Minimal Coexpression of CD34+/CD56+ in Acute Promyelocytic Leukemia Is Associated With Relapse

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ABSTRACT

Objectives: Surface CD56 expression on leukemic cells in acute promyelocytic leukemia (APML) is considered an indicator of poorer outcome even in patients receiving conventional treatment.

Methods: In the present case, at initial diagnosis, the hallmark phenotype of APML was found (strong CD33 and cytoplasmic MPO expression, absence of HLA-DR expression).

Results: Both CD34 and CD56 antigen expression was considered negative. The patient relapsed 3 years after reaching complete remission, and the hallmark surface antigen combination for APML was again found. In contrast, the leukemic cells now clearly coexpressed CD34 and CD56. Retrospective analysis revealed the presence of small CD34+ and CD56+ populations at initial diagnosis (<20%).

Conclusions: This case report suggests that the presence of a clone with minimal coexpression of CD34/CD56 in APML at initial diagnosis should not be neglected since it may be associated with earlier relapse.

Acute promyelocytic leukemia (APML) has emerged as the most curable subtype of acute myeloid leukemia (AML) in adults. Several factors have contributed to this evolution. Laboratory recognition of this subtype of AML is facilitated due to its characteristic morphologic and surface marker phenotype and a unique chromosome translocation t(15;17), which results in the formation of a hybrid promyelocytic leukemia/retinoic acid receptor (PML/RAR) α fusion protein.1 At the clinical level, the disease is characterized by frequent association with a severe bleeding diathesis as well as by a striking response to differentiating therapy with all-trans retinoic acid (ATRA).2 The introduction of this agent has dramatically improved the outcome of APML. Cure is now expected in 70% to 90% of patients when treatment includes ATRA combined with anthracycline-based chemotherapy.3 However, treatment failure may occur as a result of early hemorrhagic death or disease relapse.4

Prognostic factors that have been related to inferior outcome in APML are older age, low platelet count, and high initial WBC count.4,5 During the past 15 years, extensive research has been performed on the prognostic value of CD56 (neural adhesion factor) expression on the leukemic promyelocytes. In patients treated with ATRA plus idarubicin-derived regimens, a relationship between the expression of CD56 antigen on the surface of leukemic promyelocytes and short remission duration has been found and is considered an independent adverse prognostic factor for relapse.1,2,6 In general, CD56 expression is considered positive when it is expressed in more than 20% of the leukemic cells.1 We report on a case of an APML relapse associated with initial minimal expression of CD56 on the leukemic promyelocytes.
A 66-year-old woman who sought treatment from our hematology department had diffuse ecchymoses and hematomas, conjunctival bleeding, fatigue, lower back pain, and abdominal pain. A full blood count showed a leukocyte count of $0.67 \times 10^9/L$, hemoglobin concentration of 12.3 g/dL, and platelet count of $75 \times 10^9/L$. Fibrinogen levels were decreased (134 mg/dL) and the prothrombin time activity percentage was 65%, indicating diffuse intravascular coagulation (DIC). The bone marrow aspirate was of high cellularity, with the presence of 68.5% abnormal promyelocytes in addition to 19.5% myeloblasts. Characteristic cells containing bundles of Auer rods (“faggot cells”) were present Image 1A. Cytomorphologic and clinical evidence combined with the molecular identification of t(15;17)(q22;q21) with bcr3 led to the diagnosis of APML. No secondary cytogenetic abnormalities were noted (46,XX). Immunophenotyping (using a five-color flow cytometer [FC-500]; Beckman Coulter, Brea, CA) showed typical strong expression of CD33 and cytoplasmic myeloperoxidase. CD117 expression was variable. Partial expression was seen for CD15. There was minor expression of CD34 (<10%) and absence of HLA-DR expression Image 2. Remission induction with idarubicin and ATRA according to the PETHEMA-HOVON LPA2005 protocol was complicated by DIC and febrile neutropenia. Complete molecular remission was achieved with bcr3 PML/RARA below the detection limit and normal WT1 expression in the bone marrow aspirate 1 month after remission induction was started.

Three years and 6 months after reaching complete remission, the patient relapsed into AML, again with diffuse ecchymoses, fatigue, leukocytopenia ($1.40 \times 10^9/L$), and thrombocytopenia ($32 \times 10^9/L$) but without prothrombin time, activated partial thromboplastin time, or fibrinogen abnormalities. In contrast to the initial diagnosis, no characteristic morphologic features of APL were found. No faggot cells were found, and the predominant leukemic cells were myeloblasts (75%) with several cytoplasmic vacuoles Image 1B. Bcr3 PML/RARA could again be detected and no additional cytogenetic abnormalities could be detected. The immunophenotyping (using an eight-color flow cytometer [FACSCanto]; Becton Dickinson, Franklin Lakes, NJ) showed similar strong expression of CD33, variable expression of CD117, and absence of HLA-DR expression. However, in addition to the initial phenotype, the blasts expressed CD34 and CD56 Image 3. Retrospective analysis of the plots of the immunophenotyping at initial diagnosis revealed the presence of a minor, clearly distinguishable population (±5%) probably coexpressing CD34 and CD56 (Image 2). The latter suggests that a small clone of abnormal myeloblasts expressing CD56 probably was therapy resistant.

The patient received remission induction therapy with daily intravenous arsenic trioxide (ATO) (0.15 mg/kg) plus oral ATRA (45 mg/m²) for 35 days until hematologic complete remission. This resulted in complete remission with normal bone marrow blast count but with persisting cytogenetic abnormality and the absence of a significant molecular response.

Consolidation therapy consisted of ATO 0.15 mg/kg 5 days per week for 5 weeks and ATRA 45 mg/m² daily 2 weeks on and 2 weeks off for two courses. This resulted in complete molecular response. Because of the persistence of
t(15;17) after remission induction and the worse outcome associated with CD56 expression, the response was further consolidated with nonmyeloablative allogeneic peripheral blood stem cell transplantation with a matched unrelated donor. Conditioning consisted of fludarabine $3 \times 30$ mg/m² and 2 Gy total-body irradiation, and she received mycophenolate mofetil and cyclosporine for graft-vs-host disease prophylaxis. Thirty days after the transplant, she had full donor chimerism, and she kept her complete hematologic, cytogenetic, and molecular response. Acute graft-vs-host disease was successfully treated with systemic corticosteroids. Six months after the transplant, her quality of life is excellent, and cyclosporine and methylprednisolone are being reduced.

**Discussion**

In AML, CD56 positivity has been intensively studied and is considered a significant independent risk factor for disease-free survival and poor overall survival. In APML, CD56 positivity occurs in approximately 10% to 15% of newly diagnosed patients. This surface marker is an indicator of poorer outcome even in patients receiving conventional therapy, and the prognostic significance of CD56 expression was retained in a multivariate analysis that included WBC count and is therefore independent of this variable. To explain the worse prognosis of CD56 acute leukemia, different hypotheses have been proposed, including a greater occurrence in these patients of extramedullary involvement and a correlation between CD56 expression and multidrug resistance. Recently, CD56 expression has been associated with a higher risk of central nervous system involvement of APML. Another hypothesis states that CD56+ APML may emerge from a more immature undifferentiated and pluripotent leukemic stem cell that is less sensitive to the combination of ATRA and anthracyclines. The expression of CD56 antigen in APML is typically correlated with the bcr3 isoform. In addition, coexpression of CD56 and CD34 has been described in bcr3 APML isoforms.

In the present case, the bcr3 PML/RARA breakpoint was detected at initial diagnosis, but both CD34 and CD56 antigen expression was considered negative since less than 20% of the promyelocytes showed CD34 or CD56 expression. When the patient relapsed, the bcr3 breakpoint could again be detected, and the blasts again expressed the hallmark surface antigen expression of APML (absence of HLA-DR, strong CD33 and cytoplasmic MPO expression). In contrast, the leukemic cells now clearly coexpressed CD34 and CD56. In addition, where the morphologic presentation of the leukemic population in the bone marrow aspirate at initial diagnosis was that of a classic hypergranular M3 APML, during the relapse, atypical morphologic features were seen in the leukemic population. In contrast to the initial population, the leukemic population was hypergranular, lacked Auer rods, and did not show the typical features of M3 (variant) morphology. This was in contrast to newly diagnosed cases in which both CD34 and CD56 are correlated with the M3 variant morphology.
CD56 expression has been linked to increased WBC counts, but it has been suggested that both a WBC count greater than $10 \times 10^9/L$ and CD56 positivity are independent adverse factors for relapse. In the present case, minimal CD56 expression was seen in combination with a low WBC count at initial diagnosis. This case could confirm the independent prognostic value of CD56 expression in APML cells, and therefore it should be considered for designing future risk-adapted strategies. CD56 expression, even in a small number of APML cells, may be an indication for stratification in the high-risk group at diagnosis and for allogeneic stem cell transplantation after relapse. These strategies and the impact of drugs such as ATO and gemtuzumab ozogamicin in first-line treatment will have to be studied in clinical trials.

The retrospective analysis of the flow cytometry plots revealed small CD34+ and CD56+ populations at initial diagnosis. However, these populations were not considered relevant, since these markers were expressed on fewer than 20% of the leukemic cells. Only ±5% of the leukemic cells showed CD34 and CD56 expression. These findings suggest that even a minimal leukemic population expressing CD56 in APML can be associated with relapse. A downside of this report is that CD54 and CD34 were not included in the same sample tube at initial diagnosis, making it inconclusive whether CD34 and CD56 were coexpressed on the minimal population. However, as multicolor flow cytometry (5-10 color) is current practice in the hematology laboratory, CD34/CD56 coexpression in minimal clones can easily be detected.

References


