

ACUTE LYMPHOBLASTIC LEUKAEMIA - A STUDY OF IMMUNOPHENOTYPES

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Samina Naeem, Abdul Hayee (Department of Pathology, King Edward Medical College, Lahore.)

ABSTRACT

Immunological characteristics of leukaemic blast cells from 55 patients of acute lymphoblastic leukaemia were analysed using a panel of monoclonal antibodies and immune peroxidase technique. Among 36 children the percentage of Common-ALL was found to be low (39%) as compared to western reports, whereas that of 1-ALL was high (36%). Out of 19 adults, 52.6% were Common-ALL, 21.1% Early-B-ALL and 16% 1-ALL; the findings being consistent with western studies. The 1-ALL cases (13) were sub classified according to the stage of thymic maturation depending upon the expression of CD8 and CD4 antigens. Six were identified as early, 3 as common and 4 as late thymocyte stage (JPMA42: 83, 1992).

INTRODUCTION

The study of immunological markers is essential for the Correct diagnosis and classification of acute lymphoblastic leukaemia (ALL)¹. Till the last decade, diagnosis of acute leukaemia was based primarily on the morphology and cytochemistry of leukaemic cells whereas now the technique of immunophenotyping can be applied to confirm the diagnosis as well as to further subcategorize the disease². The discovery of monoclonal antibodies (McAbs) by Kohler and Milstein in 1975³ has made it possible to define the precise stages of differentiation of haemopoietic Cells and resulted in tremendous advancement in the Classification of leukaemia⁴. Using cytological and cytochemical methods leukaemias designated as acute undifferentiated leukaemia, can now be appropriately classified by McAbs specific for lymphoid, myeloid, erythroid and megakaryocytic cells^{1,2,5,6}. In addition to specificity, the sensitivity of the diagnostic tests has also been augmented by the use of McAbs. It is now possible to establish an earlier diagnosis of recurrence of leukaemia in the bone marrow or the detection of lymphoblasts in other sites such as CSF or testicular stroma^{2,7}. ALL blast cells can be immunophenotyped into either B-lineage or T-lineage. B-lineage-ALL includes (i) Early-B-ALL: Common ALL antigen (CALM) negative, previously called Null-ALL; (ii) Common-ALL: CALM positive; (iii) Pre-B-ALL cytoplasmic 'mu' chains present and (iv) Classical B-ALL: mature, surface immunoglobulin positive blasts. These immunotypes represent progressively maturing stages of B-lineage cells⁴. The significance of ALL immunotype as an independent predictor of response to treatment has been reported. Common-ALL has the most favourable prognosis both in children and adults. T-ALL has a significantly poorer prognosis than non T-ALL excluding the classical B-ALL which has the worst prognosis^{4,8-10}. Therefore the precise characterization of leukaemic blasts is critical to establish an accurate prognosis and optimal therapeutical approach. It permits the use of more intensive treatment protocol in those patients who have been identified as having an unfavourable outcome at the time of diagnosis¹¹. The present study was designed to identify the immunological markers on the lymphoblasts of ALL in order to see the frequency of various immunophenotypes of ALL in our country.

PATIENTS AND METHODS

Selection of patients:

This study includes 55 patients, 36 children (< 15 years) and 19 adults (> 15 years). All were newly diagnosed, untreated cases selected from Mayo Hospital and other government hospitals of Lahore. The diagnosis of ALL was made according to the French- American British (FAB) criteria by examining giemsa stained peripheral blood and bone marrow smears supplemented, by special stains which included myeloperoxidase, PAS and estrases¹².

Immunophenotyping:

Direct bone marrow smears were obtained at diagnosis and airdried for 6 to 24 hours prior to immunolabelling. When required, the smears were stored at -20°C wrapped in aluminium foil. Following.

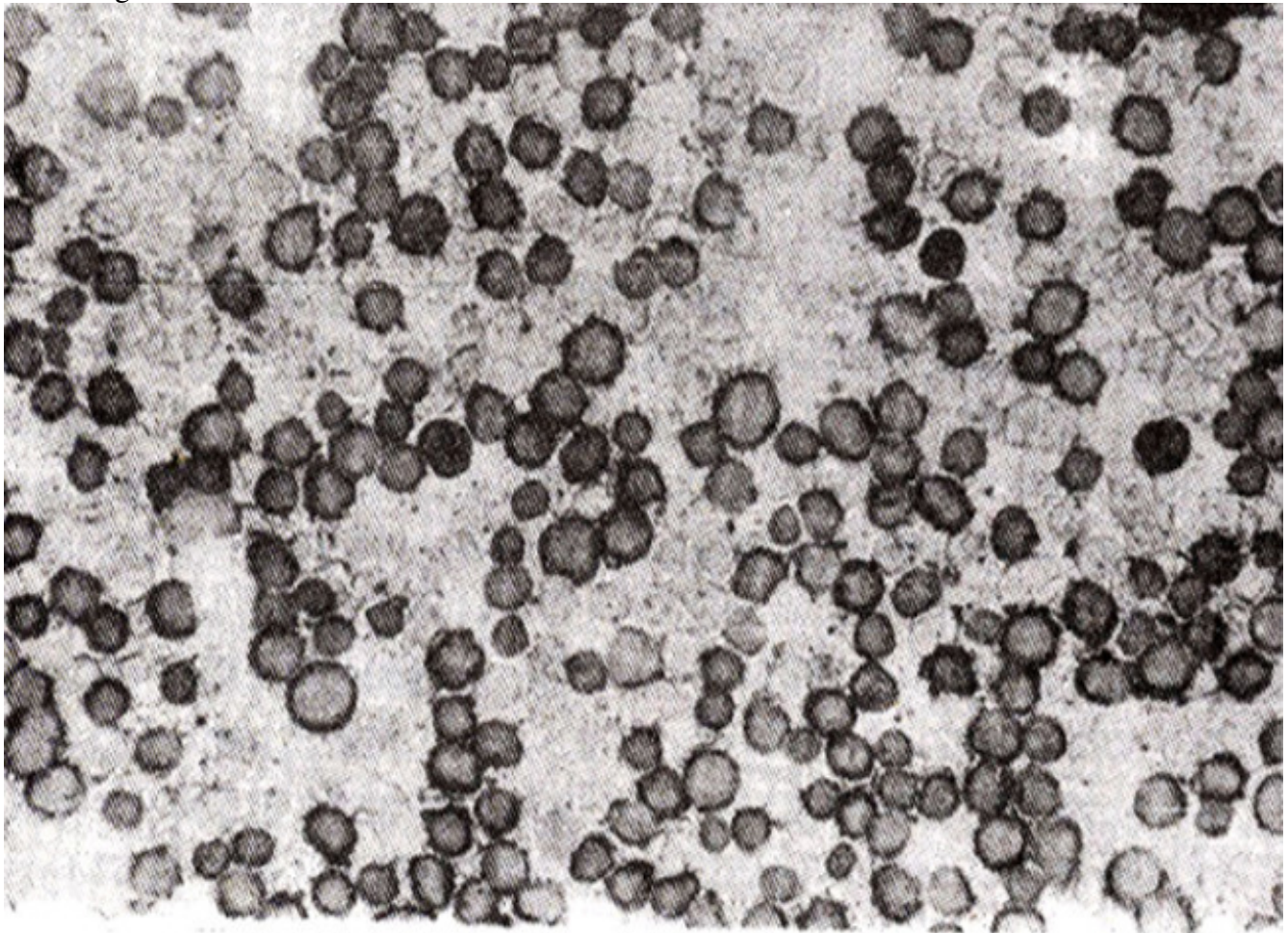


Figure 1. Positive immunoperoxidase staining of blast cells with OKB-CALLA McAb in a case of Common-ALL x 500.

McAbs were used:

OKDr, OKBCALLA (CD10), OKB7, OKT11 (CD2), OKT8 (CD8) and OKT4 (CD4) available from Ortho Diagnostics.

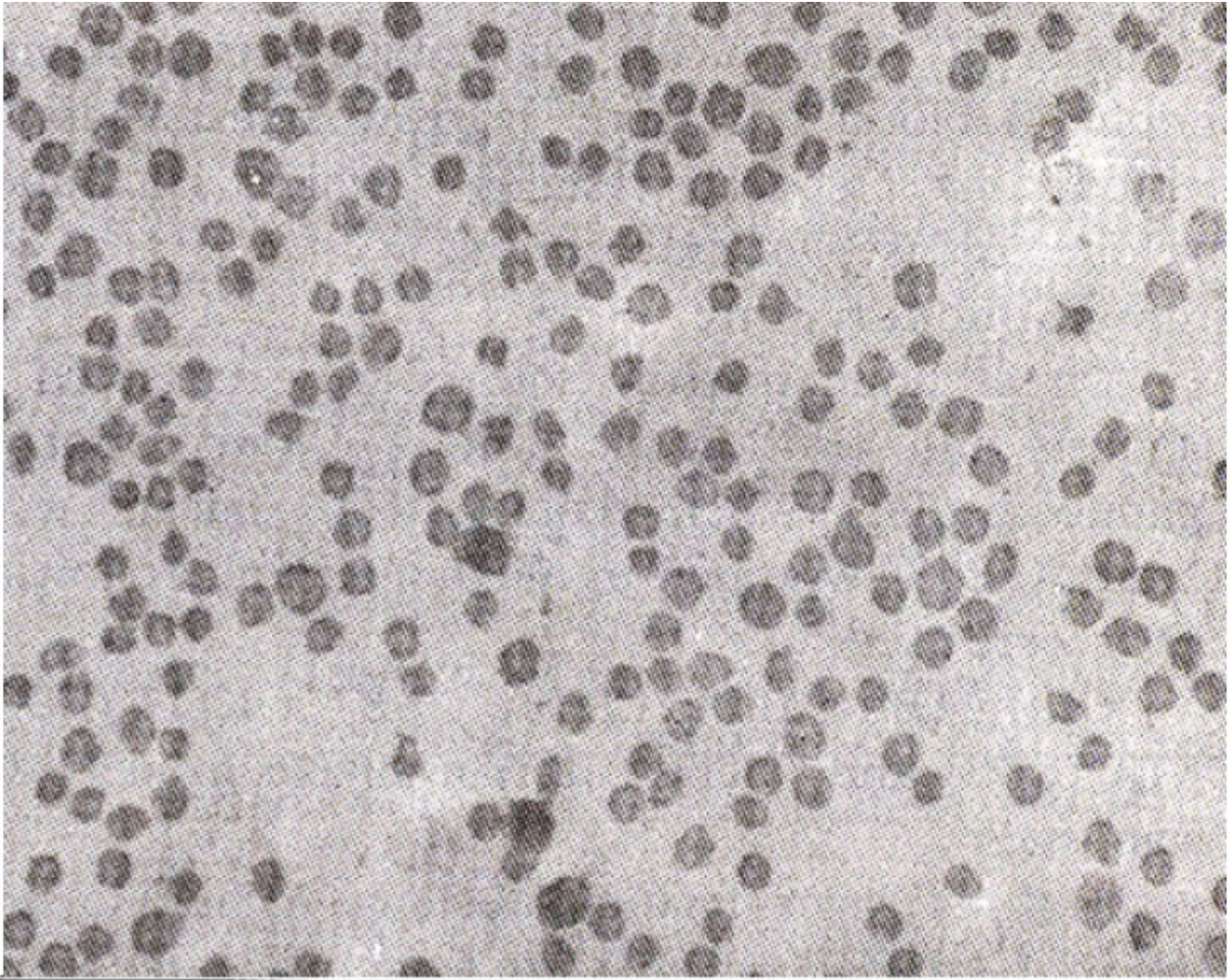


Figure 2. Negative immunoperoxidase staining of blast cells with OKB-CALLA McAb in a case of Common-ALL x 500.

A three layer immunolabelling technique was employed. Airdried smears were fixed in acetone for 10 min. They were incubated first with the primary McAb (Mouse Ig), at adequate dilution, for 20 min. at room temperature. Sequentially the smears were incubated for 20 mm. with the linking antibody (goat-anti-mouse Ig) and then with the labelling antibody (peroxidase labelled mouse Ig). The chromogenic substrate used to reveal the immunological reaction contained 3-amino 9-ethyl carbazol (AEC) and H₂O₂. The linking antibody, labelling antibody and chromogen system were available together in the Universal Immunoperoxidase Staining Kit (Ortho diagnostics). The smears were counterstained with Mayer's haematoxylin and coverslipped with glycerol gel. Cases were considered positive when 20% or more of the blast cells were labelled with the specific McAb. Clinical and haematological parameters of all patients were registered at diagnosis including age, sex, lymphadenopathy, hepatomegaly, splenomegaly, Hb, WBC and platelet count.

RESULTS

Depending upon the reactivity pattern of the blast cells with the McAbs used, the 55 ALL patients were classified into different immunological groups (Table I).

TABLE I. Immunophenotypes of 55 ALL cases and their reactivity pattern with monoclonal antibodies.

Immunophenotype	Cases		Reactivity with monoclonal antibodies					
	No.	%	OKDr	OKBCALLA	OKB7	OKT11	OKT8	OKT4
Early-B-ALL	9	16.4	+	-	-	-	-	-
Common-ALL	24	43.6	+	+	-	-	-	-
B-ALL	1	1.8	+	-	+	-	-	-
T-ALL	16	29.1	-	*Variable	+Variable	+	++Variable	++Variable
Undefined immunotype	5	9.1	-	-	-	-	-	-

* Positive reaction in 9 cases

+ Positive reaction in 7 cases

++ Reactivity dependent upon the thymic stage of maturation

Nine (16.4%) cases expressed only the Dr antigen (B-lineage marker) and were immunotyped as Early-B-ALL. Twenty four (43.6%) expressing both Dr and Common-ALL antigen (CALLA) were classified as Common-ALL. One (1.8%) case showed positivity with OKDr as well as OKB7 (identifies the antigen expressed on Sig positive B cells and some T cells) and was immunotyped B-ALL. T-ALL group included 16 (29.1%) cases positive for the sheep-erythrocytose antigen identified by OKT11 and negative for Dr antigen. In this immunotype reactivity with OKT8 and OKT4 McAbs varied according to the thymic stage of maturation; a positive reaction to OK BCALLA was seen in 9 cases and to OKB7 in 7. Blast cells of 5 (9.1%) patients gave a negative reaction with all six of the McAbs used and were grouped as undefined immunotype. The frequency of the immunological subgroups was different in children and adults (Table II).

TABLE II. Frequency of ALL immunophenotypes in children and adults.

Immunophenotype	Number of patients			
	Children ($< / = 15$ years)		Adults (> 15 years)	
	No.	%	No.	%
Early-B-ALL	5	13.9	4	21.1
Common-ALL	14	38.9	10	52.6
B-ALL	1	2.8	0	0.0
T-ALL	13	36.1	3	15.8
Undefined immunotype	3	8.3	2	10.5
Total	36	100	19	100

Among 36 children (≤ 15 years) a high proportion i.e., 13 (36%) showed T-ALL immunotype whereas only 14 (38.9%) were Common-ALL. The rest included 5(13.9%) Early-B-ALL, 1 (1.8%) B-ALL and 3 (8.3%) cases of undefined immunotype. Of the 19 adult patients (> 15 years) only 3 (15.8%) were T-ALL, whereas 10 (52.6%) were Common-ALL, 4 (21.1%) Early-B-ALL and 2 cases remained undefined.

Subclassification of T-ALL

This was based upon the reactivity pattern of T-ALL lymphoblasts (OKDR, OKT11⁺) with OKT8 and OKT4 McAbs (Table III).

TABLE III. Subclassification of 13 T-ALL cases according to the Thymic State of Maturation

Thymic Stage of Maturation	Cases		Reactivity with McAbs	
	No.	%	OKT8	OKT4
Early Thymocyte Stage	6	46.2	-	-
Mid Thymocyte Stage	3	23.0	+	+
Late Thymocyte Stage	4	30.8	+	-

Thirteen T-ALL cases were included in the subclassification, out of these 6 cases were identified as Early thymocyte stage (OKT8⁻, OKT4⁻); 3 as Mid thymocyte stage (OKT8⁺, OKT4⁺) and 4 as Late thymocyte stage (OKT8⁺, OKT4⁻). Due to incomplete pattern of reactivity, 3 of the 19 T-ALL cases could not be included in the above subclassification. In 2 of these OKT8 was positive and OKT4 was not used and in one OKT8 was not used while OKT4 was positive.

DISCUSSION

A large number of studies have been carried out on the immunophenotypes of ML in western countries^{8,10,13-18}. In these reports Common-ALL represents the majority of cases in children, i.e., 70-80% and about 50% in adults; T-ALL constitutes 10-20% of ALL cases in children and adults; Early-B (Null) ML is more common in adults - upto 40% than Children - upto 15% and B-ALL is rare comprising less than 2% of childhood and less than 5% of adult cases. In the present study the frequency of ALL immunotypes in adults i.e., Common-ALL 52.6%, T-ALL 15.8% and Early-B-ALL 21.1% is in accordance with the above mentioned reports. The findings in children, on the other hand, are different. Frequency of T-ALL is much higher i.e., 36.1% vs 15% while that of Common-ALL is much lower i.e., 38.9% vs 75%. A high frequency of T-ALL (31%) has been reported in India and in Asian residing in U.K.¹⁹. There maybe a common factor accounting for this finding in India and Pakistan. A high frequency of T-ALL was also reported in the Gaza Strip of Israel²⁰ and according to this study the pattern of lymphoid malignancy in children is very dependent upon the environment and relates particularly to socio-economic conditions. The frequency of T-ALL has been reported from the southern²¹ and northern²² parts of Pakistan in previous studies in which the diagnosis of T-ALL was based upon a strongly positive localized acid phosphatase reaction of the blast cells. The study carried out in Karachi²¹ on 50 children shows a high frequency of T-ALL i.e., 32% and our findings, conform to this study. A low frequency of T-ALL has been reported from Rawalpindi²², only 9.2% of the 65 ALL cases being diagnosed as T-ALL. These findings show a distinct variation between the northern areas and other parts of the country. Identification of cytoplasmic 'mu' chains was not carried out in this study, hence the Common-ALL group is inclusive of pre B-ALL which constitutes about 20% of Common-ALL cases²³. Thirteen cases of ALL were subclassified for the thymic stage of maturation; of these 46.2% did not express either of the antigens identified by CD8 and CD4 McAbs and were included in early thymocyte stage; 23% expressed both these antigens simultaneously and were grouped in mid. thymocyte stage; remaining 30.8% expressed only one of these two antigens and were included in late thymocyte stage. A wider panel of anti-T-cell McAbs including CD5, CD3 and CD1 could not be employed, therefore, anomalous phenotypes which do not clearly fall into any one of the three categories of intrathymic maturation cannot be excluded. Previous studies show variable frequencies of T-ALL subclasses; being reported as 70% early, 20% mid and 10% late thymocyte stage

in one study²⁴ and as 33%, 37% and 30% respectively in another²⁵. The immunophenotypes of ALL have a definite relationship with prognosis. Excluding the small percentage of B-ALL cases; Common-ALL has the best prognosis and T-ALL the worst^{5,8-11}. T-ALL patients experience significantly shorter duration of complete clinical remission, a higher relapse rate and more frequent relapses in CNS^{8,14}. In general the rank, order of favourable to unfavourable prognosis is: Common-ALL > Early-BALL > Pre-B-ALL > T-ALL > B-ALL⁵. The high frequency of T-ALL may be an important factor in the poor response of childhood ALL to usual therapeutic protocol in our country. It is important to identify the poor prognosis of T-ALL patients who may need modifications in their treatment. The finding of a high proportion of T-ALL immunotype in the present and previous studies in Pakistan may be related to an indigenous aetiological or predisposing factor - genetic and/or environmental. The present study highlights the need to carry out a more elaborate analysis of ALL immunotypes in our country by including a greater number of patients as well as their follow-up to confirm the prognostic significance of various immunophenotypes.

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