Relative Contributions of Enzyme Cytochemistry and Flow Cytometric Immunophenotyping to the Evaluation of Acute Myeloid Leukemias With a Monocytic Component and of Flow Cytometric Immunophenotyping to the Evaluation of Absolute Monocytoses

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Abstract

We evaluated the contributions of enzyme cytochemical stains and flow cytometric immunophenotyping (FCI) data to detection of monocytic cells (MCs) in acute myelomonocytic and acute monocytic leukemias (AMMLs and AMoLs) and compared FCI findings in AMoL, chronic myelomonocytic leukemia (CMML), and normal peripheral blood (PB) and bone marrow (BM) monocytes to classify and evaluate absolute monocytoses (AMs). We reviewed 10 AMMLs and 6 AMoLs with α-naphthyl-acetate esterase (ANAE) and α-naphthyl-butyrate esterase stains and a complete FCI profile and compared FCI data for 6 AMoLs, 7 CMMLs, 2 AMs, and normal monocytes.

We confirmed increased sensitivity of ANAE staining to FCI data in detecting MCs in AMML and AMoL. CD14 was insensitive for confirming MCs; other characteristic markers of MCs were absent or partially lost in AMML and AMoL. Aberrant expression of CD56 (detected in 50% of AMMLs and AMoLs), CD34, and CD117 indicated malignancy. The mature MCs of the CMMLs revealed variable FCI abnormalities (partial loss of CD13, CD14, and CD15; expression of CD56), as in the monoblasts of AMoL. These FCI abnormalities in morphologically mature MCs might indicate markers for CMML. AMs revealed FCI abnormalities, indicating clues to their correct classification as CMML.

Acute myeloid leukemias (AMLs) with a monocytic component (acute myelomonocytic leukemia [AMML] and acute monocytic leukemia [AMoL]) have varying components of mature and immature monocytic cells (MCs). They are defined by the French-American-British and World Health Organizations (WHO) classifications as having more than 20% MCs and more than 80% MCs (the majority of which are immature) detected by nonspecific esterase (NSE) staining, in AMML and AMoL, respectively.1,2 With the advent of flow cytometric immunophenotyping (FCI) techniques, MCs may be identified by relatively characteristic cluster designation markers (CD11b, CD13, CD14, CD15, CD33, and CD64).

Although CD14 is highly specific for the detection of MCs, there is controversy in the literature regarding its sensitivity. In a previous study of AMML and AMoL, NSE staining was shown to be more sensitive than CD14 in detecting the monocytic component; however, the particular NSE stain analyzed (ie, α-naphthyl acetate esterase [ANAE] vs α-naphthyl butyrate esterase [ANBE]) was not stated, and the gating analysis of MCs by FCI was not described.3 Other studies have indicated CD14 as commonly expressed in AMML and AMoL.4

By performing a complete FCI analysis of MCs in AMML and AMoL and analysis of ANAE and ANBE stains, it might be possible to clarify the relative contributions of enzyme cytochemistry and FCI techniques in the detection of the monocytic component in these AMLs. In addition, FCI abnormalities of the immature leukemic MCs possibly might be detected when compared with the FCI profile of normal monocytes and aid in the detection of an abnormal monocytic clone. These findings might be particularly useful in the
evaluation of an absolute monocytosis and establishing a diagnosis of chronic myelomonocytic leukemia (CMML).

CMML is defined as a persistent peripheral absolute monocytosis (≥1,000/µL [1.0 × 10⁹/L]) for at least 3 months with exclusion of other causes. In the WHO classification, CMML is divided morphologically into CMML-1 and CMML-2, based on the percentages of blasts in the peripheral blood (PB) and bone marrow (BM). CMML-1 has fewer than 5% blasts in the PB and fewer than 10% blasts in the BM; CMML-2 has 5% to 19% blasts in the PB and 10% to 19% blasts in the BM. Of note, promonocytes are counted as blasts for diagnostic (CMML vs AMoL) and subtyping (CMML-1 vs CMML-2) purposes. In addition, morphologically, the PB and BM might appear mature without any atypical features. FCI abnormalities possibly might aid in establishing a diagnosis of CMML.

To our knowledge, there is only 1 relevant, previously reported FCI study, and it revealed the presence of CD56 on the monocytes of patients with myelodysplastic syndrome.⁵ There are no previous studies analyzing and comparing the FCI findings in CMML, AMLs with a monocytic component (particularly AMoL, which reveals a significant population of immature MCs), and normal monocytes. Comparative FCI analysis of normal monocytes, AMLs with a prominent immature monocytic component (AMoL), and CMML may provide clues to the accurate classification of an absolute monocytosis (AM).

The purposes of the present study were to evaluate the relative contributions of enzyme cytochemistry and FCI data to the detection of the monocytic component in AMML and AMoL and to compare FCI findings in CMML, AMoL, and normal monocytes in an attempt to accurately classify and evaluate AMs.

Materials and Methods

We identified and retrospectively reviewed 10 AMMLs and 6 AMoLs (M5a, 1; M5b, 5) (French-American-British and WHO classifications) with ANAE (Sigma, St Louis, MO, and 6 AMoLs (M5a, 1; M5b, 5) (French-American-British Materials and Methods evaluate AMs.

normal monocytes in an attempt to accurately classify and AMoL and to compare FCI findings in CMML, AMoL, and monocytosis (AM).

The monocytes region was defined in these cases by CD45 expression and side light scatter properties combined with CD45 staining.

A marker was considered positive if expressed by at least 20% of analyzed cells. Significant partial loss of antigen expression was defined by more than 20% of cells without expression of the marker.

The FCI data for the 6 AMoLs; 7 CMMLs (CMML-1, 5; CMML-2, 2; defined by WHO criteria); 2 cases with AM, highly suggestive of CMML; and samples of normal PB (5 samples) and BM (3 samples) monocytes were compared. The CMMLs were composed of 5 BM and 2 PB specimens and the 2 cases of absolute monocytosis of 1 BM and 1 PB specimen each.

Results

Enzyme Cytochemical Stain Results for AMML and AMoL

All AMMLs revealed more than 20% ANAE+ BM cells; however, only 6 (60%) revealed more than 20% ANBE+ cells. Of the 6 AMoLs, 5 (83%) revealed more than 80% ANAE+ BM cells, whereas only 1 (17%) revealed more than 80% ANBE+ cells. One case of AMoL revealed more than 80% monocytic cells by ANBE staining and fewer than 80% monocytic cells by ANAE staining.

FCI Data

Acute Myelomonocytic Leukemias

FCI analysis of the 10 AMMLs revealed the percentage of monocytes by flow cytometry ranged from 0% to 16%. None had more than 20% MCs. The cells characterized as “blasts” by side light scatter properties and CD45 staining revealed partial loss or absence of CD11b in 10 (100%) and CD13 in 7 (70%) cases, absence of CD14 in 10 (100%) cases, and partial loss or absence of CD15 in 9 (90%), CD33 in 7 (70%), and CD64 in 10 (100%) cases. CD34 was expressed by 4 (40%), CD117 by 7 (70%), CD56 by 5 (50%), and dim CD2 by 10 (10%) cases.

Acute Monocytic Leukemia

The monoblasts (MBs) of the AMoLs had invariable expression of CD13 and CD33 with more variable expression of CD64, CD15, CD11b, and CD14 Table 1. CD34 and CD117 each were expressed by the MBs in 2 (33%) of the 6 AMoLs; CD56 was expressed in 3 (50%).

Image 1. Image 2, and Image 3 depict cases of AML with varying monocytic components (ie, AMML and AMoL) as detected by comparative NSE staining (ie, ANAE and ANBE) and pertinent accompanying FCI data.

CMML, AMs, and Normal Monocytes

By FCI techniques, 2 of 7 CMMLs (1 each CMML-1 and CMML-2) revealed a blast population (2% and 16%, respectively) in addition to a significant MC population (Table 1). The MCs of the CMML cases revealed invariable expression of CD33, CD11b, and CD64 and partial loss of CD13.
### Table 1
Comparison of FCI Data of Normal Monocytes, Monocytic Cells, and Monoblasts of AMoL, CMML, and Absolute Monocytoses

<table>
<thead>
<tr>
<th>Monocytic Disorder/Cell Analyzed</th>
<th>2</th>
<th>11b</th>
<th>13</th>
<th>14</th>
<th>15</th>
<th>33</th>
<th>34</th>
<th>56</th>
<th>64</th>
<th>117</th>
</tr>
</thead>
<tbody>
<tr>
<td>None/normal PB and BM monocytes (n = 8)</td>
<td>0 (0)</td>
<td>8 (100)</td>
<td>8 (100)</td>
<td>8 (100)</td>
<td>8 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>8 (100)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>AMoL (n = 6)/MBs</td>
<td>0 (0)</td>
<td>4 (67)</td>
<td>6 (100)</td>
<td>3 (50)</td>
<td>5 (83)</td>
<td>6 (100)</td>
<td>2 (33)</td>
<td>3 (50)</td>
<td>5 (83)</td>
<td>2 (33)</td>
</tr>
<tr>
<td>CMML (n = 7)</td>
<td>MCs 1 (14)</td>
<td>7 (100)</td>
<td>5 (71)†</td>
<td>6 (86)†</td>
<td>5 (71)†</td>
<td>7 (100)</td>
<td>0 (0)</td>
<td>3 (43)</td>
<td>7 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>MBs (n = 2)‡</td>
<td>0 (0)</td>
<td>1 (50)</td>
<td>2 (100)</td>
<td>0 (0)</td>
<td>1 (50)</td>
<td>2 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>2 (100)</td>
</tr>
<tr>
<td>AM (n = 2)/MCs</td>
<td>1 (50)</td>
<td>2 (100)</td>
<td>1 (50)†</td>
<td>2 (100)</td>
<td>0 (0)†</td>
<td>2 (100)</td>
<td>0 (0)</td>
<td>1 (50)</td>
<td>2 (100)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

AM, absolute monocytosis; AMoL, acute monocytic/monoblastic leukemia; BM, bone marrow; CMML, chronic myelomonocytic leukemia; FCI, flow cytometric immunophenotypic; MBs, monoblasts; MCs, monocytic cells; PB, peripheral blood.

* Expression of a marker was defined as positivity by greater than 20% of cells analyzed. Data are given as number of cases (percentage).

† Cases not included in these percentages had a significant partial loss of the indicated CD marker (ie, >20% of cells without expression of the marker).

‡ Two of the 7 CMMLs revealed a blast population by FCI data (representing 2% and 16% of cells, respectively).

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**Image 1** This acute myelomonocytic leukemia revealed 58% blasts in the bone marrow (BM) aspirate smear (A, Wright, ×400). The monocytic component was identified by the α-naphthyl-acetate esterase (ANAE) stain, which revealed >20% positive cells in the bone marrow aspirate (B, ANAE, ×400); however, the α-naphthyl-butyrate esterase (ANBE) stain revealed only rare scattered cells with positivity (C, ANBE, ×400). Flow cytometric analysis of the BM smear revealed only 8% of cells within the monocyte region; however, the smear of the flow cytometry specimen did not reveal spicules, and, thus, the flow cytometry specimen was hemodiluted.
CD14, and CD15. In addition, the MCs of the CMML cases revealed variable expression of CD56 and lack of expression of CD34 and CD117. The blast population in the 2 CMMLs revealed loss of expression of CD11b, CD14, CD64, and CD15 and revealed expression of CD34 and CD117.

The 2 AMs, highly suggestive of CMML, revealed invariable expression of CD11b, CD14, CD33, and CD64; variable expression of CD56; partial loss of expression of CD13 and CD15; and lack of expression of CD34 and CD117 (Table 1). One of the AMs revealed dim aberrant expression of CD2.

In comparison, the normal PB and BM monocytes revealed invariable, bright expression of CD11b, CD14, CD15, CD33, CD64, and HLA-DR with lack of expression of CD34, CD56, CD117, and CD2 (Table 1).

One case of CMML-2 (14% immature and 26% mature MCs) revealed no “blast population” by FCI techniques but did reveal 54% MCs with an abnormal immunophenotype (loss of expression of CD13, partial loss of expression of CD15, and dim aberrant expression of CD2).
Image 3 This acute monocytic leukemia (AMoL) revealed 75% blasts in the bone marrow aspirate smear (A, Wright, ×400). The immature cells were easier to evaluate in the peripheral blood smear (B, Wright, ×600). In contrast with the α-naphthyl-acetate esterase (ANAE) and α-naphthyl-butyrate esterase (ANBE) staining patterns seen in the great majority of the acute myelomonocytic leukemias and AMoLs, the predominant monocytic component in this AMoL was detected by the ANBE stain (D, ANBE, ×400), with a lesser percentage detected by the ANAE stain (C, ANAE, ×400). By flow cytometric analysis, the immature cells did not express CD14 or CD11b.

Discussion

AMML and AMoL represent AMLs with varying components of mature and immature monocytic cells, which traditionally have been detected by NSE staining. However, FCI offers the ability to analyze several CD markers that are expressed by monocytic cells, including CD11b, CD13, CD14, CD15, CD33, and CD64 (with CD14 being the most specific monocytic marker). Although CD14 is highly specific for monocytic differentiation by FCI, there is controversy in the literature regarding its sensitivity. Although CD14 has been touted as expressed commonly in AMML and AMoL, it also has been demonstrated that CD14 is negative in most cases of AMML and AMoL. This study by Eshoa et al concluded that
although mature monocytes strongly express CD14, most immature leukemic monocytes lack CD14 expression, and NSE staining is more sensitive for monocytic differentiation than CD14.

Our study results confirmed an increased sensitivity of ANAE staining but not ANBE staining to FCI data in detecting MCs in AMML and AMoL. However, both NSE stains should be performed routinely because there might be occasional to rare cases that reveal a higher percentage of MCs detected by ANBE staining than by ANAE staining, as in our study.

The present study also revealed that FCI analysis was not a sensitive method to detect MCs for the purpose of subtyping AMLs with a monocytic component. CD14 clearly was an insensitive marker for confirming MCs. In addition, other markers characteristically expressed by MCs (CD11b, CD13, CD15, CD33, and CD64) were absent or at least partially lost in AMML and AMoL.

In addition to revealing loss or absence of characteristic markers of monocytic cells in AMML and AMoL, our study revealed that CD56 might be expressed aberrantly in 50% of AMML and AMoL cases. This marker, as well as CD34 and CD117, were indicative of monocytic malignancy.

CMML might be a difficult diagnosis to establish, particularly when there are no significant immature forms of MCs and no cytogenetic abnormalities. FCI studies have not been reported on the MCs in this disorder. In our study, the blast populations of 1 CMML-1 case (2% blasts) and 1 CMML-2 case (16% blasts) revealed FCI findings similar to those of the MBs of the AMoL cases. Of particular interest, our study revealed that the FCI abnormalities detected in the MBs of the AMoL cases also were detected in the mature MCs of the CMML cases. In fact, although the CMML-1 cases were composed of mature MCs, the FCI data revealed variable abnormalities (partial loss of CD13, CD14, and CD15 and aberrant

**Image 4** This acute monocytic leukemia (AMoL; French-American-British classification, M5b) demonstrated 98% immature monocytic cells (blasts and promonocytes) by morphologic examination (as seen in Image 2). The flow cytometric immunophenotypic data revealed the “immature cells” invariably expressed CD13 (A), CD15 (B), and CD33 (C) with complete loss of expression of CD11b (B) and CD14 (A) and partial loss of CD64 (D) with associated dim expression of CD117 (D) and strong expression of CD56 (E). FITC, fluorescein isothiocyanate; PE, phycoerythrin.
expression of CD56), as in the MBs of AMoL and not seen in normal PB and BM MCs. Of particular interest, 1 CMML-2 case (with 14% immature and 26% mature MCs by morphologic examination) revealed no “blast population” by FCI techniques. However, by FCI techniques, all of the cells within the increased population of “MCs” revealed an identical abnormal immunophenotype, indicating that morphologically mature MCs might have abnormalities detected by FCI techniques. In addition, the 2 cases of AM, highly suggestive of CMML by morphologic examination, revealed FCI abnormalities, indicating clues to their correct classification as CMML.

**Image 5** This chronic myelomonocytic leukemia-1 was composed predominantly of (A, Wright, ×600) mature monocytes, demonstrated in the CD45 and side scatter (SSC) plot as green dots (B). The flow cytometric immunophenotypic data revealed bright expression of CD33 (C) and CD11b (D) with partial loss of expression of CD13 (E), CD14 (+56%; E), CD15 (D), and CD64 (+72%; F). FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin chlorophyll protein.
This chronic myelomonocytic leukemia-2 was morphologically composed of (A, Wright, ×400) 14% immature monocytic cells (blasts and promonocytes) and 26% mature monocytes. The flow cytometric immunophenotypic data revealed 54% of cells within the “monocytic” region (B; orange), which revealed uniform bright expression of CD11b (C), CD14 (D), and CD33 (E) with moderate expression of CD64 (F), partial loss of expression of CD15 (C), complete loss of expression of CD13 (D), and aberrant dim expression of CD2 (G). FITC, fluorescein isothiocyanate; PE, phycoerythrin.
This case with an absolute monocytosis, highly suggestive of chronic myelomonocytic leukemia (CMML), revealed 23% mature monocytes by morphologic examination (A, Wright, ×400). The flow cytometric immunophenotypic data revealed uniform bright expression of CD13 and CD14 (B), CD33 (C), and CD64 (D); moderate to bright expression of CD11b (E); and partial loss of expression of CD15 (E) with associated partial expression of CD56 (+33%; F) and dim aberrant expression of CD2 (G). FITC, fluorescein isothiocyanate; PE, phycoerythrin.
The samples of normal monocytes all revealed the same immunophenotype: uniform bright expression of CD11b and CD15 (A), CD13 and CD14 (B), CD33 (C), and CD64 (D) with no associated expression of CD34 (E), CD56 (+<20%; E), CD117 (D), or aberrant expression of CD2 (F). FITC, fluorescein isothiocyanate; PE, phycoerythrin.

References