Mixed-Phenotype Acute Leukaemia: Bridging the Gap Between Myeloid and Lymphoid Lineages

Muhammad Zain Arshad[1], Muhammad Hussain[1], Muhammad Omair Riaz[1], Mustajab Alam[1], Muhammad Aftab Hassan[1] and Maymoona Sohail[2]

[1]*Department of Immunology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan*
[2]*Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan*

ABSTRACT

Objective: To characterise mixed-phenotype acute leukaemia (MPAL) using flowcytometric immunophenotyping in a flow cytometric local population of Pakistan.

Study Design: A descriptive, cross-sectional study.

Place and Duration of the Study: Department of Immunology, Armed Forces Institute of Pathology, National University of Medical Sciences, Rawalpindi, Pakistan, from November 2021 to October 2023.

Methodology: A total of 1,115 patients (728 males and 387 females) with peripheral blood, bone marrow, or cerebrospinal fluid samples referred for immunophenotyping due to suspected acute leukaemia were included in the study. Cells were stained with lineage-specific fluorochrome-labelled monoclonal antibodies. Acquisition of cell suspension was done on the BD FACS Canto II multi-parameter (flow cytometer), and analysis was done on the BD FACS Diva software. Qualitative variables (gender, diagnosis, or positivity of CD markers) were expressed as frequency and percentages, and quantitative variables (age) were expressed as mean ± SD. The Chi-square test was used to compare positivity in both male and female patients.

Results: Among 875 patients with acute leukaemia, 11 cases (1.25%) were diagnosed as MPAL, with a mean age 32 ± 28 years. Of the MPAL cases 9 (81.8%) were males, and 2 (18.2%) were females ($p = 0.25$). The most common MPAL subtype was B-myeloid, found in 7 out of 11 cases (63.6%), followed by T-myeloid in 3 cases (27.3%) and B-T MPAL in 1 case (9.1%). The aberrant expression of a third lineage was present in 2 out of 11 cases, (18.2%), while 9 cases (81.8%) were biphenotypic, and 2 cases (18.2%) were bilineage. A fatal outcome occurred in 3 out of 11 cases (27.3%) and the average diagnostic delay was 5.5 weeks.

Conclusion: In a local Pakistani population, B/Myeloid MPAL is the most prevalent immunophenotype, followed by T/Myeloid MPAL, with an average diagnostic delay of about five weeks. It is more common in males and can occur at any age, from infancy to old age.

Key Words: *Acute leukaemia of ambiguous lineage, Bilineage acute leukaemia, Biphenotypic acute leukaemia, Mixed-phenotype acute leukaemia*.

How to cite this article: Arshad MZ, Hussain M, Riaz MO, Alam M, Hassan MA, Sohail M. Mixed-Phenotype Acute Leukaemia: Bridging the Gap Between Myeloid and Lymphoid Lineages. *J Coll Physicians Surg Pak* 2025; **35(09)**:1190-1195.

INTRODUCTION

Acute leukaemia mostly originates in either myeloid or lymphoid cells. Rarely, the same blast population may co-express lineage specific antigens of various lineages (biphenotypic), or separate blast populations may have different immunophenotypes (bilineal). Both are categorised as mixed-phenotype acute leukaemia (MPAL). MPAL is included as a category within acute leukaemia of ambiguous lineage (ALAL) as per 5th edition of the World Health Organization (WHO) classification of haematopoietic and lymphoid tumours. It is commonly further sub-classified as B-myeloid, T-myeloid, B/T lymphoid, or rare B/T myeloid.[1,2]

Correspondence to: Dr. Muhammad Hussain, Department of Immunology, The Armed Forces Institute of Pathology, Rawalpindi, Pakistan
E-mail: capthussainkk@yahoo.com

Received: August 10, 2024; Revised: January 15, 2025; Accepted: February 06, 2025
DOI: https://doi.org/10.29271/jcpsp.2025.09.1190

MPAL accounts for approximately 1-3% of all acute leukaemia cases, with an annual incidence of 0.35 cases per 1,000,000 person-years. Among MPAL, the B-myeloid is the most frequent, accounting for approximately 59%. T-myeloid, B/T, or B/T myeloid subtypes represent 35%, 4%, and 2% of cases, respectively.[3-6] MPAL was first recognised in the WHO classification of haematopoietic and lymphoid tumours in 2001. The two subcategories are recognised under the umbrella of acute leukaemias of ambiguous lineage. The first category is termed bilineal acute leukaemias, defined by the detection of a dual population of blasts, each expressing specific markers from different lineages. The second category is biphenotypic acute leuka-emia, in which a single blast population co-expresses lineage-specific markers of two or more lineages.[5,7] The European Group for the Immunological Characterisation of Acute Leukaemias (EGIL) suggested a scoring system for each antigen (marker) associated with B lymphoid, T lymphoid, and myeloid cell lineages to aid in the diagnosis of MPAL. According to this system, a diagnosis of MPAL is made when an acute leukaemia scores greater than two for antigens from two or more lineages.[8]

Multi-lineage antigen expression in MPAL indicates worse response to chemotherapy compared to either acute myeloid leukaemia (AML) or acute lymphoid leukaemia (ALL), and the five-year survival rate remains low, at around 20%.[9,10] The proposed reasons include chemoresistance of MPAL blasts due to slow replication. Furthermore, these blasts can adapt to therapy by switching phenotypes, and some MPAL cases express high levels of multidrug resistance proteins.[11] Treatment strategies for MPAL remain a matter of debate due to the absence of standardised protocols tailored specifically to this elusive condition. While therapeutic approaches often draw from AML and ALL regimens, there remains a critical need for targeted therapies that address the unique challenges posed by MPAL. Understanding the underlying molecular mechanisms and identifying novel therapeutic targets are paramount to improving patient prognosis.[12]

In the vast realm of haematological malignancies, this rare and complex form of acute leukaemia defies conventional classification, although morphology, with the advancements in diagnostic methodologies, including flow cytometry and genetic profiling, results in earlier interventions and improved outcomes. The available literature provides a limited understanding of the clinical characteristics of MPAL and mutations driving the pathogenesis of this disease. This article aimed to study the demographic, morphological, immunophenotypic, and molecular characteristics of MPAL and its subtypes in the local population, with the goal of raising awareness among clinicians for timely diagnosis and effective management of this medical condition.

METHODOLOGY

This descriptive cross-sectional study was carried out at the Department of Immunology, Armed Forces Institute of Pathology, National University of Medical Sciences, Rawalpindi, Pakistan, from November 2021 to October 2023. It was approved by the Institutional Review Board (IRB/2218). Samples from patients of either gender and age, with acute leukaemia on initial workup, were included. Patients diagnosed with other haematological malignancies were excluded from the study.

Sample size (n) was calculated using the OpenEpi online sample size calculator, based on the following assumptions; a 3.4% prevalence of MPAL among acute leukaemia, a 95% confidence level, and ±5% margin of error. Adequate sample size came out to be 51; however, 875 diagnosed cases of acute leukaemia were included to cater for design effect and better generalisation of results. A non-probability consecutive sampling technique was used.[13,14]

A total of 1,115 patients (728 males and 387 females) with peripheral blood, bone marrow, or cerebrospinal fluid samples referred for immunophenotyping due to suspected acute leukaemia were included in the study. Specimens were collected in EDTA tubes for peripheral blood/bone marrow aspirate or in plain tubes for cerebrospinal fluid (CSF), with a processing time of 12 hours. Monoclonal antibodies used for cell staining were obtained from Becton Dickinson (BD) Biosciences, in San Jose, CA, USA. The primary antibody panel included CD3 (cluster of differentiation), CD5, and CD7 for T lineage cell blasts; CD19 and cCD79a for B lineage blasts; and CD13, CD33, CD117, and myeloperoxidase (MPO) for myeloid lineage blasts. Additional antibodies included CD45, CD10, CD14, CD34, and HLA-DR. The isotype control involved mouse anti-IgG1-FITC or IgG2-PE. When needed, the primary panel extended to a secondary panel, introducing antibodies against cytoplasmic (cyt) CD3, CD4, CD8, CD2, terminal deoxynucleotidyl transferase (TdT), and CD20, CD64, CD41, CD61, CD42a, CD42b, and glycophorin (CD235a). These monoclonal antibodies were labeled with fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP), phycoerythrin-cyanine7 (PE-Cy7), or pacific blue. The cell staining followed a recommended lyse-wash procedure as per the manufacturer's instructions. The stained samples were examined on the BD FACS Canto II flow cytometer using BD FACS Diva software. A minimum of 20,000 cells were selected for analysis using forward scatter/side scatter (FSc/SSc) and CD45/Side scatter gating techniques. Expression of different CD markers was assessed via quadrant application, labeling as positive if over 20% for surface and 10% for cytoplasmic CD marker positivity on leukaemic blasts events exceeded the isotype control fluorescence threshold; otherwise, they were deemed negative. Flow cytometry dot plots were examined independently by two experienced observers. Consensus was achieved through collaborative discussion to resolve discrepancies.

Data were recorded on a pre-designed proforma. The percentages of abnormal cells, with the expression of analysed CD markers, were recorded and processed using SPSS version 23.0. The data were subsequently scrutinised for the frequencies/percentages of MPAL and its immunophenotypic characteristics. Qualitative variables (gender, diagnosis or positivity of CD markers) were expressed as frequencies and percentages, and quantitative variables (age) were expressed as mean ± SD. The Chi-square test was used to compare positivity in male and female patients. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Total of 1,115 patients suspected of acute leukaemia were tested for two years, of which 728 (65.3%) were males and 387 (34.7%) were females, with mean age 28 ± 21 (range 1 week - 87 years). Out of total tested, 875 (78.5%) patients of acute leukaemia were diagnosed, among which 11 of 875 cases (1.25%) were diagnosed as MPAL, with a mean age 32 ± 28 years. Of the MPAL cases 9 (81.8%) were males, and 2 (18.2%) were females ($p = 0.25$). The most common MPAL subtype was B-myeloid MPAL, found in 7 of 11 cases (63.6%), followed by T-myeloid in 3 cases (27.3%) and B-T MPAL in 1 case (9.1%, Figure 1, 2). Aberrant expression of third lineage was present in 2 of 11 MPAL cases (18.2%). The aberrant expression of CD 7 was present in 1 of 7 cases (14.3%) of B-myeloid MPAL and CD33 in 1 case (100%) of B-T MPAL. Out of total 11 MPAL cases, 9 (81.8%) were biphenotypic, and only 2 (18.2%) were bilineage.

Table I: Clinicopathological characteristics of the MPAL patients (n = 11).

Parameters	Results
Age	Children = 4 cases, adolescents = 1 case, adults = 4 cases, old adults = 2 cases
Gender	9 Males and 2 females
Ethnicity	3 Punjabi and 2 Pathan
Family history	Unremarkable
Presentation	Shortness of breath = 3 cases, epistaxis = 2 cases, fever = 6 cases, pneumonia = 1 case, weight loss = 1 case, gum bleed = 2 cases, cervical lymphadenopathy = 1 case, toothache = 1 case, cough = 1 case, pain abdomen = 1 case, loose stool = 1 case
Miscellaneous symptoms	Weight loss = 1 case, mediastinal lymphadenopathy = 3 case, menorrhagia = 1 case, anasarca = 1 case, hepatosplenomegaly = 2 cases
Physical examination	Pallor in all cases, lymphadenopathy in 2 cases, bruises in 2 cases, splenomegaly in 2 cases
Diagnostic Delay	5.5 Weeks
Comorbids	Smoking in 1 case, DM in 2 cases, HTN in 2 cases, IHD in 1 case
Complete blood counts	
TLC	Higher in 5 cases, low in 2 cases, normal in 4 cases
PB film blasts	<50% = 2 cases, ³50% = 9 cases
Haemoglobin	≤10 g/dl = 10 cases, >10g/dl = 1 case
Platelets	≤10 x 10^3/ul = 2 cases, 11-50 x 10^3/ul = 5 cases, 51-100 x 10^3/ul = 3 cases, >100 x 10^3/ul = 1 case
Morphological characteristics	
Blast Percentage	50-75% = In 2 cases, 76-100% = In 9 cases
Microscopy	Heterogeneous population in 9 cases, dual population of blasts in 2 cases
Cellularity	Hypercellular in all cases
Haematopoiesis	Depressed tri-lineage in all
Iron	Present in 1 and increased in 10 cases
Immunophenotyping findings	
Sample	Peripheral blood in 1 case, BM aspirate in 10 cases
Blast population	³50% = 9 cases, <50% = 2 cases
Opinion	B-T MPAL = 1 case, B-myeloid MPAL = 7 cases, T-myeloid MPAL = 3 cases
MPAL type	Biphenotypic = 9 cases, bilineage = 2 cases
Aberrancy	CD33 in one case of B-T MPAL, CD7 in one case of B-myeloid MPAL
EGIL score (average)	
B lineage	3.93
T lineage	4.3
M lineage	3.85
Bone marrow trephine biopsy findings	
Cellularity	Hypercellular in all cases
Architecture	Effaced in all cases
Haematopoiesis	Depressed in all cases
Blasts infiltration	Diffuse sheets in all cases
Reticulin	MF-II in 2 cases, MF-I in 5 cases, MF-0 in 3 cases, focal-I in 1 case
Immunohistochemistry of blasts	
CD3	+ in 4
TdT	+ in 11
CD19, CD79a	+ in 8
CD117	+ in 10
MPO	+ in 10
CD34	+ in 11
Cytochemistry	
Sudan black	+ in 10
ANAE	+ in 5
PAS	+ in 7
ACP	+ in 4
MPO	+ in 10
Cytogenetics	Normal male 46 XY = 9, normal female 46 XX = 2
Gene markers	
ALL and AML panel	Positive in one case of B-Myeloid MPAL including BCR-ABL1 (p210)
Final diagnosis	7 cases of B-Myeloid MPAL, of which one case with aberrant CD7; 3 cases of T-Myeloid MPAL; 1 case of B-T MPAL with aberrant CD33
Outcome	Fatal in 3 cases, treated 2 cases, under treatment 6 cases

The mean EGIL score for the B lymphoid lineage and myeloid lineage in B-myeloid MPAL cases was 3.9 ± 0.42 and 4 ± 1, respectively. For T-myeloid MPAL cases, the mean EGIL score for the T-lymphoid lineage and myeloid lineage was 4 ± 1 and 3.3 ± 0.5, respectively. Out of all MPAL cases, 3 (27.3%) had fatal outcomes within six months. The average diagnostic delay was 5.5 weeks, and the average blast percentage was 69% (56.5-81.5). Detailed medical history, demography, haematological, and immunological findings of all patients are summarised in Table I.

DISCUSSION

ALAL is sub-classified into two categories: one defined by genetic abnormalities and the other based on immunophenotypic characteristics. MPAL cases are immuno-phenotypically and clinically diverse. Immunophenotypic analysis by flow cytometry, immunohistochemistry and cytochemistry is required for precise diagnosis.

It remains unclear whether MPAL should be managed with single or combined chemotherapy for both immunopheno-

types, and whether bone marrow or peripheral blood stem cells should be used for transplantation.[15,16]

A study conducted by Khurshid *et al*. showed that the overall incidence of MPAL was 3.4% among acute leukaemia cases. Among 23 of 680 cases, 16 (70%) were males and 7 (30%) were females. Male-to-female ratio was 2.3:1.

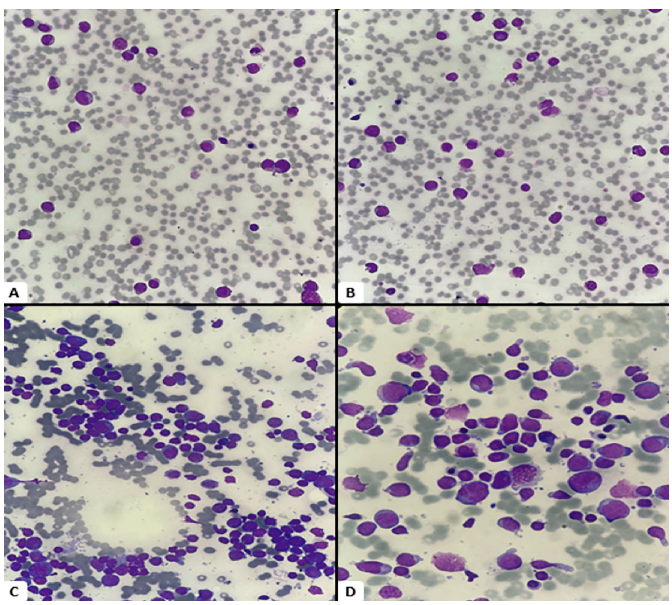

Figure 1: Blasts of B-T MPAL with aberrant expression of CD33 (A, B), and B-Myeloid MPAL with aberrant expression of CD7 (C, D).

Among the total cases, 19 cases (83%) were biphenotypic and 4 cases (17%) were bilineage.[13] These findings were only partially consistent with the present study, in which 11 of 875 cases (1.25%) were of MPAL (p = 0.006). Nine patients (81.8%) were males, and two (18.2%) were females. Out of the total 11 MPAL cases, 9 (81.8%) were biphenotypic, and only 2 (18.2%) were bilineage. The difference in MPAL frequency may be due to the use of different CD marker panels.

Tipu *et al*. in their study revealed that out of the total 151 cases of acute leukaemia, only 10 cases were diagnosed as MPAL (p <0.0001). Among these 6 (60%) were children and 4 (40%) were adults.[17] However, as per the current study, out of 11 cases diagnosed with MPAL, 7 (63.3%) were adults and only 4 (36.7%) were children.

Another study conducted by Jamal *et al*. demonstrated the frequency of MPAL as 13 out of 1,379 cases (0.9%, p = 0.464). Among these, B-myeloid accounted for 6 cases (46.15%), T-myeloid for 4 cases (30.76%), and B/T lymphoid for 3 cases (23.1%). In B/T lymphoid MPAL, aberrant expression of myeloid markers, CD13 (2 out of 3 cases, 66.6%), and CD33 (1 out of 2 cases, 50%), was also observed.[18] These findings were partially coherent with the present study, in which the most common was B-myeloid MPAL found in 7 out of 11 cases (63.6%), followed by T-Myeloid 3 cases (27.3%) and B-T MPAL in 1 case (9.1%). Aberrant expression of CD7 was observed in 1 out of 7 (14.3%) cases of B-myeloid MPAL, while aberrant expression of CD33 was observed in the only (100%) case of B-T MPAL.

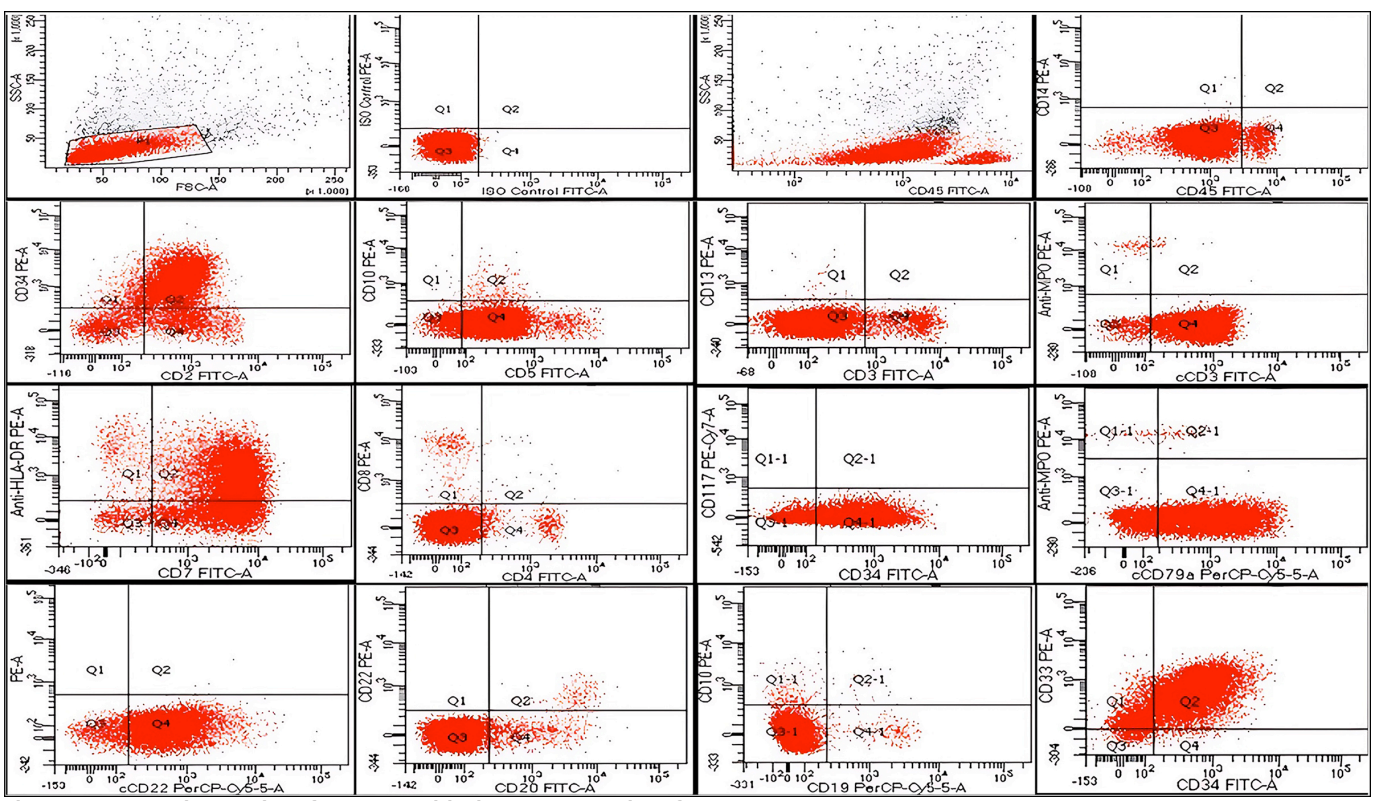

Figure 2: Immunophenotyping of B-T MPAL with aberrant expression of CD33.

Koulmane Laxminarayana *et al*. in their study showed the frequency of MPAL as 9 out of 218 cases (4.1%), of which 8 (88.9%) were males and 1 (11.1%) was female (p = 0.006).[19] These findings were partially consistent with this study, in which among the total 11 MPAL cases, 9 (81.8%) were males and 2 (18.2%) were females.

Olga *et al*. revealed that a total 61 patients were diagnosed with MPAL, including 36 (59%) males and 25 (41%) females. However, the present study revealed that among total 11 MPAL cases, 9 (81.8%) were males and 2 (18.2%) were females (p = 0.190).[20]

Due to its extremely rare occurrence, limited information is available about MPAL. Therefore, further multi-centre studies are required to have a better understanding of this phenotype and its pathogenesis, clinical course, and prognosis. Due to resource constraints, selected common CD markers were used in the present study; however, diverse panels may be used in future studies.

CONCLUSION

B-myeloid MPAL emerges as the most prevalent immunophenotype, followed by T-myeloid MPAL among the Pakistani population, with an average diagnostic delay of about five weeks. Unlike other acute leukaemia, MPAL can occur at any age, from infancy to old age, and it is more common among males.

ETHICAL APPROVAL:
Approval was obtained from the Institutional Review Board of the Armed Forces Institute of Pathology, Rawalpindi, Pakistan (IRB/2218).

PATIENTS' CONSENT:
Informed consent was obtained from all the patients.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
MZA: Concept, design, data collection, and drafting.
MH: Report analysis and drafting.
MOR: Critical revision of the manuscript.
MA: Interpretation of data and intellectual content.
MAH: Data collection and data acquisition
MS: Data analysis and critical revision.
All authors approved the final version of the manuscript to be published.

REFERENCES

1. Mi X, Griffin G, Lee W, Patel S, Ohgami R, Ok CY, *et al*. Genomic and clinical characterization of B/T mixed phenotype acute leukaemia reveals recurrent features and T-ALL like mutations. *Am J Hematology* 2018; **93(11)**:1358-67. doi: 10.1002/ajh.25256.
2. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, *et al*. The 5th edition of the World Health Organisation classification of hematolymphoid tumours: Myeloid and histiocytic/dendritic neoplasms. *Leukemia* 2022; **36(7)**: 1703-19. doi: 10.1038/s41375-022-01613-1.
3. George BS, Yohannan B, Gonzalez A, Rios A. Mixed-phenotype acute leukemia: Clinical diagnosis and therapeutic strategies. *Biomedicines* 2022; **10(8)**:1974. doi: 10.3390/biomedicines10081974.
4. Abdo Y, Gibson GD, Jain SP, Milner CP, Hilal T. Treatment of relapsed B/T-cell mixed phenotype acute leukaemia with blinatumomab. *Cureus* 2023; **15(6)**:e40661. doi: 10.7759/cureus.40661.
5. Qasrawi A, Ramlal R, Munker R, Hildebrandt GC. Prognostic impact of Philadelphia chromosome in mixed phenotype acute leukemia (MPAL): A cancer registry analysis on real-world outcome. *Ame J hematol* 2020; **95(9)**:1015-21. doi: 10.1002/ajh.25873.
6. Porwit A, Bene MC. Acute leukaemias of ambiguous origin. *Am Clinical Pathol* 2015; **144(3)**:361-76. doi: 10.1309/AJCPSTU55DRQEGTE.
7. Awasthi NP, Singh P, Agrawal N. Mixed phenotype acute leukaemia, B/Myeloid with t(9; 22): Diagnostic and therapeutic challenges. *Indian J Hematol Blood Trans* 2019; **35(1)**:179-81. doi: 10.1007/s12288-018-1020-3.
8. Maruffi M, Sposto R, Oberley MJ, Kysh L, Orgel E. Therapy for children and adults with mixed phenotype acute leukemia: A systematic review and meta-analysis. *Leukemia* 2018; **32(7)**:1515-28. doi: 10.1038/s41375-018-0058-4.
9. Batra S, Ross AJ. Pediatric mixed-phenotype acute leukaemia: What's new? *Cancers* 2021; **13(18)**:4658. doi: 10.3390/cancers13184658.
10. McCormack MP. T/myeloid MPAL: Origin and pathogenesis. *Blood* 2022; **139(3)**:313-5. doi: 10.1182/blood.2021014129.
11. Lee HG, Baek HJ, Kim HS, Park SM, Hwang TJ, Kook H. Biphenotypic acute leukemia or acute leukemia of ambiguous lineage in childhood: Clinical characteristics and outcome. *Blood Research* 2019; **54(1)**:63-73. doi: 10.5045/br.2019.54.1.63.
12. Yu J, Jiang PYZ, Sun H, Zhang X, Jiang Z, Li Y, *et al*. Advances in targeted therapy for acute myeloid leukemia. *Biomark Res* 2020; **8(1)**:17. doi: 10.1186/s40364-020-00196-2.
13. Khurshid A, Ahmed Khan S, Altaf C, Saeed Malik H, Mukarram Bashir M, Altaf M. Immunophenotyping pattern in mixed phenotype acute leukaemias: Mixed phenotype acute leukaemias. *Pak Arm Forces Med J* 2018; **67(6)**:883-9.
14. Sullivan KM, Dean A, Soe MM. OpenEpi: A web-based epidemiologic and statistical calculator for public health. *Public health Rep* 2009; **124(3)**:471-4. doi: 10.1177/003335490912400320.
15. Zheng X, Shen H, Zhu M, Shi Y, Wang H, Chen Z, *et al*. Mixed phenotype acute leukemia with PML-RARα positive: A case report and literature review. *Mole Cytogenet* 2021; **14(1)**:10. doi: 10.1186/s13039-021-00530-9.

16. Salama M, Ahmed S, Soliman S, El-Sharkawy N, Salem S, El-Nashar A, *et al*. Characteristics, treatment complexity, and outcome of mixed-phenotype acute leukaemia in children in a low-middle-income country. *Front Oncol* 2022; **12**:941885. doi: 10.3389/fonc.2022.941885.
17. Tipu HN, Muhammad MB, Altaf C, Noman M, Malik HS. Spectrum of acute leukemias and aberrant markers expression based on flowcytometry in a tertiary care center. *Pak Arm Forces Med J* 2018; **68(3)**:450-4.
18. Jamal S, Meraj F, Mansoor N, Parveen S, Shaikh A, Jabbar N. Distribution of subtypes and immunophenotypic characterization of 1379 cases of paediatric acute leukaemia. *Pak J Med Sci* 2021; **37(3)**:805-11. doi: 10.12669/pjms.37.3.3552.
19. Koulmane Laxminarayana SL, Madireddy N, Manohar C, Udupa K. Multiparametric flow cytometry in mixed phenotype acute leukaemia. *Indian J Hematol Blood Trans* 2019; **35(3)**:451-8. doi: 10.1007/s12288-019-01101-0.
20. Weinberg OK, Seetharam M, Ren L, Alizadeh A, Arber DA. Mixed phenotype acute leukemia: A study of 61 cases using World Health Organization and European group for the immunological classification of leukaemias criteria. *Am J Clin Pathol* 2014; **142(6)**:803-8. doi: 10.1309/AJCPPVUPOTUVOIB5.