

Expression of CD117 and CD11b in Bone Marrow Can Differentiate Acute Promyelocytic Leukemia From Recovering Benign Myeloid Proliferation

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Abstract

The morphologic characteristics of bone marrow aspirates from patients recovering from acute agranulocytosis may be closely similar to the pattern observed in cases of acute promyelocytic leukemia (APL). The clinical manifestation also can be ambiguous in a substantial number of cases. The immunophenotypic features of bone marrow from 5 patients recovering from acute agranulocytosis, showing an increase in the percentage of promyelocytes (26%-66%), were compared with the immunophenotype of 31 consecutive patients with APL whose diagnosis was confirmed by PML-RAR alpha gene rearrangement. All markers were similarly expressed, except for CD117 and CD11b. CD117 was positive in 24 (77%) of the APL cases and in none of the acute agranulocytosis cases. On the other hand, CD11b was positive in 5 (100%) of the acute agranulocytosis cases and in only 2 (6%) of the APL cases. Thus, the CD117-CD11b+ phenotype was detected in all patients recovering from agranulocytosis and in only 1 (3%) of 31 APL cases. Therefore, we suggest that the combination of both markers is helpful in the differentiation of APL from recovering benign myeloid proliferation.

The morphologic characteristics of bone marrow aspirates from patients recovering from acute agranulocytosis may be closely similar to the pattern observed in cases of acute promyelocytic leukemia (APL).¹ Bone marrow is replenished with promyelocytes in both eventualities, a picture occasionally referred to as *promyelocytic marrow*.² Although the morphologic characteristics of promyelocytes in APL are slightly different from those in agranulocytosis,¹ the distinction is highly challenging in some cases.

In addition, clinical manifestations of acute agranulocytosis and APL can be ambiguous in a substantial number of cases, since fever, leukopenia, and associated infections are observed in both diseases.^{3,4} Although cytopenias other than leukopenia are much more common in APL, they also can be observed in acute agranulocytosis, mainly in the context of severe infections. The prognosis in acute agranulocytosis is favorable, since complete hematologic recovery is the rule.³ On the other hand, in most cases APL is associated with disseminated intravascular coagulation, and prompt treatment with all-*trans*-retinoic acid and chemotherapy can be lifesaving. Thus, an early and correct diagnosis is critical. Although cytogenetic and molecular genetic studies are the “gold standard” for the diagnosis of APL, only morphologic, cytochemical, and immunophenotypic data usually are available at the time when the initial treatment decision needs to be made.⁵ Studies demonstrating the rapid diagnosis of APL using monoclonal antibodies against the PML protein, by immunofluorescence or immunocytochemical analysis, have been reported,⁶ but the characteristic pattern of intracellular protein distribution in APL has been a matter of debate,^{7,8} and a false-negative rate up to 8% has been reported.⁹

To the best of our knowledge, there are no data about the immunophenotype of bone marrow from patients recovering from acute agranulocytosis. In the present study, the immunophenotyping features of bone marrow from 5 patients recovering from acute agranulocytosis with the characteristic promyelocytic marrow pattern were compared with the immunophenotype of samples obtained from 31 consecutive cases of APL diagnosed in our laboratory and confirmed by *PML-RAR alpha* gene rearrangement.

Materials and Methods

Acute Agranulocytosis

Case 1

A 25-year-old previously healthy man sought care because of asthenia, odynophagia, and fever for 30 days. Symptoms had worsened in the last 7 days, and several drugs had been used to relieve symptoms. The physical examination showed whitish plaques on both pharyngeal tonsils, and the liver was palpable 2 cm below the right costal margin. No adenopathy or splenomegaly was observed. The RBC count was $5.04 \times 10^6/\mu\text{L}$ ($5.04 \times 10^{12}/\text{L}$). Other laboratory findings are shown in **Table 1**. The follow-up of this patient disclosed an acute HIV infection.

Case 2

A 30-year-old pregnant woman was admitted to another hospital with a history of use of gentamicin and dipyrrone for treatment of a urinary tract infection. On the fifth day of treatment, her pregnancy evolved to spontaneous labor and to delivery of a term infant. After delivery, the infectious picture worsened to sepsis accompanied by disseminated intravascular coagulation and pancytopenia. Antibiotics were changed to cephalothin and oxacillin. The infection and the platelet count improved, but the WBC count remained low. Laboratory findings are given in Table 1. A bone marrow aspirate was performed, and results were considered consistent with APL. A second bone marrow sample, collected 7

days after the first and sent to our hospital for review and flow cytometric studies, was considered consistent with recovery from acute agranulocytosis.

Case 3

A 42-year-old man with chronic alcoholic liver disease and malnutrition sought care because of a 2-day history of tremors, diaphoresis, and insomnia. He had been in complete alcohol abstinence for 6 days. The physical examination revealed vascular spiders on the trunk and palmar erythema. Fine crackles were heard in the lower right hemithorax. The RBC count was $3.75 \times 10^6/\mu\text{L}$ ($3.75 \times 10^{12}/\text{L}$); the mean corpuscular volume, $108 \mu\text{m}^3$ (108 fL); and the reticulocyte count, 1.68% (0.017). Other laboratory findings are given in Table 1. Abstinence syndrome and pneumonia were diagnosed, and broad-spectrum antibiotics were properly started. *Streptococcus* species was detected in 3 bottles of hemocultures. Although the initial interpretation of the bone marrow aspirate was considered consistent with APL, review of the smears (S.L.R.M. and R.P.F.) showed the marrow aspirate to be consistent with acute agranulocytosis in a recovery phase.

Case 4

A 4-year-old girl with Down syndrome who had been taking valproic acid on a long-term basis had been examined at another hospital because of a history of fever for 15 days, impetigo, and scarlet fever. She was being treated with cephalothin and dipyrrone. Except for skin lesions, the physical examination was unremarkable. The RBC count was $3.25 \times 10^6/\mu\text{L}$ ($3.25 \times 10^{12}/\text{L}$). Other laboratory values are given in Table 1. The bone marrow aspirate was obtained in the context of a workup for pancytopenia, and APL was diagnosed. A sample was sent to our hospital for flow cytometric studies and review by one of us (R.P.F.). Findings on the slides and the immunophenotypic data were considered consistent with bone marrow recovery from agranulocytosis.

Case 5

A 41-year-old man with schizophrenia was admitted to our hospital to start clozapine therapy. Weekly CBC counts were scheduled and showed no alterations while the drug

Table 1
Summary of Clinical Data From 5 Patients With Acute Agranulocytosis

Case No./Sex/Age	Hemoglobin, g/dL (g/L)	Hematocrit, %	WBC Count, $\mu\text{L} (\times 10^9/\text{L})$	Neutrophils, %	Platelet Count, $\times 10^3/\mu\text{L} (\times 10^9/\text{L})$
1/M/25	14.6 (146)	41 (0.41)	1,300 (1.3)	4 (0.04)	69 (69)
2/F/30	13.0 (130)	NA	1,100 (1.1)	70 (0.70)	25 (25)
3/M/42	13.1 (131)	40 (0.40)	1,000 (1.0)	83 (0.83)	54 (54)
4/F/4	8.1 (81)	25 (0.25)	1,100 (1.1)	8 (0.08)	75 (75)
5/M/41	15.3 (153)	45 (0.45)	900 (0.9)	7 (0.07)	141 (141)

NA, not available.

doses were being slowly increased. On the 17th day of a daily clozapine dosage of 700 mg, the patient had unexplained fever. The findings of a physical examination were unremarkable except for extremely poor dental hygiene. The RBC count was $5.16 \times 10^6/\mu\text{L}$ ($5.16 \times 10^{12}/\text{L}$). Additional laboratory findings are given in Table 1. Acute agranulocytosis was diagnosed, and the clozapine therapy was properly changed to other medications.

All patients showed complete hematologic recovery within a period of days to weeks from diagnosis. Case 3 recovered as early as 3 days after diagnosis.

Acute Promyelocytic Leukemia Cases

Thirty-one bone marrow specimens from patients diagnosed with APL in our laboratory, whose *PML-RAR alpha* gene rearrangement was detected by reverse transcriptase–polymerase chain reaction, were used for comparison with the cases of acute agranulocytosis. Immunophenotypic data were available for all cases, and the technical procedures were the same as described in the following sections.

Morphologic Examination

Bone marrow aspirates were obtained at the initial examination from the 31 patients with APL and from all patients with acute agranulocytosis. Bone marrow smears were stained with the Leishman stain and for the myeloperoxidase reaction. None of our patients underwent a bone marrow core biopsy.

Immunophenotyping

Bone marrow samples were studied for surface antigen expression using a panel of monoclonal antibodies directly conjugated with fluorochromes. Each tube contained 1×10^6

nucleated cells after adjustment. Cells were stained with 5 μL of various combinations of fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-labeled monoclonal antibodies against the following antigens: CD45-FITC, HLA-DR-FITC, CD4-PE, CD13-PE, CD11b-PE, CD19-PE, CD33-PE, CD34-PE, CD56-PE (Becton Dickinson, San Jose, CA), and CD117-PE (DAKO, Santa Barbara, CA). Negative controls were incubated with FITC/PE-conjugated immunoglobulins of irrelevant specificity (Becton Dickinson). After incubation, erythrocyte lysis was performed with FACS Lysing Solution (Becton Dickinson).

All samples were analyzed with a FACScan flow cytometer (Becton Dickinson) equipped with an argon ion laser with a wavelength of 488 nm by collecting 10,000 ungated list-mode events per tube. A gate was selected in the combination of forward scatter (FSC) and side scatter (SSC), and analysis was performed on cells with the most appropriate promyelocyte gate (intermediate-to-high SSC and intermediate-to-high FSC). Cell Quest software (Becton Dickinson) was used for data acquisition and analysis. Results were considered positive if 20% or more of the cells expressed a particular antigen.

Results

Clinical and laboratory findings for the 5 cases of agranulocytosis are summarized in Table 1. Bone marrow data are summarized in **Table 2**. The morphologic characteristics of the typical bone marrow cells are shown in **Image 1**. All specimens were filled mainly with promyelocytes (26%-66%), with a profile that had to be differentiated from APL in all cases. The median of the myeloid/erythroid (M/E) ratios was 3.45 (range, 1.8 to 6.0) in acute agranulocytosis

Table 2
Bone Marrow Data for 5 Cases of Acute Agranulocytosis*

	Case Number					
	1	2 [†]	2 [‡]	3	4	5
Blasts	3	7	1	5	6	6
Promyelocytes	52	50	26	42	60	66
Myelocytes	24	8	17	24	11	9
Metamyelocytes	4	15	17	8	5	5
Bands	0	4	14	2	1	5
Segmented neutrophils	1	2	18	1	0	1
Lymphocytes	8	4	3	5	15	6
Monocytes	1	0	0	0	1	1
Eosinophils	4	7	4	11	0	0
Basophils	0	0	0	0	0	0
Plasma cells	3	3	0	1	1	1
Myeloid/erythroid ratio	4.4:1	6.0:1	2.7:1	2.0:1	4.2:1	1.8:1

* The count was performed on 300 nonerythroid cells. Data are given as percentages.

[†] Sample from 7 days before immunophenotyping.

[‡] Same sample as used for immunophenotyping

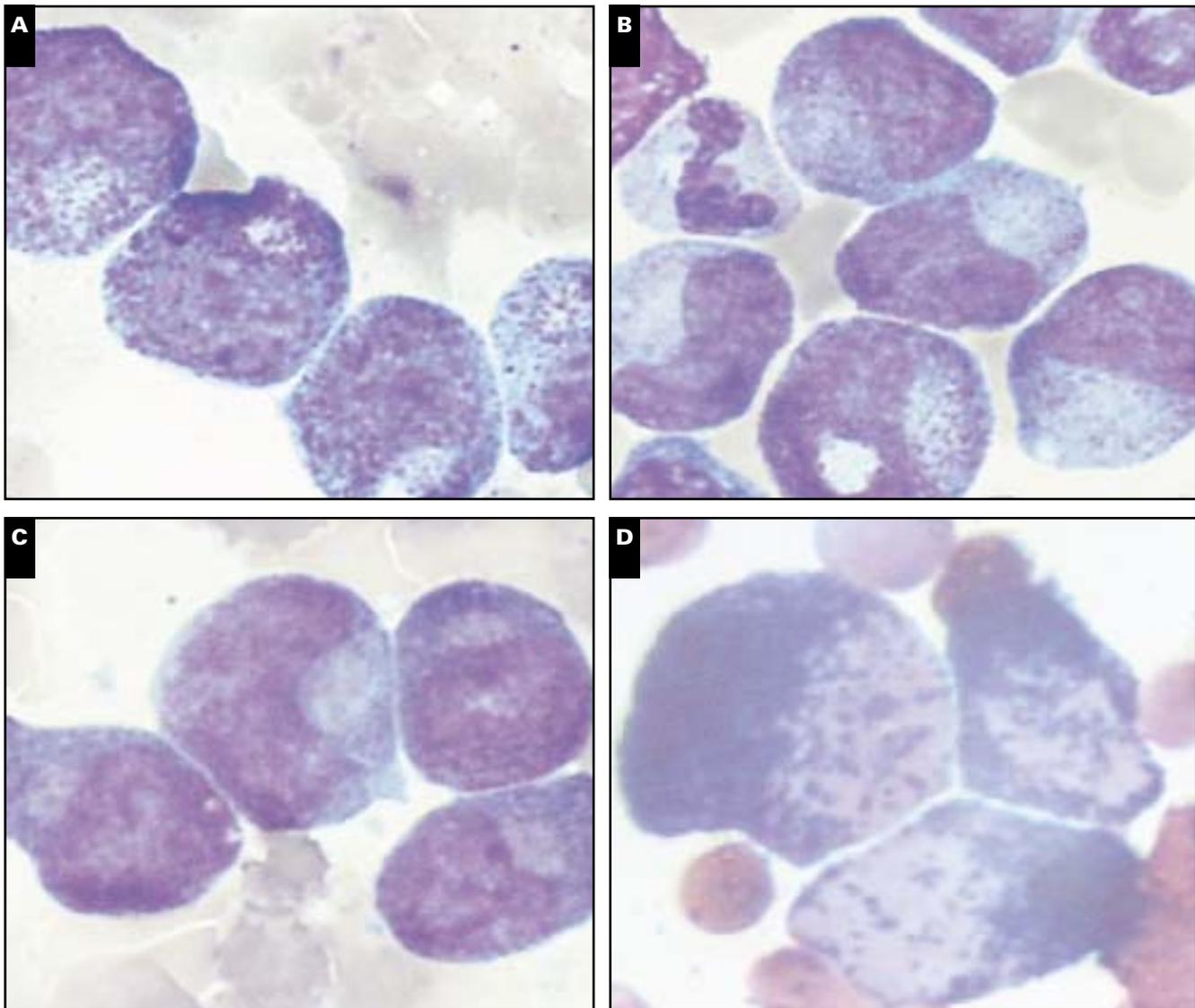


Image 1 Bone marrow smears. **A**, Case 1 (Leishman, $\times 630$). **B**, Case 2 (Leishman, $\times 630$). **C**, Case 3 (Leishman, $\times 630$). **D**, Case 3 (myeloperoxidase, $\times 630$).

and 11.0 (range, 1.1 to 50.0) in APL. In all smears, abundant azurophilic granules were observed, and the myeloperoxidase reactivity was very strong (Image 1D).

Examples of the FSC/SSC dot plots with the gating strategy used in both diseases are shown in **Image 2**, where the cells gated also are represented in a CD45/SSC dot plot for comparison. In **Table 3**, the immunophenotypic features of the bone marrow from patients recovering from acute agranulocytosis were compared with the immunophenotypic profile of 31 patients with APL. Except for the expression of CD117 and CD11b, the patterns of expression of other myeloid antigens (CD13, CD33) and immaturity markers (CD34, HLA-DR) were similar in both diseases. Markers of other hematological lineages (lymphoid B or T) also revealed no significant difference in expression. CD117 was expressed in 24 APL cases

(77%) but was not expressed by promyelocytes of patients recovering from acute agranulocytosis. By contrast, CD11b was positive in all cases of acute agranulocytosis but in only 2 (6%) of the APL cases. The typical patterns of expression of CD117 and CD11b in both diseases are illustrated in **Image 3**.

The CD117-CD11b+ immunophenotype was observed in 5 patients (100%) recovering from acute agranulocytosis and in only 1 (3%) of 31 cases of APL. The opposite combination, CD117+CD11b-, was the characteristic phenotype in APL, being observed in 23 samples from patients with APL (74%) and in none of the samples from 5 patients recovering from acute agranulocytosis. Finally, the double-positive CD117+CD11b+ phenotype was observed in 1 case of APL (3%), and the double-negative CD117-CD11b- phenotype was detected in 6 cases of APL (19%).

Discussion

The purpose of this study was to determine whether the immunophenotype of bone marrow promyelocytes is helpful in the differential diagnosis of cases in which the clinical and morphologic features are shared between recovery from acute agranulocytosis and APL. The importance of this differentiation was illustrated clearly by the fact that 3 of 5 cases of acute agranulocytosis were erroneously diagnosed as APL, and the bone marrow specimens were referred to our laboratory just for flow cytometric studies.

Three of our patients had severe infectious diseases, but they also had taken several drugs by the time of diagnosis. Two others had less severe infections and had taken some drugs (dipyrrone, clozapine) with a well-known potential to cause agranulocytosis.¹⁰ It was not our purpose to make a precise etiologic diagnosis, since the maturational arrest at the promyelocyte stage typical of the promyelocytic marrow is a feature observed in both infection-induced and drug-induced agranulocytosis. Other groups have reported difficulties in collecting the data and clarifying the agents that caused drug-induced agranulocytosis. This is often very difficult, mainly owing to lack of information about the drugs that patients ingested or because of the simultaneous ingestion of many drugs.³ Most of our patients had taken several drugs before the bone marrow aspirates were obtained, and it was impossible to unequivocally find the culprit (infection or a specific drug) in all cases, except for 1 patient, who was taking clozapine exclusively at the time of diagnosis.

Leishman-stained bone marrow smears from all 5 cases of agranulocytosis showed the typical pattern of benign myeloid proliferations previously reported in this setting,¹ with several azurophilic granules surrounding the Golgi zones and complete absence of Auer rods. Since all 5 cases of acute agranulocytosis had febrile diseases, the fever possibly had contributed to the increased number of azurophilic granules observed in the promyelocytes. Although most of the promyelocytes in APL are characterized by an abundant cytoplasm almost completely filled with azurophilic granules and by the presence of Auer rods, there are rare cases of APL in which this pattern has not been fully developed and in which the morphologic features of the promyelocytes resemble those of normal promyelocytes, with rarely observed or even absent Auer rods. We found no Auer rods in only 2 of 31 cases of APL, which is in agreement with previously reported data.¹¹ Only 1 case of APL had morphologic features slightly resembling agranulocytosis (concerning the Golgi zones), but this case had Auer rods. Therefore, although the morphologic features of the bone marrow specimens in recovery from an agranulocytic episode could be easily confounded with APL, the

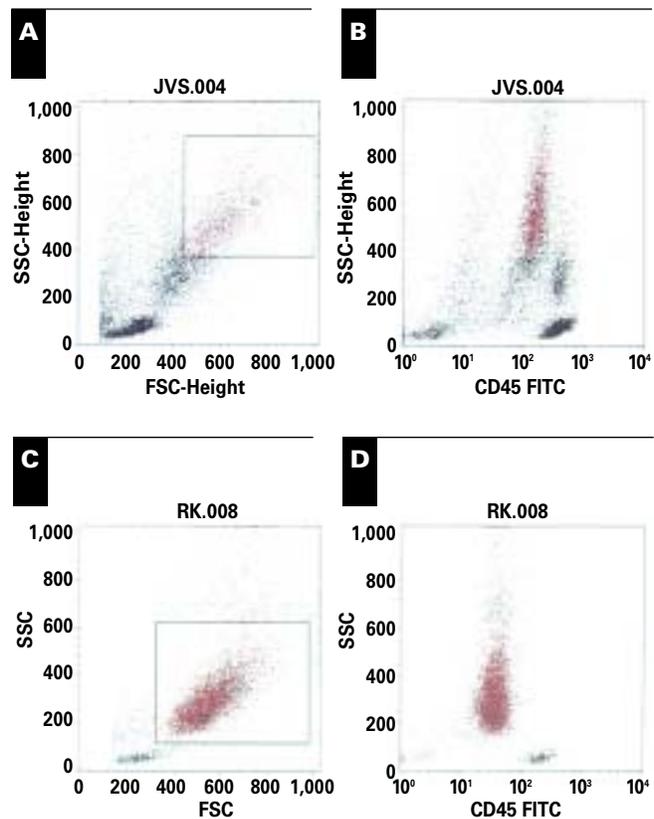


Image 2 Forward scatter (FSC)/side scatter (SSC) and CD45/SSC dot plots in acute agranulocytosis (**A** and **B**) and in acute promyelocytic leukemia (**C** and **D**). FITC, fluorescein isothiocyanate.

Table 3
Immunophenotypic Features of 5 Cases of Acute Agranulocytosis and 31 Cases of Acute Promyelocytic Leukemia*

Antigen	Agranulocytosis	Acute Promyelocytic Leukemia
CD13	5 (100)	31 (100)
CD33	5 (100)	31 (100)
CD117	0 (0)	24 (77)
CD11b	5 (100)	2 (6)
HLA-DR	0 (0)	1 (3)
CD34	0 (0)	3 (10)
CD4	2 (40)	13 (42)
CD56	0 (0)	3 (10)
CD19	0 (0)	4 (13)
CD117+CD11b-	0 (0)	23 (74)
CD117-CD11b+	5 (100)	1 (3)

* Data are given as number (percentage) of cases with positive results.

opposite situation is observed rarely, mainly owing to the presence of Auer rods and the absence of fully developed Golgi zones in APL.

Myeloperoxidase-stained smears also were very similar, with a strong reactivity in both diseases. In the typical APL cases, the M/E ratios usually were higher than

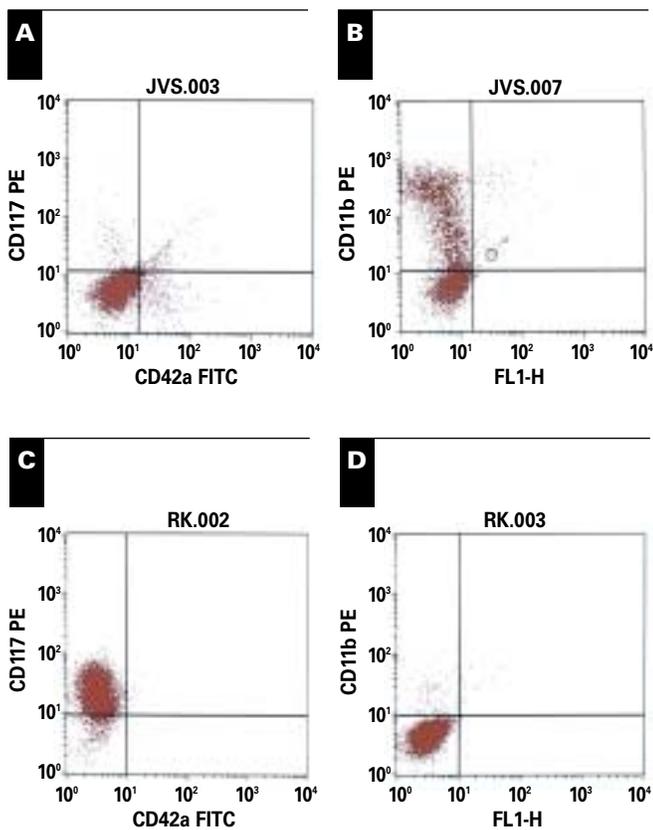


Image 3 Typical pattern of expression of CD117 and CD11b in acute agranulocytosis (**A** and **B**) and acute promyelocytic leukemia (**C** and **D**). FITC, fluorescein isothiocyanate; PE, phycoerythrin.

in the cases of agranulocytosis. However, there was substantial overlap in the range of M/E ratios observed in both diseases (data not shown).

Although our study seems to be limited by the use of only 2-color flow cytometry, we considered that a gating strategy with CD45/SSC, in the setting of a promyelocytic marrow, would not be more helpful in specifically analyzing the populations of cells of interest, as can be observed by the comparison of the promyelocytes gated in an FSC/SSC dot plot and analyzed in a CD45/SSC dot plot as in Image 2. If the gate had been performed in the CD45/SSC dot plot, the cells analyzed would be exactly the same.

The immunophenotypic features of the bone marrow from patients recovering from acute agranulocytosis were identical to the features observed in the cases of APL in many aspects, including the typical APL immunophenotype, with expression of the myeloid markers CD13 and CD33 and lack of expression of CD34 and HLA-DR. Thus, the characteristic immunophenotypic pattern of APL showing negativity for CD34 and HLA-DR, which usually is considered a confirmatory finding in some cases of morphologic overlapping with

other acute myeloid leukemias, is exactly the same phenotype as observed in the recovery from acute agranulocytosis, representing a chance for misdiagnosis. A definitive diagnosis of APL must be determined on the basis of cytogenetic and molecular biologic data, but such procedures usually are not available at initial diagnosis, when treatment decisions need to be made. Among the markers we compared, only CD117 expression and CD11b expression were clearly different in the 2 diseases. CD117 was expressed in 77% of the cases of APL (24/31) and in none of the 5 cases of acute agranulocytosis. CD11b was positive in 6% of the cases of APL (2/31) and in 100% of samples from the 5 patients recovering from acute agranulocytosis. By simple mathematical reasoning, it is possible to assume that the CD117–CD11b+ combination present in samples from all patients recovering from acute agranulocytosis would be present in only 1.6% of the APL cases. In fact, among our 31 cases of APL, only 1 (3%) had this phenotypic combination and in that case, CD11b was present in only 27% of the blasts, in contrast with the samples from patients recovering from acute agranulocytosis that always had a high expression of this marker, being positive in more than 40% of gated cells in all cases, and with 87% and 95% positivity in 2 cases.

It has been shown that the reactivity for CD117 is rare in normal bone marrow and that the positivity is characteristic of the early stages of myeloid differentiation.¹² The CD117+CD34+ subset represents myeloid precursors whose expression of CD34 will be lost after the colony-forming unit–granulocyte-monocyte stage, whenever the CD117 expression starts to decline progressively.^{13,14} We believe that the immunophenotype of the promyelocytes of patients in the recovery phase from acute agranulocytosis is in this stage of differentiation, since CD34 and CD117 already have been lost. The expression of CD117 in acute leukemias is associated with myeloid lineage,¹⁵ and a high proportion of patients with acute myeloid leukemia (23%-87%) have a variable number of CD117+ blasts.¹⁴ Positivity for CD117 in 77% of our cases of APL is in agreement with this data.

Expression of CD11b occurs late during granulocytic differentiation, beginning at the promyelocyte stage of maturation, after CD117 expression has been lost.^{16,17} The phenotypes observed in our cases of recovery from agranulocytosis are compatible with the normal granulocytic differentiation.¹⁶ Conversely, the phenotype of most APL cases seems to represent a frozen step in the maturational process, maintaining the expression of CD117 and lacking the acquisition of CD11b. In fact, CD11b, like HLA-DR, has been reported to be an antigen characteristically negative in APL.¹⁸

The expression of CD11b in association with immaturity markers, mainly CD34, has been reported as an example of asynchronous antigen expression characteristically observed in acute myeloblastic leukemia (AML).¹⁹ The expression of

CD11b in AML usually is associated with the expression of CD117, except when the blasts have a monocytic differentiation.¹⁷ To the best of our knowledge, there is no study specifically determining the incidence of the CD11b+CD117– combination in AML. Indirectly, one can infer that this combination is observed in a low proportion of AML cases, as the expression of CD117 has been demonstrated in a high percentage in AML,¹⁵ and some groups have reported an inverse relationship to the expression of CD11b.^{20,21}

Although the immunophenotypic features of benign myeloid proliferations are expected to show a varying spectrum of patterns depending on the phases of differentiation of the myeloid precursors, we observed that these features are very consistent, at least in the stage at which the bone marrow consists mainly of promyelocytes. Since it is at this stage that differential diagnosis with APL is required, the usefulness of flow cytometry in this setting is plausible. Therefore, we suggest that the analysis of the expression of CD117 and CD11b in bone marrow is helpful in the differentiation of APL and recovering benign myeloid proliferation.

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