Journal of Korean Medical Science
Vol. 7, No. 4, 377-384 December 1992

Acute Leukemias with Unusual Immunophenotypes

Myoung Hee Park, M.D., Yoon Sun Yang, M.D., Han Ik Cho, M.D.,
Byoung-Kook Kim, M.D.*, Seonyang Park, M.D.*, Hyo Seop Ahn, M.D.**,
Hee Young Shin, M.D.**, Hee Jung Kang, M.D., Won Il Oh, M.D.,
and Sang In Kim, M.D.

Departments of Clinical Pathology, Internal Medicine, and Pediatrics***
Seoul National University College of Medicine, Seoul, Korea

Over a two-year period, immunophenotypic patterns of 266 acute leukemia cases were analyzed using a panel of tests including TdT, SmIg and 9 surface antigens by the immunofluorescence stains for the assessment of the incidence and grade of phenotypic ambiguity (lineage infidelity) and the possible clinical significance of unusual immunophenotypes. Immunophenotypes were classified into four groups according to the degree of ectopic antigen expression. We classified as Group A (91.7%, 244 of 266 cases) those expressing conventional pattern without ectopic antigen. Group B (3.0%, 8 of 266 cases) was defined to have at least two lineage specific markers and single ectopic antigen. Such a "low grade deviation" did not prevent a definite immunodiagnosis. Group C (4.2%, 11 of 266 cases) revealed a promiscuous coexpression of markers related to different lineages, including two cases (0.8%, 2 cases) of biphenotypic leukemia. Group D (1.1%, 3 cases) included unclassifiable immunophenotypes with no antigen or HLA-DR only expression. Both patients with biphenotypic leukemia and one patient with unclassifiable immunophenotypes failed to respond to induction chemotherapy, suggesting a poor prognosis in these patients. The incidence of acute myelogenous leukemia (AML) cases with one or more ectopic surface antigens was 10 (8.1%) of the 124 AML cases. Ectopic antigen expression was seen in 5 (4%) of the 125 B-lineage acute lymphoblastic leukemia (ALL) cases and 3 (25%) of the 12 T-ALL cases.

It is concluded that nearly 95% of cases of acute leukemia cases can be diagnosed accurately with immunophenotyping alone including patients with a mild degree of deviation from expected antigenic patterns. Unclassifiable (1.1%) and biphenotypic leukemias (0.8%) seem to be rather rare according to strict criteria. The clinical significance of unusual immunophenotypes should be further clarified with a larger study population.

Key Words: *Acute leukemia, Immunophenotype, Ectopic antigen expression, Lineage infidelity*

INTRODUCTION

Immunophenotyping of leukemia has become widely used in routine diagnostic screening, making a critical contribution to the final diagnosis of leukemias (Browman et al., 1986; Neame et al., 1986; San Miguel et al., 1986; Foon, & Todd 1986; Foon et al., 1986; Drexler et al., 1986; Drexler et al., 1988; Kristensen et al., 1988; Kaplan et al., 1989; MIC cooperative study group, 1988). In most cases, immunophenotypic patterns are typical, and are easily interpretable to diagnose accurate types of acute leukemias. However, some cases show an unusual antigenic expression, which might have potential biologic and clinical significance as well as presenting diagnostic difficulties

Address for correspondence: *Myoung Hee Park, Department of Clinical Pathology, Seoul National University Hospital 28 Yongon-dong, Chongno-gu, Seoul, 110-744, Korea.*
This study was supported by a research grant from Seoul National University College of Medicine (1991).

(Kaplan et al., 1989; Mirro et al., 1985; Sulak et al., 1990; Gale & Ben-Bassat, 1987; Ben-Bassat & Gale, 1984; Cho et al., 1987; Bradstock et al., 1989; Vecchio et al., 1989; Park et al., 1991). Therefore, further clarification of the nature and incidence of unusual immunophenotypic subsets of acute leukemias is clearly warranted.

In this report, we have analyzed immunophenotypic patterns in 266 cases of acute leukemias over a 2 year period. The purpose of this study was to assess the incidence and grade of phenotypic ambiguity, and the possible clinical significance of unusual antigen expression.

MATERIALS AND METHODS

Patients

Over a 2 year period between January 1990 and December 1991, 266 acute leukemia cases (adult 162 cases, child 104 cases) were submitted for immunophenotyping studies at Seoul National University Hospital. Most cases were studied at the time of first diagnosis before treatment and others at relapse. Bone marrow smears were examined with the use of Wright-Giemsa stain and cytochemical stains, and classified morphologically using the criteria of the French-American-British (FAB) cooperative group in 210 cases. Cytochemical stains including peroxidase, Sudan black B, periodic acid Schiff (PAS), alpha naphthyl acetate esterase and acid phosphatase were performed according to conventional methods. Final diagnosis was established according to FAB classification (Bennet et al., 1976) and immunophenotyping. Fifty-six samples were referred for immunophenotyping from other hospitals, therefore, we had no available hematologic and clinical data in these cases.

Table 1. Markers used for immunophenotyping of acute leukemia

Test	Source	CD	Specificity
TdT	BRL		Immature lymphoid & myeloid
SmIg	Cappel		B cell
Leu9	Becton Dickinson	CD7	T cell
T1	Coulter	CD5	T cell
T11	Coulter	CD2	T cell (E-rosette receptor)
I2	Coulter		HLA-DR, Ia-like antigen
B4	Coulter	CD19	B cell (immature ~ mature)
J5	Coulter	CD10	CALLA
MY9	Coulter	CD33	Myeloid
MY7	Coulter	CD13	Myeloid
GPIIIa	Dako	CD61	Platelet glycoprotein IIIa

CD: cluster of differentiation
TdT: terminal deoxynucleotidyl transferase
BRL: British Research Laboratory

Table 2. Standard immunophenotypes of acute leukemia categories

Immunophenotype**	Non-lineage		B-lineage			T-lineage			Myeloid-lineage		
	TdT	HLA-DR	CD19	CD10	SMIg	CD2	CD5	CD7	CD13	CD33	CD61
Non-T ALL											
Group I	+	+	−	−	−	−	−	−	−	−	−
Group II	+	+	+	−	−	−	−	−	−	−	−
Group III, IV, V	+	+	+	+	−	−	−	−	−	−	−
Group VI	−	+	+	−/+	+	−	−	−	−	−	−
T-ALL	+	−/+	−	−	−	+	+	+	−	−	−
AML	−/+	+/−	−	−	−	−	−	−	+/−	+/−	+/−
Unclassified	−/+	+/−	−	−	−	−	−	−	−	−	−

* Immunological markers given as cluster of differentiation (CD), or as abbreviation: TdT, terminal deoxynucleotidyl transferase; SMIg, surface membrane immunoglobulin.

** modified from references (Foon & Todd, 1986; Foon et al., 1986; Nadler et al., 1984)

Immunologic marker studies

Cell suspensions of mononuclear cells were prepared from bone marrow or peripheral blood specimens (EDTA anticoagulated) with the use of Ficoll-Hypaque^R density gradient technique. Direct or indirect immunofluorescence stains were performed with a panel of monoclonal and polyclonal antibodies listed in Table 1. Most patients were typed with the entire panel of antibodies including TdT, SmIg, CD7, CD5, CD2, HLA-DR, CD19, CD10, CD33, CD13, and CD61. CD7 was used in 45 cases after September 1991.

Surface membrane immunoglobulin (SmIg) was stained using F (ab')$_2$ fragment of FITC-conjugated goat-antihuman immunoglobulin (Cappel, USA) according to a direct immunofluorescence protocol in all cases classified as B-lineage ALL. For staining of terminal deoxynucleotidyl transferase (TdT), cytocentrifuge preparations were used with rabbit anti-calf TdT antibody and F (ab')$_2$ fragment of FITC conjugated goat anti-rabbit IgG by indirect immunofluorescence technique (British Research Laboratory, USA). For the staining of nine surface membrane antigens, cell suspensions were incubated with monoclonal antibodies (Table 1) (Coulter, USA and Dako, Denmark) and F (ab')$_2$ fragment of FITC conjugated anti-mouse IgG (Cappel, USA) in a 96 well microplate. As negative control, isotypic controls of IgG1, IgG2a and IgG2b were used.

Immunofluorescence studies were evaluated by fluorescence microscopy, using a Nikon fluorescence microscope. When 20% or more of the cells were positive for a particular marker, the case was considered to be positive for that marker. Those cases were considered TdT positive if 10% or more of the cells were stained for TdT.

A panel of tests including TdT, SmIg and 9 surface antigens detected by monoclonal antibodies was selected to determine the predominent cellular identity of leukemic blast cells. First, cases were immunophenotypically classified according to the criteria of standard immunophenotypes (Table 2) [modified from references (Foon & Todd, 1986; Foon et al., 1986; Nadler et al., 1984)]. In this study, there was no case belonging to Group I of Non T-ALL and we classified ALL into B-lineage ALL and T-ALL. As for lineage specificities, CD2, CD5 and CD7 were considered as T cell markers; CD10 and CD19 antigen were used as B cell markers; and CD13 and CD33 were used as myeloid markers. CD61 antigen was employed as a megakaryocytic lineage specificity. HLA-DR was not considered as lineage-specific in spite of a preferential reactivity to B-lineage and myeloid leukemias.

Criteria for classification of subgroups

Antigenic constellations were classified into four patterns (Group A,B,C,D) having different degress of ectopic antigen expression as proposed by Vecchio et al (1989) (Table 3). We classified group A leukemias with conventional antigenic patterns in the absence of cross-lineage markers. Group B included leukemias with at least two lineage specific markers and single ectopic surface antigen expression. Group C included leukemias with a promiscuous coexpression of markers related to different lineages, showing a balanced (i.e., one lineage vs. one cross-lineage antigen, two vs. two, etc.) or a more complex coexpression of markers. Group D included "unclassifiable" phenotypes, characterized by no antigen, or DR only expression.

RESULTS

Of the 266 acute leukemia samples, 124 were given a final immunophenotypic diagnosis of AML, 125 were considered to be B-lineage ALL and 12 were T-ALL. Two cases were diagnosed as biphenotypic leukemia and three cases remained unclassifiable.

Ectopic antigen expression in AML, B-lineage ALL and T-ALL

The incidence of ectopic antigen expression is summarized in Table 4. A number of variants were seen in the AML group, with coexpression of the TdT, or one or more of the lymphoid lineage antigens CD2, CD5, CD7 and CD19. TdT was positive in 14 (11.5%) of the 122 AML cases, and these cases were not included as unusual immunophenotypes in this study. CD2 was present in 3 (2.5%) of the 122 AML cases, CD5 in 1 (0.8%) of the 121 AML cases and CD7 in 2 (4.4%) of the 45 AML cases. CD19, the B cell marker, was present in 4 (3.3%) of the 123 AML cases. No CD10 positive AML case was present. The incidence of AML cases with one or more ectopic surface antigens was 10 (8.1%) of the 124 cases. In 125 B-lineage ALL, CD2 and CD5 were expressed in 1 (0.8%) case each. Myeloid antigen, CD13 and CD33 were positive in 1 (0.8%) case and 2 (1.7%) cases respectively. CD10 was positive in 2 (16.7%) of the 12 T-ALL cases and CD13 in 1 (8.3%) of the 12 T-ALL cases. Overall incidence of ectopic antigen expression was seen in 5 (4%) of the 125 B-lineage ALL cases and 3 (25%) of the 12 T-ALL cases. Two cases were finally diagnosed as biphenotypic leukemia by the aid of FAB classification as well as immunophenotyping. Three cases remained unclassifiable immunophenotypes, all of the cases characterized by DR-only expression.

Classification of leukemia immunophenotypes into subgroups

Table 5 illustrates the distribution of acute leukemia cases classified into 4 immunophenotypic groups (A,B,C,D; Table 3) according to the grade of unusual surface antigen expression. Most cases belonged to Group A (conventional pattern without ectopic antigen) comprising 114 (92%) of the 124 cases. Group B (at least two lineage specific markers and single ectopic antigen) was identified in 5 (4.0%) cases of AML, 2 (1.6%) cases of B-lineage ALL and 1 (8.3%) case of T-ALL. In Group A and B, immunophenotyping attributed unequivocal lineage designation of leukemic cells, coinciding with the final diagnosis. Group C (a promiscuous coexpression of antigens, showing a balanced or more complex coexpression of markers) was observed in 5 (4.0%) cases of AML, 3 (2.4%) cases of B-lineage ALL and 1 (8.3%) case of T-ALL. Group D phenotype, characterized by no antigen or "DR-only"

Table 3. Criteria for subclassification of immunophenotypes of acute leukemia according to the grade of unusual surface antigen expression

Group	Patterns of surface antigen expression
A	Leukemias with conventional antigenic patterns in the absence of cross-lineage markers
B	Leukemias with at least two lineage specific markers and single ectopic surface antigen expression
C	Leukemias with a promiscuous coexpression of markers related to different lineages, showing balanced (i.e., one lineage vs. one cross-lineage antigen, two vs. two, etc.) or more complex coexpression of markers
D	Leukemias with unclassifiable immunophenotypes, characterized by no antigen or DR-only expression

Table 4. Incidence of acute leukemia cases with unusual immunophenotypes

Immunophenotype (no. of cases)	unusual markers	No. of cases tested	No. of cases positive	incidence (%)
AML (n=124)	TdT+	122	14	11.5
	CD2+	122	3	2.5
	CD5+	121	1	0.8
	CD7+	45	2	4.4
	CD19+	123	4	3.3
B-lineage ALL (n=125)	CD2+	125	1	0.8
	CD5+	125	1	0.8
	CD13+	121	1	0.8
	CD33+	121	2	1.7
T-ALL (n=12)	CD10+	12	2	16.7
	CD13+	12	1	8.3
Biphenotypic (n=2)				
Unclassifiable (n=3)				
Total (n=266)				

Table 5. Distribution of acute leukemia cases according to the grade of unusual surface antigen expression

Group	AML No. (%)	B-lineage ALL No. (%)	T-ALL No. (%)	Biphenotypic No. (%)	unclassifiable	Total No. (%)
A	114 (92.0)	120 (96.0)	10 (83.4)			244 (91.7)
B	5 (4.0)	2 (1.6)	1 (8.3)			8 (3.0)
C	5 (4.0)	3 (2.4)	1 (8.3)	2 (100.0)		11 (4.2)
D	0 (0.0)	0 (0.0)	0 (0.0)		3 (100.0)	3 (1.1)
total	124 (100.0)	125 (100.0)	12 (100.0)	2 (100.0)	3 (100.0)	266 (100.0)

* Group: same as in Table 3.

expression, was identified in three patients. In C (11 cases) and D (3 cases) groups, the unexpected expression or the absence of lineage specific surface antigens prevented an unequivocal immunophenotypic characterization; final diagnosis was possible in 9 out of 14 cases on the basis of FAB classification. Two cases remained biphenotypic and three cases unclassifiable.

Figure 1 shows a detailed analysis of all antigenic combinations observed in Group B, C and D, revealing a considerable variety of surface membrane mosaics. In Group B and C, 13 (69%) of the 19 cases (69%) demonstrated unique immunophenotypes.

Cases of biphenotypic leukemia and unclassifiable leukemia are shown in detail in Table 6 and 7. In case 1 of biphenotypic leukemia, blasts were positive for TdT, HLA-DR, CD19, CD10 and CD13, each marker positive in more than 50% of blasts, demonstrated a dual morphology and a complex positivity on cytochemical stains, positive for peroxidase, Sudan black B, alpha naphtyl acetate esterase and PAS in block pattern. The other case of biphenotypic leukemia also demonstrated a dual morphology and blasts were positive for HLA-DR, CD19, CD10, CD33 and CD61. In this case, blasts were 40% of all nucleated cells, and therefore CD61 (positive cell 11%) was considered to be positive (Table 6). Case 1 and 2 of unclassifiable immunophenotype were characterized by DR-only expression and negativity for all cytochemical stains. It was interesting that the third case contained CD61 positivity in 7% of the blasts and localized positivity for PAS and acid phosphatase stains, suggestive but not definitive of megakaryocytic lineage (Table 7).

Clinical outcome

Due to the presence of referral cases from other hospitals and refusal of treatment in some patients, clinical data were not sought on all patients with unusual irnmunophenotypes. The clinical outcome of patients with unusual immunophenotypes is shown in Table 8. Clinical significance could not be conclusively deter-

Final diagnosis	Immunologic pattern	TdT	DR	CD2	CD5	CD7	CD19	CD10	CD13	CD33	CD61	No. of patients
AML (n=124)	Group B	+	+	+					+	1		2
			+		+				+	+		1
			+			+			+	+		2
	Group C		+	+					+			1
			+				+			+		1
		+	+				+		+			1
			+				+		+			2
B-lineage ALL (n=125)	Group B	+	+	+			+	+				1
		+			+		+	+				1
	Group C	+	+				+		+			1
		+	+				+			+		1
			+				+			+		1
T-ALL (n=12)	Group B	+		+	+			+				1
	Group C	+	+	+	+			+	+			1
Biphenotypic (n=2)	Group C	+	+				+	+	+			1*
			+				+	+		+	+	1
Unclassifiable (n=3)	Group D		+									3

Fig. 1. Individual patterns of surface antigen expression from cases with unusual immunophenotypes. Definitions of immunophenotypic patterns of Group B, C, and D are same as in Table 3. A considerable variety of surface membrane mosaics is demonstrated. Dotted areas indicate appropriate positivity; white areas indicate ectopic antigen expression. DR positivity on T-ALL samples was not considered an extra-activity. * Peroxidase, Sudan black B and PAS (block pattern) positive blasts with dual morphology: CD19 76%, CD10 64%, CD13 65%

Table 6. Hematologic and clinical findings of patients with biphenotypic leukemia

Case No.	Age/sex	Positive immunologic markers	Cytochemistry*	Morphology	Final Diagnosis (immunophenotype+FAB)	Induction Response
1	24/F	TdT, HLA-DR, CD19, CD10, CD13	+ + + + −	dual morphology	Acute leukemia, biphenotypic	failed
2	2/F	HLA-DR,CD19,CD10,CD33,CD61	− − − + +	dual morphology	Acute leukemia, biphenotypic	failed

* Crytochemistry: Peroxidase, Sudan black B, Periodic acid Schiff, Alpha-naphthyl acetate esterase, Acid phosphatase

Table 7. Hematologic and clinical findings of acute leukemia cases with unclassifiable immunophenotypes

Case No.	Age/sex	Positive immunologic markers	Cytochemistry*	Morphology	Final Diagnosis (immunophenotype+FAB)	Induction Response
1	42/F	HLA-DR	− − − − −	unclassified	Acute leukemia, undifferentiated	no follow-up
2	67/M	HLA-DR	− − − − −	unclassified	Acute leukemia, undifferentiated	no follow-up
3	54/F	HLA-DR, (CD61 7%)	− − + − +	unclassified	Acute leukemia, unclassified	failed

* Crytochemistry: same as in Table 6.

Table 8. Clinical outcome of acute leukemia patients with unusual immunophenotypes

Immunophenotype	Cases with unusual markers	No.of cases followed up	Induction response		
			CR	Failed	Died*
AML	TdT (+) (n=14)	9	3	2	4
	CD2 (+) (n=3)	2	1	1	
	CD5 (+) (n=1)	1		1	
	CD7 (+) (n=2)	2	1	1	
	CD19 (+) (n=4)	4	4		
B-lineage ALL	CD2 (+) (n=1)	1	1		
	CD5 (+) (n=1)	1			
	CD13 (+) (n=1)	0			
	CD33 (+) (n=2)	2	1	1	
T-ALL	CD10 (+) (n=2)	1	1		
	CD13 (+) (n=1)	0			
Biphenotypic	n=2	2		2	
Unclassifiable	n=3	1		1	

CR: complete remission

*: died before, or during induction chemotherapy

mined due to the small number of cases. However, both patients with biphenotypic leukemia and one patient with unclassifiable immunophenotype failed to respond to induction chemotherapy.

DISCUSSION

In this report, we analyzed the immunophenotypes from 266 cases of acute leukemia patients with particular emphasis on the expression of "ectopic" antigens. The incidence and the distribution of unusual immunophenotypes according to the grade of lineage infidelity were documented. A number of patterns of ectopic antigen expression were seen in AML, including expression of one or more lymphoid antigens of CD2, CD5, CD7 and CD19. The T cell differentiation antigen CD7 was expressed in 4.4%, CD2 in 2.5%, and CD5 in 0.8% of the AML cases. The B cell marker CD19 was expressed in 3.3% of AML cases. CD10 positive AML was not found, which has been reported to be 1-2% in other reports (Bradstock et al., 1989; Vecchio et al., 1989; Park et al., 1991). Overall incidence of lymphoid antigen expression of AML (8.1%) in this report is somewhat lower than that of others (15-30%) (Browan et al., 1986; Ben-Bassat & Gale, 1984; Cho et al., 1987; Bradstock et al., 1989; Vecchio et al., 1989; Cross et al., 1988). Cross et al.,

(1988) reported that CD2 positive AML cases responded well to ALL therapy rather than AML protocol and that overall survival was poor. But, we have not attempted to examine the prognostic significance of this finding due to the small number of cases.

Although the major AML subcategory of unusual immunophenotype was the TdT+ group, observed in 11.5% of the cases, we excluded TdT in this context because it is not exclusively confined to the lymphoid lineage (San Miguel et al., 1986; Vecchio et al., 1989; Kaplan et al., 1987; Parreira et al., 1988; Swirsky et al., 1988). We have not attempted to examine the prognostic significance of TdT+ AML in this study. Some groups reported a poor outcome to treatment in the TdT+ AML (Jani et al., 1983; Benedetto et al., 1986), and others revealed no significant clinical difference between TdT positive and negative groups of AML (Swirsky et al., 1988).

The major manifestation of lineage infidelity seen in ALL patients was expression of one or more of the myeloid antigens. Myeloid positive ALL was initially described by Mirro and colleagues (1985), where 18 (19%) of the 95 children ALL cases showed expression of one or more myeloid antigens; CD11b, CD13, CD14 and CD15. Sobol et al., (1987) reported a higher incidence (35%) of myeloid antigen expression in adult ALL, with a significantly poorer outcome. In this report, we used only CD13 and CD33 as myeloid antigens. CD13 was expressed in 1 (0.8%) out of 121 B-lineage ALL and 1 (8.3%) out of 12 T-ALL. CD33 was positive in 2 (1.7%) of 121 B-lineage ALL, and none of 12 T-ALL. This comparatively lower incidence is thought to be due to the limited number of myeloid antigens used and the use of fluorescence microscopy instead of more sensitive flow cytometry. The additional tests for other myeloid antigens, CD11b, CD14, CD15 and VIM2 are considered for the improvement of diagnostic sensitivity in detecting AML and of the incidence of ectopic myeloid antigen expression in ALL. Concerning T cell antigen expression in B-lineage ALL, CD2 was observed in 1 (0.8%) out of 125 cases, and CD5 also in 1 (0.8%) out of 125 cases. B cell antigen CD10 was relatively frequently observed in T-ALL, being positive in 2 (16.7%) out of 12 cases.

In all three cases of unclassifiable immunophenotypes, without any evidence of myeloid or lymphoid differentiation detected, final diagnosis was also "unclassified" leukemia. Only one of the three cases could be followed up, who failed to respond to induction chemotherapy. Two cases of biphenotypic leukemia also failed, without any response to repeated chemotherapy at all. These findings suggested that at least leukemias of these patterns might have a poorer clinical outcome.

The main purpose of our study was to assess the incidence and grade of phenotypic ambiguity of acute leukemias on the basis of the presence and number of inappropriate or cross-lineage antigens, and we could identify groups of patients, revealing different levels of interpretative difficulties. As shown in Table 5, the immunophenotypes of most leukemia cases (91.7%) were strictly unilineage in the absence of cross-lineage specificity. A small number of cases (3%, 8 cases) in the Group B showed a slight limited deviation from the expected antigen pattern. Most investigators agree that leukemias expressing two or more lineage specific markers and only one unexpected marker are operationally considered unambiguous and diagnosable. In cases with a balanced coexpression and/or with two or more aberrant markers, the issue is unresolved. Vecchio et al. (1989) suggested that most such cases are true hybrid leukemias. However, other investigators are more conservative. Several groups recommend using various combinations of cytochemical, gene rearrangement, karyotypic, and immunophenotypic markers, with some tests weighing greater than others (Gale & Ben-Bassat, 1987; Hurwitz & Mirro 1990). In our study, only 2 of 11 cases belonging to Group C were given a final diagnosis of biphenotypic leukemia. Although we could not perform the karyotypic and gene rearrangement study in these cases, we believe that two aberrant immunophenotypic markers alone are not usually sufficient for diagnosis of biphenotypic leukemia. Therefore, more detailed studies described above should be included at diagnosis of biphenotypic leukemia. As shown in Figure 1, a considerable heterogeneity in ectopic antigen expression was demonstrated in the B and C groups.

In summary: 1) Overall about 95% of acute leukemia could be diagnosed with immunophenotyping alone in our study including patients showing a mild degree of deviation from expected antigenic patterns. 2) Less than 1% of acute leukemias were biphenotypic in our study and the incidence of true biphenotypic leukemia seems to be very low according to strict criteria corresponding to other reports. 3) The clinical significance of unusual immunophenotypes could not be determined due to the inadequate number of cases, but patients of biphenotypic or unclassifiable leukemia appeared to have a poor prognosis, which should be further clarified with a larger number of cases.

REFERENCES

Ben-Bassat I, Gale RP: *Hybrid acute leukemia. Leuk Res 8:929-36, 1984.*

Benedetto P, Mertelsmann R, Szatrowski TH et al.: *Prognostic significance of terminal deoxynucleotidyl transferase activity in acute nonlymphoblastic leukasemia. J Clin Oncol 4:489-95, 1986.*

Bennet JM, Catovsky D, Daniel M-T et al.: *Proposals for the classification of acute leukemias French-American-British (FAB) co-operative group. Br J Haematol 33:451-8, 1976.*

Bradstock KF, Kirk J, Grimsley PG, Kabral A, Hughes WG: *Unusual immunophenotypes in acute leukaemias: incidence and clinical correlations. Br J Haematol 72:512-8, 1989.*

Browman GP, Neame PB, Soamboonsrup P: *The contribution of cytochemistry and immunophenotyping to the reproducibility of the FAB classification in acute leukemia. Blood 68:900-5, 1986.*

Cho HI, Park MH, Kim SI et al.: *Classification of acute lymphoblastic leukemia in Korean. Seoul J Med 28:109-14, 1987.*

Cross AH, Goorha RM, Nuss R et al.: *Acute myeloid leukemia with T lymphoid features: a distinct biologic and clinical entity. Blood 72:579-87, 1988.*

Drexler HG, Gignac SM, Minowada J: *Routine immunophenotyping of acute leukaemias. Blut 57:327-39, 1988.*

Drexler HG, Menon M, Gignac SM, Misra B, Minowada J: *Diagnostic value of immunological leukemia phenotyping. Acta Haematol 7:1-8, 1986.*

Foon KA, Gale RP, Todd III RF: *Recent advances in the immunologic classification of leukemia. Sem Hematol 23:257-83, 1986.*

Foon KA, Todd III RF: *Immunologic classification of leukemia and lymphoma. Blood 68:1-31, 1986.*

Gale RP, Ben Bassat I: *Hybrid acute leukemia. Br J Haematol 65:261-4, 1987.*

Hurwitz CA, Mirro J Jr: *Mixed lineage leukemia and asynchronous antigen expression. Hematol Oncol Clin North Am 4:767-94, 1990.*

Jani P, Verbi W, Greaves MF, Bevan D, Bollum F: *Terminal deoxynucleotidyl transferase in acute myeloid leukemia. Leuk Res 7:17-29, 1983.*

Kaplan SS, Penchansky L, Krause JR, Basford Re, Zdziarski UE: *Simultancous evaluation of terminal deoxynucleotidyl transferase and myeloperoxidas in acute leukemias using an immunocytochemical method. Am J Clin Pathol 85:732-6, 1987.*

Kaplan SS, Penchansky L, Stolc V, Contis L, Krause JR: *Immunophenotyping in the classification of acute leukemia in adults. Cancer 63:1520-7, 1989.*

Kristensen JS, Ellegaard J, Hansen KB, Clausen N, Hokland P: *First-line diagnosis based on immunological phenotyping in suspected acute leukemia: a prospective study. Leuk Res 12:773-82, 1988.*

Mirro J, Zipf TF, Pui C-H et al: *Acute mixed lineage leukemia. Clinicopathologic correlations and prognostic significance. Blood 66:1115-23, 1985.*

Nadler LM, Korsmeyer SJ, Anderson KC et al.: *B cell origin of non-T cell ALL. J Clin Invest 74:332-40, 1984.*

Neame PB, Soamboonsrup P, Brown GP et al.: *Classifying acute leukemia by immunophenotyping: A combined FAB-immunologic classification of AML. Blood 68:1355-62, 1986.*

Park MH, Oh WI, Kang HJ et al.: *Immunologic marker analysis in acute myeloid leukemia. Kor J Hematol 26:253-61, 1991.*

Parreira A, Pombo de Oliveria MS, Matutes E, Foronoi L, Morilla R, Catovsky D: *Terminal deoxynucleotidyl transferase positive acute myeloid leukaemia: an association with immature myeloblastic leukaemia. Br J Haematol 69:219-24, 1988.*

San Miguel JF, Gonzalez M, Canizo MC, Anta JP, Zola H, Borrasca AL: *Surface marker analysis in acute myeloid leukaemia and correlation with FAB classification. Br J Haematol 64:547-60, 1986.*

Second MIC cooperative study group: *Morphologic, immunologic and cytogenetic (MIC) working classification of the acute myeloid leukemias. Br J Haematol 68:487-94, 1988.*

Sobol RE, Mick R, Royston I et al.: *Clinical importance of myeloid antigen expression in adult acute lymphoblastic leukemia. New England J Med 316:1111-7, 1987.*

Sulak LE, Clare CN, Morale BA, Hansen KL, Montiel MM: *Biphenotypic acute leukemia in adults. Am J Clin Pathol 94:54-8, 1990.*

Swirsky DM, Greaves MF, Gray RG, Rees JKH: *Terminal deoxynucleotidyl transferase and HLA-DR expression appear unrelated to prognosis of acute myeloid leukaemia. Br J Haematol 70:193-8, 1988.*

Vecchio LD, Schiavone EM, Ferrara F et al.: *Immunodiagnosis of acute leukemia displaying ectopic antigens: Proposal for a classification of promiscuous phenotypes. Am J Hematol 31:173-80, 1989.*