Clinical and Pathologic Features of Secondary Acute Promyelocytic Leukemia

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Key Words: Acute promyelocytic leukemia; APL; Therapy-related acute myeloid leukemia; Therapy-related myeloid neoplasm; Acute myeloid leukemia with myelodysplasia-related changes; Flow cytometry

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Abstract

Acute promyelocytic leukemia (APL) is a relatively common form of acute myeloid leukemia (AML) that has an excellent prognosis. In contrast, secondary acute myeloid leukemias, including therapy-related AML and AML with myelodysplasia-related changes, have a relatively poor prognosis. We identified 9 cases of APL at our institution in which there was a history of chemotherapy, radiotherapy, chronic immunosuppression, or antecedent myelodysplastic syndrome. The clinical and pathologic findings in these cases of secondary APL were compared with the clinical and pathologic findings in cases of de novo APL. We found that secondary and de novo APL had abnormal promyelocytes with similar morphologic and immunophenotypic features, comparable cytogenetic findings, comparable rates of FMS-like tyrosine kinase mutations, and similar rates of recurrent disease and death. These data suggest that secondary APL is similar to de novo APL and, thus, should be considered distinct from other secondary acute myeloid neoplasms.

Upon completion of this activity you will be able to:
- discuss the clinical characteristics of secondary acute promyelocytic leukemia (APL).
- discuss the phenotypic and morphologic characteristics of APL.
- discuss the prognosis of primary and secondary APL.

Acute promyelocytic leukemia (APL) is defined as an acute myeloid leukemia (AML) with a predominance of abnormal promyelocytes and retinoic acid receptor α (RARα) gene rearrangements. Most cases of APL contain the fusion of the promyelocytic leukemia (PML) gene on chromosome 15 with the RARα gene on chromosome 17, although occasional cases have variant translocations. The fusion messenger RNA PML-RARα encodes a chimeric protein that impairs normal differentiation at the promyelocyte stage. The PML-RARα fusion gene product is specifically targeted by the drug all-trans retinoic acid, which promotes granulocytic differentiation. As a result, APL is considered one of the most treatable AMLs and typically has an excellent prognosis.

In contrast, AMLs that follow chemotherapy (referred to as therapy-related myeloid neoplasms in the World Health Organization 2008 classification) or AMLs arising from myelodysplastic syndrome (AML with myelodysplasia-related changes) have an unfavorable prognosis. Cases of therapy-related AML (t-AML) and AML with myelodysplasia-related changes (MDS-AML) tend to have characteristic pathologic features, including background dysplasia, and cytogenetic abnormalities such as partial loss of chromosome 5 or 7 or a complex karyotype. These secondary myeloid neoplasms often do not respond well to treatment with standard chemotherapy. Although all of these secondary neoplasms are typically considered to have a poor prognosis, the clinical course and prognosis of patients with t-AML with recurrent genetic translocations has not been extensively studied.

Some patients with the balanced translocation t(15;17) also have a history of cytotoxic chemotherapy or radiation therapy. Despite the presence of a recurrent genetic...
abnormality, the patients are still considered to have t-AML. The prognosis of patients with therapy-related APL (t-APL) has been reported to be better than the prognosis of patients with other t-AMLs, although it has also been reported that patients with t-APL have similar survival times to patients with other t-AMLs. Moreover, patients with t-APL have been reported to have a shorter median survival time than patients with de novo APL. 

Although several clinical studies have investigated the clinical outcomes in patients with t-APL, there has been little work comparing other biologic characteristics of the disease in patients with t-APL vs de novo APL. Thus, it is not entirely clear whether t-APL should be considered a unique entity.

To identify possible differences between secondary APL and de novo APL, we evaluated the clinical, laboratory, and pathologic findings in all new cases of APL at our institution during the last 10 years. Among 65 well-characterized cases of APL at our institution, we identified 9 that did not arise as the usual de novo APL. One case occurred in a patient with a history of myelodysplastic syndrome (MDS-APL), 6 cases arose following chemotherapy for malignant neoplasms (t-APL), and 2 cases arose in chronically immunosuppressed (CI-APL) patients who were treated with an antimitabolite cytotoxic agent. Although the CI-APL cases are considered separately, these cases also meet the criteria for a therapy-related myeloid neoplasm.

We report that the abnormal promyelocytes in secondary APL are morphologically and immunophenotypically indistinguishable from abnormal promyelocytes of de novo APL and that there was no difference in the occurrence of additional cytogenetic abnormalities. In addition, in this limited series, the prognosis of patients with t-APL was not significantly different from the prognosis of patients with de novo APL.

Flow cytometric immunophenotyping was performed on fresh bone marrow aspirates or specimens of peripheral blood. The material was collected in EDTA or heparin anticoagulant and processed routinely. Cell suspensions were incubated with combinations of 4 monoclonal antibodies (Becton Dickinson, San Jose, CA) that were used at concentrations titrated for optimal staining. In most cases, the panel included antibodies specific for CD45, CD71, HLA-DR, CD33, CD13, CD2, CD3, CD5, CD7, CD19, CD22, CD10, CD34, CD56, CD38, CD14, CD64, CD61, CD11b, CD15, and CD117, although occasional specimens with low WBC counts were subjected to an abbreviated panel. Selected antibody combinations were conjugated to fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein, and allophycocyanin (APC) fluorochromes. Isotypic controls were used for FITC (mouse IgG2a), PE (mouse IgG1), and APC (mouse IgG2a) on all but 2 cases of de novo APL and 1 case of t-APL. Specimens were analyzed on a BDLS FACScalibur flow cytometry system (Becton Dickinson). List mode data files were acquired and analyzed for each specimen using CellQuest and Paint-A-Gate software programs, respectively (Becton Dickinson).

An antigen was considered positive if the leukemic cells showed a homogeneous distribution with the median intensity at least 20 log channels above that seen in the control or if there was a heterogeneous distribution of antigen expression such that a subpopulation of cells was above that seen in the control. Cell populations were classified as dim, moderate, and bright based on their intensity compared with normal promyelocytes.

All flow cytometric data were reviewed by two of us (A.S.D. and M.V.-R.). Clinical data were reviewed by one of us (A.S.D.) per the institutional review board-approved protocol, NA_00043498.

Materials and Methods

All specimens from The Johns Hopkins Hospital, Baltimore, MD, with a diagnosis of APL between January 2000 and July 2010 were included in the study. The pathology database was searched using the key words “acute promyelocytic leukemia,” “promyelocytic,” “promyelocyte,” “microgranular variant,” “microgranular,” and “APL.”

We identified 89 specimens that were diagnosed as APL, and 67 of these cases represented the initial occurrence of the disease. We were unable to find confirmation of appropriate gene rearrangements using cytogenetic or molecular methods in 2 cases of newly diagnosed APL, and these cases were eliminated from the data set. Aspirates from pretreatment bone marrow biopsies were submitted for flow cytometric analysis in all but 1 case. Five specimens had only flow cytometric analysis of the peripheral blood or bone marrow and lacked a corresponding bone marrow biopsy.

Results

Clinical Findings

The 65 new diagnoses of APL included 37 females and 28 males with an average age of 47.5 years (range, 15-83 years). The diagnosis of APL was verified by cytogenetic and/or molecular methods in all cases; 1 case had a variant translocation [t(11;17)] and the remainder had t(15;17). Of the patients, 56 had de novo APL with no history of treatment for another malignancy, chronic immunosuppression, or MDS. The patients with de novo APL had an average age of 46.8 years (range, 15-83 years) and included 24 males and 32 females.

In 6 cases, there was a history of a solid neoplasm that was treated with cytotoxic chemotherapy and/or radiation therapy. The patients with t-APL had an average age of 50.3 years (range, 29-45 years) and included 3 women and 3 men. The 3 women had a history of breast cancer, and the other patients had a history of prostate, testicular, or pancreatic cancer.
The 2 patients who had testicular cancer and prostate cancer were treated with radiation therapy alone and developed t-APL 16 months and 5 years after radiation therapy. One patient with breast cancer was treated with chemotherapy alone including an alkylating agent (cyclophosphamide), a topoisomerase II inhibitor (doxorubicin), trastuzumab, and tamoxifen. She developed t-APL approximately 4 years after chemotherapy. The 3 other patients were treated with chemotherapy and radiation therapy. Of these patients, the patient with pancreatic cancer was treated with the antimetabolites gemcitabine and capecitabine and developed t-APL 1 year after treatment for carcinoma. The other 2 patients had breast cancer and developed t-APL approximately 3 and 11 years after treatment for carcinoma. The patient with t-APL that occurred approximately 3 years after treatment for breast cancer was treated with a topoisomerase II inhibitor (an anthracycline) and trastuzumab. The details of the other patient’s chemotherapy are unknown.

Two patients diagnosed with APL in this series had a history of immunosuppression; one was a 39-year-old woman with a history of a cardiac transplant, and the other was a 69-year-old man with a history of membranous glomerulonephritis. In the first case, APL developed 3.5 years after cardiac transplantation, during which time the patient had been treated with several immunosuppressants, including tacrolimus, sirolimus, cyclosporine, mycophenolate mofetil, and prednisone. In the second case, the patient was treated with mycophenolate mofetil for approximately 4 years before APL developed.

A 69-year-old woman with newly diagnosed APL had been given a diagnosis of MDS with a normal karyotype approximately 1 year before the leukemia developed. The patient was treated supportively with transfusions, but did not receive chemotherapy.

The clinical picture in patients with secondary APL was typical and included easy bruising, fatigue, dyspnea on exertion, sore throat, and rectal bleeding. The median peripheral blood counts at diagnosis in patients with de novo APL and in patients with t-APL are shown in Table 2.

Two of the 56 patients with de novo APL were treated with growth factors prior to diagnosis, which precluded classification as the hypergranular (M3h) or microgranular variant (M3v) of APL; however, in the remaining 54 cases of de novo APL there were 38 (70%) cases of M3h and 16 (30%) cases of M3v.

### Table 2
**Clinical and Pathologic Features in Nine Cases of Secondary APL**

<table>
<thead>
<tr>
<th>Underlying Disorder</th>
<th>Sex/Age at APL Diagnosis (y)</th>
<th>Therapy for Underlying Disorder</th>
<th>Time to APL Diagnosis</th>
<th>FLT3 Status</th>
<th>Cytogenetic Findings</th>
<th>Follow-up After APL Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testicular cancer</td>
<td>M/29</td>
<td>Radiotherapy</td>
<td>18 mo</td>
<td>M3v</td>
<td>ITD (t(15;17))</td>
<td>Alive (75 mo)</td>
</tr>
<tr>
<td></td>
<td>M/59</td>
<td>Radiotherapy</td>
<td>~5 y</td>
<td>M3h</td>
<td>ND (t(15;17) and t(9;11))</td>
<td>Alive (75 mo)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>F/53</td>
<td>Chemotherapy (alkylating agent and topoisomerase II inhibitor)</td>
<td>~4 y</td>
<td>M3h</td>
<td>ITD (t(15;17))</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>F/45</td>
<td>Radiotherapy and chemotherapy (topoisomerase II inhibitor)</td>
<td>~1 y</td>
<td>M3v</td>
<td>ITD (t(15;17) and t(6;12))</td>
<td>Died of metastatic breast cancer (2.5 y)</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>F/65</td>
<td>Radiotherapy and chemotherapy (chemotherapeutic agents unknown)</td>
<td>~11 y</td>
<td>M3h</td>
<td>ND (t(15;17))</td>
<td>Died of rejection/grant failure (8 mo)</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>M/51</td>
<td>Radiotherapy and chemotherapy (antimetabolites)</td>
<td>14 mo</td>
<td>M3h</td>
<td>ND (t(15;17))</td>
<td>Alive at 2 y; no additional follow-up available</td>
</tr>
<tr>
<td>Cardiac transplant</td>
<td>F/39</td>
<td>Immunosuppression (multiples agents, including an antimetabolite)</td>
<td>3.5 y</td>
<td>M3v</td>
<td>ITD (t(15;17) and t(18;10))</td>
<td>Died of rejection/grant failure (1 y)</td>
</tr>
<tr>
<td>Membranous glomerulonephropathy</td>
<td>M/69</td>
<td>Immunosuppression (antimetabolite)</td>
<td>4 y</td>
<td>M3h</td>
<td>ND (t(15;17))</td>
<td>Alive at 1 y; no additional follow-up available</td>
</tr>
<tr>
<td>Myelodysplastic syndrome</td>
<td>F/69</td>
<td>None (supportive transfusions)</td>
<td>1 y</td>
<td>M3v</td>
<td>ITD (t(15;17))</td>
<td>Alive at 1 y; no additional follow-up available</td>
</tr>
</tbody>
</table>

APL, acute promyelocytic leukemia; ITD, internal tandem duplication; NA, not available; ND, not done.

### Table 2
**Laboratory Findings in Cases of De Novo and Secondary APL**

<table>
<thead>
<tr>
<th></th>
<th>WBC Count (/μL)</th>
<th>Platelet Count (× 10^9/μL)</th>
<th>Hematocrit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>De novo APL (n = 56)</td>
<td>3,843 (360-126,000)</td>
<td>29 (7-219)</td>
<td>25.6 (15.5-35.8)</td>
</tr>
<tr>
<td>t-APL (n = 6)</td>
<td>1,870 (210-41,930)</td>
<td>17 (9-109)</td>
<td>27.3 (23.9-33.4)</td>
</tr>
<tr>
<td>CI-APL (n = 2)</td>
<td>6,660 (1,990-11,930)</td>
<td>38 (16-59)</td>
<td>34.6 (33.2-35.9)</td>
</tr>
<tr>
<td>MDS-APL (n = 1)</td>
<td>9,030</td>
<td>12</td>
<td>24.4</td>
</tr>
</tbody>
</table>

APL, acute promyelocytic leukemia; CI-APL, chronic immunosuppressive therapy-related APL; MDS-APL, APL with history of myelodysplastic syndrome; t-APL, therapy-related APL.

* Values are given as median (range) in conventional units. Conversions to Système International units are as follows: WBC count (× 10^9/L), multiply by 0.001; platelet count (× 10^9/L), multiply by 1.0; hematocrit (proportion of 1.0), multiply by 0.01.
of M3v. Of the 6 cases of t-APL, 4 (67%) had the M3h variant and 2 (33%) had M3v. Of the 2 CI-APL cases, 1 had M3v (50%), and the case of MDS-APL also was M3v (Table 1).

There were 7 cases of recurrent APL (13%) in the 56 patients who were originally diagnosed with de novo APL. Of these patients with de novo APL, 8 (14%) died of disease, and the patients who died were on average 61 years of age (range, 49-85 years). None of the patients with secondary APL died of leukemia or ever had evidence of recurrent disease. Two patients with secondary APL died of other causes; a patient with metastatic breast cancer died 31 months after the diagnosis of APL, and the patient with CI-APL who had undergone a cardiac transplantation died of acute graft rejection and failure 8 months after the APL diagnosis. One patient with breast cancer was lost to follow-up, and, at last follow-up, the remaining patients were alive and free of leukemia from 1 to more than 5 years from the time of diagnosis.

Genetic Findings

In 45 cases of de novo APL, a karyotype was performed on the leukemic cells. In 13 cases (29%), there were cytogenetic abnormalities in addition to the t(15;17) or t(11;17). Additional cytogenetic abnormalities seen among the de novo APL cases included +8 (3 cases) and partial del9q (2 cases). In the remaining cases there were a variety of nonrecurring abnormalities. One case had t(15;17) and a robertsonian translocation identified as der(13;14), but it was not determined whether the robertsonian translocation was constitutive or disease-related. Of the 6 t-APL cases, 2 had additional cytogenetic abnormalities, specifically t(9;11) and t(6;12). Of the 2 CI-APL cases, 1 had a der t(8;10), and the case of MDS-APL had no karyotypic abnormalities with the exception of t(15;17) (Table 1). Overall, the secondary APL cases had a similar rate of additional cytogenetic abnormalities, 33% (3/9), as the de novo APL cases.

In 22 cases of de novo APL, FMS-like tyrosine kinase 3 (FLT3) mutational analysis was performed, and 13 (59%) had mutations; in all 5 cases of secondary APL in which this testing was done, internal tandem duplication (ITD) mutations were found (P = .14, Fisher exact test; Table 1). Of the de novo APL cases, 11 had ITD mutations, 1 had the D835 point mutation only, and 1 had D835 and ITD mutations. As expected,17,18 the cases of M3v tested for FLT3 mutations had a higher percentage of mutations (7/8 [88%]) than did the cases of classic APL (6/15 [40%]).

Morphologic and Flow Cytometric Findings

The percentage of abnormal promyelocytes in the marrow did not differ significantly between de novo APL (median, 84%; average, 61%; range, 24%-98%) and secondary APL (median, 90%; average, 75%; range, 8%-94%). There were no morphologic differences noted between the abnormal promyelocytes in de novo vs secondary APL cases other than differences between M3v and classic APL. The abnormal promyelocytes in therapy-related acute promyelocytic leukemia (APL), chronic immunosuppressive therapy–related APL, and myelodysplastic syndrome (MDS)-related APL have morphologic features similar to those of the atypical promyelocytes in de novo APL. Representative aspirates/touch preparations (modified Wright-Giemsa, ×100).
marrow in the case of MDS-APL did not show background dysplastic changes, although morphologic assessment of residual hematopoesis was complicated by a relatively high percentage of abnormal promyelocytes (92%) and a dry tap.

All leukemias were evaluated at diagnosis by flow cytometry using samples from bone marrow (62 cases) or peripheral blood (3 cases), although 8 of the APL cases were evaluated with an abbreviated antibody panel owing to low WBC counts. All cases of APL exhibited moderate to very high side scatter in the abnormal promyelocytes. We also found that the vast majority of abnormal promyelocytes exhibited increased autofluorescence with isotypic controls in the PE and FITC channels, as has been previously reported.19

Bright expression of CD33 with concurrent dim or absent expression of HLA-DR is typical of APL, and this was seen in the de novo and secondary cases of APL. The majority of cases of de novo APL had abnormal promyelocytes that at least partially expressed CD34 (30/56 [54%]), with a higher proportion of the microgranular variant cases expressing this antigen (13/16 [81%]).20 Of 9 cases of secondary APL, 6 expressed CD34, including all 4 of the microgranular variant cases. The cases of de novo APL expressed a range of CD13, as did the cases of secondary APL (Image 2). CD56 was expressed on a similar number of de novo APL cases (6/55 [11%]) and secondary APL cases (1/6 [17%]). The T-cell antigen CD2 was expressed on the majority of M3v abnormal promyelocytes in secondary and de novo APL. Other T-cell antigens, including CD3, CD5, and CD7, were not expressed on any of the abnormal promyelocytes.

Image 2 Representative flow cytometry plots from therapy-related acute promyelocytic leukemia (APL), subtype M3h (A); chronic immunosuppressive therapy–related APL, subtype M3v (B); and myelodysplastic syndrome–related APL, subtype M3v (C). Background autofluorescence from isotypic controls is shown in green. FITC, fluorescein isothiocyanate; M3h, hypergranular variant; M3v, microgranular variant; PE, phycoerythrin; PerCP, peridinin-chlorophyll protein; SSC, side scatter.
Overall, the range of immunophenotypes was similar in de novo and secondary APL, and no distinguishing characteristics were seen in the immunophenotype of the leukemic abnormal promyelocytes in secondary APL (Figure 1).

**Discussion**

Although therapy-related myeloid leukemias most often are composed of myeloblasts or monoblasts, secondary APL is well recognized. The development of t-APL is seen in patients with solid tumors and lymphoma and is particularly associated with prior treatment with topoisomerase II inhibitors. Several studies have shown that the prognosis of t-APL is significantly better than other t-AMLs, although some studies suggest that the prognosis of t-APL is slightly less favorable than the prognosis of de novo APL. Similar findings have been reported in patients with multiple sclerosis who were treated with the topoisomerase II inhibitor mitoxantrone and APL subsequently developed. Although these studies investigated the clinical features of secondary APL, they did not include a detailed comparison of the laboratory features of de novo and secondary APL.

In this 10-year retrospective review of new admissions for APL at our institution, we examined not only clinical findings but also laboratory findings in patients with secondary APL, including the immunophenotype and morphologic features of the abnormal promyelocytes and molecular and cytogenetic findings. We found that secondary APL is not uncommon and accounted for 14% of newly diagnosed APL cases in this series. The median interval between treatment for the initial neoplasm and the development of t-APL was 2.8 years, which is in keeping with previous research that has found that many cases of t-APL arise within 3 years. Cases of secondary APL did not have laboratory values or pathologic findings that distinguished them from cases of de novo APL. None of the patients in this series with secondary APL developed recurrent disease or died of leukemia, although 2 patients died of the primary disease and 1 patient was lost to follow-up. Although this series is small, our results agree with the general notion that secondary APL does not have as unfavorable a prognosis as other forms of secondary AML.

This review of the morphologic features and immunophenotype of abnormal promyelocytes in de novo and secondary cases of APL demonstrated that there were no specific findings that were characteristic or diagnostic of secondary APL. The M3h and M3v variants of secondary APLs were morphologically indistinguishable from de novo APL. The abnormal promyelocytes in secondary APL demonstrated a range of immunophenotypes similar to those seen in de novo APL. Of note, the expression of CD56 on the abnormal promyelocytes in APL has been shown to be an independent adverse prognostic factor for relapse. In this series, CD56 was expressed on abnormal promyelocytes in 11% of de novo APL cases (6/55) and 17% of secondary APL cases (1/6). Despite the association between CD56 expression and poor prognosis, none of the patients with CD56+ abnormal promyelocytes died of disease or had disease that later recurred.

The secondary APLs also did not show any characteristic cytogenetic findings compared with de novo APL. The finding that t-APLs do not have higher rates of additional chromosomal abnormalities agrees with previous research that showed similar rates of additional karyotypic abnormalities in de novo and t-APL. In addition, none of the karyotypic...
abnormalities seen in the cases of secondary APL were one of the mutations typically associated with t-AML such as complete or partial loss of chromosome 5 or 7. The frequent identification of FLT3 mutations in t-AML was expected, as many cases of APL exhibit mutations in this tyrosine kinase. In addition, the cases of M3v had more frequent FLT3 mutations than did cases of classic APL, which has been previously described.17,18 While FLT3 mutations are considered a poor prognostic indicator in most AMLs, in APL, the presence of an FLT3 mutation seems to suggest a somewhat poorer prognosis, but not to the same degree as in other forms of AML.26,27 In the small sample in this study, FLT3 mutations did not adversely affect the prognosis of secondary APL.

In 2 cases in this series, secondary APL developed in the setting of chronic immunosuppression. Both involved patients were treated with the cytotoxic antimetabolite mycophenolate and, thus, met the criteria for a treatment-related myeloid neoplasm. Mycophenolate was implicated in the development of therapy-related hematologic neoplasms in the 2008 World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues, and a recent case report described the development of secondary APL following renal transplantation in a patient treated with mycophenolate and other immunosuppressants.12,28 We did not identify any characteristic clinical or pathologic features in the 2 cases of secondary APL that arose in chronically immunosuppressed patients treated with mycophenolate.

This cohort of patients with secondary APL was no more likely to experience disease recurrence or die of leukemia than patients with de novo APL. Furthermore, we did not identify any morphologic, laboratory, or pathologic findings that were characteristic of secondary APL, and the range of phenotypes and genetic anomalies was similar to those seen in de novo APL. While this data set is limited, our findings suggest that secondary APL may not be biologically distinct from de novo APL and may not have an adverse prognosis and, therefore, should be considered as distinct from other treatment-related myeloid neoplasms. Further study of this or other specific subgroups of therapy-related myeloid disorders may increase our understanding of the heterogeneity of these diseases and lead to their further subclassification.

References


