ORIGINAL ARTICLES

IMMUNOPHENOTYPING PATTERN IN MIXED PHENOTYPE ACUTE LEUKAEMIAS

Ayesha Khurshid, Saleem Ahmed Khan*, Chaudhry Altaf, Hamid Saeed Malik, Muhammad Mukarram Bashir, Maria Altaf**

Armed Forces Institute of Pathology (AFIP)/National University of Medical Sciences (NUMS) Rawalpindi Pakistan, *Army Medical College/ National University of Medical Sciences (NUMS) Rawalpindi Pakistan, **Combined Military Hospital Sargodha/ National University of Medical Sciences (NUMS) Pakistan

ABSTRACT

Objective: To evaluate Immunophenotyping patterns in Mixed-Phenotype Acute Leukemias (MPAL).
Study Design: Descriptive study.
Place and Duration of Study: This study was carried out in the department of Hematology, Armed Forces Institute of Pathology Rawalpindi, from 1st Jan 2013 to 31st Jan 2017.
Material and Methods: After taking informed consent from the patients fulfilling the inclusion criteria, detailed history was taken and blood samples were drawn for blood complete picture. The patients suspected to have acute leukemia were subjected to bone marrow examination (aspiration and trephine biopsy) for further cytochemical staining (SBB) and Immunophenotyping.
Results: Total 680 new cases of acute leukemias on initial workup of either gender age were included. Patients of other haematological disorders were excluded from the study. Among 680 new cases of acute leukaemia, 23(3.4%) cases were of MPAL immunophenotyping using scoring system proposed by EGIL (European Group for the Immunological Characterization of Leukemias) classification. Among MPAL, 19(83%) cases were Biphenotypic [13(57%) cases of My/B-ALL, 5(22%) cases of My/T-ALL, and 1(4%) case of T/B-ALL]. 4(17%) cases were Bilineage (My/B-ALL). Most of the cases were diagnosed at less than 10 years of age.
Conclusion: My/B-ALL is the most common immunophenotype followed by My/T-ALL. Therefore immunophenotyping is indispensable for diagnosis and for therapy decisions of MPAL.

Keywords: Acute leukemia, Immunophenotype, Lineage, Lymphoid, Myeloid, Mixed phenotype acute leukemia.

INTRODUCTION

Acute leukemia of ambiguous lineage are the type of leukemias in which the blasts demonstrate no clear evidence of differentiation along a single lineage[1]. This group includes those acute leukemias in which there is no associated expression of lineage-specific antigens (acute undifferentiated leukemia), as well as those in which there is expression of antigens associated with more than one lineage (mixed phenotype acute leukemias). The diagnosis of acute leukemias (AL) is based on clinical features, systemic examination bone marrow aspiration and trephine biopsy findings, immunophenotyping, cytogenetic and molecular investigations. Acute leukemias are classified as having myeloid or B-lymphoid or T-lymphoid lineage, based on morphological features, as well as the expression of surface or cytoplasmic antigens on blast cells[2]. But there are rare cases of AL in which it is very difficult to classify the blasts, as they have morphologic, cytochemical and immunophenotypic characteristics of both myeloid and lymphoid lineages. This type of entity was defined as mixed-phenotypic AL (MPAL). Previously called biphenotypic acute leukemias (BALs), these neoplasms have been renamed mixed-phenotype acute leukemia (MPAL)[3]. BAL is characterized by one blast population that co-expresses several myeloid and lymphoid antigens in the same cells. In contrast, bilineage AL there are two separate blast populations, with each population expressing markers of a distinct lineage.

Correspondence: Dr Ayesha Khurshid, Department of Haematology, AFIP Rawalpindi Pakistan
Email: ayeshafaisal82@yahoo.com
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Mixed-phenotype acute leukemia (MPAL) is an uncommon clinical entity arising from a haemopoietic pluripotent stem cell, it is also known as mixed-lineage or hybrid acute leukemia[4,5]. Its incidence among acute leukemia account for approximately less than 5% of all acute leukemia[6]. Occurs in all age groups but are more frequent in adults[7]. Signs and symptoms of biphenotypic acute leukemias are similar to acute leukaemia. The diagnostic criteria were based on the scoring system proposed by the European Group for the Immunological characterization of Leukemias (EGIL) classification, that was adopted by the WHO 2001 classification[8], as shown in table-I and by the 2008 WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues[9-11].

EGIL is based on lineage specific and lineage associated markers. Two marks are given to each of lineage specific markers and 0.5 and 1 mark is given to each of lineage associated markers. According to this scoring system, a case is considered as MPAL when point values are greater than two for the myeloid and one of the lymphoid lineages.

It is not unusual to identify two distinct blast populations in the same patient, one of small size with a high nucleus/cytoplasm ratio resembling lymphoblasts and the other larger with more abundant cytoplasm with or without granulation resembling myeloblasts.

Immunophenotyping is essential for the diagnosis of mixed-phenotype acute leukaemia. MPAL is subdivided into four groups according to the expression of lymphoid and myeloid markers on the blasts. The most common are those in which the blasts co express myeloid and B-lymphoid, less often is myeloid and T-lymphoid antigens[12,13]. Trilineage differentiation with expression of B, T and myeloid markers is rare and coexistence of blasts expressing only B and T cell markers is very uncommon[14]. Most cases are terminal deoxynucleotidyl transferase (TdT) positive and express early hemopoietic markers such as CD34 and HLA-DR expression[15,16].

The aim of our study is to evaluate Immunophenotyping patterns by flow cytometry in Mixed-Phenotype Acute Leukemias (MPAL) using scoring system proposed by EGIL.

MATERIAL AND METHODS

This study was carried out in the department of Hematology, Armed Forces Institute of Pathology Rawalpindi. It was a descriptive, cross sectional study. The study was completed over a period of 4 years, from 1st Jan 2013 to 31st Jan 2017. Samples from patients of acute leukaemia on initial workup of either gender or age were included. Patients of other hematological malignancies were excluded from the study. A total of 680 patients of acute leukemia were included in this study. After taking informed consent from the patients fulfilling the inclusion criteria, detailed history was taken and blood samples were drawn for Blood complete picture. Sampling technique was non-probability consecutive sampling. In all cases MPAL we analyzed the laboratory values: white blood cell (WBC) count, hemoglobin (Hb) level, platelet count (Plt) and blast percentage. Morphologic examination of peripheral blood (PB) and bone marrow (BM) smear and cytochemical staining (SBB) was performed after staining by standard technique with Leishman stain. Immunophenotyping of the patients suspected to have acute leukaemia was done.

Immunophenotyping

Three ml of whole blood/0.5 ml bone marrow was collected and TLC, DLC was performed by Sysmex KX 21 automated hematology analyzer. Blood smear was examined for DLC and lymphocyte morphology. Carefully check the antibody panel required for the procedure. The monoclonal antibodies (MoAb) used for diagnosis of AL were: for B-lineage: CD19, CD22, CD20, CD10, for T-lineage: CD2, CD3, CD5, CD7, CD4, CD8, for myelo-monocytic lineage: CD13, CD33, CD11c, CD64, CD14, other cells surface markers: CD34, CD117, HLA-DR

and intracellular MoAb: CD79a, CD3 and MPO. Each test tube (Falcon, BD) was labeled properly and placed in sequence. 10 µl of antibody was added in each tube as per defined panel, then 50 µl of whole blood/diluted bone marrow was added in each tube and thoroughly mixed. It was incubated in dark for 30 minutes at room temperature. Dilution (1:10) of FACSLyse solution in distilled water was made. 2 ml of diluted FACSLyse was added in each tube. This was incubated in dark for 5 minutes at room temperature. Centrifuge at 300 g for 5 minutes at room temperature. Supernatant was discarded after centrifugation. 2 ml of RPMI 1640/PBS was added to each tube. Supernatant was discarded after centrifugation. Formalin (0.5 ml of 3.3%) was added to each test tube and kept at 4ºC until analysis on flow cytometer. The blast gate was identified on side scatter/CD45 dot plot. Data analysis were performed with Paint-a-Gate software. Surface antigen expression was considered positive if at least 20% of blasts showed a positive labeling. For cytoplasmic antigen expression, the threshold was 10%.

Data Analysis

All the collected data were entered and analyzed in Excel 2007. Median and range were calculated for quantitative variable like age, WBC count, haemoglobin, platelet count, percentege of blasts. Frequencies and percentages were calculated for qualitative variables like gender. Effect modifiers like age and gender were controlled by stratification. Post stratification chi-square test was applied, keeping *p*-value <0.05 as significant. Final data was shown by tables.

RESULTS

Among 680 new cases of acute leukaemia, 23 cases were diagnosed with MPAL using scoring system proposed by EGIL classification. The overall incidence was 3.4% of MPAL. Among 23 patients of MPAL, 19 (83%) cases were biphenotypic [13(57%) cases of My/ B-ALL, 5 (22%) cases of My/T-ALL, and 1(4%) case of T/B-ALL]. 4(17%) cases were bilineage (My/B-ALL). In our study the CD34 was positive in 21 (91%) cases and negative in 2(9%) cases. Tdt was positive in 14(61%) patients, negative in 6 (26%) patients and not determined in 3 (13%) patients.

Table-I: European group for the immunological characterization of Leukemias (EGIL) classification.

Scoring	B lineage	T lineage	Myeloid Lineage
2 Points	CD79a, CD22, cyt IgM	CD3	MPO
1 Point	CD19, CD10, CD20	CD2, CD5	CD13, CD33, CD117, CD65
0.5 Point	TdT	TdT, CD7	CD14, CD15, CD64, CD11b, CD11c

Table-II: Clinical and laboratory features of patients at diagnosis.

	Minimum	Maximum	Median
Age (yrs)	2	65	22
WBC (X109/L)	2.29	471	9.87
Hemoglobin mg/dl	4.8	12.5	9.4
Platelet count(X109/L)	5	392	33
Blast%	25	97	76

Table-III: Comparison between My/B-ALL and My/T-ALL immunophenotype for age and sex.

	My/B-ALL (n=17)	My/T-ALL (n=5)	*p*-value
Age (<30 yrs)	8	5	0.097
Age(>30 yrs)	9	0	
Male	10	5	0.228
Females	7	0	

Immunophenotypic findings in patients of My/B-ALL, My/T-ALL, and T/B-ALL are shown in fig-1, 2 & 3 respectively.

Among the 23 cases of MPAL, 16 were males (70%) and 07 were females (30%). Male to female ratio was 2.3:1. Most of the cases were diagnosed at less than 10 years of age.

Most common symptom were fever and pallor. Hepatosplenomegaly was found in 20 (87%) patients and cervical lymphadenopathy was found in 10 (43%) patients. Anemia was present in 14 (56%) patients at the time of presentation.

All patients had variable number of blasts in the peripheral blood film and in bone marrow aspirate. Morphologically, in 8 (35%) cases the blasts had lymphoblastic morphology, in 11(48%) cases the blasts had myeloblastic morphology and 4(17%) cases had a dual population of small and large blasts difficult to classify by morphology. SBB positivity was seen 11 (48%), along with 3 weak positive cases, and 12 (52%) patients were SBB negative. Trephine biopsy sections showed diffuse and interstitial infiltration by blast cells in all the cases.

Focal and Diffuse fibrosis was seen in 20 (87%) cases, ranging from grade I to III by reticulin staining and unremarkable in 3 (13%) cases. The main clinical and laboratory features are summarized in table-II.

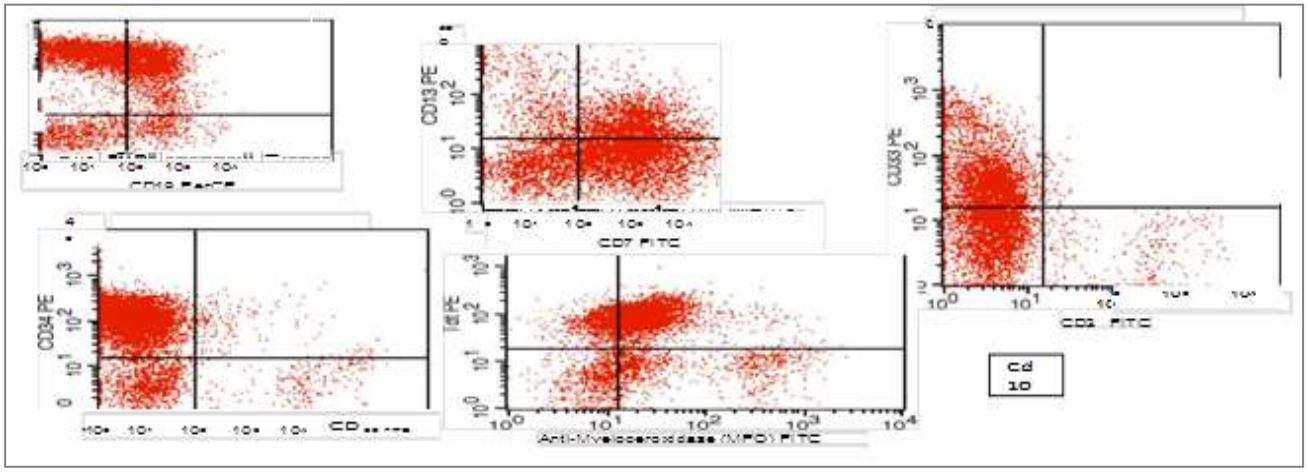

Figure-1: My/B-ALL Case 3: The blats are positive for B-lymphoid markers: CD 19, CD 10 and CD20, myeloid markers: CD33, CD13, MPO and for CD34.

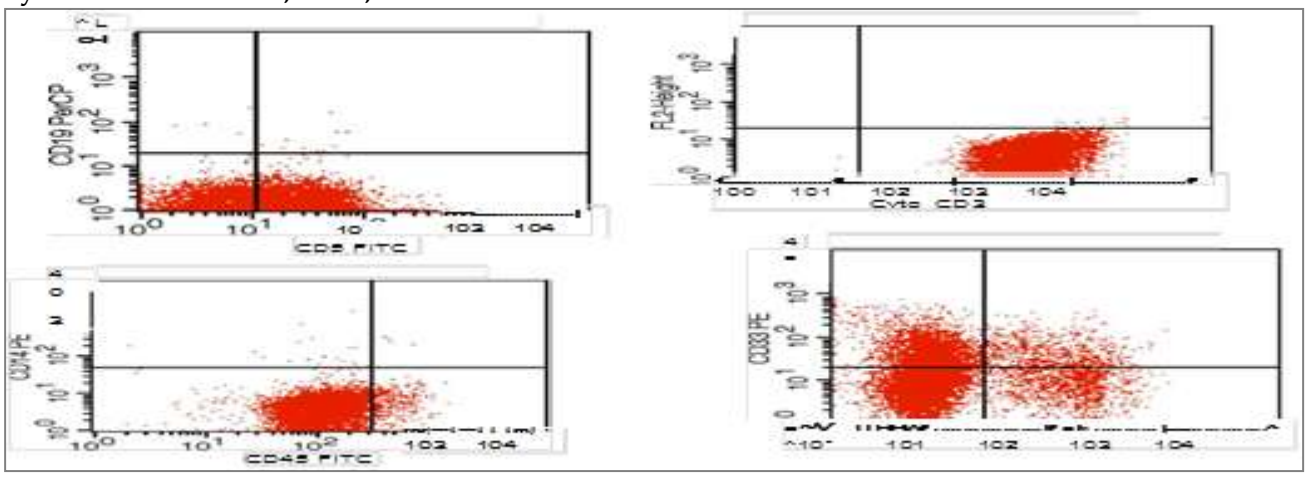

Figure-2: My/T-All Case 10: The blasts are positive for T lymphoid marker Cyto CD3 and Myeloid marker CD33 and negative for CD19.

There were no significant differences between My/B-ALL and My/T-ALL immunophenotypes for age <30 and >30 years (*p*-value: 0.09) and gender (*p*-value: 0.228), shown in table-III. However one patient with the B/T-ALL immunophenotype was child (age 4 years). All 5 patients with a My/T-ALL were males and all females were of My/B-ALL immunophenotype.

DISCUSSION

Mixed-phenotype acute leukemia (MPAL) is a rare entity that comprises 0.5–4% of all acute leukemias[17], in our laboratory 3.4% of acute leukemias were identified as MPAL. Prevalence of MPAL according to study by Xu et al, Owaldah et al, Mi et al is 4.6%, 3.4% and 3.4% respectively, which is comparable to our study. However in a study by Legrand et al prevalence of MPAL is 8% which is higher as compare to our study. There were heterogeneous population of blasts in our patients similar to other studies[18].

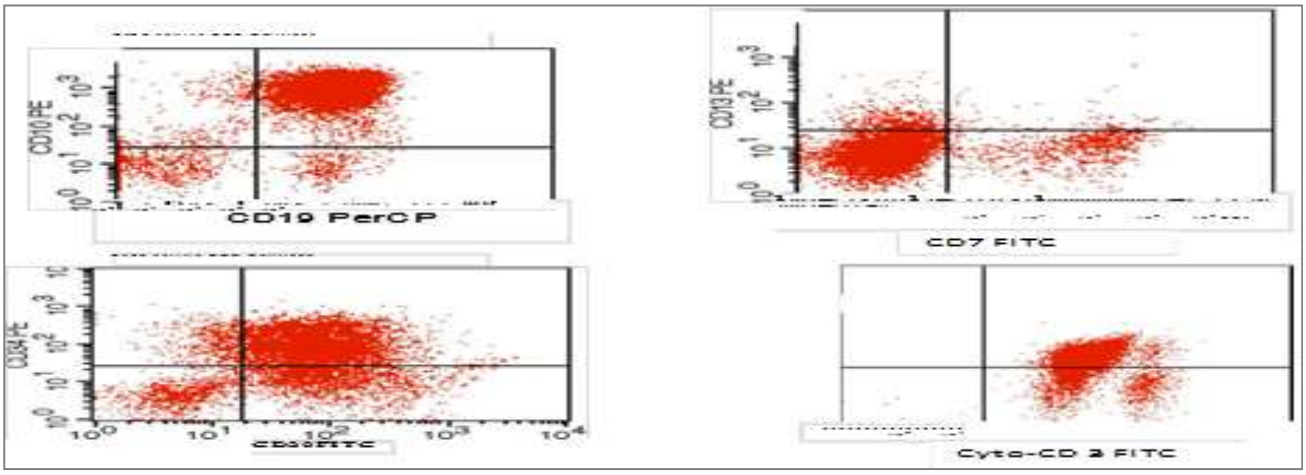

Figure-3: B/T-All Case 5: The blasts are positive for B-lymphoid markers: CD19, CD10 and CD20, T lymphoid markers: Cyto CD3.

Our study showed that out of total 680 patients of acute leukaemia 23(3.4%) were MPAL. Among MPAL, 16 were males (70%) and 07 were females (30%). Male to female ratio was 2.3:1. The male predominance in our study population is comparable with Legrand et al (France) (2.3:1)[18], Mi et al (China) (2.2:1)[19] and Aribi et al (USA) (2:1)[20]. In Xu et al (China) (1.3:1), Owaidah et al (Kingdom of Saudi Arabia) (1.6:1)[21], Lee et al (Korea) (1.4:1)[22], Weir et al (USA)(1.7:1)[23] and other international studies male predominance is less significant. In his study (Xu et al), out of total 21 patients 12 (57%) were males and 9 (43%) were females. Male to female ratio was 1.3:1. However Matutes et al (UK), in their study has shown that out of total 100 patients 62(62%) were males and 38 (38%) were females[7]. Male to female ratio was 1.6:1.

The age of the patients with MPAL in our study ranged from 2 to 65 years with median age was 18 years. Xu et al (China) in their study has shown that age of patients with MPAL ranged from 15 to 73 years, with a median age of 41 years. In another study conducted in UK by Matutes et al (Blood 2011), twenty-eight (28%) patients were children, 2 of whom were infants (<1-year old) and 68 (68%) were adults (<15-years old) i-e; mostly the patients were adults but in contrast, in our study most of the patients were children <10 years old. In our population majority of MPAL patients are seen at younger age group, probably because Pakistan generally harbors younger population or there might be some biological variation of the disease in the sub-continent.

Median WBC count in the MPAL in our study was 9.87 x 10^9/L. Minimum WBC was 2.29 x 10^9/L and maximum was 471 x 10^9/L. Xu et al showed median WBC of 19.4 x 10^9/L. Minimum WBC was 0.7 x 10^9/L and maximum was 450 x

10^9/L, which is comparable to our study. A study done by Lee et al[22] showed that median WBC of 7.4 x 10^9/L. Minimum WBC was 0.5 x 10^9/L and maximum was 520 x 10^9/L, which is also comparable to our study.

The most frequent immunophenotype was B-lymphoid + myeloid followed by T-lymphoid + myeloid immunophenotype. In a study from China (Xu et al) author compared, 9 other published studies containing from 19 to 63 cases of MPAL. They concluded that the most frequent type of MPAL involves the co-expression of markers of myeloid and B-lineage, between 47 and 72% (My/B-ALL). MPAL with myeloid and T-lineage markers are next in frequency (24%) (My/T-ALL), while both B/T and triple myeloid/B/T BAL are rare.

In Matutes et al, immunophenotyping showed that 59 (59%) cases had a B-lymphoid + myeloid immunophenotype (My/B-ALL), 35 (35%) had T-lymphoid + myeloid immunophenotype (My/T-ALL), 4 (4%) had B + T-lymphoid immunophenotype (B/T-ALL), and in the remaining 2 cases (2%) there was evidence of trilineage concomitant expression (myeloid, B, and T lymphoid) (My/B/T-ALL).

In our study My/B-ALL immunophenotype was 13(57%), My/T-ALL was 5(22%) and B/T-ALL was only 1(4%). Four (17%) cases were bilineage (My/B-ALL). No case of trilineage expression (myeloid, B, T lymphoid) is reported in our study. The results of our study are comparable to international studies. The origin of blasts cells in MPAL is unknown, most probably this leukemia arises in a very early hemopoietic progenitor cells with potential to undergo either lymphoid or myeloid differentiation or rarely B- and T-cell differentiation[7]. Most cases of MPAL show expression of early hemopoietic stem cell marker CD34[17], MPAL without CD34 are rare. Beata et al[2] showed that out of total 8 cases of MPAL, CD34 was positive in 7 (88%) cases and 1 (12%) was negative for CD34[3]. In a study by Xu et al 17 (81%) patients were positive for CD34 and 4 (19%) patients were negative for CD34. In our study the CD34 was positive in 21 (91%) cases and negative in 2 (9%) cases. Thus CD34 positivity in our study is similar to international studies.

In Xu et al, Tdt was positive in 15 (71%) patients and negative in 6 (29%) patients. In Matutes et al, Tdt was positive in 81 (89%) out of 91 cases and negative in 10 (11%) cases. In our study Tdt was positive in 14 (61%) patients, negative in 6 (26%) patients and not determined in 3 (13%) patients, similar to international studies.

CONCLUSION

My/B-ALL is the most common immunophenotype followed by My/T-ALL. Therefore immunophenotyping is indispensable for diagnosis and for therapy decisions of MPAL.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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