Flow Cytometry Rapidly Identifies All Acute Promyelocytic Leukemias With High Specificity Independent of Underlying Cytogenetic Abnormalities

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Key Words: Flow cytometry; Acute promyelocytic leukemia; Cytogenetics; HLA-DR; CD11b; CD11c; CD117

Abstract

Acute promyelocytic leukemia (APL) is a highly aggressive disease requiring prompt diagnosis and specific early intervention. Immunophenotyping by flow cytometry (FCM) facilitates a rapid diagnosis, but commonly used criteria are neither sufficiently sensitive nor specific. With an antibody panel for diagnostic screening in routine practice, we found all 149 APL cases in this study exhibited a unique immunophenotypic profile, ie, a characteristic CD11b– myeloid population and absent CD11c expression in all myeloid populations; 96.6% of cases also lacked HLA-DR expression. These distinctive features allowed recognition of all unusual cases phenotypically resembling the regular myeloblasts (CD34+/HLA-DR+) or granulocytes (CD117–/CD34–/HLA-DR–). FCM effectively identified all 19 APL cases with variant translocations, including cases with a normal karyotype due to a cryptic submicroscopic t(15;17)(q22;q21), t(11;17)(q23;q21) that escaped the detection by fluorescence in situ hybridization for t(15;17) and der(15)ider(17)(q10) that lacked a simple reciprocal t(15;17). When APL-associated profiles were validated against 107 AML cases of non-APL subtypes, including 51 HLA-DR– cases, the diagnostic specificity and positive predictive value were 98%. FCM effectively provides independent detection of APL during diagnostic workup and harmonizes with the subsequent molecular cytogenetic diagnosis.

Acute promyelocytic leukemia (APL) constitutes 6% to 8% of all adult acute myeloid leukemia (AML) in the United States and 20% to 25% of AML cases in Latin America. If untreated, APL typically confers an aggressive disease course with a tendency toward life-threatening coagulopathy. The ability to rapidly establish the diagnosis before disease progression becomes irreversible is crucial for triggering specific early intervention, which has led to markedly decreased mortality and vastly improved survival. The t(15;17)(q22;q21) PML-RARA defines the molecular hallmark of APL and serves as the molecular basis of the highly effective therapy with all-trans retinoic acid. Because early death is the major contributor to the overall mortality, early introduction of all-trans retinoic acid at the first suspicion of an APL diagnosis before it is confirmed genetically is considered an important factor in management.

Diagnosis of APL traditionally relies on the morphologic identification of the leukemic cells, followed by confirmatory molecular cytogenetic detection of the t(15;17) and other rare variant translocations by karyotyping, fluorescence in situ hybridization (FISH), and reverse transcriptase–polymerase chain reaction (RT-PCR). Unfortunately, a definitive morphologic diagnosis may be difficult in many community practice settings, especially when dealing with inadequate aspirate smears and morphologic variants. Furthermore, specific FISH and RT-PCR analyses for detection of t(15;17) are typically performed only on suspected cases. Cytogenetic analysis is relatively time-consuming. When there are poor culture and suboptimal resolution of chromosomes, the results will require further FISH confirmation. For cases that have a normal karyotype or a negative FISH result targeting only t(15;17),
CD33, and myeloperoxidase. In addition, FCM is also used in the context of myeloid antigen expression, such as CD13, CD11c, CD18, CD45RO, CD105, and CD133 in a subset of APLs with a possible poor prognosis.

However, despite a large number of publications, there has been no unified APL phenotypic profile across the spectrum. Most published immunophenotypic features of APL based on FCM, as a whole, demonstrated only moderate reliability. They are not found in all APL cases and are insufficient for separating APL from other types of AML, particularly from those lacking HLA-DR expression. For instance, expression of CD34 has been demonstrated in 20% to 41% of APL cases. Expression of HLA-DR has been documented in up to 9% of APL cases; it is also absent in 19% to 24% of other AML subtypes. Lack of both CD34 and HLA-DR has been reported in 10% of non-APL AML cases as well. In several studies, only 50% to 78% of APL cases were documented to have CD117 expression, despite CD117 expression being described for nearly all cases in another study. Of all published studies, a surrogate profile based on the lack of HLA-DR, CD11a, and CD18 expression seems to be relatively more sensitive and specific when used in a specific APL study of the Eastern Cooperative Oncology Group. However, the study was not designed and performed to screen unbiased samples, and the value of CD11a and CD18 expression has not been well documented to be useful in broad diagnostic workups for hematopoietic malignancies other than APL. Thus, reliably identifying all APL cases by an FCM screening panel remains a challenge.

In this article, we report that an aberrant immunophenotypic profile of maturing myeloid cells recognizes 100% of APL cases with high specificity, including cases lacking typical flow cytometric features of APL and cases lacking straightforward cytogenetic results owing to aberrant translocations. While an accurate diagnosis always requires a multimodal approach, a flow cytometric diagnosis of APL based on the widely used findings of CD34– and HLA-DR– blasts is inexpedient.

### Materials and Methods

The study was conducted in accordance with the Declaration of Helsinki. Cases in this study were randomly obtained from routine clinical diagnosis after a screening FCM analysis between 2000 and 2005. The final diagnosis of each case was based on the identification of t(15;17) or other variant translocations by molecular cytogenetic analyses. A cohort of 132 APL cases was randomly collected initially and retrospectively analyzed for immunophenotypic profiles. Because only 2 cases with a variant translocation were initially found and not all cases had a complete karyotype in addition to FISH and PCR results, we expanded the study to search for more cases with variant translocations from an independent cytogenetic database. An additional 17 cases with variant translocations and concurrent FCM results were identified, which brought the total number of APL with FCM results to 149 cases in this retrospective study; a total of 19 cases had variant translocations.

Four-color FCM analysis was performed for all cases using FACSCalibur, and the data were analyzed with CellQuest software (BD Biosciences, San Jose, CA) in accordance with the international consensus recommendations on flow cytometric analysis of hematolymphoid neoplasia. The antibodies in our routine panel included CD34, CD117, HLA-DR, CD2, CD11b, CD11c, CD13, CD14, CD16, CD33, CD56, and CD64 (BD Biosciences, San Jose, CA). In conjunction with other antibodies against lymphoid antigens, they constituted our standard screening panel for diagnosis of all acute and chronic leukemias, lymphoproliferative disorders, myelodysplastic syndromes, and chronic myeloproliferative disorders.

For the purpose of comparing our results with the literature, we arbitrarily defined in this study staining of 20% or

### Table I

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Conjugate</th>
<th>Antibody Clone</th>
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<tbody>
<tr>
<td>HLA-DR</td>
<td>FITC</td>
<td>TU36</td>
</tr>
<tr>
<td>CD34</td>
<td>PE-Cy7</td>
<td>8G12</td>
</tr>
<tr>
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<td>PerCP-Cy5.5</td>
<td>104D2</td>
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<tr>
<td>CD2</td>
<td>PE-Cy7</td>
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<td>HL10a</td>
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<td>APC</td>
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<td>PE-Cy7</td>
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<tr>
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<td>P67.6</td>
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<td>FITC</td>
<td>MY31</td>
</tr>
<tr>
<td>CD64</td>
<td>PE</td>
<td>10.1</td>
</tr>
</tbody>
</table>

APC, allophycocyanin; Cy, cyanin; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin chlorophyll protein.

* All antibodies were purchased from BD Biosciences, San Jose, CA.
more of leukemic cells for CD34 and HLA-DR as strongly positive and 5% to 20% of cells as partially or weakly positive. The basic gating strategy was based on the intensity of CD45 staining vs characteristics of right-angled side scatter channels (SSC) using a standard template, so myeloid populations could be separated into blast, granulocyte, and monocyte regions. An open-gate strategy was also used in parallel for all cases to analyze antigen expression in blastic and myeloid populations. We then analyzed the immunophenotypic profiles of these cases in relation to individual antigen expression and karyotype findings. Since our study was aimed at improving the ability to identify all APL cases using FCM, antigens known to be expressed in only a subset of APL (eg, CD2 and CD56) will not be discussed.

The immunophenotypic results of the randomly collected 132 APL cases in this study were further compared with a separate series of AML cases that comprised 107 cases of other subtypes, for which a diagnosis of APL was definitively excluded. This control series was a collection pooled from other studies and composed of a significant number of cases with immunophenotypic features resembling APL, including 51 cases of HLA-DR– (47.7%) and 58 cases of CD34– AML (54.2%). Further subtyping of this group was not pursued in detail because of a lack of morphologic data in many cases.

Routine molecular cyogenetic studies included metaphase cytogenetic analysis, FISH, and RT-PCR for APL-specific t(15;17) PML-RARA. The cytogenetic analysis was performed by standard G-banding techniques on unstimulated culture. Karyotypic annotation was based on the International System for Human Cytogenetic Nomenclature guidelines. The FISH procedure was performed according to the standard protocol using a dual-color, dual-fusion probe set for t(15;17)(q22;q21) from Abbott, Downers Grove, IL. An additional 17q21 break-apart probe set (Abbott) was also used in selected cases to capture variant translocations disrupting the RARA locus. The real-time RT-PCR assay for diagnosis and follow-up detected 3 variant PML-RARA transcripts, namely long, short, and variant forms (bcr1-bcr3).32

**Results**

**Common Immunophenotypic Profile Associated With the Majority of APL Cases**

APL cells characteristically displayed highly variable SSC across wide ranges spanning from the blast region to the granulocyte region Image 1A. In a minority of cases, the extent of SSC was narrowed to be within the blast region similar to typical myeloblasts Image 1B, though appreciable heterogeneity remained. A small number of cases displayed unusually high SSC that completely spared the blast region, indistinguishable from the profile of normal peripheral blood dominated by granulocytes Image 1C. As a whole, the most common finding unique to APL was the absence of a distinct blast population separable from the rest of myeloid cells (Image 1).

An analysis of the 149 APL cases revealed that APL cells typically expressed CD117 at least partially (94.6%) but lacked detectable HLA-DR and CD34 in 96.6% and 76.5% of the cases, respectively. When combined, only 76.5% had the triad of CD117+/CD34–/HLA-DR–. All cases expressed myeloid antigen in an invariable pattern of CD33 stronger than CD13 (100%) and lacked expression of mature myeloid antigen CD10 and CD16; 71.2% of cases had variable expression of CD64. Of cases with variable expression of CD2 or CD56, 77.6% of CD2+ and 47.4% of CD56+ cases were also CD34+.

**Combination of Absent CD11b, CD11c, and HLA-DR Identifies 100% of APLs**

In addition to lack of HLA-DR in a vast majority of cases, all APL cases (149/149) invariably lacked CD11b and CD11c expression Table 2. Specifically, the APL cells were best viewed as a homogeneous CD11b– myeloid population in the granulocyte region, which was distinctively segregated from the normal myeloid cells in the scattergram (Image 1). The detection of such an abnormal CD11b– myeloid population was independent of morphologic variation and whether the immunophenotypic features were “blastic” (CD34+/CD117+/HLA-DR+ and low SSC; Image 1B) or “granulocytic” (CD34+/CD117–/HLA-DR– and high SSC; Image 1C). In contrast, normal granulocytes in the peripheral blood always had bright CD11b, and normal bone marrow cells always had variable CD11b displaying a top-heavy pattern. Maturing myeloid cells and blasts in APL also lacked CD11c, whereas normal maturing myeloid cells and blasts in AML of most other subtypes regularly expressed CD11c.

**Immunophenotypic Features Identify APL at Different Stages of Maturation**

The distinctive double negative (CD11b–/CD11c–) or triple-negative profiles (HLA-DR–/CD11b–/CD11c–) enabled us to identify 2 groups of APL (8.7% of all cases) with unusual immunophenotypic features in addition to the classical immunophenotype (Image 1A). APL cells might resemble the conventional myeloblasts confined within the blast region with low SSC and characteristically express HLA-DR and strong CD34 (5/149 [3.4%]). A case with strong expression of HLA-DR and CD34 is shown in Image 1B. Notably, all HLA-DR+ cases were also CD34+ and CD117+, consistent with features of early myeloid progenitor cells. Rare cases of APL were also dominated by immunophenotypic features of granulocytes (8/149 [5.4%]) characterized by high SSC.
Table 2

<table>
<thead>
<tr>
<th>Immunophenotypic Features of APL*</th>
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<tr>
<td><strong>No. of Cases</strong></td>
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<td>------------------</td>
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<tr>
<td><strong>Total</strong></td>
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<tr>
<td>149</td>
</tr>
<tr>
<td>Random collection (total)</td>
</tr>
<tr>
<td>Blastic variant</td>
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<tr>
<td>Classical variant</td>
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<tr>
<td>Granulocytic variant</td>
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<td>Additional cases with variant translocations</td>
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APL, acute promyelocytic leukemia.

* Data are given as number (percentage).

† Selected from a separate cytogenetic database that had flow cytometric results.
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and the complete absence of CD117, CD34, and HLA-DR (Image 1C). There was no evidence for increased blasts or immature cells in these cases based on SSC or antigen expression alone.

Since we have included the lack of CD11b for APL diagnosis for many years, we found in this retrospective study that a vast majority of cases were reported initially to be at least “suspicious” for APL. The exceptions included the following: 1 case with strong CD34 and HLA-DR, which was described as AML without further classification; 2 cases with a small population of CD34+ blasts that were reported to be suspicious for myelodysplasia; and 4 cases with a granulocytic immunophenotype without CD117 expression for which a correct flow cytometric diagnosis was missed initially. These 7 cases invariantly had loss of CD11b and CD11c, which was easily recognized in the retrospective review but overlooked initially. Of note, an adequate blood or marrow aspirate smear was not available for any of these cases at the time of flow cytometric analysis.

Immunophenotypic Profile of APL Is Independent of Underlying Cytogenetic Findings

Despite the fact that complex karyotypes with various numeric or structural chromosomal changes were detected in approximately one third of APL cases in addition to the pathognomonic t(15;17)(q22;q21), they had no observable impact on the overall immunophenotype in our study. A total of 19 cases with variant translocations were identified in our random cohort (2 cases) and the extended search (17 cases). Five cases were initially reported to have a normal karyotype due to a submicroscopic t(15;17)(q22;q21) PML-RARA, which was detected subsequently by FISH analysis. Eleven cases were characterized by an unbalanced translocation der(15)der(17)(q10) without a classical reciprocal t(15;17). FISH analysis for t(15;17) detected classic dual fusion signals in all cases. Three cases had a t(11;17)(q23;q21), which was not detected by the expedited FISH targeting t(15;17). A diagnosis of APL was confirmed by subsequent karyotyping.

FISH analysis using the 17q21 break-apart probe was also performed on 2 of 3 cases, which detected a translocation disrupting the RARA locus. In all 19 cases, FCM effectively provided the preliminary diagnosis based on the signature immunophenotype of APL despite the initial negative cytogenetic or FISH results.

Phenotypic Distinctions Between APL and Other Subtypes of AML

We further compared the immunophenotypic features between the 132 APL cases in the random series and 107 cases of non-APL AML. Figure I summarizes the value of antigens used in the differential diagnosis of APL by FCM. Of the routinely described antigens, the lack of HLA-DR was a highly sensitive marker for APL (sensitivity, 96.2%). The presence or absence of CD34 and/or CD117, alone or

\[
\begin{array}{|c|c|c|}
\hline
& APL (n = 132) & Non-APL (n = 107) \\
\hline
CD11b– (P < .0001) & 100 & 13 \\
CD11c– (P < .0001) & 100 & 11 \\
CD11b+/CD11c– (P < .0001) & 96 & 2 \\
HLA-DR–/CD34+ (P = .0060) & 96 & 2 \\
CD34+ (P = .1740) & 94 & 2 \\
HLA-DR– (P = .0010) & 94 & 94 \\
HLA-DR–/CD11b– (P < .0001) & 74 & 48 \\
HLA-DR–/CD11b–/CD11c– (P < .0001) & 74 & 48 \\
CD11b–/CD11c– (P < .0001) & 74 & 48 \\
CD11b– (P < .0001) & 74 & 48 \\
CD11c– (P < .0001) & 74 & 48 \\
\hline
\end{array}
\]

Figure I Expression frequency of antigens used in immunophenotypic profiles for differential diagnosis between acute promyelocytic leukemia (APL) and acute myeloid leukemia of non-APL subtypes. The phenotypic features are listed on the left, and the percentages of APL (black bar, n = 132) and non-APL (white bar, n = 107) cases exhibiting these features are shown on the right. The \(P\) values depict the statistical significance between the 2 groups based on the Fisher exact test.
combined, improved neither the diagnostic sensitivity nor the specificity. Instead, the detection of a distinct CD11b– myeloid population and the lack of CD11c expression in blasts and myeloid cells stood out as more reliable parameters in a correct context. The detection of a distinct CD11b– myeloid population combined with the absence of CD11c had a sensitivity and negative predictive value of 100%.

However, the lack of HLA-DR was also seen in 51 cases (47.7%) of our non-APL control series. Thus, it had a relatively low specificity (52.3%) and a positive predictive value of only 71.3%. In comparison, the lack of CD11b or CD11c alone was seen in only 13.1% and 11.2% of non-APL cases; both had a positive predictive value of better than 90%. The detection of combined CD11b– myeloid population and CD11c– myeloblasts improved specificity and positive predictive value to more than 98%. Such a finding was independent of HLA-DR expression and was particularly crucial for excluding HLA-DR– non-APL cases and supporting the diagnosis of rare HLA-DR+ APLs. Lack of both HLA-DR and CD11b was seen in 12.1% of cases in our control non-APL series, for which the expression of CD11c became especially useful to help distinguish APL from other AML subtypes. Still, we found 2 non-APL cases (1.9%) that had the triple-negative profile. These 2 cases were not only phenotypically identical to APL, but also had APL-like hypergranular cytoplasm. However, a t(15;17) or other translocation involving the RARA locus was not detected by karyotyping and FISH analyses in either case.

**Discussion**

In this study, we demonstrated that all APL cases in a large cohort could be rapidly identified by FCM using a routine screening panel (sensitivity, 100%), irrespective of their differentiation stages, morphologic variations, and karyotypic complexity. Equally important, we also validated the APL immunophenotypic profile against a series of AML cases and achieved a diagnostic specificity and positive predictive value greater than 98%. Although detection of t(15;17) and variant RARA translocations should serve as the “gold standard” for directing specific therapy, FCM provides rapid early recognition of APL that overcomes uncertainty of the morphologic diagnosis early on and confusion resulting from an initial negative cytogenetic or FISH result during the diagnostic process. Thus, besides rendering a rapid preliminary diagnosis for typical APL cases, the results of FCM maximize the benefits of molecular cytogenetic testing by expediting additional studies and limiting the use of expensive tests when they are unnecessary.

Our study emphasizes for the first time that APL immunophenotypic profiles are independent of the underlying cytogenetic variations. FCM is capable of identifying APL with cryptic translocations unrecognizable by metaphase G-banding cytogenetic analysis, such as those masked in a normal karyotype, chromosome derivatives, or those that are beyond the detection range of commonly expedited FISH analysis for t(15;17). The presence of other numeric or structural abnormalities in the context of t(15;17)(q22;q21) seems to have no impact on the APL immunophenotype. Because of the remarkable consistency, FCM results should be valued as an independent parameter for the APL workup, even when initial cytogenetic results are negative.

Our unbiased diagnostic screening found that APL consistently displays a unique immunophenotypic profile. Contrary to the common belief that blasts in APL are characterized by CD117 expression in the absence of CD34 and HLA-DR, we and others found that these features are by no means specific or sensitive. It is the aberrant profile of maturing myeloid cells that is the most reliable diagnostic indicator. The diagnostic criteria should include the following: (1) the presence of a distinct CD11b– myeloid population, especially among cells in the granulocyte region; (2) the lack of HLA-DR and CD11c in all myeloid cells, including myeloblasts; (3) CD117 expression in myeloid populations; and (4) myeloid cells with highly variable side scatters without a distinct blast population based on the CD45 and SSC display. While an expanded myeloid population expressing CD117 is typical of AML, the lack of CD11b, CD11c, and HLA-DR expression in myeloid cells, including blasts, is typical of APL. To some degree, our results are in agreement with a surrogate marker profile for APL featuring the lack of CD11a, CD18, and HLA-DR and the findings of another study on adhesion molecules, since CD11a, CD11b, CD11c, and CD18 are all members of the β2-integrin family expressed in mature myeloid cells. However, CD11b and CD11c used in our study are also integral components of common FCM panels proven to

<table>
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<tr>
<th>APL-Associated Features</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
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<tr>
<td>CD11b– myeloid population</td>
<td>100</td>
<td>86.9</td>
<td>90.4</td>
<td>100</td>
</tr>
<tr>
<td>CD11c</td>
<td>100</td>
<td>88.8</td>
<td>91.7</td>
<td>100</td>
</tr>
<tr>
<td>CD11b/CD11c–</td>
<td>100</td>
<td>98.1</td>
<td>98.5</td>
<td>100</td>
</tr>
<tr>
<td>CD117–</td>
<td>100</td>
<td>98.1</td>
<td>98.5</td>
<td>100</td>
</tr>
<tr>
<td>HLA-DR–</td>
<td>96.2</td>
<td>90.6</td>
<td>92.0</td>
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</tr>
<tr>
<td>CD34–</td>
<td>96.2</td>
<td>52.3</td>
<td>71.3</td>
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<td>CD18–</td>
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<tr>
<td>CD11c–</td>
<td>73.5</td>
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<td>65.0</td>
</tr>
<tr>
<td>HLA-DR–</td>
<td>73.5</td>
<td>61.7</td>
<td>70.3</td>
<td>65.3</td>
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</table>

APL, acute promyelocytic leukemia; NPV, negative predictive value; PPV, positive predictive value; +, positive; –, negative.
be important for the diagnosis of other hematopoietic malignancies, such as myelodysplasia,7 T-large granular lymphocytic leukemia,38 and hairy cell leukemia,39 that represent significant clinical differential diagnoses of panmyelopения.

Our study revealed that 9% of APLs were diagnostically challenging owing to unusual immunophenotypic variations beyond the common definition in the current literature. APL may be reminiscent of common myeloblasts with strong CD34 and HLA-DR, imposing difficulties for AML classification. APL may also lack detectable blast antigens, hardly distinguishable from neutrophils. This is particularly important for cytopenic patients with few recognizable leukemic cells in blood smears. Because high SSC together with the lack of CD34, CD117, and HLA-DR are normal for granulocytes in the peripheral blood, these cases can be easily viewed as nonneoplastic, a high risk of misdiagnosis that has not drawn enough attention in the literature.

Expression of CD117 traditionally serves as the indicator of myeloid immaturity in APL, but it varies among cases. The detection rates range from 50% to 78%,10,13,28 to more than 99% of APL cases in the literature. Our data indicate a vast majority of APL (94.6%) was variably positive for CD117. It is imperative to analyze all myeloid populations with open gates because an analysis of cells in only the blast region will risk missing a majority of CD117+ APL cells in cases with high SSC. However, rare exceptions exist. Our study identified 5.4% (8/149) of APL cases with no evidence of CD117 expression. The lack of CD117 expression was not due to technical issues or arbitration in cases with low-level (low-intensity) CD117. Four of these cases were missed at the initial diagnosis. On reanalysis, the characteristic pattern of the unique CD11b– myeloid population rose as the most reliable diagnostic indicator.

Normal myeloblasts and promyelocytes do not express CD11b, a myeloid antigen expressed at high levels on terminal maturation. Expression of CD11b is heterogeneous in the normal bone marrow owing to the spectrum of myeloid maturation but is homogeneously bright in the normal peripheral blood owing to the dominance of terminally differentiated granulocytes. Because accumulation of uniform CD11b– promyelocytes never occurs during the dynamic process of normal maturation, a homogeneous CD11b– population distinctively isolated from the rest of myeloid components tends to reflect a clonal nature of APL cells with attenuated downstream maturation. We emphasize that the lack of CD11b in APL refers to an isolated CD11b– myeloid population in the granulocyte region rather than CD11b– blasts per se. It is universally present in APL cases and is of paramount importance for diagnosis. This feature is not only independent of morphologic and genetic variations but also effectively distinguishes APL from other nonleukemic conditions with heterogeneous CD11b down-regulation, such as myelodysplasia after treatment with filgrastim and erythropoietin, and folate and vitamin B12 deficiencies.

HLA-DR is normally expressed in myeloblasts and distinguished during maturation at the stage of promyelocyte. The lack of HLA-DR and CD11b provides a narrow window for the detection of promyelocytes. Only 1 (0.7%) of 149 cases of APL in our study had strong HLA-DR expression, and another 4 (2.7%) of 149 cases had weak HLA-DR expression. Our results reiterated the notion that the lack of HLA-DR is one of the most sensitive findings associated with APL, even though HLA-DR expression was reported in up to 9% of APL cases in the literature.16 However, it should be kept in mind that lack of HLA-DR alone is not APL-specific and has been well documented in up to 20% of cases of other AML subtypes.26-28,40 Our AML control series comprised 47.7% of cases without HLA-DR and 54.2% without CD34 expression, which imposed even bigger challenges to our ability to recognize APL and further validated our study results. A distinct CD11b– myeloid population rose again as the key feature preserved in all HLA-DR+ APL cases, and yet the feature was absent in a vast majority of AML cases of other types.

CD11c is regularly expressed in immature and mature myeloid cells. In our study, the absent CD11c expression added crucial diagnostic specificity for APL. Rare cases of AML without maturation lack HLA-DR and CD11b, display heterogeneous high SSC, and have a mixture of blasts with hypogranular and azurophilic hypergranular cytoplasm in aspirate smears. These features are nearly identical to those of APL and may lead to the claim that APL and other AMLs were indistinguishable by FCM and morphologic studies alone.51 Unlike APL, however, they consistently express CD11c in a majority of cells and do not exhibit RARA translocations (H.Y.D. et al, unpublished data). In addition, identifying CD11c– and HLA-DR– blasts (CD117+) helps distinguish residual APL from normal regenerating blasts using a routine FCM panel, a feature useful for patient follow-up.

It is necessary to view the profile as a whole in analyzing leukemic cells because antigenic aberrancies occur frequently in AML cells.42 Microgranular variants of APL may express a higher percentage of CD34 together with aberrant CD2 and/or CD56 expression.21,43 Normal blasts and most AMLs do not express CD11b; thus, lack of CD11b in the blast population only has limited diagnostic value. However, aberrant CD11b expressed in AML may be seen as well. Since completion of this study, we have also seen an APL case with a distinct blast population on CD45/SSC plot and a case comprising only a uniform population of CD34+ blasts (not shown). Thus, relying on any single feature for diagnosis should always be avoided.

All APL cases can be reliably recognized by a routine FCM screen. FCM analysis overcomes complications of other tests that may miss the diagnosis. The results of FCM also
help discriminate APL from other AML subtypes in a vast majority of cases. Therefore, swift flow cytometric analysis should be considered as an independent component of APL workup complementary to cytogenetic testing, even in the rapidly evolving molecular era.

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References


