RESEARCH ARTICLE

Aberrant expression of myeloid antigens in patients of acute lymphoblastic leukaemia

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Abstract

Objective: To determine the immunophenotypic pattern and aberrant expression of myeloid antigens in newly diagnosed patients of acute lymphoblastic leukaemia(ALL).

Methods: This descriptive cross-sectional study was carried out in Haematology / Pathology department, Army Medical College, National University of Medical Sciences (NUMS) in collaboration with Immunology and Haematology departments of Armed Forces Institute of Pathology (AFIP), Rawalpindi from 1st January, 2019 to 31st December, 2019. Seventy-three (73) recently diagnosed patients of Acute Lymphoblastic leukaemia of all age groups and both genders were included in the study. A proforma was used to note demographic data. CBC, cytochemical stains and bone marrow examinations were carried out and assessed for morphology and percentage of blasts using a microscope. Flow cytometry was used to perform immunophenotyping on samples of peripheral blood and bone marrow, using a standard panel.

Results: The most commonly expressed markers were weak CD45, TdT, CD19, CD10 and HLA-DR. Weak CD45 was present in almost all blast cells and there was no remarkable difference in its positivity among various subtypes of ALL. Myeloid expression was observed in 13 (17.8%) cases. CD13 and CD33 were aberrantly expressed in 11 and 12.3 of all cases of ALL respectively.

Conclusion: Expression of aberrant myeloid CD markers in acute lymphocytic leukaemia has prognostic significance and should be documented during lineage assignment of acute leukaemias while performing immunophenotyping.

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Introduction

Acute lymphoblastic leukaemia is defined as a malignant transformation in extramedullary sites of progenitor lymphoid cells in the bone marrow. Eighty percent (80%) of acute lymphoblastic leukaemia occurs in childhood but it has devastating effects when it occurs in adults.[1] Its incidence follows a bimodal peak, the first one occurring in the paediatric age group and the second peak occurring around 50 years of age.[2] The disease originates in lymphoid cells which express various antigens thus giving rise to B- or T- cell leukaemia or sometimes mixed lineage leukaemia.[3] Growth and differentiation of the progenitor cells are regulated by specific cytokines, growth factors, and their corresponding receptors. By analyzing these receptors, the grade of differentiation and lineage of the progenitor cells can be determined.[4]

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Early systems of classification for acute leukaemia used morphological features and cytochemical stains, however recently classification systems are using immunophenotyping to achieve greater accuracy in establishing the haematopoietic lineage and stage of differentiation in a particular type of leukaemia.[5] Multiparametric flow cytometry is an important and established tool for diagnosis, prognosis as well as monitoring minimal residual disease in acute leukaemias.[6] It is the method of choice to determine the lineage of blasts and to detect aberrant antigenic expression.[7] It improves both the accuracy and precision of classification of acute leukaemias.[8]

Blasts of acute lymphocytic leukaemia express molecular markers and surface antigens that help in identifying their lineages. Some cases of acute lymphoblastic leukaemias demonstrate those surface antigens that are associated with myeloid leukaemias are co-expressed with surface antigens that are associated with T or B lymphocytes. This phenomenon is termed lineage infidelity or mixed-lineage expression.[9] There is a proposed scoring system according to EGIL Criteria[10] for lineage assignment. Diagnosis of Multi phenotypic acute leukaemia (MPAL) is made when the scores in EGIL criteria for both lymphoid and myeloid lineages are more than two; sometimes tri-lineage differentiation may be seen. Myeloid markers expressed in lymphoid leukaemias and lymphoid markers expressed in

myeloid leukaemias, but not reaching more than two points according to criteria are termed as aberrant expression.[11] Myeloid antigen expressed in ALL patients can be used independently as a predictor of poor chemotherapeutic response and can be used for risk stratification.[9,12]

Rationale: This study was conducted to identify aberrant expression of myeloid antigens in new cases, diagnosed with acute lymphoblastic leukaemia using flow cytometry so that patients could be stratified into prognostic groups by pattern and intensity of antigen expression. The objective of this study is to determine the immunophenotypic patterns in recently diagnosed patients of acute lymphoblastic leukaemia by flow-cytometry and to correlate aberrant myeloid antigens with other CD markers and complete blood counts.

Materials and Methods

This descriptive cross-sectional study was conducted in the Haematology Department of Army Medical College in collaboration with the Immunology & Haematology departments of Armed Forces Institute of Pathology (AFIP), Rawalpindi. The study was conducted from 1st January to 31st December 2019.

Ethical approval was obtained from the Ethical Review Committee of Army Medical College, with IRB Number ERC/ID/14. Participants' information was kept confidential. Every patient was allotted a code number.

WHO sample size calculator was used to calculate the sample size[13] Confidence interval was 95% and 5% absolute precision was used. The minimum sample required to conduct the study was 31.[14] However, 73 newly diagnosed patients of acute lymphoblastic leukaemia were included in the study, regardless of gender and age. Diagnosis of a patient was made keeping in view the morphological characteristics, cytochemical stains and immunophenotyping.

All newly diagnosed cases of ALL, diagnosed on the basis of morphology, of all ages and both genders were included in the sample. Patients of other leukaemias for example secondary leukaemias evolved from myeloproliferative neoplasms and myelodysplastic syndromes, patients of AML and those patients being treated for ALL were excluded from the cohort. Patients who were fulfilling all the conditions of the inclusion criteria were explained and briefed about the study and informed consent was obtained. All the particulars of the patient were endorsed on a proforma.

Non probability convenience sampling technique was used for the study.

Three ml of blood was taken in tripotassium ethylene di-amine tetra acetic acid (EDTA) tube and a complete blood count was carried out using an automated Sysmex-KX-21 haematology analyzer. Peripheral blood and bone marrow aspirate were inspected under a microscope for blast percentage and morphology. Three ml of bone marrow sample was taken in EDTA tube. Samples for immunophenotyping were processed within 6 hours of collection. A primary panel constituted antibodies against TdT CD3, CD5, CD7 for T lineage cells blasts, CD19, CD20 for B lineage blasts and MPO, CD13, CD33, CD117 for myeloid lineage blasts. It also included antibodies against CD45, CD10, CD34 and HLA-DR. Mouse antiIgG1 FITC/ IgG2 PE was used as isotype control. Wherever indicated the primary panel was extended up to (secondary panel) antibodies against cytoplasmic cCD3, CD4, CD8, CD79a, cCD22 and glycophorin. Labelling of monoclonal antibodies was done using either phycoerythrin, fluorescein isothiocyanate or peridinin chlorophyll protein. Cells were stained using the standard protocol. Immunophenotyping was performed by a flow cytometer called BD FACS Calibur. At least 10,000 cells were analysed through a forward scatter/side scatter gating method in the flow cytometer. The expression of different CD markers or their absence was assessed using isotype control by quadrant application. Cell populations were defined as positive for a particular CD marker if more than 20% of the leukaemic blasts events were above the isotype control threshold, otherwise, they were defined as negative. Each test tube was labelled properly and was sequentially placed. Ten ul of monoclonal antibody in each test tube, followed by 50ul of peripheral blood or diluted bone marrow in each test tube. The contents in the tube were mixed thoroughly and then placed in a dark room for incubation for half an hour at room temperature. FACS Lyse was prepared in distilled water in 1:10 dilution. Two ml of FACS Lyse was added to each test tube and placed in incubation for 5 minutes at room temperature in a dark room. The contents of the tube are centrifuged for five minutes at 3000 RPM at room temperature and after wasting the supernatant, the residual fluid was mixed thoroughly for re-suspension of cells. This was followed by adding 2ml of phosphate buffered saline in each test tube, centrifuged for 5 minutes at 3000 RPM. The supernatant was again wasted. The residual was mixed and 0.5 ml of 3.3% formalin was added to each test tube. The samples were stored at 4°C for further analysis on FACS Caliber.

The percentages of abnormal cells analyzed for CD markers were entered in the Statistical Package for Social Sciences (SPSS) version 21. The data was studied statistically for frequencies and percentages for different variables. Mean, median, mode and standard deviations were calculated for

quantitative variables like age, white blood cell count and monoclonal antibodies. Effect modifiers like age and gender were controlled by stratification. These were also individually calculated for paediatric and adult age groups. Expression of aberrant markers CD13, CD33 and CD117 were calculated in all patients of each age groups. Pearson correlation coefficient was used to measure the strength of correlation between variables.

Results

A total of 73 patients of ALL were diagnosed during these study period. Of these, 56 (76.7%) were males and 17 (23.3%) were females. Male to female ratio was 3.2:1. The age of the cohort ranged from 9 months to 75 years (median age=12 years, Mode=4 years). Out of 73 ALL cases 56 (76.7%) were diagnosed as B-ALL and 17(23.2%) were diagnosed as T-ALL. The median haemoglobin level was 8.6 g/dl (IQR=4.5). The range of haemoglobin level was from 3.8 g/dl to18.2 g/ dl. The median of white cell count was 11.8×10^9/L (IQR=32.2), the lowest being 0.42×10^9/L seen in B-ALL and the highest being 586.6×10^9/L seen in T-ALL. The median value of platelet count was 32×10^9/L (IQR=47). The lowest Platelet count 2×10^9/L was seen in B-ALL and the highest platelet count being 339×10^9/L seen in T-ALL. Pancytopenia was found in 13 (17.8) patients. The minimum percentage of blasts cells seen in bone marrow was 20% and the highest was 99%. Among the 47 paediatric patients (< 18 years of age), the mean blast percentage was 83.2±16.9. Among adults (>18 years of age), the mean blast percentage was 74.55±26.1. Table-1 shows the frequency of positive CD markers in acute lymphoblastic leukaemia. Positive expression of weak CD 45 was seen in 97.3 % of cases and there were no substantial variations in between the different sub-types. Expression of HLA-DR was positive in 49 out of 73 patients. The positivity was strongest in B-ALL and weakest in T-ALL. TdT showed mean positivity of 76.5% among all cases.

Table-1: Frequency of positive CD marker expression in ALL patients.

CD markers	B cell ALL cases n (%)	T cell ALL cases n (%)
Weak CD 45	56 (100)	15 (88)
HLA-DR	42 (75)	7 (41.1)
TdT	43 (76.7)	13 (76.4)
CD34	41 (71.4)	6 (35.2.1)
CD10	50 (89.2)	4 (24)
CD 19	51 (91)	2 (12)
CD20	12 (21.4)	-
cCD22	6 (10.7)	1 (5.8)
CD23	1 (1.7)	-
CD79a	4 (7)	-
CD3	10 (17)	12 (71)
CD4	-	2 (12)
CD5	10 (17)	17 (100)
CD7	10 (17)	17 (100)
CD8	-	3 (18)
cCD3	-	15 (88)
CD13	7 (12.5)	1 (5)
CD14	-	-
CD33	7 (12.5)	3 (17.6)
MPO	-)	-
CD117	-	-

ALL: Acute lymphocytic laeukemia, CD: Cluster of differentiation

Table-2: Expression and distribution of abberant myeloid markers in subtypes of ALL.

	Abberant marker	Paediatric group (n=47) n (%)	Adults n (%)	Total n (%)
Precursor B	13+33	3(6.3)	2(7.6)	5(11.3)
ALL	13	-	2(7.6)	2(4.5)
(n=44)	33	-	2(7.6)	2(4.5)
Pre B- ALL	13+33	-	-	
(n=10)	13	-	-	
	33	-	-	
B-ALL (n=2)	13+33	-	-	
	13	-	-	
	33	-	-	
T-ALL (n=17)	13+33	-	1(3.8)	1(5.8)
	13	-	0	
	33	-	3(11.5)	3(17.6)
Total		3 (6.3)	10(38.4)	13(17.8)

ALL: Acute lymphoblastic leukaemia.

Table-3: Blood counts of patients with and without myeloid antigen expression.

	Myeloid antigen positive cases (n=13)	Myeloid antigen negative cases (n=60)	*p*-value
WBC (mean rank)	31.7	38.1	0.32
HB (mean rank)	42.1	35.8	0.33
PLT (mean rank)	42.3	35.8	0.32
Blasts (mean rank)	32	38.1	0.33

WBC: white blood cells. HB: haemoglobin, Plt: platelet

Out of the total 73 cases of ALL, 13(17.8%) patients co-expressed one or more of the myeloid markers (CD13 and CD 33). The remaining 60 (82.1%) patients did not show any aberrant myeloid antigen expression. On further analysis of 56 cases of B-ALL, 9(16%) showed positive aberrant expression for myeloid antigen and 47(83.9%) were myeloid negative, while out of 17 T- ALL cases 4 (23.5%) showed aberrant myeloid expression whereas 13 (76.4%) were negative for myeloid antigen expression. Based on flow cytometry, the B-ALL is further divided into precursor B-ALL, pre B-ALL and mature B-ALL. Figure shows the bar chart of the frequency of different sub-types of ALL according to age.

In table-2, it is evident that there is a strong relationship between myeloid aberrance and increased age (chi-square =11.769, df =1, $p<0.05$). Out of 73 cases, 7 expressed at least 1 myeloid positive antigen (CD13 or CD33) and 6 patients expressed both. No patient showed positivity for CD 117

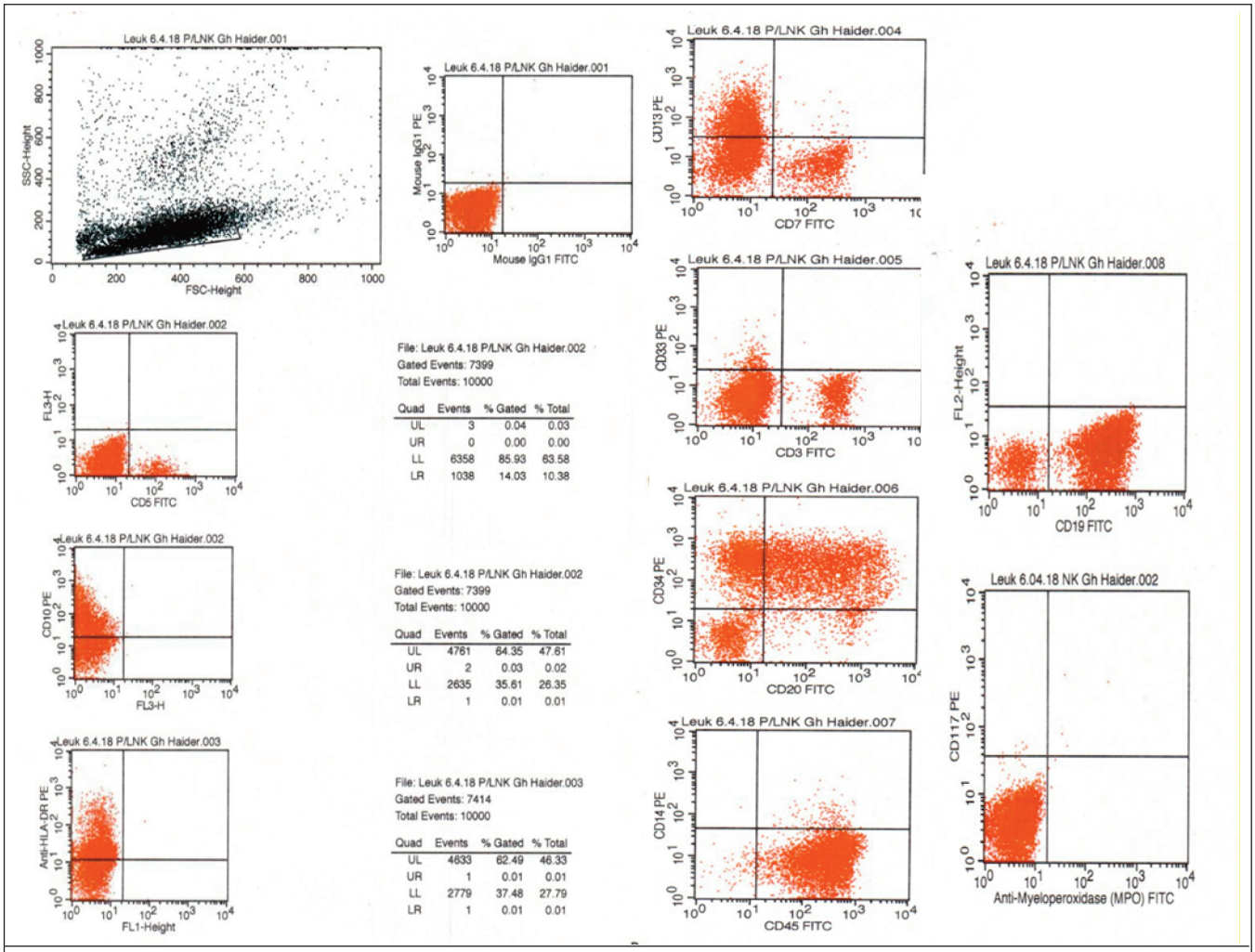

Figure: Dot-plot for precursor B-ALL patient showing positive expression of aberrant CD 13 marker.

and CD14.

Table-3 shows a correlation between TLC, Hb and platelet count between patients who were with aberrant myeloid expression and those who were without aberrant myeloid expression.

According to Mann Whitney U test performed on the above data, there was no statistical difference in TLC, Hb, platelet and blast percentages between aberrant myeloid antigen-positive and negative cases in our study.

Discussion

In the classification of acute leukaemia, immuno-phenotyping is considered to have a recognized role that helps in diagnosis and stratifying patients into prognostic groups. It is also helpful in identifying the maturational stage of blast cells.[15] Acute lymphoblastic leukaemia is the most common malignancy in the paediatric age group, representing 32% of all malignancies diagnosed in Pakistan.[16]

The median age in our study was 12 years, with 65% cases younger than 18 years of age similar to the study by Faris and Jafer in Iraq, in which 62% were less than 18 years,[17] while in other studies carried out in the USA,[18,19] 80% cases were younger than 18 years. This difference in our study can be due to racial differences in our cohort.

There is a predilection for the male gender in ALL, especially T-ALL.[16] Our study showed that the male to female ratio was 3.2:1 similar to the study conducted in Morocco,[20] while in other studies conducted in Egypt it is 2.6:121. In our study 76.7% of cases were B- ALL and 23.3% were T-ALL, similar to Faris and Jafar study, where 72% were B-ALL and 28% were T-ALL.[16] While the study by Hamed and El-Deen from Iraq, reported that 86% were B-ALL and 14 % were T-ALL.[21]

For further analysis, subjects were divided into subgroups

of B-ALL that are: precursor B-ALL, pre-B-ALL and mature B-ALL. Hence, in our study precursor B-ALL was found to be most common with 44(60.3%) cases out of 73 cases of ALL in our study. Pre B-ALL was diagnosed in 10(13.7%) patients and mature B-ALL in 2 (2.7%) patients. The results of our study were comparable to the study conducted in Morocco by Lahjouji A et al in which precursor B-ALL is the predominant ALL followed by mature B-ALL and T-ALL[20] and National Cancer Institute, US guidelines that showed early B cell lineage ALL to be 80%, mature B–ALL to be 5% and T-ALL 15%.[22]

In our study, 13 (17.8%) patients out of 73 showed at least one aberrant myeloid expression (CD13 & CD33) which is in comparison to a study conducted in Iraq by Faris in which 20% cases showed myeloid expression17 but against another study conducted in Jordan that showed 29% incidence of aberrant expression in all cases of ALL.[23] This difference can be due to racial and genetic factors which might influence the antigenic expression.

Our study showed significant statistical evidence of increasing myeloid aberrance in patients with increasing age ($p<0.05$), with 38.4% patients showing myeloid aberrance in adults and 6.3% in the paediatric age group. This is similar to a study conducted in India by M. Sharma and colleagues, which showed a similar increase in myeloid aberrant cases in the adult age group.[24] But against the study conducted in India by Monika et al, who found no correlation between myeloid aberrance and age.[25]

Although, the platelet counts in patients of acute lymphoblastic leukaemia with aberrant myeloid expression are low, according to our results no statistical difference was present when it is compared with the non-aberrant group ($p>0.05$) which is against the study conducted by Lopes in Brazil,[12] but in accordance to the study conducted by Monika et al.[25]

Aberrant antigen expression has a prognostic significance and has a poor outcome on overall survival, clinical response and remission rate in patients with acute lymphoblastic leukaemia.[12,23] Hence, clinicians should keep it in mind while prognostic stratification of patients for treatment.

Conclusion

Flow cytometry analysis of acute lymphoblastic leukaemia patient is a standard workup which determines the cell lineage and detects any aberrant antigenic expression in the leukaemia cells. In our study, there is a less number of patients who showed aberrant myeloid expression (17.8%), but this is important for risk stratification and treatment planning. Further studies are needed for validation of the role of myeloid aberrance in the prognosis of our patients, using a larger sample size and patient follow up.

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