Acute Myeloid Leukemia Immunophenotyping by Flow Cytometric Analysis

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KEYWORDS

• Flow cytometry • Acute myeloid leukemia • Mixed phenotype acute leukemia
• Minimal residual disease

KEY POINTS

- Immunophenotyping by flow cytometry plays a critical role in the diagnosis and classification of AML allowing for blast identification, lineage assignment, and immunophenotypic characterization.
- Flow cytometry can provide useful clues to underlying genetics, reliably distinguish AML from other precursor neoplasms that may be in the differential diagnosis, and provide insight to prognosis.
- AML MRD assessment has been demonstrated to be a particularly powerful tool in AML prognostication.

INTRODUCTION

Over the past 20 years, immunophenotyping by flow cytometry has become an essential laboratory tool in the diagnosis and classification of several hematologic malignancies. The role of this technology in evaluating acute leukemia and lymphoma is particularly well established and widely implemented.[1,2] In acute myeloid leukemia (AML), flow cytometry is crucial for detection of leukemic blasts, blast lineage assignment, and identification of aberrant immunophenotypic features that allow distinction of abnormal blast populations from normal progenitors.

With the elucidation of the antigen expression pattern of a given hematopoietic cell lineage during normal maturation,[3–5] assignment of individual cells to a specific lineage and stage of maturation is achieved with high confidence using a unique combination of markers. Leukemic blasts frequently show immunophenotypic deviation

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from the antigenic expression pattern seen on normal hematopoietic progenitors of similar lineage and maturation stage. This basic principle allows for the recognition of leukemic blasts, determination of the immunophenotype, and subsequent classification in AML.

GENERAL APPROACH TO GATING

Population Identification

Specimens typically assessed by flow cytometry for involvement by AML include peripheral blood and bone marrow. In these specimens, the neoplastic blasts are often present in a background of normal hematopoietic progenitors. CD45 versus side scatter (SSC) gating is an initial strategy for blast identification in many clinical laboratories and provides a useful starting point to distinguish major hematopoietic cell populations (Fig. 1).[6,7] Based on the different intensity of CD45 expression and SSC on the hematopoietic lineages, this method allows for reliable identification of mature lymphocytes, monocytes, maturing granulocytes, myeloid blasts, and lymphoid blasts (hematogones). Although CD45 versus SSC gating is useful to guide initial assessment and allows tracking of a population of interest between tubes, the populations discriminated by this strategy are often not pure and additional lineage-specific markers must be used for definitive population identification.

Panel for Immunophenotyping

The expanded myeloid blasts in AML are often first recognized in a CD45 versus SSC defined "blast gate" with decreased CD45 expression and intermediate SSC.[4] However, several other populations fall within "blast gate" in addition to blasts, including basophils, plasmacytoid dendritic cells, hypogranular myeloid cells, and early monocytic cells. Therefore, additional markers are required to differentiate blasts from other populations in the blast gate.

The antibodies included in a panel used for the diagnosis of AML should be capable of confirming the immaturity of the suspected blast population, assigning the lineage, and delineating the aberrant antigenic expression pattern that distinguishes neoplastic blasts from normal progenitors. For myeloid blasts, a combination of CD34 and CD117 can usually be used to define immaturity. Accurate lineage assignment is a critical component in the diagnosis of acute leukemia. For this reason, the Bethesda International Consensus Conference recommends that the initial assessment of a new acute leukemia includes evaluation of myelomonocytic and lymphoid markers.[8] Myeloid lineage is suggested by expression of antigens including CD13, CD15, CD33, and MPO, whereas monocytic lineage is suggested by expression of antigens including CD4, CD14, bright CD33, and CD64. Assessment of lymphoid markers should be included in the initial evaluation of AML to confirm correct lineage assignment and to evaluate for aberrant expression of a nonlineage marker on an abnormal myeloid blast population. In this regard, a panel for acute leukemia evaluation should include B-cell markers (ie, CD19, CD22, and/or cytoplasmic CD79a) and T-cell markers (ie, CD2, surface and cytoplasmic CD3, CD5, and CD7). Once lineage is confidently assigned, a comprehensive assessment of antigens expressed by the blast population should be made to determine how the aberrant blast immunophenotype differs from that of normal progenitors. Aberrant antigenic expression on the leukemic blasts may include the following[4]: overexpression, underexpression, or loss of an antigen typically expressed by a myeloid blast; asynchronous antigen expression; cross-lineage antigen expression; and abnormally homogeneous antigen expression.