


## RESEARCH ARTICLE

# Mean platelet component and mean platelet volume as useful screening markers for myelodysplastic syndrome

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**Abstract**

**Background:** Hematologic disorders, including myelodysplastic syndrome (MDS), are difficult to identify in routine hematologic examinations using automated hematology analyzers. However, the practical uses of mean platelet component and mean platelet volume (MPV) measured by these analyzers as screening markers for MDS, remain unclear.

**Methods:** Mean platelet component and MPV values were measured in the peripheral blood of patients with MDS, aplastic anemia, idiopathic thrombocytopenic purpura, myeloproliferative neoplasms, and in healthy controls using an automated hematology analyzer. Cutoff values for discriminating between the MDS group and healthy controls were determined by recursive partitioning analysis.

**Results:** Mean platelet component was significantly lower in MDS patients compared with controls, while MPV was significantly higher. Combined cutoff values for MDS diagnosis of <25.3 g/dL for mean platelet component and >10.0 fL for MPV showed a specificity and positive predictive value of 99.9% and 99.1%, respectively. These cutoff values also differentiated between MDS and diagnoses of aplastic anemia, idiopathic thrombocytopenic purpura, and myeloproliferative neoplasms.

**Conclusion:** Mean platelet component and MPV may, thus, be useful and convenient screening markers for MDS.

**KEYWORDS**

MDS, MPC, MPV, screening test

## 1 | INTRODUCTION

Myelodysplastic syndrome (MDS) is a heterogeneous syndrome of disorders that cause ineffective hematopoiesis as a result of the occurrence and proliferation of abnormal hematopoietic stem cell clones in the bone marrow, inducing pancytopenia or hematocytic dysplasia.<sup>1</sup> Myelodysplastic syndrome comprises 7 subtypes: MDS with single lineage dysplasia (MDS-SLD), MDS with ring sideroblasts, MDS with

multilineage dysplasia (MDS-MLD), MDS with excess blasts-1 (MDS-EB1), MDS with excess blasts-2 (MDS-EB2), MDS unclassifiable (MDS-U), and MDS with isolated del(5q).<sup>2</sup> Each of these subtypes has its own diagnostic criteria defined by combinations of peripheral blood and bone marrow abnormalities.<sup>1</sup>

Morphological abnormalities of peripheral blood cells, such as false Pelger nuclear neutrophils, degranulated neutrophils, poikilocytes, and degranulated giant platelets, are often found in

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peripheral blood smears from MDS patients.<sup>3</sup> Given that these abnormal peripheral blood cells may be derived from abnormal bone marrow cells in MDS patients, morphological abnormalities of the cellular components of the peripheral blood may provide clues for diagnosing MDS.

Rocco et al studied peripheral blood cell abnormalities using automated hematology analyzers, and concluded that flag “dysplasia” detected by this method was sufficiently specific and sensitive to act as a screening test for MDS<sup>4</sup> and thus proposed using automated hematology analyzers as a screening tool for MDS.<sup>4</sup> However, Koenders et al subsequently reevaluated the specificity and sensitivity of the flag dysplasia detected by automated analysis for screening of MDS<sup>5</sup> and reported that the sensitivity was significantly lower than that reported by Rocco et al, but the specificity for MDS screening were comparable.<sup>5</sup> Other studies have also demonstrated the usefulness of automated hematology analyzers as screening tools for discriminating between MDS patients and normal controls and/or patients with other hematologic disorders.<sup>6,7</sup> Most previous studies, however, have investigated abnormalities of erythroid, myeloid, and/or lymphoid cells in the peripheral blood, and none have considered platelet abnormalities. Parameters other than morphological abnormalities may offer more objective indicators.

Hematology analyzers can measure various parameters, among which several platelet parameters are good candidate indicators for MDS.<sup>8,9</sup> However, to the best of our knowledge, no practical studies have investigated the value of platelet parameters in peripheral blood of MDS patients obtained using automated hematology analyzers, as screening tools.

In the current study, we investigated the platelet parameters obtained using an automated hematology analyzer (ADVIA 2120i). The ADVIA 2120i can measure the mean platelet volume (MPV), which shows the average size of platelets, and the mean platelet component (MPC) concentration, which shows the intrathrombocytic protein concentration.<sup>9</sup> Mean platelet component is an original parameter of the ADVIA, indicating loss of granules and contents in the platelet. We show that these parameters reflect the morphological characteristics of platelets in the peripheral blood of MDS patients and are useful and convenient screening markers for MDS. We also report on the appropriate analyzer settings for the screening diagnosis of MDS.

## 2 | MATERIALS AND METHODS

### 2.1 | Subjects

In this study, all samples for platelet research were handled as recommended by Harrison and Goodall throughout all procedures, from blood collection to operation of the hematology analyzers.<sup>10</sup> In brief, all specimens were measured within 2 hours after blood sampling. Peripheral blood samples from 1305 subjects (851 male, 454 female; mean age, 70 y; range, 18-93 y) undergoing health checks, with complete blood cell counts within the normal range and normal biochemical results, were used as controls. Normal range was decided as the mean  $\pm$  2 standard deviations. The characteristics of

the healthy controls were as follows: mean white blood cell count  $5.56$  (range,  $3.30$ - $8.20$ )  $\times 10^9/L$ ; mean red blood cell count  $4.37$  (range,  $3.75$ - $5.30$ )  $\times 10^{12}/L$ ; mean hemoglobin concentration  $13.9$  (range,  $11.5$ - $16.4$ ) g/dL; mean platelet count  $223$  (range,  $162$ - $329$ )  $\times 10^9/L$ ; mean blood urea nitrogen  $5.4$  (range,  $1.8$ - $12.5$ ) mmol/L; mean lactate dehydrogenase  $188$  (range,  $130$ - $250$ ) IU/L; mean C-reactive protein  $800$  (range,  $<100$ - $2500$ )  $\mu\text{g}/L$ ; mean creatinine  $74.3$  (range,  $44.2$ - $106.1$ )  $\mu\text{mol}/L$ ; and mean alanine aminotransferase  $22$  (range,  $5$ - $35$ ) IU/L.

All of the samples analyzed in this study came from patients with no past histories of any treatment such as platelet transfusions or chemotherapies for at least 3 weeks before diagnosis and were collected at the initial visits of the patients to our hospital.

Myelodysplastic syndrome patients ( $n = 57$ ) were classified based on the WHO classification 2016 revision<sup>2</sup> as follows: MDS-SLD ( $n = 8$ ), MDS-MLD ( $n = 12$ ), MDS-EB1 ( $n = 10$ ), MDS-EB2 ( $n = 12$ ), MDS-U ( $n = 8$ ), therapy-related MDS ( $n = 4$ ), and MDS with isolated del(5q) ( $n = 3$ ). Refractory cytopenia of childhood (RCC) ( $n = 5$ ) and acute myeloid leukemia with myelodysplasia-related changes ( $n = 3$ ) were also recognized as similar diseases.

Peripheral blood samples from patients with aplastic anemia (AA) ( $n = 20$ ), idiopathic thrombocytopenic purpura (ITP) ( $n = 25$ ), and myeloproliferative neoplasms (MPN) ( $n = 35$ ), which also show morphological or numerical platelet abnormalities,<sup>2</sup> were also analyzed (Table 1). Myeloproliferative neoplasms was classified as follows: essential thrombocythemia (ET) ( $n = 20$ ), primary myelofibrosis ( $n = 10$ ), and polycythemia vera ( $n = 5$ ).

### 2.2 | Measurements of platelet parameters and observations of morphologic characteristics

Peripheral blood samples were analyzed and platelet parameters were obtained using an ADVIA 2120i Hematology System (Siemens Healthcare Diagnostics, Tarrytown, New York).

We confirmed if MPV and MPC reflected the morphologic characteristics as mean values for individual platelet size (volume) and azurophil granules (component) by staining peripheral blood smears with May-Grünwald Giemsa solution. We measured maximum platelet size in 100 counts of platelets from healthy controls chosen at random ( $n = 20$ ) and in patients with MDS, AA, ITP, MPN, RCC, and acute myeloid leukemia with myelodysplasia-related changes, using an all-in-one fluorescence microscope (BZX-700; Keyence, Osaka, Japan) and its associated software, according to the manufacturer's instructions. We classified the platelets into 6 patterns according to size (normal,  $<4$   $\mu\text{m}$ ; large,  $4$ - $8$   $\mu\text{m}$ ; and giant,  $>8$   $\mu\text{m}$ ) and staining of the platelet azurophil granules (normal or degranulation) (Figure 1A).

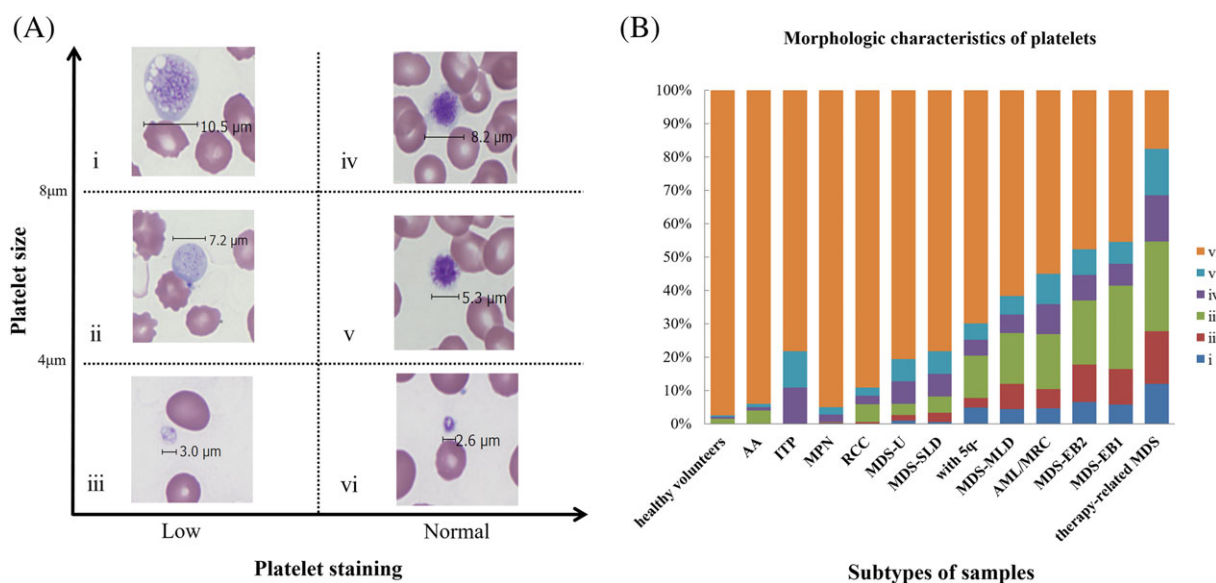
### 2.3 | MPC and MPV values in patients and controls

Reference values were calculated based on the mean  $\pm$  2 standard deviations of control values. We compared MPC and MPV values at initial diagnosis between patients with MDS, AA, ITP, MPN, and the reference values.

**TABLE 1** Platelet parameters in MDS, AA, ITP, MPN, and control subjects

Groups	Healthy Control Mean ± 2 SD	MDS Mean ± 2 SD	AA Mean ± 2 SD	ITP Mean ± 2 SD	MPN Mean ± 2 SD
Age	70 ± 26 (range, 18-93)	74 ± 22 (range, 21-85)	54 ± 41 (range, 13-78)	52 ± 44 (range, 6-76)	73 ± 26 (range, 38-93)
PLT ( $\times 10^9$ L)	223 ± 79 (range, 162-329)	88 ± 182 (range, 6-542)	35 ± 45 (range, 7-76)	30 ± 42 (range, 6-90)	689 ± 831 (range, 58-1835)
MPV, fL	8.1 ± 1.5 (range, 162-329)	12.0 ± 5.0 (range, 7.2-21.0)	8.9 ± 2.9 (range, 6.7-12.1)	9.7 ± 4.4 (range, 5.5-14.5)	9.0 ± 2.7 (range, 7.3-13.5)
MPC, g/dL	27.0 ± 2.4 (range, 21.5-30.5)	23.1 ± 2.9 (range, 20.4-27.1)	24.4 ± 3.5 (range, 20.7-27.4)	26.6 ± 3.8 (range, 21.6-29.3)	24.5 ± 3.2 (range, 21.0-27)
n	1304	57	20	25	35
Male/ female	851/454	38/19	8/12	8/17	17/18

Abbreviations: AA, aplastic anemia; ITP, idiopathic thrombocytopenic purpura; MDS, myelodysplastic syndrome; MPC, mean platelet concentration; MPN, myeloproliferative neoplasms; MPV, mean platelet volume; PLT, Platelet.



**FIGURE 1** Morphological classification of platelets in relation to size and staining intensity in healthy controls and myelodysplastic syndrome (MDS) cases. A, Different platelet sizes and staining intensities. Giant ( $>8 \mu\text{m}$ ), low staining = category i (panel i); large ( $4\text{--}8 \mu\text{m}$ ), low staining = category ii (ii); normal ( $<4 \mu\text{m}$ ), low staining = category iii (iii); giant ( $>8 \mu\text{m}$ ), normal staining = category iv (iv); large ( $4\text{--}8 \mu\text{m}$ ), normal staining = category v (v); and normal ( $<4 \mu\text{m}$ ), normal staining = category vi (vi). B, Percentage of platelets in each category (i-vi) in healthy controls and various subtypes of MDS. Most platelets in healthy controls were category vi. There were more category (i) and (ii) platelets in samples derived from MDS patients than in those from healthy controls, which were predominantly category iv

## 2.4 | Ethics

Informed consent was obtained from all patients in this study. The study protocol was approved by Osaka Medical College Hospital Ethics Committee and adhered to the Declaration of Helsinki.

## 2.5 | Statistical analysis

Data were analyzed using JMP12 software (SAS Institute Inc, Cary, North Carolina), receiver operating characteristic curve (ROC) analysis, and partition analysis, as described previously.<sup>11</sup>

Partition analysis is a nonparametric multivariate analysis based on the decision tree method, using a cutoff value. A classification tree is constructed with decision rules for assigning samples to categories based on a series of consecutive decisions in partition analysis. Samples were then assigned to the MDS or normal group depending on whether value of an individual predictor was greater or less than the selected cutoff value.

We set the appropriate cutoff values for MDS diagnosis by ROC analysis using MPV and MPC alone and combined. We also performed recursive partitioning analysis to diagnose MDS based on MPV and MPC. The sensitivity, specificity, and positive- and negative-predictive values were calculated for all tested cutoff values.

Significant differences ( $P < .05$ ) in MPV and MPC between MDS patients and controls were detected using the 2-tailed Mann-Whitney  $U$  test.

## 3 | RESULTS

### 3.1 | Platelet parameters and morphologic characteristics

Several giant platelets were present in the peripheral blood of MDS patients with MDS-EB2 (Figure 1B) but none in the peripheral blood of healthy controls. May-Grünwald Giemsa staining of smears of

peripheral blood from MDS patients with high MPV and low MPC values showed numerous giant ( $>8 \mu\text{m}$ ) platelets with decreased azurophil granules under light microscopy (Figure 1A). The numbers of giant and degranulated platelets varied according to the subtype of MDS: Therapy-related MDS, MDS-EB1, and MDS-EB2 were associated with more giant and degranulated platelets in the peripheral blood than other subtypes (Figure 1B).

### 3.2 | MPC and MPV in patients and controls

Platelet parameters in the controls and in patients with various diseases are shown in Table 1. Mean platelet component was significantly lower in MDS patients (mean, 23.1 g/dL) compared with controls (mean, 27.0 g/dL) ( $P < .001$ , Mann-Whitney  $U$  test), while MPV was significantly higher in MDS patients (mean, 12.0 fL) compared with controls (mean, 8.1 fL) ( $P < .001$ , Mann-Whitney  $U$  test). Then we confirmed that the decreased MPC values in MDS samples were not associated with platelet activation (Figure S1).

Among cases with high MPV values ( $>10.0$  fL), 89.4% (51 of 57) were MDS patients, with only 0.9% of controls (24 of 1305) having high MPV values (Figure 2A). Mean platelet component values showed a wide range of distribution in controls, from normal to low values (Figure 2A).

### 3.3 | Cutoff value based on combination of MPV and MPC for diagnosis of MDS

We determined the specific cutoff value based on a combination of MPC and MPV. Cutoff values determined by ROC analysis of  $<25.5$  g/dL for MPC and  $> 9.3$  fL for MPV gave the largest areas under the curve, of 0.971 and 0.959. This combination had sensitivity, specificity, and positive- and negative-predictive values of 89.5%, 99.4%, 86.4%, and 99.5%, respectively.

Similarly, cutoff values of  $<25.3$  g/dL for MPC and  $>10.0$  fL for MPV determined by recursive partitioning analysis, gave sensitivity,

specificity, and positive- and negative-predictive values of 78.9%, 99.9%, 97.8%, and 99.1%, respectively (Table S1).

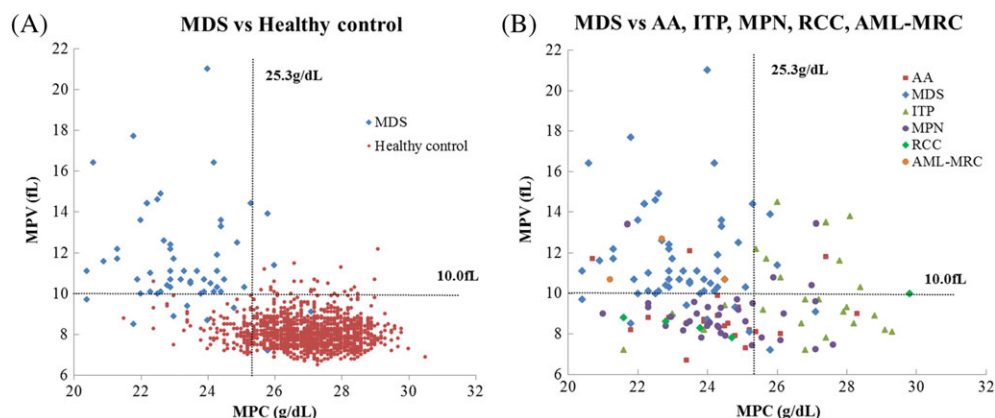
We, therefore, adopted the cutoff values of MPC  $< 25.3$  g/dL and MPV  $> 10.0$  fL as the most specific cutoff values, with the emphasis on specificity as the most important factor. When this cutoff value ( $<25.3$  g/dL for MPC and  $>10.0$  fL for MPV) was used, 21% of MDS (12 of 57) were not judged to be MDS (MDS-SLD,  $n = 3$ ; MDS-MLD,  $n = 1$ ; MDS-EB1,  $n = 2$ ; MDS-2,  $n = 2$ ; MDS-U,  $n = 2$ ; 5q-syn-drome,  $n = 2$ ).

The mean MPC and MPV in ITP patients were 26.6 g/dL and 9.7 fL, respectively, and no ITP cases (0%) were judged to be MDS using these cutoff values. The mean MPC and MPV in the 20 AA cases were 24.4 g/dL and 8.9 fL, respectively, and 10% of AA cases (2 of 20) were judged as MDS using these cutoff values. The MPC and MPV among the 35 patients with MPN were 24.5 g/dL and 9.0 fL, respectively, and one MPN case (3%), who had primary myelofibrosis, was judged as MDS using these cutoff values (Figure 2B).

## 4 | DISCUSSION

Myelodysplastic syndrome is characterized by ineffective erythropoiesis caused by abnormalities in hematopoietic stem cells.<sup>1</sup> Bone marrow cell dysplasia has been recognized morphologically in MDS.<sup>1,2</sup> We investigated the values of MPC and MPV obtained using an ADVIA 2120i automatic analyzer as useful and convenient indicators for MDS screening.

Platelets become activated when they are collected in EDTA blood-collecting vessels, with cell surface expression of the platelet-activation marker CD62P, while MPV and MPC also change over time.<sup>12,13</sup> Harrison et al thus suggested that appropriate sampling conditions were necessary for experiments involving MPV.<sup>10</sup> We, therefore, conducted a preliminary experiment to examine the time course of changes in platelet parameters to determine the appropriate sample conditions and confirmed that MPC decreased and MPV



**FIGURE 2** Flow cytometry analysis of mean platelet component (MPC) and mean platelet volume (MPV) in healthy controls and in myelodysplastic syndrome (MDS), aplastic anemia (AA), idiopathic thrombocytopenic purpura (ITP), myeloproliferative neoplasms (MPN), refractory cytopenia of childhood (RCC), and acute myeloid leukemia with myelodysplasia-related changes (AML/MRC) determined using ADVIA2120i. A, MPC and MPV values in MDS cases and healthy controls and B, in MDS, AA, ITP, MPN, RCC, and AML/MRC cases. Few healthy controls had an MPV  $> 10.0$  fL, while MPC values exhibited a wide range (21.5–30.5 g/dL) (A). Several ITP cases had high MPV values, but MPC values were within the reference value (B). Several MPN and AA cases had MPV values within the reference value, but MPCs above the reference value. A few ITP, MPN, or AA cases had high MPV and low MPC values, which were distinctive features seen in MDS cases

increased over time, as described before.<sup>12,13</sup> We concluded that all procedures, including measurements using the ADVIA 2120i analyzer, should be performed within 2 hours after blood collection based on the results of confirmative experiments (data not shown).

Mean platelet volume reflects the size of the platelets, and high MPVs are found in ITP, disseminated intravascular coagulation, MDS, and congenital giant thrombocytopathy.<sup>14</sup> Mean platelet component has been reported to be low in MDS cases with degranulated platelets<sup>9</sup> and may, thus, seem to be a promising candidate indicator of MDS. However, the value of MPC as an indicator of MDS is limited by its wide variability.

We hypothesized that using cutoff values for both MPC to reflect the density of platelets and MPV to express their size, might improve the detection of giant and/or degranulated platelets in MDS patients.

The cutoff values determined using statistical analysis software JMP12 according to recursive partitioning analysis were <25.3 g/dL for MPC and >10.0 fL for MPV. This cutoff value detected 78.9% (45 of 57) of MDS patients based on peripheral blood analysis; however, no patient with RCC was judged to have MDS despite being a type of MDS. Refractory cytopenia of childhood is a refractory childhood syndrome involving decreased blood corpuscles, similar to severe AA.<sup>15</sup> Refractory cytopenia of childhood is not usually accompanied by peripheral blood cell dysplasia, and it was, therefore, difficult to judge RCC as MDS. Although it is not surprising that these cases were not judged as MDS with these cutoff values, the reason for the absence of giant platelets in these MDS cases is unknown. In contrast, only 2 of the 1305 controls were judged as MDS using these cutoff values, but their details were also unknown.

We also investigated the MPC and MPV in patients with ITP, MPN, and AA using the determined cutoff values. Giant platelets have been reported to occur in the peripheral blood of patients with ITP; however, platelet production is enhanced in these patients, and granular abnormalities do not usually occur.<sup>14</sup> These conditions may contribute to the apparently normal MPC values in patients with ITP, thus allowing discrimination between ITP and MDS cases.

Only one of 20 patients with ET had an MPV value >10.0 fL. We speculated that, even if a few giant platelets occurred in the peripheral blood of ET patients, the MPV value provided a population average value and would, therefore, not be elevated because of the large number of normal-sized platelets.

Two of 20 cases of AA were judged as MDS using the combined cutoff values, though the details of these cases are unknown. The distinction between AA and hypoplastic MDS is generally unclear and their differentiation is difficult.<sup>16,17</sup> Furthermore, Bessho et al reported that 7.7% of AA cases transformed into MDS.<sup>18</sup> It is, therefore, necessary to follow up patients with AA in case they progress to MDS, and platelet parameters including MPC and MPV may be useful indicators to detect this transformation.

In this study, we used peripheral blood from patients with MDS because degranulated giant platelets give high MPV values. However, MPV values obtained by the electrical resistance method do not always match those obtained by optical methods,<sup>19,20</sup> and no standard method of measuring MPV values has yet been established. More appropriate criteria or cutoff values for MPV are, therefore, needed. Furthermore, a standard procedure that emphasizes time management

is required to account for the fact that MPV and MPC values change with time after blood collection.

## 5 | LIMITATIONS OF THE STUDY

This study had some limitations. First, the sample size was relatively small, and further studies at other institutions are needed. Second, we focused on MDS, and confirmation of the results will be required for other blood or drug-induced hematological disorders. Third, although MPC can reflect the platelet staining very well, MPC does not directly correlate with components of platelets since MPC is measured based on the scattering light method.

## 6 | CONCLUSION

The results of the current study demonstrated that MPC and MPV values obtained using an automated hematology analyzers may be useful and convenient indicators for MDS diagnosis, particularly in screening procedures, and may help to overcome the problem of morphological abnormalities being missed by manual inspection.

## ACKNOWLEDGEMENTS

We thank Susan Furness, PhD, from Edanz Group ([www.edanzediting.com/ac](http://www.edanzediting.com/ac)) for editing a draft of this manuscript in English.

## CONFLICT OF INTEREST

The authors declare no competing financial interests.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Masutani R, Ikemoto T, Maki A, et al. Mean platelet component and mean platelet volume as useful screening markers for myelodysplastic syndrome. *Health Sci Rep.* 2018;1:e50. <https://doi.org/10.1002/hsr2.50>