Leukemias Resembling Acute Promyelocytic Leukemia, Microgranular Variant

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Abstract

Acute promyelocytic leukemia (APL) should be distinguished from other subtypes of acute myeloid leukemia (AML) because of the increased risk of disseminated intravascular coagulation (DIC) and its response to arsenic compounds and retinoids. Some cases of AML seem morphologically similar to the microgranular variant of APL (French-American-British [FAB] AML-M3v) but lack the t(15;17). We evaluated 8 cases of APL-like leukemias for subtle morphologic, cytochemical, immunophenotypic, and cytogenetic differences compared with 5 cases of promyelocytic leukemia/retinoic receptor alpha (PML/RARalpha)-positive APL (FAB AML-M3v). We also evaluated both groups for the presence of DIC. No differences among the groups were noted in blast size, chromatin pattern, nuclear morphologic features, intensity of myeloperoxidase staining, or presence of Auer rods. Immunophenotypes were similar; both types of cases lacked CD34 and HLA-DR and were CD13+ and CD33+. Two cases of APL-like leukemias also were CD56+. DIC was present in 2 patients with M3v. Our study shows that there are no definitive morphologic, cytochemical, or immunophenotypic findings that can distinguish these cases from PML/RARalpha-positive APL.

Distinguishing acute promyelocytic leukemia (APL) from the other subtypes of acute myeloid leukemia (AML) is important because APL responds to treatment with all-trans-retinoic acid1 and arsenic compounds.2 The diagnosis of the microgranular variant of APL can be difficult because the morphologic, cytochemical, and immunophenotypic features often are nonspecific. As a result, APL can be confused with other AMLs, such as acute myelomonocytic (AML-M4) or acute monocytic leukemia (AML-M5).3,4 Definitive diagnosis of the classic form of APL rests with identification of the characteristic reciprocal translocation between chromosomes 15 and 17 and/or recognition of the promyelocytic leukemia/retinoic receptor alpha (PML/RARalpha) transcript.

Chemotherapy often needs to be initiated rapidly in many patients suspected of having APL. This may be accompanied by a significant risk of hemorrhage if all-trans-retinoic acid is not included in the induction regimen. All-trans-retinoic acid substantially reduces the incidence of early hemorrhagic death during induction therapy for APL.5 Unfortunately, molecular and cytogenetic studies may be time consuming, requiring the use of morphologic, cytochemical, and immunophenotypic studies for initial classification. While immunofluorescent staining for the PML gene product has shown promise as a method to rapidly diagnose APL, it is not used routinely in most hematology laboratories.6 Therefore, we conducted a study to determine whether there are morphologic and cytochemical differences between PML/RARalpha-positive AML-M3v as assessed by the presence of t(15;17) and AML-M3v-like cases, which do not demonstrate t(15;17), and the usefulness of the clinical manifestations and immunophenotype in determining whether these leukemias were of promyelocytic origin.
Materials and Methods

Case Selection and Review

Peripheral blood or bone marrow aspirate smears (1 peripheral blood smear and 12 bone marrow aspirates) from 5 cases of microgranular variant of APL (French-American-British type AML-M3v) demonstrating t(15;17) were compared with 8 cases of AML in which the morphologic features suggested APL; the blasts in these 8 cases had prominent nuclear indentations or folds, multiple Auer rods, or prominent myeloperoxidase activity but demonstrated no t(15;17) (APL-like leukemias). The cases were obtained for review from the Department of Pathology, University of Iowa Hospitals and Clinics (UIHC), Iowa City, and University Hospitals of Cleveland (UHC), Cleveland, OH. These cases were initially diagnosed between January 1993 and June 2000, and clinical history and follow-up, peripheral blood samples and bone marrow aspirates, and findings from cytochemical, flow cytometric, and karyotypic studies were available. Peripheral blood or bone marrow aspirate smears from the cases were prepared as unknown slides for rereview by a board-certified hematopathologist (N.R.) and a clinical laboratory scientist experienced in bone marrow pathology (G.S.). The blasts were examined for size, chromatin pattern, nuclear features, cytoplasmic features, and presence of Auer rods. Based on the morphologic features, the reviewers gave an impression (AML-M3 or AML-M3–like). The cytochemical (myeloperoxidase and alpha-naphthyl butyrate esterase), immunophenotypic, karyotypic, and molecular features (if available) also were reviewed.

Flow Cytometric, Karyotypic, and Molecular Evaluation

Flow cytometric data for the 13 cases were reviewed retrospectively. Analysis was performed initially on the FACSCalibur Flow Cytometer (Becton Dickinson, San Jose, CA) and/or the EPICS XL-MCL (Coulter, Hialeah, FL) on peripheral blood or bone marrow aspirate specimens. Antibody combinations selected for review included CD45, CD34, CD14, CD13, CD33, HLA-DR (Becton Dickinson), and CD56 (Coulter).

For karyotype analysis, cells from unstimulated peripheral blood or bone marrow aspirate specimens were arrested at metaphase with colchicine. Chromosomes were stained by the G-banding method. The chromosome number was determined by microscopic analysis, and the cells were examined for the presence or absence of detectable structural rearrangements. Karyotypes were prepared from computer-assisted images of the metaphases.

Fluorescent in situ hybridization (FISH) or reverse transcriptase–polymerase chain reaction (RT-PCR) was performed in 3 of 13 cases. FISH was performed using a PML/RARalpha dual-color translocation probe (Vysis, Downers Grove, IL). RT-PCR studies were performed at ARUP Laboratories, Salt Lake City, UT. Patient RNA was isolated, reverse transcribed into complementary DNA, and subjected to PCR amplification using oligonucleotide primers specific for the PML gene on chromosome 15 and the retinoic acid receptor alpha gene on chromosome 17. PCR products were analyzed by electrophoresis and ultraviolet transillumination of ethidium bromide–stained gels.

Clinical Evaluation

The medical records of the patients were studied to determine clinical manifestations, coagulation parameters (prothrombin time [PT], partial thromboplastin time [PTT], fibrinogen level, fibrin degradation product [FDP] level, D-dimer level), chemotherapeutic regimen, and patient outcome.

Results

The patients in this study included 5 males and 8 females with a mean age of 40 years. Five patients had AML-M3v (2 males and 3 females; mean age, 39 years), and 8 patients had AML-M3–like leukemias (3 males, 5 females; mean age, 40 years). At diagnosis, all patients had a variety of nonspecific symptoms, including cough, nasal congestion, arthralgias, fatigue, ecchymoses, and fever.

At diagnosis, most patients had moderate to marked leukocytosis (mean WBC count, 93,800/µL [93.8 × 10^9/L]). Patients with APL-like leukemia had higher mean leukocyte counts (WBC count, 119,500/µL [119.5 × 10^9/L]; range, 18,200–235,000/µL [18.2–235 × 10^9/L]) compared with patients with AML-M3v (WBC count, 53,200/µL [53.2 × 10^9/L]; range, 1,400–211,000/µL [1.4–211 × 10^9/L]). Most patients were mildly to moderately anemic at initial examination (mean hemoglobin concentration, 10.2 g/dL [102 g/L]; range, 8.0–13.5 g/dL [80–135 g/L]). Thrombocytopenia was noted in all study patients (mean platelet count, 62 × 10^9/L [62 × 10^9/L]; range, 11–120 × 10^9/L [11–120 × 10^9/L]). Blasts, abnormal promyelocytes, or both were seen in the peripheral blood of 12 of 13 patients.

Evidence of disseminated intravascular coagulation (DIC) was assessed by the prothrombin time (PT), partial thromboplastin time (PTT), and/or fibrinogen; fibrin degradation products (FDP); and D-dimer. Measurement of FDP or D-dimer was institutionally dependent; only FDP levels were measured in UIHC patients, while only D-dimer levels were measured in UHC. Two patients with AML-M3v had clinical and laboratory evidence of DIC at diagnosis (elevated PT and PTT, decreased fibrinogen level, elevated...
Elevated FDP or D-dimer levels were noted in 7 of 7 patients with APL-like leukemia, none of whom had a concomitantly decreased fibrinogen level. Two reviewers blinded to case data morphologically classified the 13 bone marrow aspirate or peripheral blood smears as AML-M3v or APL-like after assessing blast size, chromatin pattern, nuclear pattern, cytoplasmic features, and the presence of Auer rods. The results are given in Table 1. The APL and APL-like cases were characterized by predominantly intermediate-sized blasts with fine to mildly clumped chromatin, irregularly shaped nuclei with frequent folding and bilobation, and granulated basophilic cytoplasm. Two of the APL-like cases had blasts with somewhat rounder nuclei. Auer rods were noted in the blasts of 8 of 13 cases; 4 of these cases had blasts with multiple Auer rods. No cases showed Pelger-like cells. No morphologic parameter such as blast size, chromat pattern, nuclear shape, or presence of cytoplasmic vacuoles differentiated APL-like leukemias from AML-M3v. Both reviewers correctly classified only 9 (69%) of 13 cases.

Strong granular staining for myeloperoxidase was noted in 13 of 13 cases; no difference in staining intensity was identified between AML-M3 and AML-M3-like leukemias. No blasts were positive for alpha-naphthyl butyrate esterase.

Flow cytometric immunophenotyping studies were performed on the peripheral blood or bone marrow samples in all 13 cases. The blasts in APL and APL-like cases consisted of intermediate- to large-diameter, CD45 dim, CD14- cells with variable orthogonal scatter. Positivity for CD34 (hematopoietic progenitor antigen) was noted in the blasts of 2 of 5 APL-like leukemias; no CD34 positivity was identified in the AML-M3v cases. Myeloid antigens (CD13, CD33) were expressed in most APL-like leukemias and AML-M3v. Expression of HLA-DR was noted in only 2 of 8 APL-like leukemias; no HLA-DR positivity was identified in the AML-M3v cases. In 2 APL-like leukemia cases, the blasts expressed CD56 (natural killer cell marker).

Karyotypic analysis was performed on all 13 cases. All 5 cases of AML-M3v expressed the characteristic translocation between chromosomes 15 and 17. Trisomy 8 was noted in 1 AML-M3v case. Normal karyotypes were noted in 6 of 8 patients with APL-like leukemias. Further search for t(15;17) was conducted through FISH in 2 cases and RT-PCR in 1 case; no cryptic t(15;17) or presence of the PML/RARalpha transcript was identified. Trisomy 4, trisomy 8, and/or trisomy 11 were noted in the 2 other patients with APL-like leukemia.

Based on morphologic, cytochemical, and immunophenotypic data, the APL-like leukemias were classified as AML-M1 (4 cases), AML-M2 vs -M3 (2 cases), and consistent with AML-M3v (2 cases). Cytogenetic studies were not available initially for all 4 UHC cases to correlate with morphologic and immunophenotypic results. The final diagnoses of 2 of these cases were signed out as consistent with AML-M3v; a comment was added recommending karyotypic studies to better classify these leukemias.

All-trans-retinoic acid was used to treat 2 of 8 patients with APL-like leukemia and 2 of 5 patients with AML-M3v. No patient with APL-like leukemia experienced a response to all-trans-retinoic acid. Multiagent induction chemotherapy with idarubicin, cytarabine, daunorubicin, etoposide, or thioguanine was used to treat 11 of 13 patients.

Seven of 8 patients with APL-like leukemia experienced remission after chemotherapy; 6 of these patients relapsed and died with disease. Most patients died within 9 months of diagnosis. Three patients with AML-M3v died of disease; 2
of these patients did not receive chemotherapy because they died within 48 hours of admission to the hospital.

**Discussion**

APL is characterized by a proliferation of abnormal promyelocytes. The disease comprises about 5% to 10% of all AMLs and is divided into the classic (hypergranular) and microgranular (hypogranular) types. The classic form of APL is characterized by t(15;17)(q22;12) or the presence of the PML/RARalpha transcript. Three alternative translocations associated with APL also have been characterized: t(11;17) (q23;q21) (promyelocytic leukemia zinc finger [PLZF]/RARalpha), t(5;17) (q35;q21) (nucleophosmin [NPM]/RARalpha), and t(11;17)(q13;q21) (nuclear mitotic apparatus [NuMA]/RARalpha). In addition to PML/RARalpha APL, NPM/RARalpha and NuMA/RARalpha APL also are responsive to retinoids when used in combination with chemotherapy.

Because patients with APL benefit from treatment with retinoids or arsenic compound therapy and have an increased risk of DIC and hemorrhage, correct classification of APL is important. Definitive diagnosis is based on cytogenetic and molecular tests; these studies are highly technical, and results often are not available in a timely manner. Pathologists often are faced with the dilemma of classifying APL based only on morphologic, cytochemical, and immunophenotypic findings. Our study confirms that there is considerable difficulty distinguishing AML-M3v from APL-like leukemias based on these parameters.

At diagnosis, most patients with the microgranular variant of APL have marked leukocytosis and laboratory evidence of DIC. Substantial leukocytosis was noted in cases of APL and APL-like leukemias. The leukocytosis was
most marked in APL-like leukemias; the significance of this finding is uncertain. Clinical and laboratory evidence of DIC (elevated PT and/or PTT, decreased fibrinogen level, elevated FDP or D-dimer level) were identified in 2 of 5 APL cases. Patients with APL-like leukemia showed no clinical evidence of DIC but had increased fibrinolysis (elevated FDP or D-dimer level).

The typical blasts of the microgranular variant of APL show a bilobed, reniform nucleus with hypogranular cytoplasm containing fine granules. Auer rods may be seen but are not as numerous as in classic AML-M3. The APL-like leukemias and the AML-M3v cases in the present study had similar morphologic features. All APL-like tumors were intensely positive for myeloperoxidase and negative for alpha-naphthyl butyrate esterase; this profile is typical of AML-M3v.

When morphologic and cytochemical classification of leukemias fail, flow cytometric analysis may be performed to further classify the disease. APL has been known to have a characteristic immunophenotype: HLA-DR– and CD13+ and/or CD33+. APL and the majority of APL-like leukemias in the present study also demonstrated this immunophenotype. The blasts in 2 of 8 cases also expressed HLA-DR. Occasional cases of AML-M3v have been HLA-DR+; Exner et al found 2 of 4 cases of AML-M3v positive for HLA-DR. Thus, HLA-DR positivity cannot always be used as an exclusionary criterion for APL. Although all APLs and 2 of 5 APL-like leukemias in the present study were CD34–, occasional APLs express CD34. Foley et al noted that 32% of APL cases expressed CD34. These patients tended to have higher leukocyte counts and earlier mortality.

Because morphologic, cytochemical, and immunophenotypic studies often are nonspecific in these leukemias, cytogenetic studies are necessary for accurate classification. Cytogenetically detectable rearrangements are identified in about 70% to 80% of cases. Additional studies such as FISH or RT-PCR may increase detectability of the translocation or transcript to almost 90% of cases. Sainty et al undertook a morphologic, immunophenotypic, cytogenetic, and molecular review of a series of cases referred to as APL lacking t(15;17) and compared them with a control group of cases of APL with t(15;17). The majority of the APL cases that did not demonstrate t(15;17) revealed underlying rearrangements of the PML/RARalpha transcript by FISH or RT-PCR. No major differences in morphologic features or

### Table 2

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<th>Diagnosis/Case No.</th>
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AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; ND, not done; +, positive; –, negative.
immunophenotype were observed between the control group and cases lacking t(15;17) with molecular evidence of PML/RARalpha rearrangements. Three cases in our study were tested with FISH or RT-PCR; no t(15;17) or transcript was detected. It is unclear whether t(15;17) would have been detected in the other 5 cases if additional FISH or molecular testing had been conducted.

A novel but sensitive method for diagnosis of APL can be obtained through analysis of the immunocytochemical pattern of the PML protein with the monoclonal antibody PG-M3. Villamor et al noted a microgranular staining pattern in 25 of 25 cases of APL; all of these leukemias exhibited the PML/RARalpha transcript by RT-PCR. Seven cases had morphologic and cytochemical findings equivocal of APL. APL was excluded from the diagnosis in 6 of 7 cases by immunostaining for the PML protein; absence of the PML/RARalpha transcript was confirmed in the excluded cases. Unfortunately, this promising staining method is not used routinely in most clinical laboratories.

With absence of t(15;17), how can these leukemias best be classified? Most of the leukemias in our study were classified as AML-M1 or AML-M2. Fenu et al suggest the possibility of a transitional M2-M3 AML subtype that demonstrates no cytogenetic or molecular evidence of t(15;17) or the PML/RARalpha. The presence of CD56 on 2 of the APL-like leukemias raises the possibility that these leukemias may be myeloid/natural killer cell acute leukemias. Scott et al described 20 cases of acute leukemia that expressed the following immunophenotype: HLA-DR−, CD33+, CD56+, and CD16−. Morphologically, the blasts in these cases demonstrated irregular nuclear contours, moderate cytoplasm with fine to moderately coarse azurophilic granules, and weak to moderate myeloperoxidase or Sudan black B positivity. Karyotypic and RT-PCR analysis showed no evidence of t(15;17) or the PML-RARalpha transcript. These leukemias showed functional natural killer cell–mediated cytotoxicity and were resistant to in vitro all-trans-retinoic acid. These findings suggest that when the diagnosis of AML-M3v is questionable, CD56 should be performed to help exclude myeloid/natural killer cell leukemia.

CD56 positivity also has been noted in the t(11;17) variant of APL exhibiting the PLZF/RARalpha transcript. Leukemic blasts in this variant exhibit regular nuclei; Pelger-like cells also are present. Although 2 of the APL-like leukemias in the present study demonstrated blasts with somewhat round nuclei, the cases did not show Pelger-like cells or CD56 expression, likely excluding a variant PLZF/RARalpha APL in these patients.

Our study reiterates the lack of specificity of morphologic, cytochemical, and flow cytometric findings in APL-like tumors. Karyotypic analysis, as well as FISH and RT-PCR, must be conducted on these cases to rule out APL. APL-like leukemias that do not demonstrate t(15;17) or the PML-RARalpha transcript may constitute a heterogeneous population of leukemias including myeloid/natural killer cell acute leukemia and PLZF/RARalpha leukemia.

References


