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Significance of Transmission Electron Microscopy in Subtyping of Monocytic Leukemia

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ABSTRACT

The objective of this paper is to produce an ultrastructural classification of acute monocytic leukemia (AML-M5) in relation to clinical behaviors. The ultrastructural characteristics of blasts of the monocytic series were analyzed in 72 M5 patients by transmission electron microscopy (TEM), in terms of their content of typical monoblasts, atypical monoblasts, atypical promonocytes, and typical promonocytes in bone-marrow aspirates. Four kinds of monocytic blasts were identified by cell size and shape, nuclear profile, nucleocytoplasmic ratio, heterochromatin content, nucleolus, granules, vesicles, and Golgi apparatus. Their characteristics of remission rate, cytochemistry, immunophenotype, and cytogenetics were also investigated. The data obtained permitted M5 patients to be divided into monoblast and promonocyte types. Monoblast-type patients expressed weaker monocytic enzymograms and specific antigen staining for CD14 and CD64, compared with promonocyte-type patients. Monoblast patients had higher CR than promonocyte patients. Therefore, TEM subclassification of patients differs from and improves upon the light microscopical criteria for distinguishing monoblasts and promonocytes and has clinical significance.

Keywords: acute monocytic leukemia, classification, differentiation, ultrastructure

The development or maturation of monocytes in the bone marrow is a dynamic process, including myeloid progenitor, monoblast, promonocyte, and monocyte stages. Circulating monocytes and various histiocytes are also recognized according to their location and function in peripheral blood and tissues. In acute monocytic leukemia (M5), monoblasts, promonocytes, and monocytes are distinguished by light microscopy, and M5 is further subdivided into M5a and M5b by the percentage of blast

types in the French-American-British (FAB) and World Health Organization (WHO) classifications [1, 2].

M5 has distinct characteristics compared with other AML subtypes in terms of immunophenotype, karyotype, and genetics. CD14, CD56, and CD64 are expressed in M5 in addition to common myeloid antigens, and the 11q23 translocation occurs more frequently in M5 than in other AML subtypes [3]. The FMS-like tyrosine kinase 3 (FLT3) mutation is also frequently found in M5 [4].

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TEM Subtyping of Monocytic Leukemia

The characteristic immunophenotype, genetics, and prognostic factors for M5a and M5b have been compared recently. Haferlach et al. [4] detected an aberrant karyotype in 75.9% of M5a and 28.8% of M5b, of which 11q23/MLL aberrations were found in 31% of M5a and 12.1% of M5b. These data suggest that M5 should be

categorized as two different groups [5]. Other clinical investigations indicated more aberrant karyotype and less FLT3 mutation in M5a compared with M5b [6]. In contrast, some studies showed no significant differences in immunophenotype, karyotype, or prognosis between M5a and M5b [7, 8].

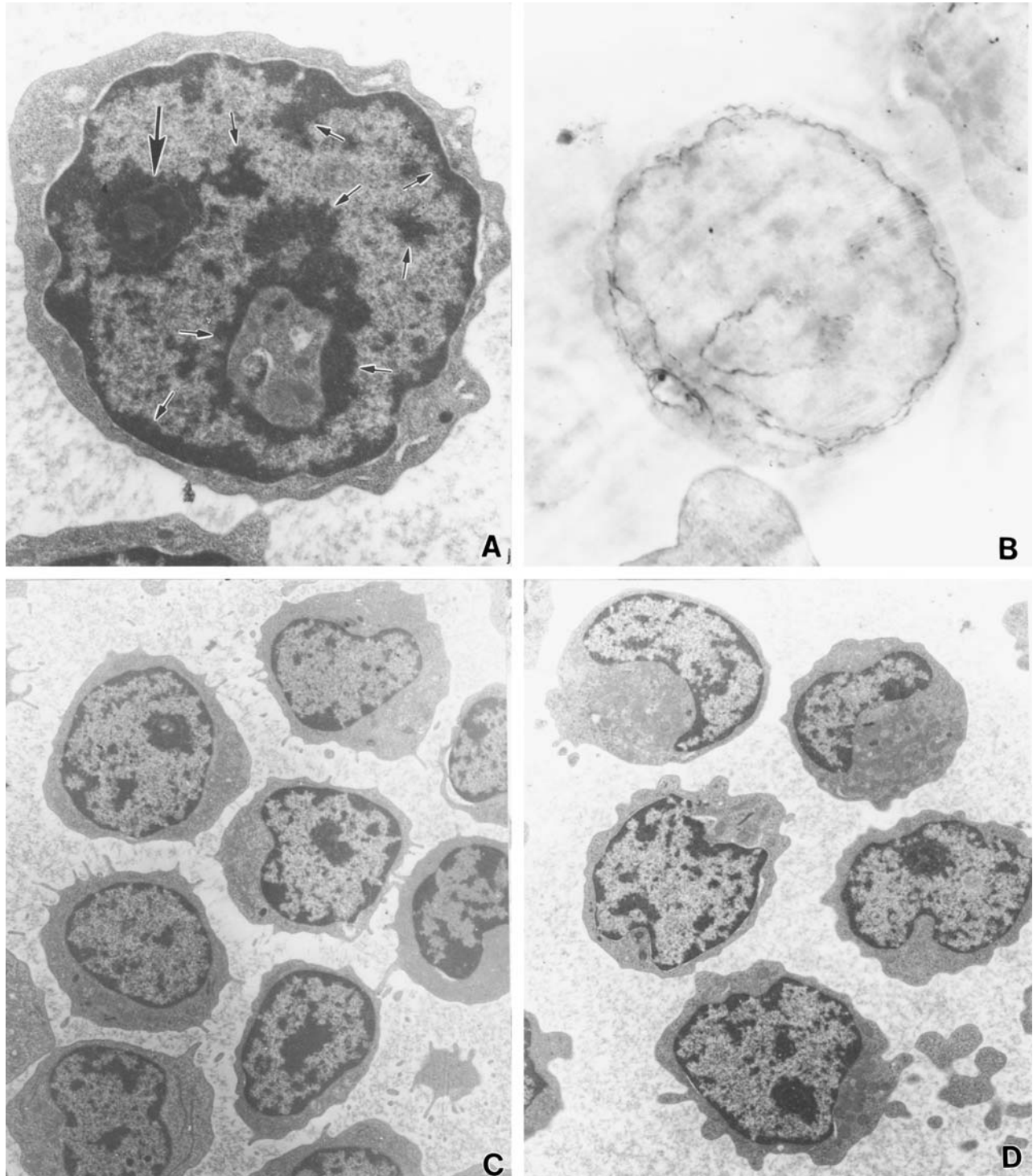


Figure 1. (A) Typical monoblast with diameter of $10\mu\text{m}$, high NCR, rounded nuclear contour, smaller nucleolus (arrow), increased heterochromatin (short arrow), scarce cytoplasm, and few organelles. $\times 10,000$. (B) Nuclear membrane positive for MPO and no positively stained granules in the cytoplasm. $\times 10,000$. (C, D) TMB subtype. $\times 4000$, $\times 3500$.

We suppose this controversy might result from uncertainties in the morphological (light microscopical) distinction of monoblasts from promonocytes. Since there are no strict criteria to distinguish monoblasts from promonocytes cytochemically, immunophenotypically, and cytogenetically, it may be necessary to introduce transmission electron microscopy (TEM) to distinguish blasts in M5, and this is the objective of the present study.

MATERIALS AND METHODS

Patients and FAB/WHO Diagnostic Criteria

Seventy-two patients with de novo M5 were admissions of the Blood Diseases Hospital from 2004 to 2006. The diagnosis was established by conventional criteria, combining results from clinical features, flow cytometry, cytochemistry, and cytogenetics. M5a and M5b were subtyped, with all patients fulfilling the following criteria: (1) At least 80% of the leukemic cells were morphologically of monocytic lineage, and included monoblasts, promonocytes, and monocytes. (2) A minor granulocytic component was present (<20%). (3) In M5a, the majority of the monocytic series were monoblasts ($\geq 80\%$), while in M5b, the majority were promonocytes ($\geq 80\%$). (4) The leukemic population usually showed intense nonspecific esterase activity with sodium fluoride (NaF) inhibition. (5) Patients had $\geq 20\%$ bone-marrow leukemic cells. The cases that were difficult to classify were designated as M5u. Fifty-four of the 72 patients accepted standard chemotherapy.

TEM Technique and Classification

The procedure for morphological TEM was as previously described [9]. Myeloperoxidase activity (MPO) was detected by the method of Roels et al. [10]. MPO-positivity was assessed in 200 blasts from each specimen, and calculated as a percentage. Four kinds of monocytic blast were ultrastructurally defined as follows. (1) *Typical monoblast (TMB)*: small size, round shape, round nucleus, high nucleocytoplasmic ratio (NCR), prominent heterochromatin, small nucleolus, few cytoplasmic organelles such as granules or vesicles, and rare Golgi apparatus (Figure 1). (2) *Atypical monoblast (AMB)*: larger size, only slightly irregular shape, large nucleus, prominent nucleolus, high NCR, reduced heterochromatin, and moderate amount of cytoplasm containing fewer cytoplasmic organelles (Figure 2A, B). (3) *Atypical promonocyte (APM)*: shape and nuclear structure as in AMB, rich in granules and vesicles and immature Golgi apparatus (Figure 2C, D). (4) *Typical promonocyte (TPM)*: irregular shape, cell-surface processes, voluminous cytoplasm containing plentiful granules and

vesicles and fully developed Golgi apparatus, lower NCR, irregular nucleus with increased heterochromatin and small or obscure nucleolus, occasional vacuoles or phagocytosis (Figure 3, Table 1). Patients were subtyped on the basis of the predominant TMB, AMB, APM, or TPM.

Karyotyping

Chromosome analysis was performed on short-term bone-marrow cultures by standard methods with at least 20 mitoses analyzed in each case.

Cytochemistry

Cytochemistry for peroxidase (POX), Sudan black B (SBB), periodic acid-Schiff (PAS), naphthol AS-D chloracetate esterase (CE), acid phosphatase (ACP), α -naphthyl butyrate esterase (NBE), α -naphthyl acetate esterase (NAE), and their inhibition by NaF (NAE + NaF) were performed according to routine procedures. The scoring was that of the International Committee for Standardization in Hematology [11].

Immunophenotyping

Immunophenotype was analyzed on a FACS Calibur flow cytometer (Becton Dickinson, San Jose, CA) equipped with Cellquest software [12]. Cytoplasmic and nuclear antigens were detected with FACS permeabilization solution (Becton Dickinson). Antigens detected were lymphoid markers CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD22, and CD79a; myeloid markers CD13, CD14, CD16, CD33, CD64, CD117, and MPO. The nonspecific markers CD34, CD56, and HLA-DR were also detected. Positivity was defined as expression in $\geq 20\%$ of blasts for surface antigens and $\geq 10\%$ of blasts for cytoplasmic and nuclear antigens.

Statistical Analyses

Age, sex, abnormal karyotype, complete remission, and immunophenotype were compared with chi-square test, respectively, and cytochemical characteristics using Variance Test in SPSS. MB (TMB + AMB) and PM (TPM + APM) types were also compared.

RESULTS

Patients

The 72 patients consisted of M5a ($n=10$), M5b ($n=51$), and M5u ($n=11$). The median age for all patients was 37 years (range, 2-67); 34 years (range,

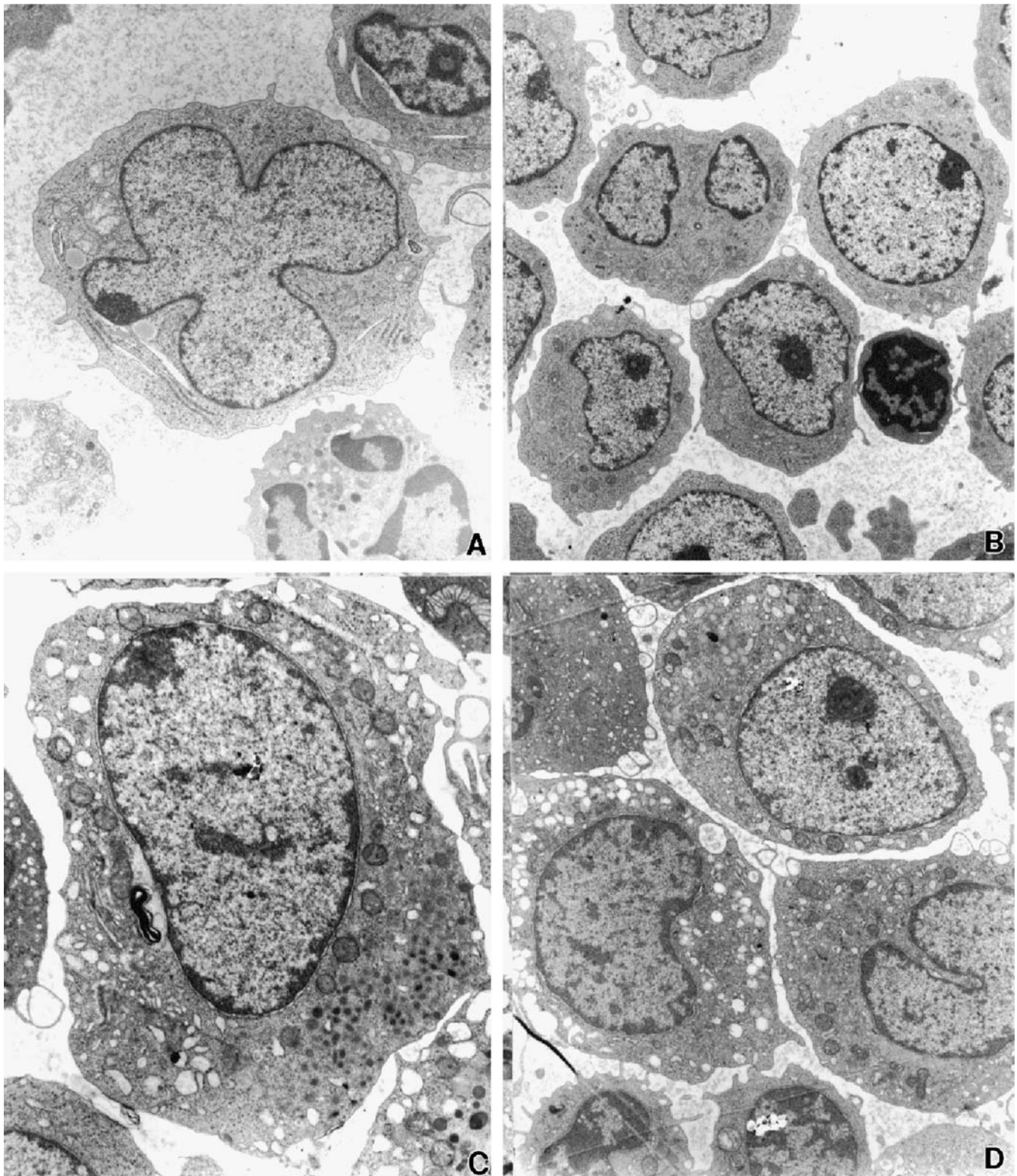


Figure 2. (A) Atypical monoblast, 15 μm in diameter, less heterochromatin, prominent nucleolus, moderate cytoplasm containing fewer organelles. $\times 5000$. (B) AMB subtype. $\times 4000$. (C) Atypical promonocyte, with a size and nuclear features like those of atypical monoblasts, but rich in granules, vesicles, and Golgi apparatus. $\times 6000$. (D) APM subtype. $\times 3000$.

12–54) for M5a, 38 years (range, 6–67) for M5b, and 41 years (range, 2–63) for M5u. Karyotyping was tested in

6 of 9 M5a patients (66%), 17 of 44 M5b (36%), and 3 of 6 M5u (50%). Complete remission (CR) was achieved

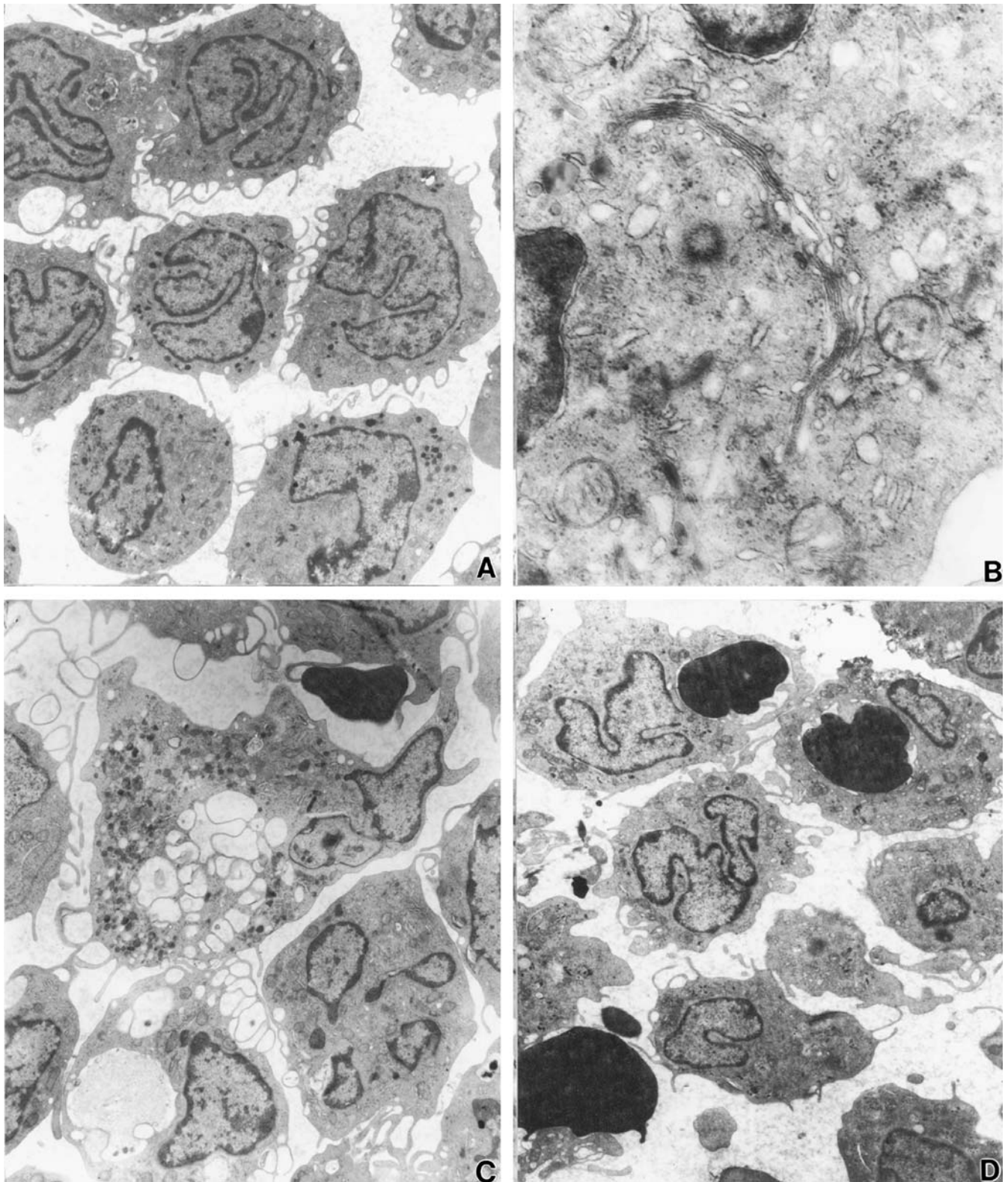


Figure 3. Typical promonocyte and TPM subtype. (A) Blasts showing irregular shape, 16 μm in diameter, many processes, lower NCR, twisted nucleus with more heterochromatin and obscure nucleolus, prominent cytoplasm containing many small granules and vesicles. $\times 4000$. (B) Blasts containing highly developed Golgi apparatus. $\times 20,000$. (C) Blasts showing erythrophagocytosis. $\times 3000$. (D) Blasts showing active features such as vacuolization and phagocytosis and longer processes. $\times 3500$.

in 4 of 7 M5a patients (57%), 24 of 41 M5b (58%), and 3 of 6 M5u (50%). There were no statistical differences

in age, sex, karyotype abnormality, or CR between M5a and M5b patients.

Table 1. Ultrastructural characteristics of the four subtypes of the monocytic series in AML-M5.

Feature	TMB	AMB	APM	TPM
Shape	Round	Round or elliptical	Irregular	Irregular
Surface	Smooth	Rounded processes	Less processes	More Slender processes
Diameter	≤11 μm	≥12 μm	≥12 μm	≥12 μm
NCR	≥1:1.2	≥1:1.5	≤1:1.5	≤1:2
Nucleus	Regular	Slightly irregular	Slightly irregular	Irregular
Nucleolus	≤3 μm	≥3 μm	≥3 μm	≤3 μm
Heterochromatin	Increased	Decreased	Decreased	Increased
Granules (number)	Few	Few	Moderate	Many
Ga Development	Little	Little	Moderate	Many
Vesicles	Few	Few	Moderate number	Many
Vacuoles	Absent	Absent	Absent	Many
Phagocytosis	Absent	Absent	Absent	Occasional

Note. NCR, nuclear-cytoplasm ratio; GA, Golgi apparatus.

TEM Ultrastructural Classification

On the basis of ultrastructural criteria, four subtypes of M5—TMB, AMB, APM, and TPM—were identified. The numbers of cases of these subtypes and their composition in terms of being M5a, b, or u are given in Table 2. No significant difference existed in age and sex between any two of the four ultrastructural subtypes, as well as between MB and PM types.

Cytogenetics and CR

Cytogenetic data were available for 59 cases. There were no statistically significant differences among them in karyotype abnormalities (KA) of TMB, AMB, APM, and TPM types or MB and PM types. CR data were available for 54 cases: 33% were TMB type, 40% AMB type, 67% APM type, and 72% TPM type. The CR of the four subtypes illustrated an increasing tendency from TMB to TPM, although no statistical difference existed between any two of them (Table 2). Patients of MB type had increased CR compared with PM type (37 versus 70%; $p < .025$).

Cytochemistry

Cytochemical data were available for all cases (Table 3). Positive indices of ACP, PAS, POX, CE, and SBB mostly increased from TMB to TPM subtype, though there was no statistical difference between them. The NBE index for TPM and APM was higher than for TMB and AMB statistically and respectively ($p < .05$). The NAE index of APM subtype was higher than for AMB subtype statistically ($p < .005$). Indices of PAS, POX, CE, SBB, NAE, and NBE for MB type were higher than for PM type and there was significant difference between them ($p < .05$).

Immunophenotyping

For the 69 patients tested, the numbers and percentages of cases positive for the relevant MoAbs in the four subtypes are listed in Table 4. CD33, HLA-DR, CD13, and CD117 were highly expressed in the four subtypes and there was no statistical difference between them. CD34, CD7, and CD19 generally decreased from TMB to TPM subtypes. CD34 was

Table 2. Clinical data of the four ultrastructural subtypes of AML-M5.

UST	Cases	MA	Sex (F/M)	KA/CT (%)	CR/TC (%)	M5a	M5b	M5u
TMB	13	39	8/5	5/10 (50)	3/9 (33)	3	7	3
AMB	21	43	5/16	8/17 (47)	6/15 (40)	5	12	4
APM	14	35	7/7	8/13 (62)	8/12 (67)	1	11	2
TPM	24	34	6/18	5/19 (26)	13/18 (72)	1	20	3
Total	72	37	26/46	26/59 (44)	30/54 (56)	10	51	11

Note. UST, ultrastructural subtype; MA, median age; F/M, female/male; KA, karyotype abnormality; CT, case tested; CR, complete remission; TC, treated cases.

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Table 3. Cytochemistry in four ultrastructural subtypes of AML-M5: positive indices.

UST	ACP	PAS	POX	CE	SBB	NAE	NBE
TMB	103 ± 70	108 ± 24	45 ± 56	14 ± 21	59 ± 61	89 ± 61	10 ± 11
AMB	100 ± 65	123 ± 37	65 ± 62	40 ± 60	90 ± 70	62 ± 62	23 ± 23
APM	119 ± 64	153 ± 52	115 ± 112	77 ± 107	138 ± 115	151 ± 121●	136 ± 147★●
TPM	122 ± 43	136 ± 41	103 ± 82	69 ± 61	136 ± 80	114 ± 80	98 ± 95★●
MB	100 ± 64	117 ± 33	58 ± 60	30 ± 50	79 ± 68	72 ± 61	15 ± 27
PM	121 ± 53	142 ± 47▲	108 ± 99▲	71 ± 85▲	136 ± 97▲	129 ± 98▲	105 ± 117▲

— Note. MB, TMB + AMB; PM, TPM + APM; ★, compared with TMB, $p < .05$; ●, compared with AMB, $p < .05$; ▲, compared with MB, $p < .05$.

Table 4. Flow cytometry in the four ultrastructural subtypes of AML-M5: positive cases (%).

UST	TC	CD13	CD33	CD117	DR	CD19	CD34	CD7	CD14	CD56	CD64
TMB	12	11 (92)	11 (91.7)	12 (100)	11 (92)	3 (25)	11 (92)	6 (50)	1 (8.3)	3 (25)	2 (16.7)
AMB	20	16 (80)	19 (95)	17 (85)	20 (100)	3 (15)	16 (80)	10 (50)	5 (25)	6 (30)	13 (65)★●
APM	14	13 (93)	14 (100)	11 (78.6)	14 (100)	2 (14.3)	8 (57)	2 (14.3)	5 (35.7)	6 (42.9)	9 (64.3)★●
TPM	23	23 (100)	21 (91.3)	17 (73.9)	18 (78.2)	2 (8.7)	14 (60.9)	5 (21.7)	13 (56.5)★	13 (56.5)	19 (82.6)★
MB	32	27 (84.4)	30 (93.8)	29 (90.6)	31 (96.9)	6 (18.7)	27 (84.4)	16 (50)	6 (18.8)	9 (28.1)	15 (46.9)
PM	37	36 (97.3)	35 (94.6)	28 (75.7)	32 (86.5)	4 (10.8)	22 (54)	7 (18.9)▲	18 (48.6)▲	19 (51.4)▲	28 (75.7)▲

TC, tested cases; ★, compared with TMB, $p < .05$; ●, compared with TPM, $p < .05$; ▲, compared with MB, $p < .05$.

higher in MB than PM type but without significant difference, while CD7 was higher in MB than PM type statistically ($p < .05$). CD14, CD56, and CD64 increased from TMB to TPM subtypes. For CD14, the differences between TMB and TPM subtypes and between MB and PM types were statistically significant ($p < .05$). CD64 was significantly decreased in TMB compared with AMB, APM, and TPM subtypes, and it was decreased in TPM compared with AMB and APM subtypes respectively and statistically ($p < .05$). Additionally, the MB and PM types were significantly different for CD56 ($p < .05$).

DISCUSSION

M5 is conventionally subdivided into M5a and M5b according to the numbers of monoblasts or promonocytes in the monocytic series by light microscopy in the FAB and WHO protocols, in which the monoblast is defined as having round nuclei, finely dispersed chromatin, prominent nucleoli, and abundant basophilic cytoplasm, while the promonocyte has irregular nuclei and less intensely basophilic cytoplasm [2]. Though the morphological identification by light microscopy is a quick, simple, and cheap method that is universally accepted, some M5 patients are still difficult to categorize as M5a or M5b. For example, the

blasts in some M5 patients do not show the typical morphological features of monoblasts or promonocytes as in the FAB description. In addition, there is interobserver inconsistency among some FAB M5 patients. In the present study, 11 cases (15.3%) were unclassified on the basis of strikingly contradictory clinical opinions. Such clinical inconsistencies may be responsible for the difficulties reported in the literature in distinguishing M5a and M5b.

In this study, we have succeeded in classifying M5 on the basis of the ultrastructurally determined predominant monocytic blasts, of which we have identified four kinds—TMB, AMB, APM, and TPM subtypes. The TMB subtypes were poorly differentiated, while the TPM subtypes were well-differentiated: AMB and APM showed an intermediate level of differentiation. These differences in differentiation are based on features such as larger size of AMB compared with TMB; numerous vacuoles and granules in TPM and APM compared with TMB and AMB; and larger nuclei and nucleoli in AMB and APM compared with TMB and TPM. These characteristics have illustrated consecutive differentiation stages, helping to make more precise definitions of monoblasts and promonocytes and refine the light microscopy criteria in M5. Therefore, this subtyping system may reveal relationships between the morphology and the clinic

significances such as karyotyping, CR, cytochemistry, and immunophenotyping.

Of 59 available cases, karyotype abnormality showed no significant difference between any two of the four subtypes or between MB and PM types, respectively. In 54 available cases, there was an increasing tendency of CR for TMB to TPM. Patients of MB type had poorer CR than those of PM type, demonstrating the correlation between higher ultrastructural differentiation and better CR rates. Our ultrastructural classification therefore shows that there is a difference prognostically between M5a and M5b, whereas other workers have sometimes found a difference and sometimes not [13].

Cytochemically, although the indices for ACP, PAS, POX, CE, and SBB increased from TMB to TPM generally, they were not statistically different among the four subtypes. NAE and NBE indices also increased

from TMB to TPM subtype and the NBE index was significantly higher in TPM and APM than in TMB and AMB subtypes, respectively. These data confirmed that NBE was more specific for monocytic differentiation but less sensitive than NAE [14]. With the exception of ACP, the indices of PAS, POX, CE, SBB, NAE, and NBE in the PM type were statistically higher than in the MB type, indicating that the enzymograms expression correlated with ultrastructural differentiation (Figure 4).

Immunophenotypically, all four subtypes showed strong positivity for the common myeloid markers, consistent with the literature, and showed no correlation with differentiation [15]. There was a gradual decrease in CD34 positivity from TMB to TPM subtype and a statistical difference between MB and PM types. Patients of MB type were more frequently positive for

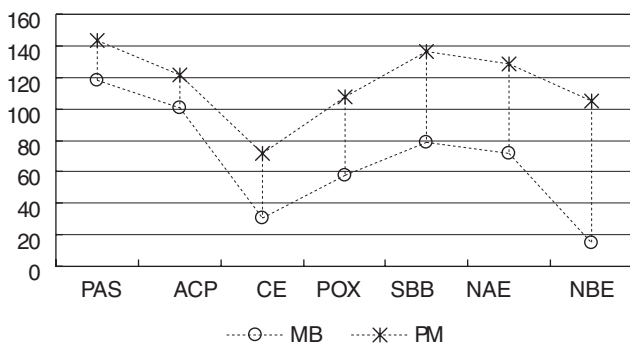


Figure 4. Comparison average cytochemical indices of MB and PM types.

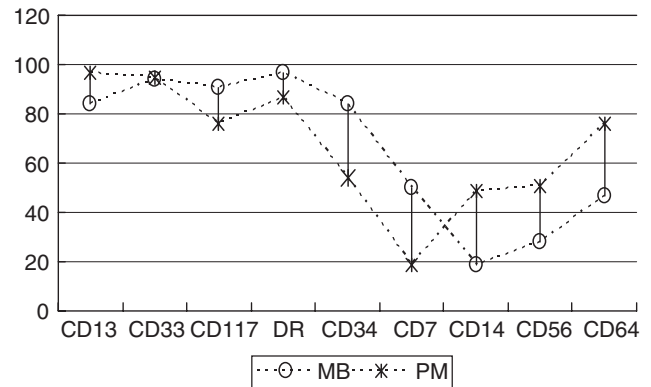


Figure 5. Percentages of cases positive for MoAbs in MB and PM types.

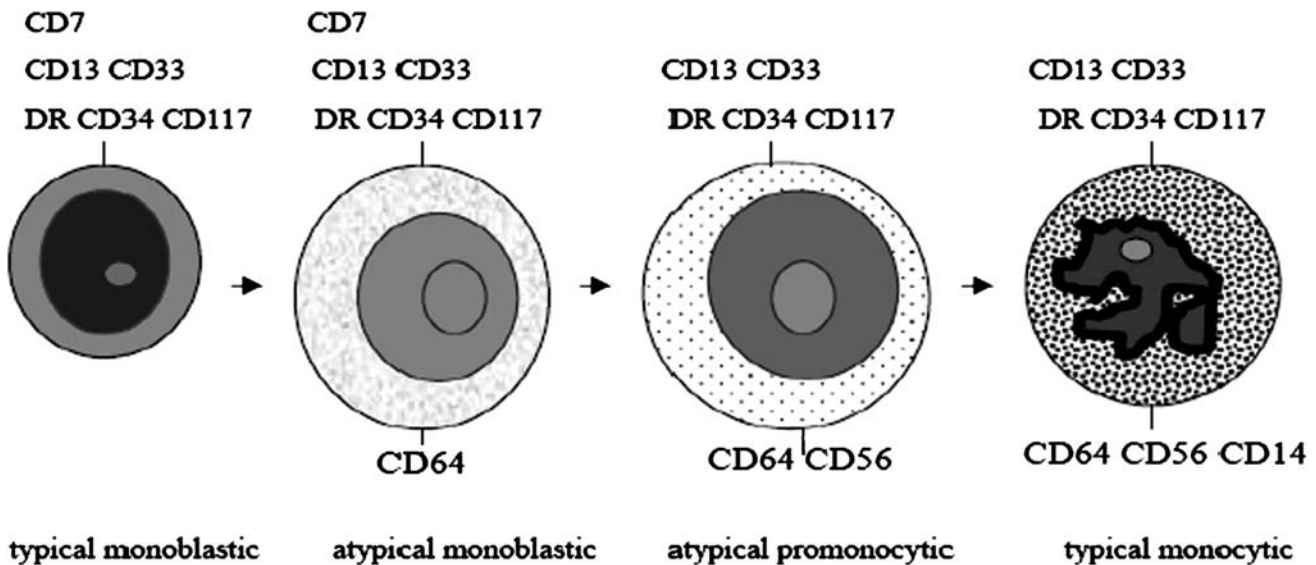


Figure 6. Correlation between ultrastructural and immunophenotype differentiation. Note development of cytoplasm, nucleus, nucleoli, heterochromatin, euchromatin, vesicles, and granules in the monocytic series.

CD7 than PM type statistically, suggesting that CD34 and CD7 were more frequently expressed in the poorly differentiated monocytic series as in other AML and may be associated with worse prognosis [16].

CD14 and CD64 are specific antigens for monocytes, although they are not completely sensitive in M5 patients [17]. Our data showed cases positive for these antigens increasing from TMB to TPM subtypes generally, and significant differences between the four subtypes. The data demonstrated that these antigens were usually expressed on well-differentiated monocytic blasts in M5. CD56 is a natural killer (NK) cell marker identified in approximately 15–20% of AML, and is associated with monocytic morphology [18]. In our study, the percentage of cases positive for CD56 also increased from TMB to TPM subtypes with a statistically difference between MB and PM types. Therefore, the ultrastructural subtypes were correlated with the antigens expression immunophenotypically (Figure 5 and 6).

CONCLUSION

Cells of the monocytic series exhibited a number of ultrastructural features in different M5 patients, enabling them to be divided into two broad groups (types)—monoblastic and promonocytic—based on features such as cytoplasmic volume, development of Golgi apparatus, and numbers of vesicles and granules. Monoblast-predominant M5 patients expressed weaker monocytic enzymograms and specific antigens, CD14 and CD64, compared with promonocyte-predominant M5 patients. CD34 and CD7 were more frequently expressed in the monoblast-predominant patients. Monoblast-predominant M5 patients had poorer CR than promonocyte-predominant patients. Therefore, the ultrastructural subclassification on basis of the predominant blast type differs from and improves on the light microscopical criteria for distinguishing monoblasts and promonocytes and has clinical significance.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

1. Bennett JM, Catovsky D, Daniel MT, et al. Proposed revised criteria for the classification of acute myeloid leukemia: a report of the French-American-British Cooperative Group. *Ann Intern Med.* 1985;103:620–625.

2. Jaffe ES, N.L. H, Stein H, Vardiman JW. Pathology and genetics. World Health Organization of tumours or haematopoietic and lymphoid tissues. Lyon: IARC Press; 2001.
3. Chang H, Brandwein J, Yi QL, et al. Extramedullary infiltrates of AML are associated with CD56 expression 11q23 abnormalities and inferior clinical outcome. *Leuk Res.* 2004;28:1007–1011.
4. Haferlach T, Schoch C, Schnittger S, et al. Distinct genetic patterns can be identified in acute monoblastic and acute monocytic leukaemia (FAB AML M5a and M5b): a study of 124 patients. *Br J Haematol.* 2002;118:426–431.
5. Kuchenbauer F, Kern W, Schoch C, et al. Detailed analysis of FLT3 expression levels in acute myeloid leukemia. *Haematologica.* 2005;90:1617–1625.
6. Haferlach T, Schoch C, Schnittger S, et al. Distinct genetic patterns can be identified in acute monoblastic and acute monocytic leukaemia (FAB AML M5a and M5b): a study of 124 patients. *Br J Haematol.* 2002;118:426–431.
7. Villeneuve P, Kim DT, Xu W, et al. The morphological sub-categories of acute monocytic leukemia (M5a and M5b) share similar immunophenotypic and cytogenetic features and clinical outcomes. *Leuk Res.* 2008;32:269–273.
8. Tallman MS, Kim HT, Paietta E, et al. Acute monocytic leukemia (French-American-British classification M5) does not have a worse prognosis than other subtypes of acute myeloid leukemia: a report from the Eastern cooperative oncology group. *J Clin Oncol.* 2004;22:1276–1286.
9. Ru YX, Wang HJ, Yang BX, et al. The ultrastructure of hybrid acute leukemia: a study of 15 cases. *Ultrastruct Pathol.* 2005;29:341–347.
10. Roels F, Wisse E, Brest BDE, Meulen J. Cytochemical discrimination between catalases and peroxidases using diaminobenzidine. *Histochemistry.* 1975;41:281–312.
11. Shibata A, Bennett JM, Castoldi GL, et al. Recommended methods for cytological procedures in haematology. *Clin Lab Haemat.* 1985;7:55–74.
12. Francis C, Connelly MC. Rapid single-step method for flow cytometric detection of surface and intracellular antigens using whole blood. *Cytometry.* 1996;25:58–70.
13. Tallman MS, Kim HT, Paietta E, et al. Acute monocytic leukemia (French-American-British classification M5) does not have a worse prognosis. *J Clin Oncol.* 2004;22:1276–1286.
14. Dunphy CH, Orton SO, Mantell J. Relative contributions of enzyme cytochemistry and flow cytometric immunophenotyping to the evaluation of acute myeloid leukemia with a monocytic component and of flow cytometric immunophenotyping to the evaluation of absolute monocytoses. *Am J Clin Pathol.* 2004;122:865–874.
15. Cascavilla N, Musto P, D’Arena G, et al. CD117 (c-kit) is a restricted antigen of acute myeloid leukemia and characterizes early differentiative levels of M5 FAB subtype. *Haematologica.* 1998;83:392–397.
16. Urbano-Ispizua A, Matutes E, Villamor N, et al. The value of detecting surface and cytoplasm antigens in acute myeloid leukaemia. *Br J Haematol.* 1992;81:178–183.
17. Khalidi HS, Medeiros LJ, Chang KL, et al. The immunophenotype of adult acute myeloid leukemia: high frequency of lymphoid antigen expression and comparison of immunophenotype, French-American-British classification, and karyotypic abnormalities. *Am J Clin Pathol.* 1998;109:211–220.
18. Delgado J, Morado M, Jimenez MC, et al. CD56 expression in myeloperoxidase-negative FAB M5 acute myeloid leukemia. *Am J Hematol.* 2002;69:28–30.

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