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Monocyte subsets to differentiate chronic myelomonocytic leukemia from reactive monocytosis

Sang Mee Hwang[1,2] 🆔 | Haejin Ahn[1] | Seungah Jeon[1] | Jun Park[1] | Yunye Chang[1] | Hyungsuk Kim[2,3] 🆔

[1]Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam, South Korea

[2]Department of Laboratory Medicine, Seoul National University College of Medicine, Seoul, South Korea

[3]Department of Laboratory Medicine, Seoul National University Hospital, Seoul, South Korea

Correspondence
Sang Mee Hwang, Gumiro 173 Beongil 82 Bundanggu, Seongnamsi, Gyeonggido, 13620, South Korea.
Email: smilemee@snubh.org

Abstract

Background: Chronic myelomonocytic leukemia (CMML) is characterized by persistent monocytosis and dysplastic features of blood cells. No specific genetic abnormalities are present in CMML, and reactive monocytosis should be excluded. An increase in classical monocytes (MO1) has been suggested as a screening tool for CMML.

Methods: We evaluated monocyte subsets in the peripheral blood of patients with CMML (n = 16), patients with reactive monocytosis (n = 19), and normal controls (n = 15) with flow cytometry using antibodies against CD14, CD16, CD56, CD24, CD45, and CD2. The cutoff of MO1 ≥94% was validated, and the optimal cutoff was analyzed with receiver operating curve analysis.

Results: The sensitivity of monocyte subset testing for screening for CMML was 0.938 (0.717-0.997), and the specificity was 0.882 (0.734 - 0.953) using the cutoff of MO1 ≥94%. Serial samples from patients who responded to hypomethylating therapy showed an MO1 < 94%. However, few patients with reactive monocytosis, including patients with nonhematologic malignancies and acute myeloid leukemia, showed an increase in the MO1 ≥ 94%. Monocyte subset results were correlated with the response to hypomethylating therapy in follow-up samples.

Conclusion: Monocyte subset analysis is useful in screening for and monitoring CMML. Harmonization of the protocols for monocyte subset analysis is required.

KEYWORDS
chronic myelomonocytic leukemia, flow cytometry, monocyte subset, screening tests

1 | INTRODUCTION

Chronic myelomonocytic leukemia (CMML) is categorized as a myeloproliferative/myelodysplastic syndrome showing peripheral blood (PB) monocytosis and dysplasia or acquired clonal genetic abnormalities in hematopoietic cells.[1,2] However, reactive monocytosis can be present in many conditions, such as other cancers and inflammation.[3,4] Cytogenetic aberrations are present in only 20% of patients, and molecular aberrancies may support the evidence of a neoplasm but cannot differentiate CMML from other myeloid malignancies.[1,5] Thus, morphology, clinical history, and cytomolecular aberrations need to be comprehensively considered for a diagnosis.

Recently, flow cytometric monocyte subset repartitioning with PB has been suggested as a screening test for the diagnosis of CMML.[6] Monocytes have been classified into classical monocytes (MO1, $CD14^{++}/CD16^-$), intermediate monocytes (MO2, $CD14^{++}/CD16^+$), and nonclassical monocytes (MO3, $CD14^+CD16^{++}$), differing in phagocytic activities and inflammatory characteristics.[7,8] An increase in classical monocytes (MO1) has been suggested in CMML, and the cutoff of MO1 ≥94% has been validated in many studies with a relatively high sensitivity and specificity,[6,9–12] and other studies have used a decrease in MO3 <1.13%.[10,11] Since CMML is a rare disease in Asia, in Korea with fewer than 100 cases diagnosed in a year,[13] monocyte subset analyses have not been evaluated for the screening of CMML in the Korean population. We assessed the usefulness of PB monocyte subset analysis in differentiating CMML from other causes of reactive monocytosis by flow cytometry and the use of monocyte subset analysis in monitoring treatment in CMML patients.

2 | MATERIALS AND METHODS

Fifty-three samples from 50 patients with CMML, patients with reactive monocytosis (> 1×10^9/L) and individuals who had undergone routine checkups with normal complete blood counts were tested for monocyte subsets in PB. A diagnosis of CMML was based on bone marrow examination (BM) with the World Health Organization criteria.[14] Analysis of the samples without obtaining informed consent from the patients was approved by the Institutional Review Board of the institution (IRB-B1807/483-301).

PB samples were collected in potassium ethylenediaminetetraacetic acid tubes and tested within 8 hours of sample collection. The antibodies used to identify monocyte subsets were APC-H7-CD45 [clone: 2D1, Beckton Dickinson (BD), San Jose, USA], APC-CD56 (clone NCAM16.2, BD), FITC-CD2 (clone: S5.2, BD), PE- CD14 (clone: RM052, Beckman Coulter, Marseille, France) PECy7-CD16 (clone: 3g8, BD), and PerCP-Cy5.5-CD24 (clone: M15, BD). In total, 300,000 events were acquired with a BD FACSCanto (BD). The gating strategies to identify monocyte subsets in PB were performed as previously reported (Figure 1).[6] Briefly, singlet gating was performed, and monocytes were roughly gated on $CD45^{high}/SSC^{int}$ cells. T cells, NK cells, B cells, and granulocytes were excluded. Then, double-negative CD14 and CD16 cells were excluded from the monocyte subset, and the monocytes were separated into MO1, MO2, and MO3. The contour plot was used for an optimal gating of MO1 identifying the valley to divide MO1 from MO2. The original cutoff suggested by Selimoglu-Buet et al of MO1 ≥94.0% [6] and another cutoff proposed, MO3 < 1.13%,[10] were verified, and the optimal cutoff for the diagnosis of CMML was evaluated. All statistical analyses were performed with GraphPad Prism 8 (GraphPad Software). Fisher's exact test was used to compare the differences. Receiver operating curve (ROC) analysis was performed to identify the optimal cutoff values for flow cytometric screening for CMML. Sensitivity and specificity values were calculated based on the results of diagnostic samples from patients with CMML and the results of samples from patients with reactive monocytosis and normal blood count as controls. P values <.05 were considered statistically significant.

3 | RESULTS

We tested 53 specimens from patients with CMML (n = 19), patients with other causes of reactive monocytosis (RM) (n = 19) and normal controls (NC) (n = 15). Among the 19 samples from patients with CMML, 14 were diagnostic samples, and 5 samples were from patients who underwent treatment with hypomethylating agents; of those samples, three were serial samples from three patients with CMML. Thus, the number of patients with CMML was 16. The clinical characteristics of the patients are shown in Table 1. The patients with reactive monocytosis included patients with other myeloid neoplasms (n = 4), nonhematologic malignancies (n = 9), and other diseases (n = 6).

The sensitivity of monocyte subset testing for the screening of CMML was 0.938 (0.717 - 0.997), and the specificity was 0.882 (0.734 - 0.953) using a cutoff of MO1 ≥ 94.0%, while the sensitivity was 0.625 (0.386 - 0.815) and the specificity was 0.824 (0.665 - 0.917) with a cutoff of MO3 < 1.13%. The optimal cutoff value identified by ROC analysis was MO1 > 94.1%, which produced a sensitivity of 93.8% and specificity of 88.2%, similar to the cutoff suggested by Selimoglu-Buet et al[6] The area under the curve (AUC) was 0.858 (0.722 - 0.993, P < .0001) with MO1. The AUC was lower with the cutoff MO3, being 0.813 (0.681 - 0.944), and the optimal cutoff for MO3 in our analysis was < 2.55. The MO1, MO2, and MO3 of RM and NC were compared by Mann-Whitney test. There was no significant difference of MO2 between RM and NC (P = .306). However, there were significant differences between MO1, MO3 of RM and NC (P = .004, 0.001, respectively), with the median of MO1 of RM being 88.30%, while 82.90% for NC and the median of MO3 being 2.60% for RM and 7.70% for NC.

Among the 14 diagnostic samples from patients with CMML, 13 (92.9%) showed an accumulation of MO1 ≥ 94.0%, while 9 (64.3%) showed decrease of MO3 < 1.13%. None of the normal controls had MO1 ≥ 94.0% or MO3 < 1.13%. Since the AUC was lower using MO3 < 1.13%, and most of the previous studies were based on MO1 ≥ 94.0%, we used MO1 ≥ 94.0% as the threshold.

One diagnostic sample, that did not show an accumulation of MO1 ≥ 94.0%, was from a patient with CMML-0. Although diagnosed as CMML-0, this patient had transfusion requirement with cytopenia of hemoglobin and platelet < 50 X 10^3/μL, and splenic infarction with splenomegaly at diagnosis, thus therapy was initiated. Among the reactive monocytosis samples, 4 samples (21.1%) showed accumulation of MO1 ≥ 94.0%. These samples were from patients with brain tumors, colon cancer, acute myeloid leukemia (AML), and immune thrombocytopenia, and a previous study reported an increase in the MO1 population in AML patients.[12]

There were follow-up samples from three patients with CMML. Two samples showed MO1 ≥ 94.0%, and these patients had persistent monocytosis despite hypomethylating therapy. The other sample did not show an accumulation of MO1, and this patient had
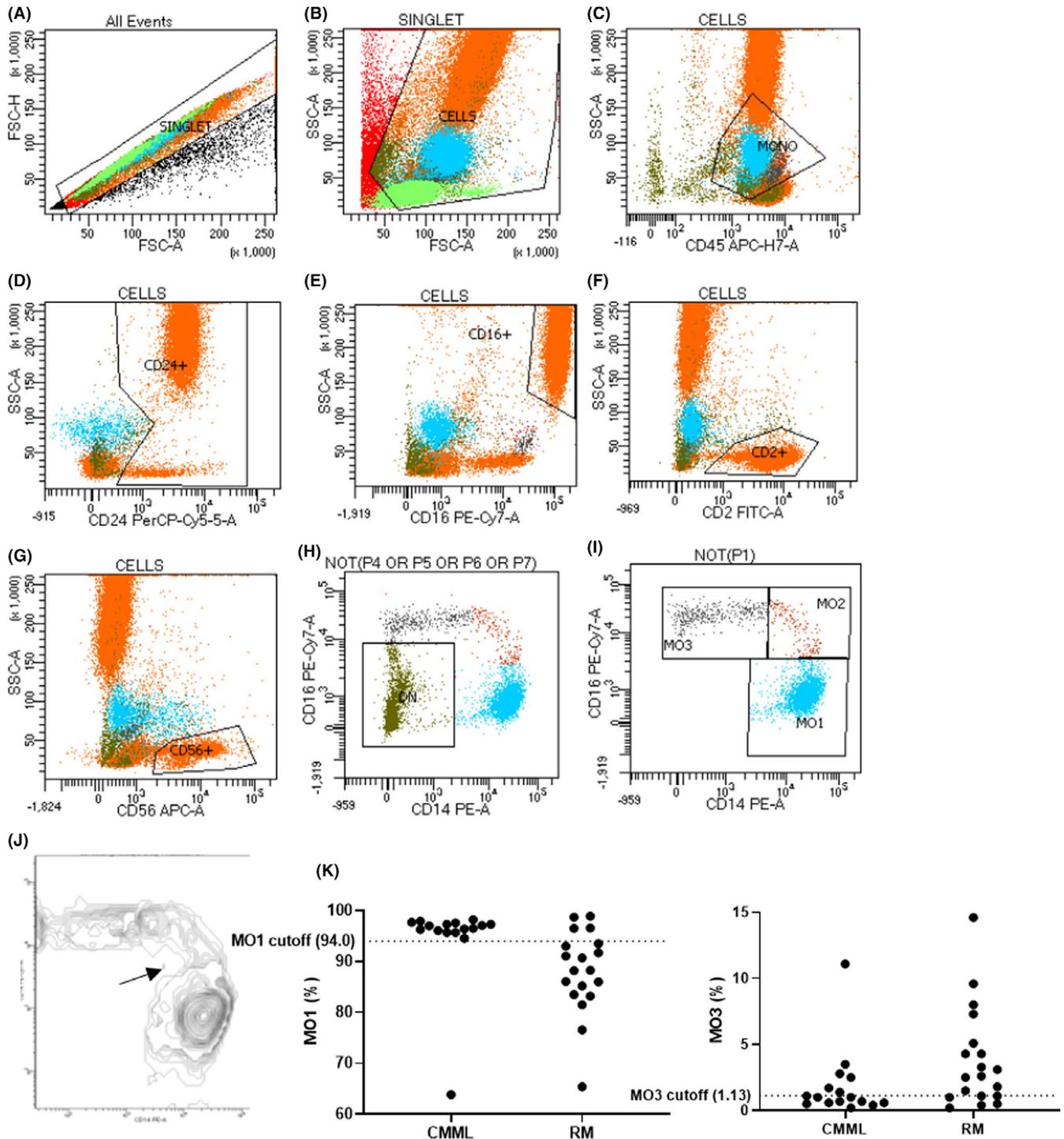
FIGURE 1 A. Singlet selection. B. Exclusion of debris based on forward scatters and side scatters (SSC). C. Rough selection of monocytes based on CD45-SSC. D. Exclusion of CD24+/SSChigh granulocytes, and CD24+/SSClow B cells. E. Exclusion of CD16+/SSChigh granulocytes. F. Exclusion of CD2+/SSClow T cells. G. Exclusion of CD56+/SSClow NK cells. H. Exclusion of double-negative cells. I. Separation of classical monocytes (MO1), intermediate monocytes (MO2), and nonclassical monocytes (MO3). J. Contour plot for an optimal gating of MO1 subset K. Dot plots of MO1 (%) and MO3 (%) in chronic myelomonocytic leukemia (CMML) and reactive monocytosis (RM).

a good response after hypomethylating agent treatment, without monocytosis. A decrease in the percentage of MO1 was observed in this patient from 94.6% to 86.6%, and the AMC after therapy was 431/μL.

4 | DISCUSSION

Monocyte subset analysis with PB using a cutoff of MO1 ≥ 94.0% showed relatively high sensitivity of 0.938 (0.717 - 0.997) and the specificity with 0.882 (0.734 - 0.953) similar to the optimal cutoff

Group	CMML (n = 16)	Reactive monocytosis (n = 19)	P-value
Age	71 (41 – 89)	68 (36 – 86)	.550
Sex (male:female)	7:9	17:2	
White blood cell count (×10^9/L)	19.36 (3.4 – 106.9)	8.76 (5.23 - 18.52)	.142
Absolute monocyte count (×10^9/L)	4.4 (1.0 – 25.7)	1.7 (1.1 - 3.78)	<.001
Hemoglobin (g/dL)	9.2 (5.4 – 13.8)	9.8 (7.2 – 12.3)	.202
Platelet (×10^9/L)	72.5 (7 – 212)	15.7 (12 – 568)	.007
Sample status			
Diagnostic	14		
Follow- up	2	19	
Diagnosis			
Hematologic malignancies	CMML-0 (7), CMML-1 (2), CMML-2 (7)	Acute myeloid leukemia (2), acute lymphoblastic leukemia (1), lymphoma (3), primary myelofibrosis (2)	
Nonhematologic malignancies		Colon cancer (1), brain tumor (2), malignant thymoma (1)	
Others		Lung cancer (2), kidney disease (1), infective spondylopathy (1), immune thrombocytopenia (1), liver cirrhosis (2)	

Abbreviation: CMML, chronic myelomonocytic leukemia.

MO1 > 94.1%, with the same sensitivity of 93.8% and specificity of 88.2%. We found that a response to hypomethylating agents was associated with a decrease in MO1 to < 94.0% in the serial samples.

We have confirmed that the well-established MO1 ≥ 94.0% cut-off can be used,[6,9,10] and the easily accessible PB is the specimen of choice, although some studies have tested BM.[12] The protocol for monocyte subset analysis is not uniform, as is the case for many flow cytometry assays. We acquired 300 000 total events with PB,[12] while others have used 50000 events in the monocyte subpopulation [6] and even fewer than 50 000 total events [10] and showed similar results.[15] Since the acquisition of 50 000 events in the monocyte subpopulation would require more time than the usual flow cytometric assays, we used a protocol with 300000 total events and were able to acquire a median of 32000 events in the monocyte subpopulation and achieved similar results. Moreover, depending on the cytometer, the same fluorescent molecules and antibodies may not be used, causing different but comparable results [11,16,17]; thus, standardization is necessary.

False-negative results have been identified in CMML with an associated inflammatory state due to the increase in intermediate monocytes [18]; thus, inflammatory conditions may have been present at diagnosis. However, the "bulbous aspect" present in CMML patients with inflammatory state was not observed in the patient with MO1 < 94.0% in our study. Adding 6-sulfo LacNac (slan) testing has been suggested to identify CMML with inflammatory conditions,[15,18] but we did not test for slan in the negative patient, and incorporation of slan should be evaluated in future studies. Increases in classical monocytes have been found in nonhematologic malignancies, and other studies have identified patients with MO1 ≥ 94.0% in non-CMML diseases, such as monoclonal gammopathy and metastatic lung cancer,[11] suggesting alteration of monocyte populations, or the transcriptomes of monocytes, in such diseases and thus warranting further studies in other cancers.[19]

As in our study, where responses to therapy correlated with the decrease in MO1 percentages, previous studies have also shown a good correlation of the results with monocyte subset analysis [6,12] and therapeutic response; thus, monocyte subsets can be useful in response monitoring in CMML without having to undergo invasive BM examination.

The major limitation of this study is the small number of CMML patients included for evaluation. Due to the low incidence of CMML in Korea,[13] we were only able to analyze a few diagnostic samples. However, our study showed similar sensitivity to previous studies, with a slightly lower specificity.[6,9,12,16] We were not able to correlate molecular abnormalities and flow cytometry results since only a few patients had undergone targeted gene sequencing, but previous studies have shown that monocyte subset results are not dependent on cytogenetic or molecular results.[6,11]

Monocyte subset analysis can be performed with PB, which is noninvasive, to help differentiate CMML from reactive monocytosis and potentially monitor therapeutic response in CMML.

ORCID

Sang Mee Hwang https://orcid.org/0000-0003-3390-1932

Hyungsuk Kim (iD) https://orcid.org/0000-0002-0574-9200

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