ± 10.57	165.75 ± 18.74	123.75 ± 9.96
Early granulated cells (mean ± SD)							
High-grade MDS	178 ± 44.89	**130 ± 5.18‡**	**131.00 ± 4.78* †**	**141.67 ± 4.89* †**	116.67 ± 6.53	105.33 ± 17.11	137.67 ± 27.46
Low-grade MDS	165.39 ± 23.33	134.22 ± 4.51	140.22 ± 5.67	148.89 ± 8.07	127.06 ± 6.49	121.78 ± 20.96	139.67 ± 17.67
Control	168.19 ± 21.43	137.19 ± 4.18	141.25 ± 9.43	151.00 ± 8.33	126.94 ± 12.09	126.69 ± 21.28	137.25 ± 10.98

Note: Significant differences between the groups are in bold.

*$p < .05$ versus low-grade MDS group.

**$p < .01$ versus low-grade MDS group.

†$p < .05$ versus control group.

‡$p < .01$ versus control group.

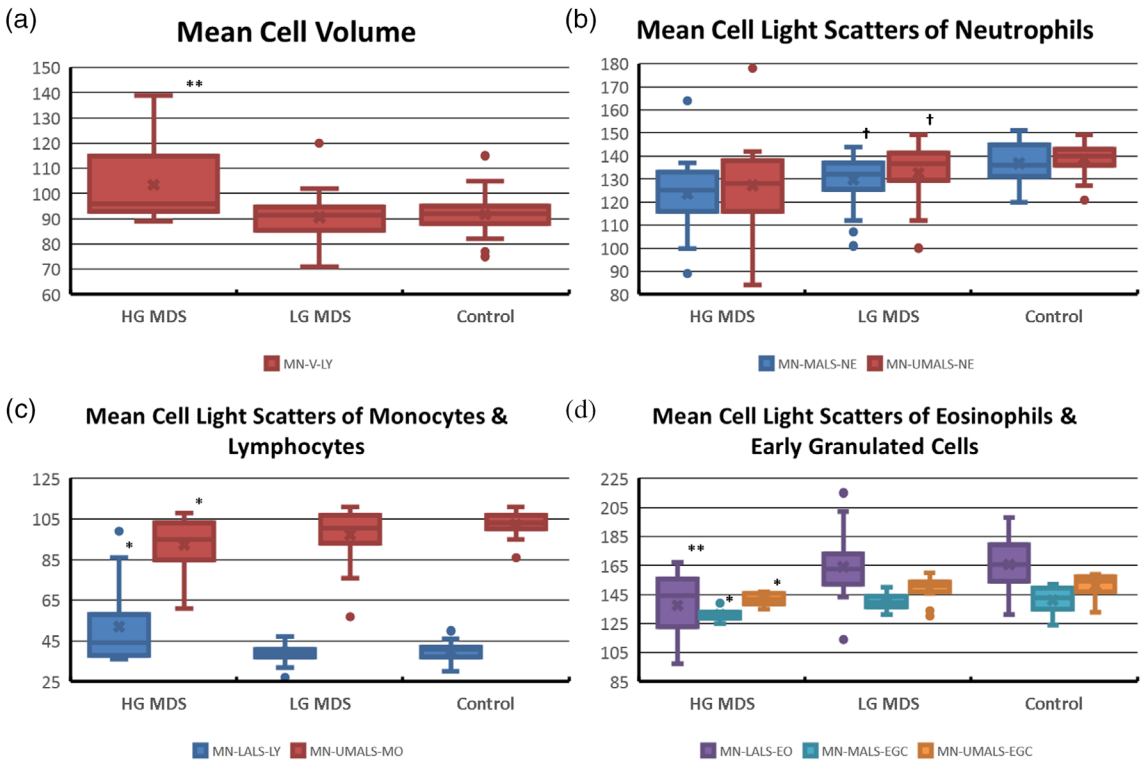

FIGURE 1 Box and whisker plots of mean values of VCS parameters in leukocytes to demonstrate significant differences among the low-grade, high-grade MDS and control groups. (a) Mean cell volume of lymphocytes (MN-V-LY) in three different groups. (b) Mean median angle light scatter of neutrophils (MN-MALS-NE) and mean upper median angle light scatter of neutrophils (MN-UMALS-NE) in three different groups. (c) Mean lower angle light scatter of lymphocytes (MN-LALS-LY) and mean upper median angle light scatter of monocytes (MN-UMALS-MO) in three different groups. (d) Mean lower angle light scatter of eosinophils (MN-LALS-EO), mean median angle light scatter of early granulated cells (MN-MALS-EGC), and mean upper median angle light scatter of early granulated cells (MN-UMALS-EGC) in three different groups. HG, high-grade; LG, low-grade. * $p<0.05$ and ** $p<0.01$ - HG MDS versus LG MDS; † $p<0.05$ - LG MDS versus Control

63%, a specificity of 67%, an accuracy of 67%, and AUC of 0.703 with a cutoff value of ≤133 (Figure 3a). The value of MN-C-EGC as a screening parameter in evaluating dysplasia showed a sensitivity of 63%, a specificity of 75%, an accuracy of 68%, and an area under the curve (AUC) of 0.734 with a cutoff value of ≤136 (Figure 3b).

Among the eight SDs in neutrophils, SD-MALS-NE and SD-UMALS-NE revealed the highest accuracy (AUC 0.80–0.90, good accuracy). SD-MALS-NE with a cutoff value ≥11.68 showed AUC 0.80 with 70% sensitivity, 71% specificity and 72% accuracy (Figure 4a). SD-UMALS-NE with a cutoff value ≥12.46 showed AUC 0.82 with 77% sensitivity, 81% specificity and 78% accuracy (Figure 4b).

After the stepwise selection procedure, SD-MALS-NE (cutoff ≥11.80, sensitivity 71%, specificity 86% and accuracy 76%) and SD-UMALS-NE (cutoff ≥11.16, sensitivity 77%, specificity 82% and accuracy 79%) were identified to be the top two predictors of MDS (Table 6). Overall, the best predictor of the MDS was a standard deviation of UMALS in neutrophils, SD-UMALS-NE (Figure 5). The other six SDs that revealed a fair accuracy in predicting MDS include SD-V-NE, SD-LMALS-NE, SD-AL2-NE, SD-MALS-MO, SD-UMALS-MO, and SD-MALS-EO (Table 6).

4 | DISCUSSION

Clinically, MDS represent a spectrum of the severity of the disease ranging from low-grade to high-grade MDS with an increased rate of progression to acute myeloid leukemia. Revised 4th edition of WHO classification utilizes blast count, degree of dysplasia and cytogenetics/molecular findings to categorizes MDS into low-risk, intermediate-risk and high-risk groups. Notably, low-risk (MDS with single lineage dysplasia, MDS with ring sideroblasts with single lineage dysplasia, and MDS with isolated 5q deletion) and intermediate-risk (MDS with multilineage dysplasia and MDS with ring sideroblasts with multilineage dysplasia) groups are characterized by blasts <2% in the peripheral blood and <5% in the bone marrow. High-risk group is defined by MDS with excess blasts (EB1 or EB2). MDS that present with subtle dysplasia, subtle cytopenias, and low/absent blast count poses a diagnostic challenge. Since this study was geared towards screening of patients with mild clinical manifestations of MDS, we thought to combine low-risk and intermediate-risk groups under the low-grade group.

The first reports that utilized the VCS parameters to help diagnose MDS reported the abnormalities based on the histogram analysis

TABLE 5 Mean values of standard deviation of VCS parameters in neutrophils

	SD-V	SD-C	SD-MALS	SD-UMALS	SD-LMALS	SD-LALS	SD-AL2
	Neutrophils (mean ± SD)						
High-grade MDS	**30.95 ± 9.84**** ‡	**7.4 ± 1.06**** ‡	**14.62 ± 3.24*** ‡	**17.15 ± 2.58**** ‡	**16.30 ± 3.04†**	**38.69 ± 6.57*** ‡	**20.12 ± 5.78**** ‡
Low-grade MDS	**22.71 ± 5.68†**	6.25 ± 2.32	**13.11 ± 2.96‡**	**13.87 ± 3.38‡**	**15.81 ± 3.23‡**	34.02 ± 5.10	**14.68 ± 3.90†**
Control	20.07 ± 5.11	5.86 ± 2.06	10.87 ± 2.0	11.61 ± 1.87	13.66 ± 3.17	32.22 ± 6.90	12.28 ± 2.72
	Lymphocytes (mean ± SD)						
High-grade MDS	**22.99 ± 8.24*** †	11.44 ± 3.09	**20.28 ± 3.02***	23.10 ± 3.48	**22.43 ± 2.63***	**19.53 ± 9.65**** ‡	15.25 ± 6.38
Low-grade MDS	17.08 ± 3.74	10.89 ± 3.52	18.85 ± 4.52	21.51 ± 3.99	21.29 ± 4.25	12.39 ± 1.84	11.79 ± 2.53
Control	18.16 ± 4.64	10.51 ± 3.91	18.04 ± 3.84	21.29 ± 4.21	20.39 ± 3.73	13.04 ± 2.51	12.17 ± 2.84
	Monocytes (mean ± SD)						
High-grade MDS	24.19 ± 8.35	8.48 ± 4.92	**12.61 ± 1.75‡**	**13.70 ± 2.55†**	14.77 ± 2.84	26.33 ± 9.35	**18.74 ± 6.17†**
Low-grade MDS	24.40 ± 6.26	8.93 ± 8.74	**12.43 ± 3.99†**	**14.14 ± 5.46†**	**14.75 ± 2.95†**	27.13 ± 6.34	16.63 ± 7.72
Control	22.08 ± 4.16	7.18 ± 3.65	11.02 ± 2.66	11.83 ± 2.61	13.58 ± 2.76	25.63 ± 4.46	13.89 ± 3.96
	Eosinophils (mean ± SD)						
High-grade MDS	**26.51 ± 9.54†**	**14.70 ± 14.10†**	**15.26 ± 7.56*** ‡**	**15.64 ± 7.04‡**	**16.46 ± 8.92†**	37.86 ± 8.19	**17.39 ± 9.37**** ‡
Low-grade MDS (n = 28[a])	20.88 ± 5.54	9.46 ± 9.39	10.15 ± 4.12	12.05 ± 5.01	11.74 ± 3.37	41.30 ± 5.74	11.34 ± 4.53
Control (n = 20)	19.51 ± 5.11	6.49 ± 4.41	9.48 ± 4.54	10.73 ± 5.44	10.88 ± 3.43	38.75 ± 6.57	9.69 ± 2.67
	Early granular cells (mean ± SD)						
High-grade MDS (n = 6)	33.61 ± 9.40	3.11 ± 0.93	8.04 ± 1.47	12.17 ± 2.53	9.32 ± 1.53	**25.1 ± 2.83***	24.32 ± 3.17
Low-grade MDS (n = 18)	27.60 ± 10.27	2.53 ± 0.95	6.92 ± 2.42	10.61 ± 3.35	8.71 ± 2.76	19.06 ± 8.33	17.66 ± 6.64
Control (n = 16)	30.07 ± 11.47	2.40 ± 0.72	6.83 ± 1.70	10.21 ± 2.28	9.95 ± 2.94	24.77 ± 9.62	19.34 ± 8.06

Note: Significant differences between the groups are in bold.
*p < .05 versus low-grade MDS group.
**p < .01 versus low-grade MDS group.
†p < .05 versus control group.
‡p < .01 versus control group.
[a]The numbers of the patients are different from that of the regular numbers of each groups (high-grade MDS, n = 13; Low-grade MDS, n = 30; Control, n = 21).

of WBCs (Orazi & Milanesi, 1990; Rappaport, Helbert, Ladd, et al., 1987). Since then, the technology of the automated hematology analyzers was used to evaluate MDS (Della Porta, Lanza, Del Vecchio, et al., 2011; Furundarena, Araiz, Uranga, et al., 2010; Haschke-Becher, Vockenhuber, Niedetzky, Totzke, & Gabriel, 2008; Inaba et al., 2011; Jafari et al., 2018; Le Roux, Vlad, Eclache, et al., 2010; Miguel et al., 2007; Mori et al., 2004; Raess et al., 2014) chronic myeloproliferative disorders (Haschke-Becher et al., 2008; Silva, Fourcade, Fartoukh, et al., 2006), acute leukemia (Yang et al., 2014), bacterial infection (Celik et al., 2012; Chaves, Tierno, & Xu, 2005; Lee & Kim, 2013), viral infection (Zhu, Cao, Tao, et al., 2013), and malaria (Briggs, Da Costa, Freeman, et al., 2006). Light scatter parameters were successfully used to differentiate MDS from control patients using Sysmex XE (Furundarena et al., 2010; Le Roux et al., 2010), and Beckman Coulter LH 780 platforms (Kim et al., 2018; Raess et al., 2014). Recently, Kim et al. (2018) used the Beckman Coulter DxH800 automated hematology analyzer to identify VCS parameters in leukocytes to discriminate MDS and other myeloid malignancies from non-clonal hematologic disorders.

The current study demonstrated that hemoglobin, hematocrit, erythrocyte and platelet count were significantly lower in low-grade MDS group than that of the control group, indicating that cytopenias of the MDS patients are mostly present in the red blood cells and platelets (Marinier, Mesa, Rawal, & Gupta, 2010). Leukocytes, except eosinophils, demonstrated tendency to macrocytosis in both low-grade and high-grade MDS groups. In addition, the lymphocytes were significantly macrocytic in high-grade MDS group than in low-grade and control groups. Increase in the size of lymphocytes in patients with MDS is an intriguing finding, but its mechanism is not fully understood. Viral infection, which induces lymphocyte activation, is one of the known factors to increase in the size of the lymphocytes (Silva et al., 2006). The size of lymphocytes may be used as an ancillary parameter to differentiate high-grade MDS from low-grade and control patients.

Although the neutrophils show dysplastic changes in MDS, their cell volume and conductivity were not different from the control group in the current study and other reports (Haschke-Becher et al., 2008; Miguel et al., 2007; Raess et al., 2014). In agreement with

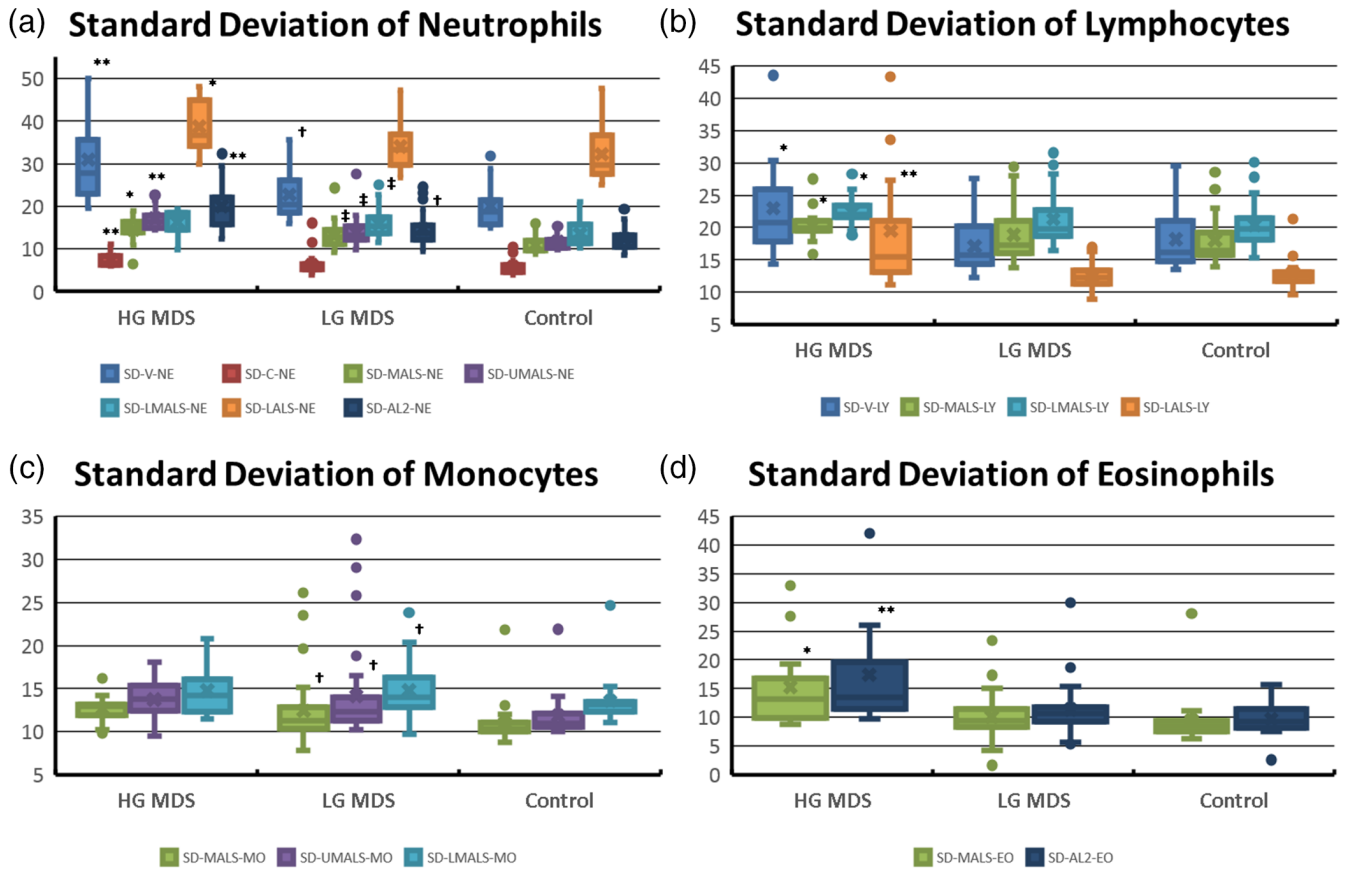

FIGURE 2 Box and whisker plots of mean values of standard deviations (SD) of VCS parameters in leukocytes with significant differences between LG MDS and the control groups and between HG MDS and LG MDS groups. (a) Standard deviation of cell volume (SD-V-NE), standard deviation of cell conductivity (SD-C-NE), standard deviation of median angle light scatter (SD-MALS-NE), standard deviation of upper median angle light scatter (SD-UMALS-NE), standard deviation of lower median angle light scatter (SD-LMALS-NE), standard deviation of lower angle light scatter (SD-LALS-NE), and standard deviation of axial light loss (SD-AL2-NE) of neutrophils in three different groups. (b) Standard deviation of cell volume (SD-V-LY), standard deviation of median angle light scatter (SD-MALS-LY), standard deviation of lower median angle light scatter (SD-LMALS-LY), and standard deviation of lower angle light scatter (SD-LALS-LY) of lymphocytes in three different groups. (c) Standard deviation of median angle light scatter (SD-MALS-MO), standard deviation of upper median angle light scatter (SD-UMALS-MO), and standard deviation of lower median angle light scatter (SD-LMALS-MO) of monocytes in three different groups. (d) Standard deviation of median angle light scatter (SD-MALS-EO) and standard deviation of axial light loss (SD-AL2-EO) of eosinophils in three different groups. HG, high-grade; LG, low-grade. * $p<0.05$ and ** $p<0.01$ - HG MDS versus LG MDS; † $p<0.05$ and ‡ $p<0.01$ - LG MDS versus Control

TABLE 6 Cutoff values determined by ROC analysis of predictors of MDS

	Cut-off	Sensitivity (%)	Specificity (%)	Accuracy	AUC (95% CI)
MN-MALS-NE	≤133	63	67	64	0.70 (0.57–0.84)
MN-C-EGC	≤136	63	75	68	0.73 (0.58–0.89)
SD-MALS-NE	≥11.68	70	71	72	0.80 (0.68–0.92)
SD-UMALS-NE	≥12.46	77	81	78	0.82 (0.71–0.93)
SD-LMALS-NE	≥13.06	81	71	78	0.73 (0.59–0.88)
SD-AL2-NE	≥13.22	72	76	75	0.77 (0.65–0.89)
SD-V-NE	≥19.44	74	62	70	0.73 (0.59–0.86
SD-MALS-EO	≥9.34	63	70	66	0.71 (0.57–0.85)
SD-MALS-MO	≥10.79	69	71	70	0.71 (0.57–0.84)
SD-UMALS-MO	≥11.82	67	71	67	0.71 (0.57–0.84)
After stepwise selection procedure					
SD-MALS-NE	≥11.80	71	86	76	0.85 (0.68–1.00)
SD-UMALS-NE	≥11.16	77	82	79	0.90 (0.76–1.00)

Abbreviations: AUC, area under the curve; CI, confidence interval.

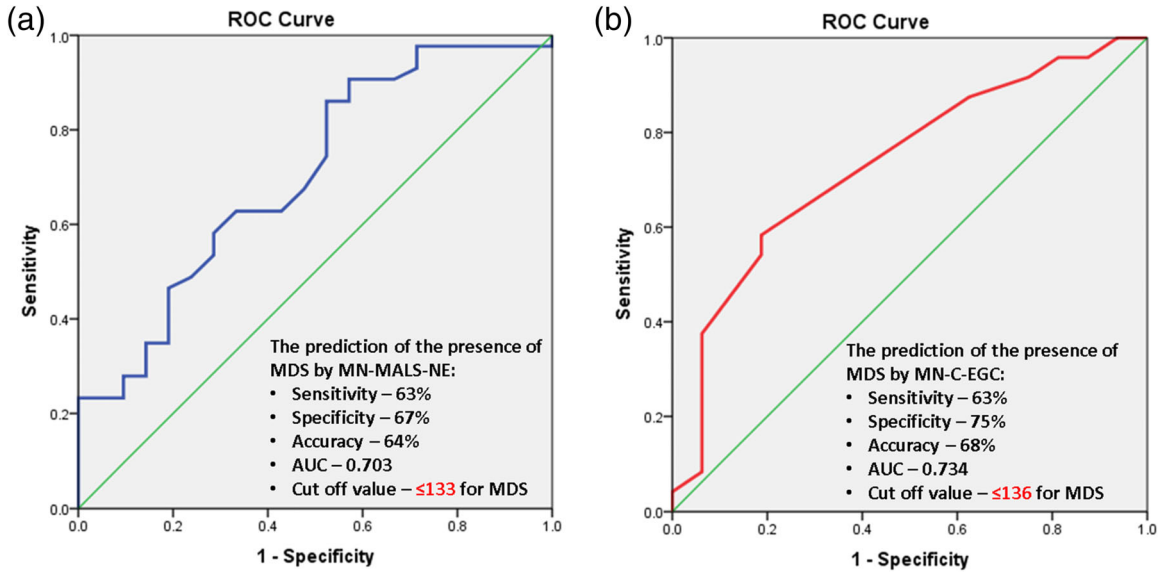

FIGURE 3 Receiver operating characteristic (ROC) curves and area under the ROC (AUC) of the means used to discriminate the myelodysplastic syndrome (MDS) from the control. (a) ROC and AUC of mean median angle light scatter of neutrophils (MN-MALS-NE), (b) ROC and AUC of mean cell conductivity of early granulated cells (MN-C-EGC) [Color figure can be viewed at wileyonlinelibrary.com]

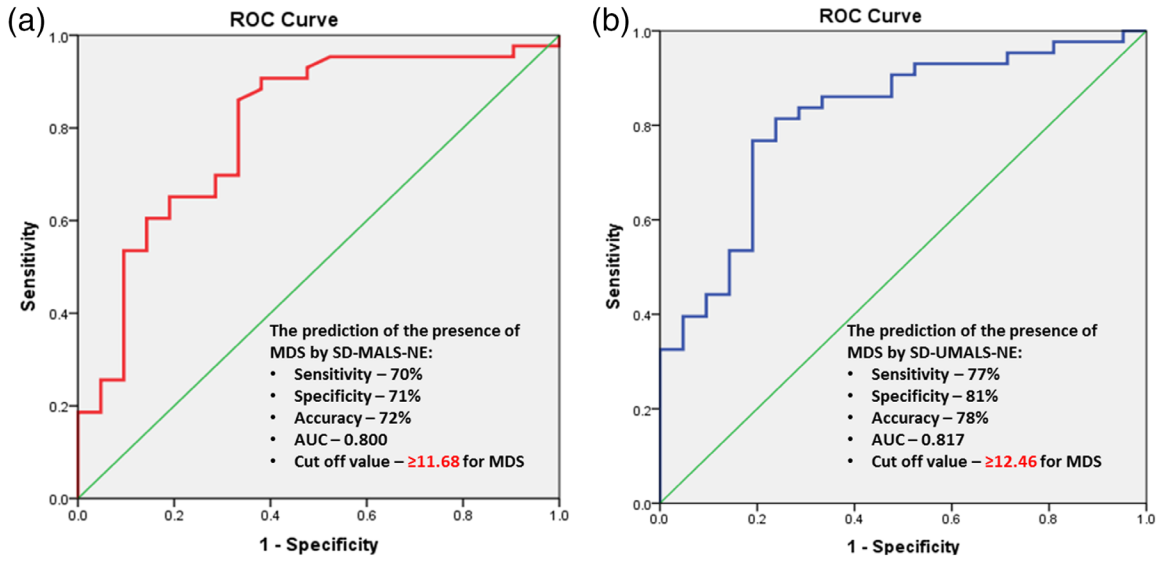

FIGURE 4 Receiver operating characteristic (ROC) curves and area under the ROC (AUC) of the mean standard deviations in neutrophils used to discriminate the myelodysplastic syndrome (MDS) from the control. (a) ROC and AUC of the mean standard deviation of median angle light scatter of neutrophils (SD-MALS-NE). SD-MALS-NE with the cut-off value 11.68, demonstrated sensitivity of 70%, specificity of 71%, and accuracy 72% to predict MDS. (b) ROC and AUC of the mean standard deviation of upper median angle light scatter of neutrophils (SD-UMALS-NE). SD-UMALS-NE with the cut-off value 12.46, demonstrated sensitivity of 77%, specificity of 81%, and accuracy 78% to predict MDS [Color figure can be viewed at wileyonlinelibrary.com]

the previous studies, the current study confirmed significantly decreased mean light scatter parameters in neutrophils in patients with MDS (Haschke-Becher et al., 2008; Kim et al., 2018; Miguel et al., 2007; Raess et al., 2014). Notably, two values of light scatter parameters in neutrophils, MALS and UMALS, were significantly lower in low-grade MDS patients than in the control group. In addition, five means of the VCS parameters in neutrophils (MN-MALS-NE, MN-UMALS-NE, MN-LMALS-NE, MN-LALS-NE, and MN-AL2-NE) were significantly lower in high-grade MDS patients than in the control group. However, there were no significant differences in these parameters between the low-grade and high-grade MDS groups. In addition, MALS of EGC, UMALS of monocytes and EGC, LALS of eosinophils and lymphocytes were significantly different between high-grade and low-grade MDS groups.

Recent study from Kim et al. (2018) reported decreases in five light scatter parameters in neutrophils using the same Beckman

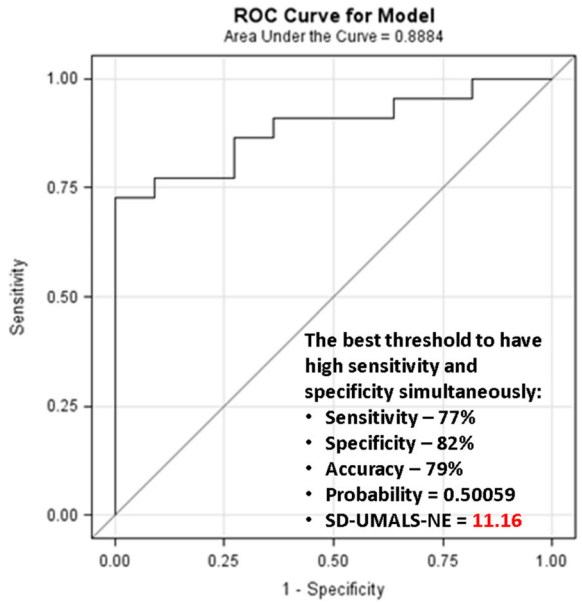

FIGURE 5 Receiver operating characteristic (ROC) curve of the mean standard deviation of upper median angle light scatter of neutrophils (SD-UMALS-NE) after the stepwise selection procedure to differentiate MDS from the control. SD-UMALS-NE with the cut-off value 11.16, demonstrated sensitivity of 77%, specificity of 82%, and accuracy 79% to predict MDS [Color figure can be viewed at wileyonlinelibrary.com]

Coulter DXH800 hematology analyzer. Similar to the current study it demonstrated that means of the light scatter parameters were significantly lower in MDS patients than in the patients without hematological malignancies. In Kim's study, a scoring system using 13 parameters including CBC data, means of VCS data and standard deviations of VCS data was implemented to achieve overall sensitivity of 92.4% and a specificity of 85.4% to predict MDS. Therefore, it is possible to suggest that the significant difference in the means of the VCS parameters between MDS patients and control group is a consequence of the dysplasia in neutrophils.

Evaluation of the variability or standard deviation, of the means of VCS parameters can be important due to the heterogeneity of the cellular dysplasia. Standard deviations of the mean values of VCS parameters were previously reported as strong predictors of MDS (Haschke-Becher et al., 2008; Kim et al., 2018; Raess et al., 2014), lymphoproliferative disorders, and infections (Silva et al., 2006). The current study reported significant increase in standard deviations of the VCS parameters in neutrophils and monocytes in low-grade MDS group in comparison to the control group. High-grade MDS group demonstrated an increase in standard deviation in some of the VCS parameters in all leukocytes. Notably, standard deviations in all VCS parameters, except SD-LMALS, were increased in neutrophils in comparison to control and low-grade MDS groups. Overall, variability of the standard deviation can serve as an objective tool to screen patients with cytopenias for MDS.

Low-grade MDS poses a diagnostic challenge due to the variable degree of cytopenias, low blast count in peripheral blood and variable dysgranulopoiesis. Focus of the current study was to establish cutoff values using VCS parameters to screen peripheral blood for low-grade MDS. To our knowledge, this is the first study to separate high-grade MDS from low-grade MDS using VCS parameters.

It is evident that the combination of the four best predictors identified in our study provided the best outcome in predicting MDS by the automated hematology analyzer on peripheral blood samples. The scoring system recommended by Kim et al. (2018) using 13 parameters (12 VCS parameters and one CBC parameter) may maximize the sensitivity and specificity of prediction of MDS, however, the process is tedious. We recommend a simpler tool to predict MDS using the combination of these four predictors on a daily basis by laboratory staff.

In summary, the patients with MDS demonstrated significant decrease in the means of the VCS parameters. In addition, the standard deviations of the means of the VCS parameters in neutrophils were increased, indicating cellular dysplasia. The degree of these changes was more pronounced in high-grade than low-grade MDS when compared to the control group. The degree of the heterogeneity that is measured by standard deviation of the VCS parameters in neutrophils was the most predictive of MDS. The combination of these predictors (SD-MALS-NE, SD UMALS-NE, MN-MALS-NE, and MN-C-EGC) can be instrumental to screen MDS patients in the daily practice. However, a prospective study is needed to validate the proposed cutoff values.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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REFERENCES

Alhan, C., Westers, T. M., Cremers, E. M., Cali, C, Ossenkoppele, G. J., & Van de Loosdrecht, A. A. (2016). Application of flow cytometry for myelodysplastic syndromes: Pitfalls and technical considerations. *Cytometry Part B, Clinical Cytometry*, *90*, 358–367.

Aul, C., Gattermann, N., & Schneider, W. (1992). Age-related incidence and other epidemiological aspects of myelodysplastic syndromes. *British Journal of Haematology*, *82*, 358–367.

Bejar, R., Levine, R., & Ebert, B. L. (2011). Unraveling the molecular pathophysiology of myelodysplastic syndromes. *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology*, *29*, 504–515.

Bennett, J. M. (2016). Changes in the updated 2016: WHO classification of the myelodysplastic syndromes and related myeloid neoplasms. *Clinical Lymphoma, Myeloma & Leukemia*, *16*, 607–609.

Bennett, J. M., Catovsky, D., Daniel, M. T., Flandrin, G., Galton, D. A. G., Gralnick, H. R., & Sultan, C. (1982). Proposals for the classification of the myelodysplastic syndromes. *British Journal of Haematology*, *51*, 189–199.

Briggs, C., Da Costa, A., Freeman, L., Aucamp, I., Ngubeni, B., & Machin, S. J. (2006). Development of an automated malaria discriminant factor using VCS technology. *American Journal of Clinical Pathology*, *126*, 691–698.

Celik, I. H., Demirel, G., Aksoy, H. T., Erdeve, O., Tuncer, E., Biyikli, Z., & Dilmen, U. (2012). Automated determination of neutrophil VCS parameters in diagnosis and treatment efficacy of neonatal sepsis. *Pediatric Research*, *71*, 121–125.

Chaves, F., Tierno, B., & Xu, D. (2005). Quantitative determination of neutrophil VCS parameters by the coulter automated hematology analyzer: New and reliable indicators for acute bacterial infection. *American Journal of Clinical Pathology*, *124*, 440–444.

Cogle, C. R., Craig, B. M., Rollison, D. E., & List, A. F. (2011). Incidence of the myelodysplastic syndromes using a novel claims-based algorithm: High number of uncaptured cases by cancer registries. *Blood*, *117*, 7121–7125.

Della Porta, M. G., Lanza, F., Del Vecchio, L., et al. (2011). Flow cytometry immunophenotyping for the evaluation of bone marrow dysplasia. *Cytometry Part B, Clinical Cytometry*, *80*, 201–211.

Foran, J. M., & Shammo, J. M. (2012). Clinical presentation, diagnosis, and prognosis of myelodysplastic syndromes. *The American Journal of Medicine*, *125*, S6–S13.

Furundarena, J. R., Araiz, M., Uranga, M., Sainz, M. R., Agirre, A., Trassorras, M., … Argoitia, N. (2010). The utility of the Sysmex XE-2100 analyzer's NEUT-X and NEUT-Y parameters for detecting neutrophil dysplasia in myelodysplastic syndromes. *International Journal of Laboratory Hematology*, *32*, 360–366.

Goasguen, J. E., & Bennett, J. M. (1992). Classification and morphologic features of the myelodysplastic syndromes. *Seminars in Oncology*, *19*, 4–13.

Goasguen, J. E., Bennett, J. M., Bain, B. J., Brunning, R. D., Vallespí, M. T., Tomonaga, M., … International Working Group on Morphology of MDS IWGM-MDS. (2016). Quality control initiative on the evaluation of the dysmegakaryopoiesis in myeloid neoplasms: Difficulties in the assessment of dysplasia. *Leukemia Research*, *45*, 75–81.

Greenberg, P. L. (2012). Molecular and genetic features of myelodysplastic syndromes. *International Journal of Laboratory Hematology*, *34*, 215–222.

Haase, D., Germing, U., Schanz, J., Pfeilstöcker, M., Nösslinger, T., Hildebrandt, B., … Steidl, C. (2007). New insights into the prognostic impact of the karyotype in MDS and correlation with subtypes: Evidence from a core dataset of 2124 patients. *Blood*, *110*, 4385–4395.

Haferlach, T., Nagata, Y., Grossmann, V., Okuno, Y., Bacher, U., Nagae, G., … Ogawa, S. (2014). Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia*, *28*, 241–247.

Haschke-Becher, E., Vockenhuber, M., Niedetzky, P., Totzke, U., & Gabriel, C. (2008). A new high-throughput screening method for the detection of chronic lymphatic leukemia and myelodysplastic syndrome. *Clinical Chemistry and Laboratory Medicine*, *46*, 85–88.

Hasserjian, R. (2017). *WHO classification of tumours of haematopoietic and lymphoid tissues. Edited by Lyon* (p. 98). France: International Agency for Research on Cancer.

Inaba, T., Yuki, Y., Yuasa, S., Fujita, N., Yoshitomi, K., Kamisako, T., … Tohyama, K. (2011). Clinical utility of the neutrophil distribution pattern obtained using the CELL-DYN SAPPHIRE hematology analyzer for the diagnosis of myelodysplastic syndrome. *International Journal of Hematology*, *94*, 169–177.

Invernizzi, R., Quaglia, F., & Porta, M. G. D. (2015). Importance of classical morphology in the diagnosis of myelodysplastic syndrome. *Mediterranean Journal of Hematology and Infectious Diseases*, *7*(1), e2015035. https://doi.org/10.4084/mjhid.2015.035.

Itzykson, R., & Fenaux, P. (2014). Epigenetics of myelodysplastic syndromes. *Leukemia*, *28*, 497–506.

Jafari, K., Tierens, A., Rajab, A., Musani, R., Schuh, A., & Porwit, A. (2018). Visualization of cell composition and maturation in the bone marrow using 10-color flow cytometry and radar plots. *Cytometry Part B, Clinical Cytometry*, *94*, 219–229.

Jean, A., Boutet, C., Lenormand, B., Callat, M. P., Buchonnet, G, Barbay, V., … Vasse, M. (2011). The new haematology analyzer DxH 800: An evaluation of the analytical performances and leucocyte flags, comparison with the LH 755. *International Journal of Laboratory Hematology*, *33*, 138–145.

Kern, W., Bacher, U., Schnittger, S., Alpermann, T., Haferlach, C., & Haferlach, T. (2013). Multiparameter flow cytometry reveals myelodysplasia-related aberrant antigen expression in myelodysplastic/myeloproliferative neoplasms. *Cytometry Part B, Clinical Cytometry*, *84*, 194–197.

Kim, S. Y., Park, Y., Kim, H., Kim, J., Kwon, G. C., & Koo, S. H. (2018). Discriminating myelodysplastic syndrome and other myeloid malignancies from non-clonal disorders by multiparametric analysis of automated cell data. *Clinica Chimica Acta*, *480*, 56–64.

Krause, J. R. (1990). Automated differentials in the hematology laboratory. *American Journal of Clinical Pathology*, *93*, S11–S16.

Le Roux, G., Vlad, A., Eclache, V., Malanquin, C., Collon, J. F., Gantier, M., … Ajchenbaum-Cymbalista, F. (2010). Routine diagnostic procedures of myelodysplastic syndromes: Value of a structural blood cell parameter (NEUT-X) determined by the Sysmex XE-2100. *International Journal of Laboratory Hematology*, *32*, e237–e243.

Lee, A. J., & Kim, S. G. (2013). Mean cell volumes of neutrophils and monocytes are promising markers of sepsis in elderly patients. *Blood Research*, *48*, 193–197.

Marinier, D. E., Mesa, H., Rawal, A., & Gupta, P. (2010). Refractory cytopenias with unilineage dysplasia: A retrospective analysis of refractory neutropenia and refractory thrombocytopenia. *Leukemia & Lymphoma*, *51*, 1923–1926.

Miguel, A., Orero, M., Simon, R., Collado, R., Perez, P. L., Pacios, A., … Carbonell, F. (2007). Automated neutrophil morphology and its utility in the assessment of neutrophil dysplasia. *Laboratory Hematology*, *13*, 98–102.

Montalban-Bravo, G., & Garcia-Manero, G. (2018). Myelodysplastic syndromes: 2018 update on diagnosis, risk-stratification and management. *American Journal of Hematology*, *93*, 129–147.

Mori, Y., Mizukami, T., Hamaguchi, Y., Tsuruda, K., Yamada, Y., & Kamihira, S. (2004). Automation of bone marrow aspirate examination using the XE-2100 automated hematology analyzer. *Cytometry Part B, Clinical Cytometry*, *58*, 25–31.

Mutlu, H., Akca, Z., Teke, H. U., & Ugur, H. (2011). Evaluation of peripheral blood smear for myelodysplasia in breast cancer patients who received adjuvant Antracycline. *Eurasian Journal of Medicine*, *43*, 173–176.

Naqvi, K., Jabbour, E., Bueso-Ramos, C., Pierce, S., Borthakur, G., Estrov, Z., … Garcia-Manero, G. (2011). Implications of discrepancy in morphologic diagnosis of myelodysplastic syndrome between referral and tertiary care centers. *Blood*, *118*, 4690–4693.

Orazi, A., & Milanesi, B. (1990). The technicon H6000 automated hematology analyzer in the diagnosis and classification of the myelodysplastic syndromes. *Haematologica*, *75*, 87–90.

Raess PW, van de Geijn G. J. M., Njo, T.L., Klop, B., Sukachev, D., Wertheim, G., … Bagg, A. (2014). Automated screening for myelodysplastic syndromes through analysis of complete blood count and cell population data parameters. *American Journal of Hematology*, *89*, 369–374.

Rappaport, E. S., Helbert, B., Ladd, D. J., & Trowbridge, A. A. (1987). Myelodysplastic syndrome: Identification in the routine hematology laboratory. *Southern Medical Journal*, *80*, 969–974.

Richardson-Jones, A. (1990). An automated hematology instrument for comprehensive WBC, RBC, and platelet analysis. *American Clinical Laboratory*, *9*, 18–22.

Sandhaus, L. M., Wald, D. N., Sauder, K. J., Steele, E. L., … Meyerson, H. J. (2007). Measuring the clinical impact of pathologist reviews of blood

and body fluid smears. *Archives of Pathology & Laboratory Medicine*, *131*, 468–472.

Schanz, J., Tuchler, H., Sole, F., Mallo, M, Luño, E., Cervera, J., … Haase, D. (2012). New comprehensive cytogenetic scoring system for primary myelodysplastic syndromes (MDS) and oligoblastic acute myeloid leukemia after MDS derived from an international database merge. *Journal of Clinical Oncology*, *30*, 820–829.

Shammo, J. M., Foran, J. M., Houk, A., Epstein, J., Narang, M., Rubinstein, P., … York, J. M. (2011). An examination of educational gaps in the diagnosis and treatment of myelodysplastic syndromes. *Cancer Control*, *18*, 65–74.

Silva, M., Fourcade, C., Fartoukh, C., Lenormand, B., Buchonnet, G., Callat, M. P., … Vasse, M. (2006). Lymphocyte volume and conductivity indices of the haematology analyser coulter GEN.S in lymphoproliferative disorders and viral diseases. *Clinical and Laboratory Haematology*, *28*, 1–8.

Sperling, A. S., Gibson, C. J., & Ebert, B. L. (2017). The genetics of myelodysplastic syndrome: From clonal haematopoiesis to secondary leukaemia. *Nature Reviews. Cancer*, *17*, 5–19.

Steensma, D. P., Bejar, R., Jaiswal, S., Lindsley, R. C., Sekeres, M. A., Hasserjian, R. P., & Ebert, B. L. (2015). Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood*, *126*, 9–16.

Tang, H., Jing, J., Bo, D., & Xu, D. (2012). Biological variations of leukocyte numerical and morphologic parameters determined by UniCel DxH 800 hematology analyzer. *Archives of Pathology & Laboratory Medicine*, *136*, 1392–1396.

Yang, J. H., Kim, Y., Lim, J., Kim, M., Oh, E. J., Lee, H. K., … Han, K. (2014). Determination of acute leukemia lineage with new morphologic parameters available in the complete blood cell count. *Annals of Clinical and Laboratory Science*, *44*, 19–26.

Zhu, Y., Cao, X., Tao, G., Xie, W., Hu, Z., & Xu, D. (2013). The lymph index: A potential hematological parameter for viral infection. *International Journal of Infectious Diseases*, *17*, 490–493.

Zini, G. (2017). Diagnostics and prognostication of myelodysplastic syndromes. *Annals of Laboratory Medicine*, *37*, 465–474.

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