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Flow cytometric analysis of childhood leukemias

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Students' Corner

Flow Cytometric Analysis of Childhood Leukemias

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Abstract

Objective: To collect demographic data for childhood (less than 15 years) leukemias in Karachi, describe the accuracy of the cell surface markers routinely used in the flow cytometric analysis of leukemic cells and arrive at an ideal panel of antibodies for analyzing leukemic samlples.

Materials and Methods: Data from 62 consecutive cases of childhood leukemias referred to the Department of Pathology, Aga Khan University Hospital, (AKUH) between January 1995 and December 1998 was analyzed using Epi Info Version 6. Flow cytometry on all samples was performed using standard protocols.

Results: The mean age of patients was 8.2 years and 49 (79%) were males. Fifty (81%) had acute lymphoblastic leukemias of which 50% were CD10 positive and 24% CD10 negative Pre-B cell leukemias.

Among all Pre B cell All 98% were positive for CD19, 96% for CD22, 89% for HLA-DR and 67% for CD10. Of the 10 AML cases, 100% were positive for CD33, 90% for CD13, 80% for CD19 and 70% for HLA-DR.

Conclusion: The mean age in this study population was significantly higher and percentage of CD10 positive Pre-B All is lower than that in the West. Both these factors might be responsible for the poorer prognosis of these patients. It is not possible to specify a minimum or maximum panel of antibodies that should be used for phenotyping all cases of childhood leukemias. A certain degree or redundancy is essential in any panel of antibodies used for flow cytometry of leukemias (JPMA 51:133;2001).

Introduction

Leukemias are the most common cancers in children less than 15 years of age in Pakistan[1]. Leukemias also comprise the majority (40%) of the childhood malignancies in the West including United States [2]. Therefore, they make up the majority of pediatric malignancies in several parts of the world. Most of these are fortunately curable[3]. The epidemiology and biology of these tumors, unlike the incidence varies from region to region[4,5]. This makes it essential to ascertain the epidemiology and biology of these tumors in our population, as has been done elsewhere [6,7]. A search of the past five years of literature reveals very limited statistics relevant to Pakistan, in this regard[8]. To arrive at these statistics was one of the objectives of this study.

Flow cytometric is highly applicable to the detection and classification of leukemias because of the ease with which single cell suspensions can be made. Composite immunophenotypic analysis is essential for classifying leukemias once the disease is detected by traditional means[9]. Ongoing efforts to standardize protocols are essential to ensure widespread applicability, together with improved understanding of the attributes and limitations of this technology.

Flow cytometric analysis of leukemic samples was started routinely at The Aga Khan University Hospital, Department of Pathology in 1995 since then there has been continuous evaluation and revision of the panel of antibodies used to analyze leukemic cells. This study also looks at the accuracy of the cell surface markers that have been used routinely for analysis of leukemic samples, since 1995 to arrive at an ideal panel of antibodies for analyzing leukemic samples.

Materials and Methods

A study question was formulated and a list of all the records of leukemias referred to The Department of Pathology, between January 1995 and December 1998 was generated. Patients in the desired age group (less than 15 years) were selected and relevant information from their records was documented on specially designed data entry forms. The data was then entered in Epi. Info Version 6.1. Analysis was performed using the same program. Student's t test and Chi square test of significancies were performed to determine the presence of a significant difference between mean age and the percentage of CD10 positive pre-B acute lymphoblastic leukemias between this study population and Western literature. Alpha value was chosen to be 0.05.

Flow cytometry was performed on all the peripheral blood and bone marrow samples by centrifuging mononuclear cells from them. These were then stained with florescence labeled antibodies (obtained from Becton Dickinson, U.S.A.) and run on FACSCAN using cell quest software. The same software was used to analyze the flow cytometry data[10].

CD19, CD20, CD22, CD10, CD3, CD5, CD7, CD13, CD33 and HLA-DR were tested on all the cases[11].

CD2 was not included in the panel because of the presence of CD3, CD5 and CD7, which are all T cell markers as its inclusion would have added no new information. CD14, CD45, Anti-Kappa, Anti-Lambda, IgG, IgM and CD61 were markers that were tested on selected cases. Positivity of a clone for a specific cell surface marker was defined as more than 30% of the cells staining with the antibody for the respective marker.

Results

Data from a total of 62 cases of childhood (less than 15 years) leukemias, on which flow cytometry had been performed was analyzed. The mean age of patients was 8.2 years with a standard deviation of 3.9 years. Most of them (49/62 or 79%) were males. The site from which the sample was taken was reported in 60 (97%) cases. Of these 32 (53.3%) were peripheral blood and 28 (46.7%) bone marrow samples.

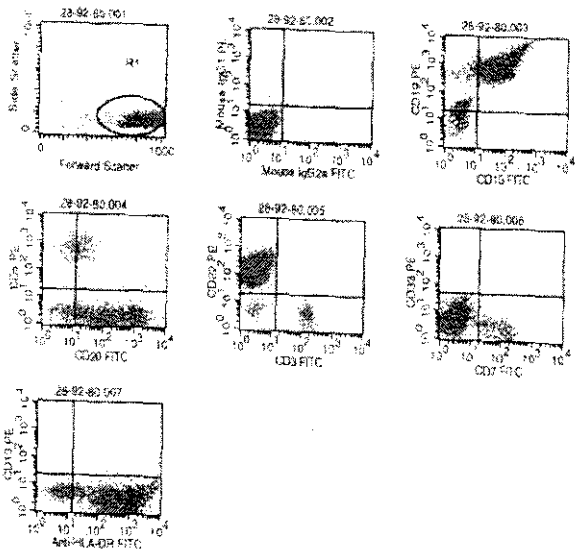

Figure 1. Flow cytometry of CD10 positive common pre-B acute lymphoblastic leukemia.

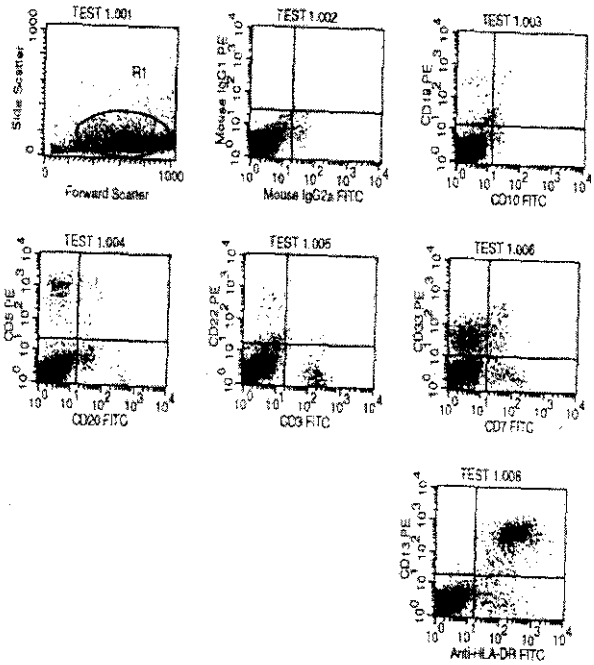

Figure 2. Flow cytometry of acute myeloblastic leukemia.

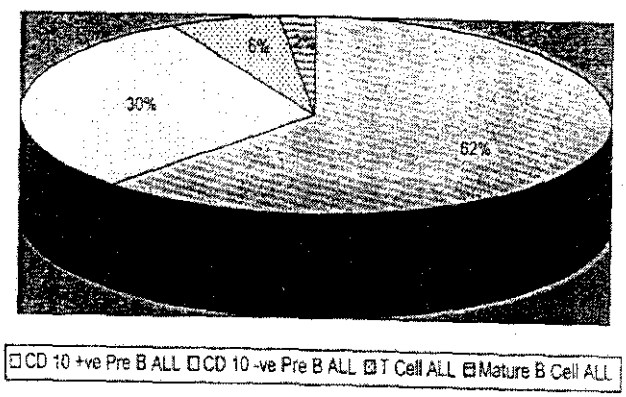

Figure 3. Immunologic phenotypes of acute lymphoblastic leukemias (ALL) (n=50).

Table 1. Positivity of different cell surface markers in various classes of leukemias.

	CD19+ve	CD20+ve	CD22+ve	CD10+ve	CD3+ve	CD5+ve	CD7+ve	CD3+ve	CD33+ve	HLA-DR+ve	Total cases
Pre-B ALL	45	21	44	31	3	5	6	3	1	41	46
T-ALL	0	0	0	0	2	3	3	0	0	0	3
Mature B Cell ALL	1	1	1	0	0	0	0	1	0	1	1
AML	8	0	0	0	1	2	2	9	10	7	10

Fifty two cases (83.9%) were acute lymphoblastic leukemias (ALL). Of these 52 cases, 59.6% were CD10 positive (Figure 1), 28.9% were CD10 negative Pre-B ALL, 5.8% were T-Cell ALL and 1.9% were mature B-Cell ALL.

Table 2. Comparison of percentage of different subtypes of Acute Lymphoblastic Leukemias.

	Our data (%)	Western data[32] (%)
Common Pre-B cell ALL	88.5	80
T cell ALL	5.8	15
Mature B cell ALL/others	7.7	5

Ten of the 62 cases were acute myeloblastic leukemias (AML) (Figure 2). Two cases were reported as inconclusive. Of the 31 CD10 positive cases, the majority (54.8%) were detected in bone marrow samples and 45.2% in peripheral blood. The frequencies of the various immunophenotypes of acute lymphoblastic leukemias are shown in Figure 3.

The cell surface markers which were positive in each immunologic phenotype of ALL and AML are shown in Table 1.

Discussion

The mean age in this study was 8.2 years with a standard deviation of 3.9 years. Peak incidence in the 5-9 years age group in males and the 10-14 year age group in females[1]. In the West the peak age of incidence is 4 years [12] which is significantly less ($p<0.05$) than that seen in our population. This could be attributed to the late presentation of these children in our setting.

It is established that ALL is more common in males than females, in the pediatric population[1,12,13]. In accordance, the majority of our patients were males. Eighty four percent of our cases were those of ALL, the remainder being AML, which is similar to the results reported elsewhere[13,14].

A comparison of the immunologic phenotypes of ALL in our population and in the West are shown in Table 2. The frequencies of gross categories are similar but there is a paucity of CD10 positive Pre-B ALL ($p<0.05$) in our series as compared to the West and Far East[15-18]. This decrease in CD10 positive Pre-B ALL, (CD10 being a good prognostic indicator[15,17,19] could account for the poorer prognosis of leukemias in our population [20].

There was no marked excess of CD10 positive cells in the bone marrow samples as compared to the peripheral blood samples. This corroborates evidence that significant homing mechanisms do not seem to be actively involved in localizing CD10 positive cells to the bone marrow[21].

Since 1995, flow cytometry has routinely been performed on leukemic samples at the Department of Pathology, AKUH.

The following is an analysis of the accuracy of markers commonly used for flow cytometric analysis of leukemias at AKUH. The denominator for this analysis is 60 cases (excluding the two cases which were reported as inconclusive). The terms "specificity" and "sensitivity" are not used in the strict statistical sense. Accuracy of various markers was calculated by working out the percentage of cells which tested positive for the markers which were expected biologically, to be present on these cells. This method of analysis has been used before, successfully[8].

CD19 was detected on 92% of B-Cell ALL and 80% of AML cases. As shown earlier, CD19 is not very specific to B Cell leukemias[10,22]. CD20 was positive in 44% of cases of B Cell ALL and was not expressed on any non B Cell malignancy. Our study confirms that CD20 is less often expressed on acute B lineage leukemias than CD19 but is more "specific" for B cell malignancies[10,23]. CD22 was found on 90% of the B cell malignancies and on none of the non B cell malignancies. A highly "sensitive" and "specific" marker, CD 22' s usefulness to detect acute B-lineage leukemias is decreased by the fact that it is often dim and is more strongly expressed in the cytoplasm of cells than on their surface[10,24]. 67% (31/46) of the cases of Pre B Cell ALL were CD10 positive. This is lower than the percentage of CD10 positive Pre-B cell ALL reported in the West and this point has been discussed above. CD10 was not detected on any case of T cell ALL (as has been reported in literature[15]. This could be due to the small number of cases of T cell ALL in our study. We hypothesize that it could also be due to the high socio-economic status of the patients reporting to the Aga Khan University Hospital since T-ALL is more common in the low socio-economic group. However our study does not have the power to test this hypothesis.

CD3 was detected on 66.7% of the cases of T cell ALL and 7% of the cases of non T cell malignancies. It is hence a "sensitive" and "specific" marker for detecting T cell ALL[23]. Its usefulness is limited by the fact that it is uncommonly expressed on the surface of T-lineage ALL. It is almost always present in the cytoplasm of these cells[10,25]. CD5 was found on all 3 cases of T cell leukemias and 12.3% of non T cell malignancies. Our study corroborates evidence that CD5 is specific for T cells and occurs in a great majority of cases, the drawback once again being that it is dimly expressed[10,26]. CD7 was seen on 4/46 cases of Pre-B cell ALL. This was seen in samples of peripheral blood for leukemia analysis, which normally has 80% T lymphocytes. This pattern is most likely reactivity to normal circulating lymphocytes. CD7 was also found on all cses of T cell ALL and on 14% of cases of non T cell ALL. It is reported in literature however, that CD7 is found in cases of AML too

and is not as "specific" for T cell ALL as our study suggests[10,27].

CD13 was found on 90% of cases of AML and 8% of non AML cases. CD33 was found on all cases of AML and on 2% of non AML cases. Both these markers are hence valuable for diagnosis of AML. Since both can occasionally be expressed on B or T lineage ALL they should be interpreted as strongly suggestive of myeloid leukemia only when expressed in the absence of lymphoid associated antigens[10,26]. Our study confirms previous reports that HLA-DR is found in most cases of AML and B lineage ALL[10,28].

In conclusion, the epidemiology of acute leukemias in our study population is similar to that reported in the West. This has also been shown in another Pakistani studies[29,30]. Late age at presentation and an excess of CD10 positive cases may lead to poor outcome.

Regarding flow cytometry, antibodies clearly vary in their degree of lineage specificity. Also, many leukemias lack one or more antigens expected to be present on normal cells of a particular lineage[9,10,31,32]. It is hence recommended, that a certain degree of redundancy be built into a panel for leukemia phenotyping.

Where possible it is recommended that lineage assignment be confirmed with an antibody from a second CD cluster which is known to react principally with the suspected cell lineage. However, failure to detect the confirmatory marker does not exclude the suspected lineage since leukemias commonly exhibit loss of various antigens. It is hence not possible to specify a minimum or maximum panel that should be used for phenotyping all cases of leukemias[10,14,33].

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