Morphologic Study of Acute Lymphoblastic Leukemia-FAB Classification and its Relationship with Age and Leukocyte Count in 257 Childhood and 57 Adult Cases

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Morphology of blast cells was studied in 314 patients of acute Lymphoblastic leukemia, they included 257 children (<=15 years) and 57 adults (> 15 years). L1, L2 and L3 morphology was seen in 191 (60.8%), 118 (37.6%) and 5 (1.6%) of childhood cases and in 32 (56%), 24 (42%) and 1(1.76%) of the adult cases respectively. In childhood ALL the prognostically unfavorable age group of 10-15 years was associated with a higher proportion of L2 cases (50.9%) than the prognostically favorable age group of 1-9.99 years (32.8%), the difference being statistically significant p=0.021. Among adults also, L2 morphology was more frequent (75%) in the prognostically unfavorable age group above 35 years than in younger patients (37.5%). Frequency of FAB types at various levels of total leukocyte count was determined. L2 was seen in 28.7% of patients with TLC below $4x10^9$/L, in 43.7% with TLC 4-$50x10^9$/L and in 38.2% in the high risk group with TLC above $50x10^9$/L, showing no increase in the frequency of L2 ALL in this group.

Key words: Acute Lymphoblastic leukemia, morphological classification

The FAB classification of acute leukemia was presented by a group of seven French, American and British haematologists[1]. This classification is based on the morphological appearance of blast cells in bone marrow and peripheral blood in Romanowsky stained films supplemented by cytochemical reactions. Acute Lymphoblastic leukemia (ALL) is classified into following three types: L1: This subgroup is characterized by a homogeneous population of small cells with a high nuclear cytoplasmic ratio and inconspicuous nucleoli. L2: heterogeneous population of cells with a low nuclear cytoplasmic ratio and prominent nucleoli characterizes it. L3: These blast cells resemble Burkitt cells. They are large and homogeneous with deep basophilic vacuolated cytoplasm, prominent nucleoli and low nuclear cytoplasmic ratio.

To facilitate the differentiation of L1 from L2 ALL, the FAB group introduced a scoring system. This ALL-Score' increased the concordance among observers from 63% to 84% [2].

L1 is the most common variety in children accounting for more than 70% of the cases[2,3,4]. L2 is the predominant variety in adults affecting about 60% of adult cases[4]. L3 is the least common type and represents less than 5% of all cases[2,3,4]. L1 and L2 bear no relation to the immunophenotype of blast cells. By contrast L3 morphology correlates well with mature B-Cell phenotype[2,4,5]. Regarding the two major morphological groups L1 and L2, L1 cases show a better prognosis than L2 both in terms of clinical remission and survival[3, 4, 6, 7, 8]. The small number of L3 ALL cases which represent mature B-ALL immunotype are associated with the worst prognosis[2,4].

Age and total leukocyte count (TLC) still remain the most important prognostic factors in ALL. Children 1 to 9.99 years of age enjoy the best prognosis while those younger than one year and older than 10 years have a worse prognosis [7,9]. Adults have a less favorable outcome than children and adults above 35 years fare worse than younger adults while those above 60 years have the worst prognosis [7,10]. TLC at presentation is a highly significant prognostic variable both in children and adults. TLC > $50x10^9$/L has been proposed for the purpose of defining high-risk category in childhood ALL[9].

The present study was carried out in childhood and adult cases of ALL to determine the frequency of L1 which is associated with a better prognosis, L2 which has a less favorable outcome and L3 which has the worst prognosis. The distribution of patients in various age groups and TLC levels of prognostic significance was studied. The frequency of FAB morphologic types in these age groups and TLC levels was determined.

Patients and Methods

Newly diagnosed cases of ALL were selected for this study. They included both children (<=15 years) and adults (>15 years). These patients presented in the Hematology section of the Department of Pathology, King Edward Medical College, Lahore and the Department of Pediatric Pathology, Mayo Hospital Lahore from January 1991 to June 1996. Clinical evaluation of the patients was done and blood and bone marrow (BM) samples taken at the time of presentation before initiation of therapy. Diagnosis of ALL was made from the morphology of blast cells in Giemsa stained BM smears and their negative staining for Sudan Black B supplemented by other stains i.e. PAS and estrases[11].

A well spread area of Giemsa stained BM smears was selected to study the morphology of blast cells. The cases were classified as L1, L2 and L3 ALL according to the criteria laid down by the FAB cooperative group[1]. L1 and L2 classification was further confirmed by using the FAB

scoring system[3].

Results

Morphology of blast cells was studied in 314 cases of ALL including 257 children (<= 15 years) and 57 adults (> 15 years) (table-1). Of the total patients 60.8% were L1 ALL, 37.6% L2 and 1.6% L3. Among 257 children L1 morphology was seen in 61.8%, L2 in 36.6% and L3 in 1.6%. In 57 adults the proportion was 56.1%, 42.1% and 1.8% respectively. Thus L1 ALL was more frequent than L2 ALL both in children as well as in adults, however the proportion of patients with L2 morphology was higher in adults as compared to children.

Table 1. Frequency of FAB morphological types in 314 total patients, 257 children and 57 adults with acute Lymphoblastic Leukemia.

	FAB Morphologic Type		
	L1 n (%)	L2 n(%)	L3 n(%)
n = 314	191(60.83)	118 (37.58)	5 (1.59)
Children (<= 15 years) n = 277	159 (61.87)	94 (36.57)	4 (1.56)
Adults (<= 15 years) n = 57	32 (56.14)	24 (42.11)	1 (1.75)

Both childhood and adult cases were divided in age groups of prognostic significance (table-2). In children the proportion of L2 cases was higher (50.9%) in the prognostically unfavorable age group of 10-15 years than in younger children (32.8%), the difference in frequency being statistically significant P=0.021 (table-2). Among adults L2 morphology was more frequent (75%) in-patients above 35 years as compared to younger adults (37.5%) (table-2).

Table 2. Frequency of FAB Morphologic Types by age groups in Acute Lymphoblastic Leukemia patients at presentation.

Age Group (years)	FAB Morphologic Type			n=
	L1	L2	L3	
	n(%)	n(%)	n(%)	
Children				
<1	1(100.00)	0	0	1
1-9.99	131(65.17)	66(32.84)	4(1.99)	201
10-15	27(49.09)	28(50.91)	0	55
Adults				
>15-35	29(60.42)	18(37.50)	1(2.08)	48
>35-60	2(25.00)	6(75.00)	0	8
>60	1(100)	0	0	1

Total cases were divided into 3 levels of TLC:- i) subnormal count <$4x10^9$/L, ii) TLC 4-$50x10^9$/L and iii) TLC above $50x10^9$/L. L2 ALL was seen in 28.7%, 43.7% and 38.2% of patients in these respective groups (table-3). Hence L2 was not found to be more frequent in the prognostically unfavorable group with TLC> $50x10^9$/L.

Table 3.FAB morphology by various levels of Total Leukocyte Count (TLC) inpatients of Acute Lymphoblastic Leukemia at the time of diagnosis.

TLC (x 10^9/L)	FAB Morphologic Type			n=
	L1	L2	L3	
	N(%)	N(%)	N(%)	
< 4	74(68.52)	31(28.70)	3(2.78)	108
4-50	83(54.97)	66(43.71)	2(1.32)	151
≥ 50	34(61.82)	21(38.18)	0	55

Discussion

In the present study, among 257 children L1 morphology was seen in 61.8%, L2 in 36.6% and L3 in 1.6%. In childhood ALL the frequency of L1 morphology is reported to be higher than L2 in studies from the Western Countries as well as from Pakistan. In the Western series the proportion of L1 cases varies from 70% to 85%, L2 17% to 36% and L3 4% to 0%[2, 3, 6]. In studies from Pakistan the frequency of L1 in children varies widely from 55% to as high as 92%, L2 8% to 40% and L3 5.4% to 0%[12, 13, 14, 15, 16].

Among 57 adults, the proportion of L1 cases was 56%, L2 42.1% and L3 1.8% in our study. Previous studies from Western Countries show a high proportion of L2 vs L1 morphology in adults[2]. In the present study however L1 ALL was more frequent than L2 although the frequency of L1 was less in adults as compared to children. The findings of Alvi et al[13] in adult ALL in our country are in accordance with the present study. Hassan et al reported 61.2% L1,34.3% L2 and 4.4% L3 cases in 67 patients[17], where as Butt and Lodhi reported 35% L1, 58% L2 and 6% L3 cases in 79 patients[18]; the frequency in children and adults was not given separately in these studies.

L2 morphology has been reported to be associated with a worse prognosis than L1 type whereas L3 represents B-ALL immunotype and is associated with the worst prognosis[2, 3, 4, 6, 7].

Distribution of FAB types was determined in prognostically significant age groups in children and adults. L2 was more frequent (50.9%) in the high risk age group of children (10-15 years) than in the standard risk group (32.8%) of younger children (1 to 9.99 years), the difference being statistically significant p= 0.021. Hassan et al (15) reported that in children 70% of L2 cases were above 5 years of age as compared to 60% of L1 cases. Relationship of FAB morphology with another important prognostic factor i.e. TLC was studied. The frequency of L2 morphology (38.2%) was not increased in the high risk group with TLC > $50x10^9$/L as compared to that (43.7%) in patients with TLC of 4-<$50x10^9$/L. However L2 morphology was least frequent (28.7%) in patients with TLC below $4x10^9$/L. Hassan et al[15] also did not find a relationship between high TLC and FAB L2 morphology, in fact 36.6% of their L1 patients vs 23.1% of L2 patients had TLC above $10x10^9$/L.

This study therefore reveals that the frequency of L1 in children is lower as compared to Western reports

Considering the prognostic significance of FAB classification i.e. association of better prognosis with L1, this is an important finding. On the other hand among adults the proportion of L1 vs L2 in the present study is higher than that reported in the West. Our study also shows the association of poor prognostic age groups with increased frequency of L2 ALL both in children and adults. However patients with high risk levels of TLC (> $50x10^9$/L) did not show an increase in the proportion of L2 ALL.

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