Chronic Myelomonocytic Leukemia Monocytes Uniformly Display a Population of Monocytes With CD11c Underexpression

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Key Words: Flow cytometry; Reactive monocytosis; Chronic myelomonocytic leukemia

DOI: 10.1309/AJCPUY0ZMG3VTLFG

ABSTRACT

Objectives: To examine the utility of CD11c expression on monocytes in normal controls and patients with chronic myelomonocytic leukemia (CMML) (n = 23) with flow cytometric immunophenotyping.

Methods: Twenty-three CMML samples and 10 control bone marrows submitted for lymphoma staging without evidence of disease were examined.

Results: Monocytes in CMML samples ranged from 4% to 35%. Expression of at least one aberrant monocytic marker was found on the monocytes in 18 (82%) of 22 evaluable cases. The most common aberrancy was underexpression of CD11c (n = 15), while none of the bone marrow controls showed underexpression of CD11c.

Conclusions: A distinct heterogeneous population of monocytic cells with underexpression of CD11c was identified in all these cases. CD11c underexpression was independent of other aberrancies, including HLA-DR underexpression (n = 14), aberrant CD56 expression (n = 11), and underexpression of CD33, CD38, and CD14 (n = 6, 5, and 5, respectively), supporting the utility of CD11c expression status on monocytes in establishing a CMML diagnosis.

The diagnosis of chronic myelomonocytic leukemia (CMML) using the World Health Organization (WHO) 2008 schema relies heavily on the documentation of persistent monocytosis and several other exclusionary criteria, including the absence of the Philadelphia chromosome and documentation of less than 20% blasts.¹ Even so, only a few cases diagnosed as CMML exhibit dysplasia or diagnostic cytogenetic abnormalities. Establishing a diagnosis of CMML based on absolute monocyte counts is further compounded by the presence of monocytosis in reactive conditions. Consequently, the identification of aberrancies in monocytic antigen expression via flow cytometry (FCM) could serve as an important surrogate of neoplastic monocytic proliferation. Indeed, several investigators in recent years have sought to examine aberrancies in myelomonocytic antigen expression in the context of myelodysplasia.²-⁴
The collective evidence from these studies, combined with the growing use of multiparameter FCM, has led to a better understanding of normal granulocytic and monocytic maturation, which demonstrates predictable and characteristic patterns using carefully chosen sets of conjugated antibodies targeting myelomonocytic antigens. Furthermore, it became apparent from these studies that the magnitude of hematopoietic abnormalities in myelodysplastic syndrome (MDS) could be reliably quantified by the deviations from known normal patterns of antigen expression.5,6

The first large-scale study to systematically evaluate myelomonocytic aberrations was conducted by Wells and colleagues, who examined 115 patients with MDS and 25 normal controls by three-color FCM. These authors developed a prognostic score based on the frequency and extent of aberrancies, which correlated significantly with outcome after allogeneic hematopoietic stem cell transplantation. However, cases of CMML were specifically excluded in this study. A subsequent study by Xu et al7 specifically examined monocytes in CMML cases using four-color FCM and identified the frequent occurrence of CD14mod populations in CMML compared with reactive monocytes. At our institution, we have anecdotally observed CD11cdim populations in CMML cases and wondered whether such populations are specific to CMML. While CD11c is widely regarded as a marker for dendritic cells and hairy cells, its expression pattern has not been examined in monocytic cells by FCM, although it is generally well known that monocytes do express CD11c. To this end, the purpose of this study was to systematically examine cases of CMML and control marrows and quantify monocyte marker aberrancies using an expanded five-color, seven-parameter FCM modality with a specific focus on the CD11c expression pattern.

Materials and Methods

Case Selection and Patient Information

We searched our database and identified 23 patients with CMML (18 males and 5 females). To ensure uniformity, only in-house cases examined using the same FCM instruments (2008 to present) were retained in this study. A diagnosis of CMML was established based on a monocyte count of more than 1,000/µL and other criteria per the WHO 2008 schema.1 The histology was reviewed by one of the authors (G.V.). Cases were subdivided further as dysplastic CMML (d-CMML) or proliferative CMML (p-CMML) based on the 2008 schema.1 The histology was reviewed by one of the authors (G.V.). Cases were subdivided further as dysplastic CMML (d-CMML) or proliferative CMML (p-CMML) based on the 2008 schema.1 The histology was reviewed by one of the authors (G.V.). Cases were subdivided further as dysplastic CMML (d-CMML) or proliferative CMML (p-CMML) based on the 2008 schema.1

Ten normal bone marrows (lymphoma staging marrows, n = 9; treated acute myeloid leukemia without evidence of disease, n = 1) and six peripheral blood samples (without monocytosis or evidence of other hematopoietic malignancy) were included as controls. In addition, we selected 10 cases of chronic myelogenous leukemia in the chronic phase and one case of acute myeloid leukemia without evidence of disease on growth factor therapy (pegfilgrastim) with significant reactive monocytosis to provide a comparison. This study was approved by the institutional review board of Loyola University Medical Center.

Flow Cytometric Immunophenotyping

Specimens with CMML (bone marrow, n = 22; peripheral blood, n = 1) were obtained and stained within 24 hours of collection with a panel of antibodies (Coulter Immunotech, Marseille, France; Becton Dickinson, San Jose, CA). Erythrocytes were lysed by incubating with lysis solution (150 mmol/L NH4Cl, 10 mmol/L KHCO3, and 0.1 mmol/L EDTA) for 5 minutes at room temperature (maintained at 21°C-23°C) at a ratio of 1:30 (volume of sample/volume of lysing solution). Specimens were then washed with phosphate-buffered saline before determining cell number. Specimens were stained for 15 minutes at room temperature (maintained at 21°C-23°C) with a cocktail of five antibodies. All cells were fixed in 1.0% paraformaldehyde after staining and analyzed immediately. Specimens were acquired with a seven-parameter, five-color FCM on the FC500 flow cytometer (Beckman Coulter, Pasadena, CA). At least 50,000 total events were acquired in most cases, including at least 2,000 monocyte events.

The following two tubes were used for specifically examining monocyte populations:
1. CD45-PC7/CD11c-PE/CD64-FITC/CD14-ECD/C8
2. CD45-PC7/CD33-PE/CD15-FITC/HLA-DR-ECD/CD34-PC5

In addition, CD2, CD56, CD38, and antibody combinations for B, T, and myeloid cells were used in other tubes. Isotypic controls were also included for each of the fluorochromes used. The specimens were stained according to Clinical Laboratory Standards Institute document H43-A recommendations.8,9 FCM list mode files were examined using CXP (Beckman Coulter, Brea, CA) software gating on the monocytes with either CD45/side scatter (SSC) or forward scatter (FS)/SSC in most cases. The CD14/CD64 combination was used as a primary gate in two cases where-in a distinct monocyte population could not be identified using the first two gates. There was no clustering of antigen expression patterns in any one of the two instruments used for analysis.

Normal lymphoid cells within specimens served as internal negative controls for many monocytic antigens (except HLA-DR), while the expression pattern on monocytoic cells from controls was used to define moderate antigen
expression. Using this system as a reference, dim expression in monocytic cells was defined as having lower antigen levels compared with that observed in control monocytes, in concordance with the 1997 US-Canadian consensus guidelines\(^\text{10}\) and the subsequent 2006 Bethesda International Consensus guidelines.\(^\text{11}\) In addition, for markers with heterogeneously rather than homogeneously decreased antigen expression, we defined cut points based on the level of antigen expression by normal monocytes in control cases and quantified the percentage of mononuclear cells expressing antigens dim in intensity in comparison with the control mononuclear cells. Aberrant mononuclear populations were defined as showing a shift by at least a half decade compared with normal monocytes (eg, CD56 or aberrant CD2). CD14 was analyzed using the criterion described by Xu et al.\(^\text{6}\) Low granulocyte SSC was determined if the top of the granulocyte cluster ellipse was apparent as a “dome.”

Basic descriptive statistics were performed, including independent sample \(t\) tests or Mann-Whitney tests for continuous variables and the \(\chi^2\) test for categorical variables. All statistical analyses were performed within the environment of Stata 11 (StataCorp, College Station, TX).

Results

CMML Cases

Of the 23 CMML marrows, 12 were d-CMML and 11 were p-CMML. Nineteen of 23 cases showed variable dysplastic morphologic changes in the megakaryocytic, myeloid, or erythroid lineages. Five cases showed cytogenetic abnormalities without evidence of the t(9;22). Overall marrow monocyte percentages in CMML samples ranged from 4% to 35% of the WBCs with predominantly myeloid, or erythroid lineages. Five cases showed cytogenetic abnormalities without evidence of the t(9;22). Overall marrow monocyte percentages in CMML samples ranged from 4% to 35% of the WBCs with predominantly mature morphology and only occasional cells identifiable as promonocytes.

In the FCM analysis, one of 23 cases was excluded in the examination of aberrancies because the granulocytic and monocytic clusters case showed significant overlap on CD45/SSC plots. In 13 of the remaining 22 cases, granulocytes showed low SSC but could still be separated from the monocyte cluster \(\text{Figure 1}\). In two cases, the monocytic populations were better separated in the CD14/CD64 plot when CD45/SSC alone was not deemed reliable (Figure 1H). The remaining seven cases showed normal high/heterogeneous granulocyte SSC. The median monocyte percentage in CMML cases by FCM was 7.59%.

Overall, 18 (82%) of 22 CMML cases showed aberrancies \(\text{Table 1}\). At least one aberrant marker expression was found on the monocytes in 18 (82%) of 22 cases. In the order of frequency, the most common aberrancy was decreased expression of CD11c (CD11c\(^{\text{dim}}\)) (15 cases; see Figure 1) followed by HLA-DR underexpression (14 cases). Other aberrancies are listed in Table 1. A population of mononuclear cells expressing dimmer CD14 (CD14\(^{\text{mod}}\), as described by Xu et al\(^\text{6}\)) was observed in a small proportion of CMML cases \(\text{Figure 2}\). When we examined the expression of CD11c on the monocytes of 10 CML marrows, seven (70%) cases showed less than 5% monocytes with decreased expression, and only one case showed a 15% CD11c\(^{\text{dim}}\) population.

When we examined the expression of CD11c on the monocytes of 10 CML marrows, seven (70%) cases showed bright homogeneous expression of CD11c, two cases showed less than 5% monocytes with decreased expression, and only one case showed a 15% CD11c\(^{\text{dim}}\) population.

In addition, we also examined one case of acute myeloid leukemia (AML) without evidence of disease after chemotherapy and pegfilgrastim administration. This case was notable for a brisk peripheral reactive monocytosis. Via FCM, there was significant underexpression of CD11c in nearly 50% of the monocytes, with distinct dim HLA-DR in around 40% of the monocytes.

To examine temporal variation in monocytic antigen expression, we selected a patient with thrombocytopenia
and suspected CMML who underwent two bone marrow biopsies 17 months apart. The more recent marrow was notable for an early/emerging CMML (absolute monocyte count ~900/µL). Using a threshold cutoff at the 25th percentile in normal marrows, this retrospective review showed a distinct and persistent CD11c<sup>dim</sup> population in both the initial and follow-up FCM studies (81.14% and 79.82% of total monocytes, respectively) \textit{Figure 3A} and \textit{Figure 3B}. In addition, plots of CD14/CD64 showed an increase in monocytes with dimmer spectrum of CD14 expression in the follow-up marrow compared with 17 months prior (16.67% and 12.85% of total monocytes, respectively) \textit{Figure 3C} and \textit{Figure 3D}. As of this writing, the patient had transferred care to a different hospital and was lost to follow-up.

\textit{Figure 1} CD11c expression on monocytes. \textbf{A}, Vertical line plot depicting the spread of CD11c expression in control marrows and chronic myelomonocytic leukemias (CMMLs): 15 CMML cases had CD11c<sup>dim</sup>– populations (defined as expression levels dimmer than the lower cutoff for CD11c in normal controls [lower line]). Using a threshold cutoff at the 25th percentile of CD11c expression in normal cases (upper line), CMML cases had significantly higher median CD11c<sup>dim</sup> monocytic cells (\(P = .01\), Mann-Whitney test) compared with controls. \textbf{B, C}, Bright expression of CD11c in two control cases. \textbf{D, E}, Two CMML cases with uniform underexpression of CD11c. \textbf{F}, CMML with heterogeneous dim to negative CD11c expression. \textbf{G, H, I}, One case of dysplastic CMML with an indistinct monocytic population (on CD45/side scatter, \textbf{G}) identified by primary gating on CD14/CD64 (gate T) with uniform underexpression of CD11c.
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Bone Marrow and Peripheral Blood Controls

Neither the bone marrow nor peripheral blood normal controls showed significant monocytosis. FCM analysis of the 10 bone marrow controls showed a median monocyte percentage of 2.31% (range, 1.02%-3.6%) with a normal granulocyte SSC pattern. Only four control marrows showed aberrancies (see Table 1). Notably, there was a significant difference between percentages of CD14mod-expressing monocytes in marrow controls (10.29%; range, 3.58%-28.58%) compared with peripheral blood control samples (6.76%; range, 1.14%-5.29%). However, only one normal marrow showed greater than 20% expression of CD14mod. Notably, CD11c expression was bright and homogeneous on both marrow and peripheral blood control monocytes, with only one control marrow showing a minor heterogeneous CD11cdim population.

Discussion

In the current study, we identified that the presence of decreased CD11c expression by monocytic cells is a sensitive and specific feature of CMML. Besides confirming previously reported aberrancies,6 such as aberrant CD56 expression and CD14mod, we also identified several other abnormalities such as CD38 underexpression in a small but significant subset of cases (five cases). Concurrently, we compared peripheral blood and bone marrow monocytes to assess differences in expression patterns, especially since marrow monocytes may contain a higher proportion of maturing monocytic cells compared with peripheral blood. We notably identified that normal marrow monocytes typically contained a significantly higher proportion of CD14mod compared with normal peripheral blood monocytes, while CD11cdim populations were infrequent in normal peripheral blood as well normal bone marrow samples. The progressive acquisition of CD14 on maturing monocytic cells exiting the marrow is consistent with the observed findings.

In one of the earlier studies to examine monocytes in CMML by FCM, Dunphy and colleagues12 examined seven CMML cases and noted that the monocytes of CMML expressed uniform CD11b, CD33, and CD64 with partial loss of CD13, CD14, and CD15, although the

![Image](https://example.com/image.png)

**Table 1**

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<th>Characteristic</th>
<th>Cases (n = 22)</th>
<th>Controls (n = 10)</th>
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<td>Expression of aberrancies, No. (%)</td>
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</tr>
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<td>14 (63.64)</td>
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</tr>
<tr>
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<td>CD2 expression</td>
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<td>Low granulocyte side scatter</td>
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**Figure 2**

A, Normal control case showing only 4.9% of monocytes underexpressing CD14 (CD14mod). B, Dysplastic chronic myelomonocytic leukemia (CMML) case showing 37% CD14mod population. C, Box plot demonstrating the absolute percentages of CD14mod populations that did not significantly differ between CMML cases and normal marrows (P = .42, Mann-Whitney test).
exact percentages of cells and numbers of cases showing each of these abnormalities were not detailed. Subsequently, Xu et al. expanded these observations in a study focused solely on identifying immunophenotypic aberrancies in CMML compared with normal and reactive monocytosis. They used 20 CMML bone marrows, 10 normal controls, and 20 marrows with reactive monocytosis, all of which were examined using three-color and four-color FCM. They concluded that a combination of monocytosis with two or more immunophenotypic aberrancies in addition to more than 20% marrow monocytes showing the CD14\textsuperscript{mod} phenotype was 67% sensitive and 100% specific for CMML. Furthermore, they noted that 80% of CMML cases demonstrated aberrant CD56 expression with associated dim expression of HLA-DR in 50% of cases. Although they proposed that the presence of CD56-expressing monocytes in conjunction with underexpression of myeloid antigens was 100% specific to CMML, this combination was present in only 40% of CMMLs. We were able to corroborate CD56 expression in a subset of CMMLs, although our frequency of detection was much less compared with the findings of Xu and colleagues. Notably, a more recent study noted that CD56 expression is more heterogeneous on marrow monocytes compared with peripheral blood monocytes.\textsuperscript{13} This may have some bearing on the subjectivity of determining thresholds for calling CD56 aberrancy in different studies. Alternatively, differences in CD56-conjugated fluorochromes used may also have affected the positivity rates. More important, while our study identified a significantly higher percentage of monocytes with dimmer CD14 expression in normal marrow compared with peripheral blood, there was no significant difference in median CD14\textsuperscript{mod} percentages between CMML and normal marrows.

More recently, Subirá and coworkers\textsuperscript{14} identified that CMML and MDS showed statistically significant differences (P < .05) in CD56 monocyte expression. CMML and myeloproliferative disorder had significant differences in CD45 myeloid distribution, myeloid antigenic profile, CD56 and CD2 monocyte expression, and B-cell development. Furthermore, this group noted that 47% of CMML cases demonstrated aberrant CD2 expression, a frequency much higher than the one observed in our study. On the other hand, Kern et al.\textsuperscript{15} found that 81.9% of CMML cases expressed CD56 and 100% of AML cases expressed CD56, whereas yet another study by Gorczyca\textsuperscript{16} described CD56 expression in only 53% of CMML and 86% of AML cases. Gorczyca concluded that aberrant CD56 expression in CMML as well as in other myeloid neoplasms is a highly characteristic feature. In contrast, other studies have documented that low expression of CD56 can be seen in normal regenerating monocytic cells.\textsuperscript{17} Equally intriguing is our finding of significant CD11c\textsuperscript{dim} populations in the case of growth factor therapy and a minor subset of CMLs (1 of 10). Hence, the specificity and utility of aberrant CD56 for a CMML phenotype requires further clarification.

On the other hand, CD11c/CD18 is found primarily on myeloid cells, where its expression is regulated during both differentiation and monocyte maturation into tissue macrophages. Monocytic maturation stage can be determined by the pattern of CD11b, CD11c, and CD14 expression. In our comparison of normal peripheral blood and marrow monocytes, we noted uniform bright expression of CD11c on monocytes without significant CD11c\textsuperscript{dim}\text偿还 populations in contrast to CD14, wherein CD14\textsuperscript{dim} populations were more apparent even in controls (data not shown). On the basis of
the collective data, we were able to define the lower limit of CD11c expression in normal cases (see Figure 1A-C). Based on this threshold, 15 CMML cases were noted to contain CD11cdim trailing populations as well as homogeneous under-expression. Although data detailing CD11c expression on monocytes are scarce, Gorczyca and colleagues\textsuperscript{6} examined 39 CMML cases and noted bright CD11c in all CMML cases in contrast. However, the specific gating strategy and fluorochromes used were not detailed in this study, although these authors noted underexpression of CD13, CD14, and HLA-DR in a subset of their cases, congruent with the findings of Xu and colleagues\textsuperscript{6} in a more recent study.

In conclusion, examining CD11c via FCM analysis affords another means of definitively establishing an aberrant monocytic population given the uniform presence of a distinct CD11cdim population in CMMLs. CD11c may add further diagnostic value in CMML if CD11c-associated abnormalities precede other well-established phenotypic abnormalities in evolving CMMls with borderline monocytosis.

Acknowledgments: We thank Maryalice Stetler-Stevenson of the National Cancer Institute for her insightful comments and suggestions in the manuscript. We also thank M. Kamran Mirza of the University of Chicago for providing pertinent clinical information relating to one of the cases included in the study.

References


