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CLASSIFICATION OF NINETY-EIGHT ADULT CASES OF ACUTE LEUKEMIAS ACCORDING TO MORPHOLOGY, IMMUNOLOGY AND CYTOGENETICS

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In the present study, 98 cases of acute leukemias (AL) were diagnosed and classified based on morphologic, immunologic and cytogenetic (MIC) features to assess their diagnostic value in AL. The results showed that: the conformity rate of cytomorphologic/cytochemical classification with MIC classification was 90.8%. For ALL, the conformity rate of immunologic classification with MIC classification was 95.6% while it was only 70.8% for AML. Of the 48 AML, 10 expressed lymphoid-lineage-associated antigens and 8 of 43 ALL expressed myeloid-lineage-associated antigens. Seven cases were diagnosed as hybrid acute leukemia according to Catovsky's scoring criterion. The clonal chromosomal aberrations were found in 70 cases, of them 46 cases showed characteristic changes including t(9; 22), t(4; 11), t(11; 14), t(8; 12), t(8; 14), 6q-, 9p- and t(15; 17), t(8; 21), inv(16), etc. These data suggested that MIC classification of acute leukemias could provide more diagnostic and biologic information than traditional FAB classification.

Key words: Leukemia diagnosis, Cytogenetics.

In the past, acute leukemias (AL) were mainly classified according to morphology- and cytochemistry-based criteria proposed by the French American British (FAB) group. More recently, immunophenotyping and cytogenetics have been routinely applied to the characterization of leukemic cells.[1-4] The so-called morphologic, immunologic and cytogenetic (MIC) working classification of AL has been proposed in an attempt to more precisely define biologically and clinically relevant entities as well as to provide new prognostic insights.

In present study, ninety-eight cases of adult AL were classified based on MIC features to evaluate the diagnostic value of MIC classification in AL.

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MATERIALS AND METHODS

Patients

Between April 1991 and April 1995, a group of 98 patients aged 14 to 76 years with newly diagnosed untreated AL were classified based on MIC features. All patients were admitted to Jiangsu Institute of Hematology.

Morphologic Examination

The diagnosis and FAB subtype of AL were determined from Wright-Giemsa-stained bone marrow (BM) and peripheral blood (PB) smears. The cytochemical reactions tested included myeloperoxidase (MPO), periodic-acid Schiff (PAS) and α-naphtylacetate esterase with or without NaF inhibition.

Immunophenotyping

Blasts in the marrow aspirates were over 70 percent. Heparinized fresh BM samples were isolated by Ficoll-Hypaque density gradient centrifugation and cell-surface antigens were detected by a standard indirect immunofluorescence assay. Two hundred cells were counted under fluorescence microscope.

The monoclonal antibodies (MoAbs) used, all available from commercial sources (Immunotech, France), were as follows: (1) B-lineage-associated antigens:CD_{10}, CD_{19} and CD_{22}; (2) T-lineage-associated antigens: CD_2, CD_3 and CD_7; (3) myeloid-lineage-associated antigens: CD_{13}, CD_{15}, CD_{33}, CD_{11b} and CD_{w65}. Other MoAbs included CD_{34}, CD_{41} and CD_{42} were used in some samples. Positivity for each MoAb was arbitrally defined as 20% or more of cells above the negative control.

Cytogenetics

Samples for cytogenetic studies were obtained form BM or PB. Cells were examined directly as well as following culture for 24 h. Chromosomes were analyzed with R-and/or G-banding methods. Karyotypes were described according to the International System for Human Cytogenetic Nomen-clature (ISCN, 1985).

Definition of Hybrid Acute Leukemia

The diagnosis of hybrid acute leukemia (HAL) was according to the scoring system proposed by Catovsky, et al..[5]

RESULTS

MIC Classification

Among 98 patients with acute leukemias (AL) diagnosed according to MIC classification, 43 cases were acute lymphoblastic leukemia (ALL) including 15 T-ALL and 28 B-ALL; Forty-eight were acute myeloid leukemia (AML); Seven cases were diagnosed as HAL including 3 T/B HAL, 2 B/M HAL, 1 each T/M HAL and T/B/M HAL.

Morphologic Classification

Based on FAB criteria, 45 cases were ALL including 18 L_1, 26 L_2 and 1 L_1; Fifty cases were AML including 3 M_1, 30 M_2, 3 M_3, 4 M_4, 8 M_5 and 2 M_6. Three cases could not be classified based on FAB criteria. The conformity rate of cytomorphologic/cytochemical classification with MIC classification was 90.8 percent.

Immunophenotyping Classification

Based on immunophenotyping features, 45 cases were ALL including 17 T-ALL and 28 B-ALL. In this study, the T-and B-ALL could not be further subtyped because of relatively few kinds of MoAbs used. For ALL, the conformity rate of immunologic classification with MIC classification was 95.6 percent. Combined with morphologic classification, 2 patients who were misdiagnosed with immunologic classification alone were diagnosed as M_2 and M_5, respectively. Among 43 patients who were finally diagnosed as ALL with MIC classification, 8 cases expressed myeloid-associated antigens (My$^+$-ALL) including 2 each expressed CD_{13} and CD_{15}, 4 expressed CD_{33}.

Among 48 patients who were finally diagnosed as AML with MIC classification, 43 (70.8%) expressed myeloid antigens. Ten cases expressed lymphoid-associated antigens (Ly$^+$-AML) including 4 expressed CD_2, 2 each CD_3 and CD_7, 1 CD_3 and CD_7. Among 14 patients who did not express myeloid antigens, 2 expressed T lymphoid antigens only, 2 CD_{34} only and another 10 cases did not expressed any antigen used.

Among 3 patients who could not be classified with morphologic classification, 2 were diagnosed as ALL according to immunophenotypic features, another one (Table 1, case 1) whose morphologic examination showed two kinds of distinctive blasts and 23% of the blasts showed MPO activity and was suspected as HAL was diagnosed as B/M HAL combined with immunophenotyping results. In another 6 HAL (Table 1), 5 could be diagnosed just depended on immunophenotypic features, case 5 was diagnosed combined immunophenotype and positive MPO.

Cytogenetics

In 98 patients, 70 (71.4%) had clonal chromosomal aberrations, of which 46 (65.7%) showed

specific changes. The main specific aberrations in AML included: t(8; 21), 12 cases, all were M_2, the frequencies in M_2 and all AML were 41.4% and 25.0%, respectively; t(15; 17), could only be seen in 3 patients with M_3; t(9; 22), 2 cases with M_1 and M_2, respectively, the frequency in AML was 4.2%.

Table. 1. MIC features of HAL

Case No.	FAB subtype	Immunophenotyping (positive cells %)										Karyotype
		CD_2	CD_3	CD_7	CD_{10}	CD_{19}	CD_{22}	CD_{13}	CD_{15}	CD_{33}	CD_{11b}	
1	ALL-L_2	95	30	33	95	95	75					t(9; 22)
2	ALL-L_2	95	24	20	80	80						t(9; 22)/N
3*	ALL-L_2	53				62	22		68		25	N
4	ALL-L_1	44	33		80	85						t(9; 22)
5	AML-M_2	24	26	35					52			t(8; 21)/N
6	AML-M_5	80	27			24	23		85			N
7	HAL?				54	70						complex change**

*CD_{41} 24%, CD_{42b} 40%, CD_{61} 28% N–normal

**45, XX, -3, -5, -13, 6p+, tan (12q21:12q12), t(12p12; 12p12), +mar1, +mar2/N.

In 43 patients with ALL, 33 (77.0%) had clonal chromosomal abnormalities (Table 2). The main specific aberrations in ALL included: t(9; 22): 15 in 43 (34.9%) cases of ALL, of them 14 (92.3%) were B-ALL and one was T-ALL. The frequency of Ph+ALL in B-ALL was 50.0% (14/28). In 15 Ph+ALL, 11 were L2 and 4 were L1; t(11; 14): 2 cases, both were T-ALL and L1. The frequency in ALL was 4.9%; others: 1 L2, T-ALL with t(8; 14), 1 L1, B-ALL with t(4; 11), 1 L1, B-ALL with t(8; 12) and 3 6q-, 2 9p-, etc. Table 1 showed that 5/7 HAL had clonal chromosomal aberrations including 3 t(9; 22), 1 t(8; 21) and 1 complex abnormality. In 14 AML patients who did not express myeloid antigens, 6 had AML specific aberrations such as t(8; 21), inv (16) etc., which further confirmed morphologic diagnosis.

DISCUSSION

In the past, FAB classification was once the most important method and now is still primary in the diagnosis of acute leukemias (AL). However, with the accumulation of the data of immunologic and cytogenetic studies on the AL, it was established that the MIC classification of AL was more accurate, objective and reproducible than traditional FAB classification. Therefore, MIC classification of AL was widely accepted by hematologists. In the present study, the conformity rate of morphologic classification with MIC classification was 90.8%.

Immunophenotyping played especially important role in the diagnosis of ALL and HAL. In this study, all the HAL were diagnosed based on immunophenotyping feature with or without combining cytochemical results. For 43 ALL, the immunophemotyping classification was completely conformed to MIC classification. However, not all the AL which expressed lymphoid-lineage-associated antigens could diagnosed as ALL, there were still the possibilities of HAL and Ly^+-AML. Myeloid-lineage-associated antigens were detected in approximately 20% of adult ALL,[6] we also found 8/43 (18.6%) of adult ALL were My^+-ALL. The diagnostic significance of immunophenotyping was relatively low in AML, but it was necessary for the diagnosis of Ly^+-AML. Like results of Chen et al.,[7] in this study, 10/48 (20.8%) of adult AML were Ly^+-AML.

Cytogenetics can better reflect the biological features of leukemias than morphology and immunophenotyping. Some AL subtypes had specific chromosomal aberrations which were very important for the diagnosis of AL, especially for AML subtypes. As

were reported in the two MIC meetings,[1,2] in this study, 71.4% of adult AL had clonal chromosomal abnormalities, of them 65.7% were specific changes which were higher than Qian et al.[3] and Wang et al..[3] In AML, t(8; 21), t(15; 17) and inv(16) were associated with FAB-M2, M3 and M_4E_0, respectively. The incidence of Ph+AML was low and was associated M_1 or M_2. Because the prognosis of M_3 was greatly improved with induction differentiation therapy which was different from other subtypes of AML, so t(15; 17) was specifically important for clinic hematologists to determine induction protocol. In ALL, t(11; 14), t(8; 14) were associated with T-ALL and t(11; 14), t(8; 12) were the specific changes of B-ALL, while 6q- and 9p- were associated with ALL. The incidence of Ph+ALL in adult ALL was 34.9% which was slightly higher than 20%–30% reported by other researchers. Almost all the Ph+ALL were B-ALL, so the t(9; 22) was important for the diagnosis of B-ALL.

Table 2. MIC features of 33 ALL with chromosome aberrations

No.	FAB	Karyotype	Immunophenotype
1	L_2	52, XY, +X, +4, +4, +5, +7, +11/N	T
2	L_1	46, XY, t(11; 14)/47, XY, +21, t(11; 14)/N	T
3	L_2	47, XX, +mar/N	T
4	L_2	48, XX, +2, t(9; 22), +Ph	B
5	L_1	46, XX, $9p^-$/N	B
6	L_2	45, X, –4, –8, –Y, –9, $1q^+$, i(7q), $+7p^+$, –12, $+9p^+$, del(11), +mar1, +mar2, +15/N	T
7	L_2	47, XY, t(9; 22), +Ph/N	B
8	L_3	47, XX, +3/49, XXX, +13, +18/N	T
9	L_2	46, XY, t(9; 22)	B
10	L_1	46, XY, t(4; 11)/48, XY, +8, t(4; 11), $+4q^-$/N	B
11	L_2	46, XX, –4, +13, –22, +mar/N	B
12	L_2	47, XX, +17, t(9; 22)/46, XX, t(9; 22)/N	B
13	L_1	48, XX, +2, –7, +i(7q), t(9; 22), +del (22)	B
14	L_1	47, XY, t(9; 22), +Ph	B
15	L_1	46, XX, $9p^-$, t(8; 12)/N	B
16	L_1	47, XY, –3, +mar1, +mar2	B
17	L_1	46, XY, i(17q)/45, X, –Y, i(17q)/N	B
18	L_1	46, Y, t(X; 1)/46, Y, t(X; 1), t(6; 21), $11p^-$/N	B
19	L_2	45, XY, -20, t(9; 22)/N	T
20	L_2	48, XY, +8, t(7; 9), t(9; 22), +Ph/N	B
21	L_2	46, XX, t(9; 22), $6q^-$	B
22	L_2	46, XX, t(6; 14)/N	B
23	L_2	47, XY, +8, $21q^+$/48, XXY, +8, $21q^+$/48, XXY, +8, t(8; 9), $21q^+$/N	B
24	L_1	46, XY, t(9; 22)	B
25	L_1	46, XY, t(11; 14)/47, XY, t(11; 14), +21/N	T
26	M_1/L_2?	46, XY, t(9; 22)/N	B
27	L_1	46, XY, $6q^-$/N	T
28	L_2/M_{5b}?	46, XY, t(9; 22)/N	B
29	L_2	46, XY, t(9; 22), $20q^-$/N	B
30	L_2	46, XY, t(11; 13)/47, XY, t(9; 22), +10, +17, -20, -20, +mar/N	B
31	L_2	46, XY, t(8; 14)/N	T
32	L_2	46, XY, 6q /N	T
33	L_1	46, XY, t(9; 22)/N	B

N=normal

In summary, we suggested that morphology, immunology and cytogenetics could complement with each other in the diagnosis of AL. The diagnostic level of AL was enhanced with MIC classification which might provide more information for individualized treatment and prognosis evaluation.

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