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Original Article

Clinicopathological features of acute promyelocytic leukemia: an experience in one institute emphasizing the morphological and immunophenotypic changes at the time of relapse

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Abstract: Acute promyelocytic leukemia (APL) has two morphological variants, namely macrogranular (M3) and microgranular (M3v). M3v, characterized by the presence of neoplastic promyelocytes with only sparse fine azurophilic granules, accounts for 10-25% of all APL and has unique biological characteristics. Relapse occurs in approximately 20% of patients with APL. The morphological type of the leukemic cells at relapse is usually identical with the primary disease, and only one case of morphological change at relapse has been reported. Here, we analyzed the clinicopathological features of APL, including 4 relapsed cases emphasizing morphological changes at the time of relapse. The unique finding of the present study is that 2 of 4 relapsed cases changed from M3 to M3v at relapse. The morphological features of these were different in each case (one had blastic features and the other resembled monocytoid leukemic cells). Cytogenetic analyses revealed the continued presence of t(15;17)(q22;q12) at the time of relapse and morphological change. Moreover, the immune phenotype of the leukemic cells changed from $CD2^-$/$CD34^-$ to $CD2^+$/$CD34^+$ at that time. These findings suggest that morphological change at relapse in APL may not be a rare event, and that the leukemic cells can show variable morphological features at the time of relapse, which could result in misdiagnosis as a different type of acute myeloid leukemia. Therefore, a comprehensive approach with emphasis on combined morphological, immunophenotypic, and cytogenetic analyses is important for diagnosis and appropriate treatment of relapsed APL.

Keywords: Acute promyelocytic leukemia, macrogranular variant, microgranular variant, relapse

Introduction

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia (AML) with characteristic clinical and molecular features, and accounts for 5-8% of all cases of AML. It is well known that APL has two morphological variants, namely macrogranular (M3) and microgranular (M3v) [1]. M3v accounts for approximately 10-25% of adult APL cases and perhaps a somewhat higher fraction in children, and has unique biological characteristics, such as a higher white blood cell count at presentation, and frequent expression of CD2 and CD34 [2-5]. This variant is characterized morphologically by the presence of neoplastic promyelocytes with only sparse fine azurophilic granules and infrequent Auer rods, or no granules and containing a bi-lobed, multi-lobed, or reniform nucleus [6].

Despite the success of current therapies for APL, relapse still occurs in approximately 20% of patients. The morphological and immunophenotypic characteristics of APL at the time of relapse have not been well-documented, although recently Dimov *et al*. analyzed 38 cases of relapsed APL [7]. Herein, we analyzed the clinicopathological features of 12 consecutive cases of APL including 4 relapsed cases in

our institute; we discuss the morphological and immunophenotypic features, especially in the relapsed cases.

Materials and methods

This retrospective study included 12 consecutive APL cases treated at the Department of Hematology of Shiga University of Medical Science from 1993 to 2012. All cases of APL were confirmed by the presence of t(15;17)(q22;q12) identified by conventional cytogenetic analysis. All cases were morphologically classified according to the French-American-British (FAB) classification.

Flow cytometric immune phenotyping analyses on bone marrow aspirates were performed using a FACSCalibur flow cytometer (Becton Dickinson Biosciences, San Jose, CA, USA). The panel of monoclonal antibodies used in this study included CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD13, CD14, CD15, CD19, CD20, CD25, CD33, CD34, CD38, CD45, CD56, and HLA-DR. These antibodies were purchased from Becton Dickinson Biosciences. For this study, a marker was considered positive if expressed by more than 20% of the analyzed cells.

The risk of relapse in APL cases was established at initial diagnosis according to the previously reported predictive model based on peripheral blood leukocyte and platelet counts [8]. Low-risk patients had a leukocyte count $\leq 10 \times 10^9$ /L and a platelet count $>40 \times 10^9$ /L; intermediate-risk patients had a leukocyte count $\leq 10 \times 10^9$ /L and a platelet count $\leq 40 \times 10^9$ /L; and high-risk patients had a leukocyte count $>10 \times 10^9$ /L.

Results

Patients' characteristics

Table 1 summarizes the clinical features of these APL cases at initial diagnosis. There were 4 males and 8 females of ages ranging from 15 to 64 years (mean 46.3 years). This study included 3 patients at high risk of relapse (2 cases had relapsed and the other was M3v), 4 intermediate relapse risk (one relapsed and 3 in complete remission (CR)), and 5 cases at low risk of relapse (one relapsed and 4 in CR).

All patients except Case 12 were treated with all-*trans*-retinoic acid (ATRA) plus chemotherapy.

Clinical outcome

In the four cases of M3 with relapse (Cases 1-4), Cases 1 and 2 had one relapse, Case 3 had two relapses, and Case 4 had three relapses. Case 1 had a skin relapse without bone marrow involvement, and the other 3 cases had relapses only in the bone marrow. Cases 1 and 2 died of disease progression, and Case 4 remains alive without progression 8 months after the third relapse. The fate of Case 3 is not known. The continued presence of t(15;17)(q22;q12) was confirmed at every time of relapse in these cases.

In the seven cases of M3 in complete remission (Cases 5-11), 5 remain alive in CR. Case 10 developed myelodysplastic syndrome (MDS) after ATRA combined chemotherapy, resulting in death on disease progression into AML. In this patient, the leukemic cells after ATRA therapy were $CD34^+$/$HLA\text{-}DR^+$ and lacked t(15;17)(q22;q12). Therefore, this case was considered as a therapy-related myeloid neoplasm (t-MN). Case 11 died of liver cirrhosis.

The M3v patient (Case 12) died of brain hemorrhage immediately after the diagnosis, before treatment could be initiated.

Morphological characteristics

In Cases 1-11, the leukemic cells at initial diagnosis showed typical features of macrogranular neoplastic promyelocytes. They had bi-lobed or folded nuclei and abundant cytoplasm with large granules (**Figure 1A**). In Case 12, the leukemic cells at initial diagnosis had bi-lobed or multi-lobed nuclei and only sparse fine azurophilic granules and infrequent Auer rods, or no granules (**Figure 1B**), which are characteristic features of M3v.

In Cases 3 and 4, the morphology of the leukemic cells changed from typical macrogranular type to microgranular variant at relapse (**Table 2**). The leukemic cells at the second relapse of Case 3 had a high nuclear/cytoplasmic ratio, scant cytoplasm, and irregular nuclear contours. The cytoplasm of these cells contained no granules, although small vacuoles were present in some of them (**Figure 1C**). The leukemic cells at the third relapse of Case 4 had a small amount of cytoplasm and irregular nuclear contours, and contained no granules (**Figure 1D**).

Table 1. Clinical features of acute promyelocytic leukemia

Case No.	Age	Gender	Leukocytes ($\times 10^9$/L)	Platelet ($\times 10^9$/L)	Relapse Risk	Treatment	Outcome
Group I (M3 with relapse)							
1	15	Female	26.2	1.1	High	ATRA combined with chemotherapy	Died of disease progression
2	64	Male	0.5	3.6	Intermediate	APL92	Died of disease progression
3	49	Female	15.2	3.1	High	APL97	Not available
4	59	Female	0.8	6.6	Low	APL97	Alive without progression
Group II (M3 with complete remission)							
5	31	Female	1	7.7	Low	APL92	Alive in complete remission
6	34	Female	0.5	2.1	Intermediate	APL97	Alive in complete remission
7	55	Female	1	0.6	Intermediate	APL97	Alive in complete remission
8	34	Female	3	1.7	Intermediate	APL204	Alive in complete remission
9	51	Male	0.8	14.8	Low	APL204	Alive in complete remission
10	58	Male	2.2	15.5	Low	APL97	Died of treatment-related myeloid neoplasm
11	58	Male	2.8	9.6	Low	APL92	Died of liver cirrhosis
Group III (M3v)							
12	48	Female	147	1.8	High	Not Done	Died of brain hemorrhage

ATRA, all-*trans*-retinoic acid.

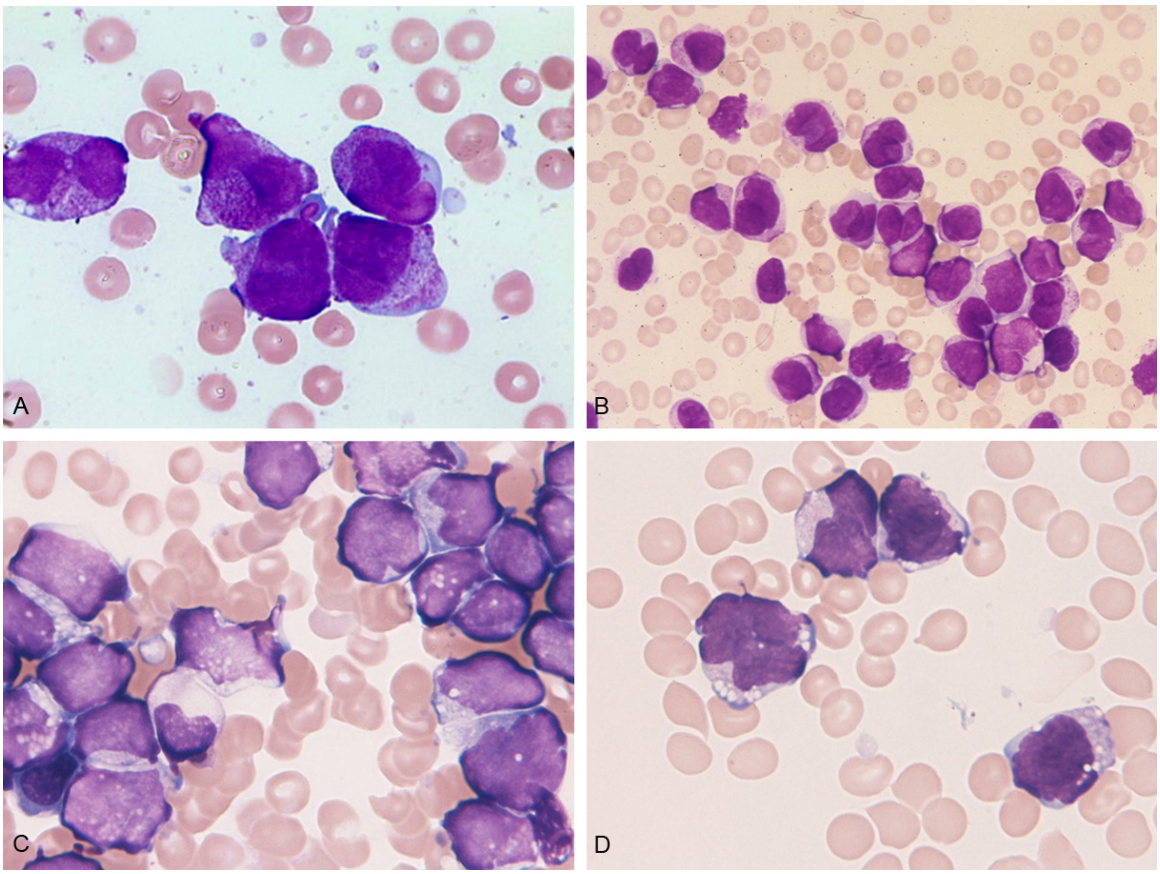

Figure 1. Bone marrow aspirate smears of acute promyelocytic leukemia. A: Typical macrogranular neoplastic promyelocytes (Case 3 at initial diagnosis). The leukemic cells have bi-lobed or folded nuclei and abundant cytoplasm with large granules. Wright-Giemsa, × 1,000. B: Microgranular variant of acute promyelocytic leukemia (Case 12). The leukemic cells have bi-lobed or folded nuclei and inconspicuous cytoplasmic granules. Wright-Giemsa, × 400. C: Case 3 at the second relapse. The leukemic cells have a high nuclear/cytoplasmic ratio, scant cytoplasm, and irregular nuclear contours. The cytoplasm of these cells contains no granules, although small vacuoles are present in some. Wright-Giemsa, × 1,000. D: Case 4 at the third relapse. The leukemic cells have a small amount of cytoplasm and irregular nuclear contours, and contain no granules. Wright-Giemsa, × 1,000.

Immunophenotypic characteristics

Table 2 summarizes the immunophenotyping of these APL cases. The data at initial diagnosis (Case 1), the first relapse (Case 2), and initial and subsequent relapse (the second and third time in Cases 3 and 4, respectively) were available for relapsed M3. They were also available for all M3 cases in CR (Cases 5-11) and M3v (Case 12).

CD2 and CD34 were expressed by the leukemic cells in Case 1, and CD2 expression was also noted in Case 12 at initial diagnosis. However, these markers were not expressed in any of the other cases at initial diagnosis. CD56 expression was observed in Case 2 (at the first relapse) and Case 12. Intriguingly, the expression of CD2 and CD34 was seen in those cases showing morphological changes at relapse (Cases 3 and 4). In both cases, at the initial diagnosis, the leukemic cells showed the typical morphology of macrogranular promyelocytes and were $CD2^-/CD34^-$. However, the leukemic cells showing microgranular morphology at the time of relapse were $CD2^+/CD34^+$. In addition, both cases also gain CD56 expression at the time of morphological change at relapse.

All cases were positive for CD13 and CD33, and negative for HLA-DR.

Discussion

Although ATRA combined with anthracycline-based chemotherapy yields a complete remission rate of approximately 90% for newly-diag-

Table 2. Morphological and immunophenotypic characteristics of acute promylocytic leukemia

Case No.	Morphology	CD2	CD34	CD56	CD13	CD33	HLA-DR
Group I (M3 with relapse)							
1 (initial)	Macrogranular	33	34	2	96	99	8
2 (relapsed, 1st)	Macrogranular	1	2	58	53	92	6
3 (initial)	Macrogranular	1	1	0	58	95	2
(relapsed, 2nd)	Microgranular	76	66	7	99	99	11
4 (initial)	Macrogranular	4	6	2	74	86	11
(relapsed, 3rd)	Microgranular	85	90	21	86	98	10
Group II (M3 with complete remission)							
5	Macrogranular	0	1	ND	65	96	1
6	Macrogranular	0	0	0	30	98	15
7	Macrogranular	1	1	0	75	98	2
8	Macrogranular	0	0	0	60	91	0
9	Macrogranular	9	4	1	86	87	5
10	Macrogranular	7	16	3	96	99	2
11	Macrogranular	11	4	ND	74	79	8
Group III (M3v)							
12	Microgranular	51	8	23	95	98	9

ND, Not done.

nosed APL, relapse frequently occurs (approximately 20%). The approach for establishing the diagnosis of relapse in APL relies on the presumption that recurrent disease will have morphology similar to pre-therapy appearance. Dimov *et al*. reported that the morphological type of leukemic cells at relapse in APL is usually stable over time. However, they did find that one of 38 relapsed cases (2.6%) showed a morphological change [7]. That case was a typical macrogranular type at initial diagnosis before therapy and relapsed as M3v, having blastic features with scant cytoplasm and no granules in the cytoplasm [7]. In our study, 2 of 4 cases of relapsed APL manifested morphological change at the time of relapse, and the leukemic cells had different morphological features in each case. At the second relapse of Case 3, the leukemic cells had a high nuclear/cytoplasmic ratio and scant cytoplasm, resembling myeloblasts. These are the same morphological features as the previously reported case by Dimov *et al*. [7]. The leukemic cells at the third relapse of Case 4 had a small amount of cytoplasm and irregular nuclear contours, and were therefore akin to monocytoid leukemic cells. Cytogenetic analyses revealed the continued presence of t(15;17)(q22;q12) in these two cases at relapse. Therefore, the leukemic cells with a changed morphology were considered to be relapsed APL. These findings indicate that the morphological change from typical macrogranular type to M3v at relapse may not be such a rare event, so that these morphological characteristics at relapse might lead to misdiagnosis as a different type of AML, such as M0, M1, M4, or M5.

Additionally, the development of t-MN can occur in patients successfully treated for APL [9, 10]. Case 10 of the present series developed MDS after ATRA therapy. Because the neoplastic cells were $CD34^+$/$HLA\text{-}DR^+$, and lacked t(15;17)(q22;q12), this case was diagnosed as t-MN secondary to APL. According to previous studies, although MDS is the most common disease of t-MN secondary to APL, AML including M0, M1, M4, and M5 has also been recognized as t-MN [9, 10]. Therefore, t-MN must be distinguished from relapsed APL showing morphological change, because the latter can manifest similar morphological features as M0, M1, M4, and M5, as seen in the present Cases 3 and 4, and previously reported [7]. Thus, cytogenetic analysis is needed for the correct diagnosis of relapsed APL. Moreover, because therapies for relapsed APL and t-MN secondary to APL are completely different, this differential diagnosis is crucial.

Typical neoplastic promyelocytes characteristically express mature myeloid markers, includ-

ing CD13 and CD33, and are usually negative for CD2 (12%), CD34 (0%), and HLA-DR (1%) [2, 3]. However, M3v is more heterogeneous with variable expression of CD2 (56%), CD34 (75%), and HLA-DR (31%), although CD13 and CD33 are also expressed in M3v [3]. Although the immune phenotype of the leukemic cells at relapse was unchanged in the previously reported case despite the morphological change from typical macrogranular type at initial diagnosis to M3v at relapse (the detailed immune phenotype was not available) [7], we saw clear expression of CD2 and CD34 at relapse in the cases showing morphological change (Cases 3 and 4). This might be related to the change of morphological features of leukemic cells in APL because CD2 and CD34 are commonly expressed in M3v, but not in macrogranular type.

In summary, this study revealed that morphological changes of leukemic cells at relapse in APL may not a rare event, and that the leukemic cells can show variable morphological features resembling myeloblasts or monocytoid leukemic cells. It is difficult to estimate by morphological, immunophenotypic, and risk-of-relapse analysis at initial diagnosis whether the morphological features will change or not at the time of relapse. Given the risk of misinterpretation, a comprehensive approach with emphasis on combined morphological, immunophenotypic, and cytogenetic analysis is important for the diagnosis and correct treatment of relapsed APL.

Disclosure of conflict of interest

None.

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