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Emerging utility of flow cytometry in the diagnosis of chronic myelomonocytic leukemia

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ABSTRACT

The diagnosis of many hematologic malignancies has shifted from being based almost exclusively on morphology and clinical data to include ancillary studies such as flow cytometry. This trend has yet to affect the diagnosis of chronic myelomonocytic leukemia (CMML) as flow cytometry, while being integral in the diagnosis of many hematologic malignancies, has no explicit role in the current WHO criteria for CMML. The absence of WHO-determined criteria for flow cytometry in the diagnosis of CMML is not due to a lack of research on the subject over the years. Herein, we review the literature concerning the use of flow cytometry in the diagnosis of CMML, focusing on recent studies showing that CMML can be differentiated from other hematologic malignancies and reactive monocytoses by the quantification of monocyte subsets by flow cytometry with high sensitivity and specificity. We also detail how this methodology could be used clinically, both as a diagnostic test and potentially as a screening test.

1. Introduction

Over the past 30 years the diagnosis of hematologic malignancies has shifted from being based almost exclusively on morphology and clinical data to include ancillary studies such as molecular studies, cytogenetics, and flow cytometry. In this regard, the diagnosis of chronic myelomonocytic leukemia (CMML) has lagged behind the diagnosis of other hematological malignancies as it relies predominantly on clinical/laboratory findings and the absence of certain disease-defining translocations. According to the 2016 revision to the WHO guidelines, the diagnostic criteria for CMML includes: persistent monocytosis ($\geq 1 \times 10^9$/L and at least 10% of the total number of leukocytes), the lack of satisfying the criteria for any other myeloproliferative neoplasm, the lack of disease-defining translocations (involving BCR-ABL, PDGFRA, PDGFRB, FGFR1, PCM1, and JAK2), less than 20% blasts, and either myelodysplasia, an acquired clonal cytogenetic or molecular genetic abnormality in hematopoietic cells, and the exclusion of other causes of monocytosis [1]. In other words, flow cytometry, while being integral in the diagnosis of many hematologic malignancies, has no explicit role in the current WHO criteria for CMML. This is in spite of the fact that there is substantial overlap between CMML and other hematological neoplasms, predominantly myelodysplastic syndrome (MDS), in both recurrent somatic mutations and clonal cytogenetic abnormalities, making them of limited use in differentiating CMML from MDS. The absence of WHO-determined criteria for flow cytometry in the diagnosis of CMML is not due to a lack of interest or effort. There has been substantial research regarding the use of flow cytometry, likely due in part to the relative success of flow cytometry as an ancillary test for MDS [2]. Unfortunately, the flow cytometric changes observed in MDS are often present in CMML cases as well and cannot be used to differentiate between the two diseases [3]. Herein, we will discuss this research, focusing first on studies regarding aberrant surface marker expression and then shifting to promising recent developments regarding the quantification of monocyte subsets as a potential diagnostic and/or screening test for CMML.

2. Aberrant expression of cell surface markers on monocytes

The most widely studied flow cytometric topic in CMML historically has been the aberrant expression of cell surface markers on monocytes, with the basic premise being that either the “ectopic” expression of a supposedly non-monocytic surface marker or a loss/decrease in expression of a monocytic marker by the neoplastic monocytes in CMML could lead to the discrimination between neoplastic monocytes and the non-neoplastic monocytes found in reactive monocytoses. Regarding the expression of non-monocytic markers by CMML monocytes, the

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marker that has received the most attention has been CD56, a marker most associated with natural killer cells. While CD56 has been repeatedly shown to be expressed on monocytes in the bone marrow and blood of CMML patients [4–6], the expression of CD56 is not specific for CMML. In a study by Xu et al., while 80% of CMML patients had CD56-positive monocytes, so too did 30% of patients with a reactive monocytosis [5]. Monocytic expression of CD56 has also been identified in a variety of other conditions, including MDS and myeloproliferative neoplasms (MPNs), albeit in a lower percentage of cases than in CMML [7,8]. In fact, it has been postulated that CD56-positive monocytes represent a normal subset of monocytes [9], and that this population is increased in autoinflammatory/autoimmune diseases such as Crohn's disease and rheumatoid arthritis as well as in the elderly [10,11].

Aberrant expression of several other markers have been investigated as diagnostic tools in CMML: CD2, commonly used as a T cell marker; CD10, a neutrophil marker that is also commonly used to assess B cell subsets; CD23, a dendritic cell marker that is also used to assess B cell subsets. Like CD56, CD2 is typically not expressed by monocytes but is expressed on the monocytes of CMML patients, albeit in a much lower percentage of cases (~80% for CD56 vs 10–40% for CD2) [5,12]. Also like CD56, CD2 is also expressed by monocytes in MDS, MPNs, and reactive monocytoses [5,8] and has been shown to be expressed by a subpopulation of monocytes in normal individuals [13]. The ectopic expression of CD10 and CD23 on monocytes in CMML has also been reported, but in much lower percentages of CMML cases (23% and 26%, respectively) than CD56 (79%) [12].

The lost and/or decreased expression of several normal monocytic markers have also been studied in CMML. The aforementioned study by Xu et al. showed that 50% of CMML cases had monocytes with decreased HLA-DR expression. However, much like with CD2 and CD56 expression, this was not unique to CMML, as 35% of reactive monocytoses showed a similar decreased expression of HLA-DR [5]. The underexpression of CD11c on monocytes has been shown to be highly specific for CMML (100% in one study), suggesting its potential as a confirmatory test for CMML, but has a sensitivity of only ~70% [14]. Other monocytic markers reported to show decreased expression in CMML monocytes include CD13, CD14, CD15, CD33, CD36, CD38, CD45, and CD64, with CD14 and CD15 showing significant differences between CMML and controls in various reports [5,6,14–16]. Unfortunately, the use of decreased expression of these individual surface markers as a diagnostic test for CMML is limited by relatively low sensitivity.

As all of the markers discussed above lack either specificity, sensitivity, or both as a biomarker for CMML, there have been attempts to use various combinations in order to increase the likelihood of correctly identifying CMML cases. Studies have shown that utilizing the presence of more than one aberrant monocytic marker as criterion for CMML led to less "false positives" amongst reactive monocytoses [5,14], and Xu et al. showed that when the aberrancies were monocytic CD56 expression and decreased expression of a normal monocytic marker, the false positives were eliminated. However, this strict criterion led to decreased sensitivity (only 40% of CMML cases would be considered positive) [5]. In sum, the use of surface marker expression aberrancies on monocytes in order to diagnose CMML lacks either sensitivity and/or specificity, regardless of the marker(s) employed.

3. Aberrant expression of cell surface markers on other hematopoetic cells

As myelodysplasia is a hallmark of CMML, it is not surprising that alterations in surface marker expression on granulocytes have been found in CMML. These alterations are similar to those found in MDS and include aberrant/increased expression of CD117, CD36, CD56, and HLA-DR and decreased expression of CD10 on granulocytes [3,4,15,17]. Further, abnormal granulocytic expression of CD11b, CD13, CD15, and CD16 has also been reported in CMML [12,18]. Unfortunately, while these aberrancies tend to be relatively specific for CMML when compared to reactive conditions, they lack sensitivity. For instance, in one report, aberrant granulocyte expression of CD56 and HLA-DR were found in only 18% and 11% of CMML cases, respectively [15]. Further, as the same granulocyte aberrancies are also found in MDS, they are of limited clinical utility.

The blast population have also been investigated for abnormalities in surface marker expression. Blasts in bone marrows of CMML patients were likely to have abnormal surface marker expression, including aberrant CD7 and CD56 expression, decreased CD45 expression, increased CD34 and CD117 expression, and alterations (increase or decrease) in CD13, CD33, and HLA-DR [15,19]. Moreover, there was a significant difference in the number of abnormalities in the blasts per case when the CMML patients were compared to control subjects [15]. Unfortunately, the use of blast immunophenotype in the diagnosis of CMML is limited by the relatively low percentage of blasts in CMML specimens, particularly in cases of CMML-0 (< 2% blasts in blood, < 5% blasts in the bone marrow), the cases in which ancillary testing would most likely be beneficial.

A relatively recent report indicated that there is an association between myeloid dendritic cells and CMML [20]. While most attention regarding dendritic cells in CMML has been focused on plasmacytoid dendritic cells [1], Meyerson et al. showed that CMML blood and bone marrow specimens were more likely to have an increased percentage of CD1c-positive myeloid dendritic cells than. However, this identified at most 50% of CMML cases, limiting the usefulness of increased percentage of CD1c-positive myeloid dendritic cells as a diagnostic test for CMML [20].

4. Monocyte subset distribution

Despite efforts by the many groups cited above, the detection of aberrant surface markers, whether individual or multiple, has not led to a diagnostic test for CMML with both high sensitivity and specificity. A new and fruitful avenue of research focuses on the distinct distribution of monocyte subsets in CMML. Based on CD14 and CD16 expression, circulating monocytes can be separated into three subsets, classical/MO1 ($CD14^{bright}CD16^{-}$), intermediate/MO2 ($CD14^{bright}CD16^{+}$), and nonclassical/MO3 ($CD14^{dim}CD16^{+}$) (Fig. 1a) [21]. Over the last few years, several studies have shown that changes in the percentages of the subsets can differentiate CMML cases from both normal controls, reactive monocytoses, and other hematological malignancies (summarized in Table 1). The MO1 subset, which comprises the vast majority of the circulating monocyte pool, has received the most attention to date. In 2015, Selimoglu-Buet et al. showed that there is an increased percentage of the MO1 subset (MO1 / total monocytes) in the peripheral blood and bone marrow of CMML patients compared to both healthy controls and patients with other hematological malignancies and that a cutoff value of > 94% MO1 in blood identified CMML cases with a sensitivity of > 90% and a specificity of > 95% [22]. This increase in MO1% was independent of specific molecular and cytogenetic changes [22,23]. Subsequent studies showed that this increase in MO1% can, with high specificity and sensitivity, differentiate CMML from MDS [24] and MPN [25] and predict which MDS cases are likely to evolve into CMML [26]. Interestingly, while the 2015 Selimoglu-Buet et al. report focused on the increased MO1% in CMML as a diagnostic tool, they also state that the increase in the total number of MO1s comes at the expense of the MO3 population in CMML [22]. We have recently shown that while MO2% was not useful in identifying CMML cases, a decreased MO3%, like an increased MO1%, could also be used to identify CMML cases with sensitivity and specificity at least comparable to that of increased MO1% (Fig. 1b) [27]. In fact, in this study, a decreased MO3% showed better sensitivity than an increased MO1% at identifying CMML cases in both blood and bone marrow specimens. This is at least in part due to correctly classifying a subset of CMML cases that have an increased MO2% and are incorrectly classified as