Frequency and Aberrant CD Marker Profile of Acute Leukaemias

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ABSTRACT

Objective: To determine the frequency of different types of acute leukaemia and their subtypes along with associated aberrant CD markers.

Study Design: Descriptive 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: All samples received for flow cytometric immunophenotyping with suspicion of acute leukaemia were included in the study. Cells were stained with fluorochrome labelled monoclonal antibodies against lineage-specific cluster of differentiation (CD) markers through a lyse-wash procedure. Acquisition and analysis were done using multi-parameter BD FACS Canto II Flow cytometer and BD FACS Diva software, respectively. Data were entered and analysed using SPSS v 23.0.

Results: Over a period of 2 years, a total of 1,115 suspected patients were tested for acute leukaemia. Among them, 728 (65.3%) were males and 387 (34.7%) were females, with mean age 28 ± 21 years, ranging from 1 week to 87 years. Among a total of 875/1115 (78.5%) diagnosed cases of acute leukaemia, AML was the most common leukaemia present in 408/875 (46.6%) patients followed by B-ALL and T-ALL in 384/875 (43.8%) and 70/87 (8%) patients, respectively ($p = 0.5712$). Aberrant CD markers were detected in 109/875 (12.5%) leukaemias ($p = 0.0628$). The most common aberrant CD markers in B-ALL were CD13 and CD33 present in 30/384 (7.8%) cases separately. Among AML and T-ALL most common aberrant CD markers were CD7 and CD33 present in 25/408 (6.13%) and 7/70 (10%) cases, respectively.

Conclusion: Special consideration should be given to the presence of aberrant CD markers when assigning lineages to acute leukaemias. They may be important diagnostic, prognostic, and management tools for institution of immunotherapy.

Key Words: *Aberrant CD markers, Acute leukaemia, CD Markers, Flow cytometry, Immunophenotyping.*

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INTRODUCTION

Acute leukaemia is characterised by the abnormal proliferation of either lymphoid or myeloid progenitors, leading to acute lymphoblastic leukaemia (ALL) and acute myeloid leukaemia (AML) respectively and represents a significant burden in the realm of haematological malignancies.[1] The complex pathogenesis of acute leukaemia involves various genetic and molecular aberrations, among which the identification of specific CD markers has emerged as a crucial step in refining classification, diagnosis, and guiding the management.[2]

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There are two prevalent classification systems used in the categorisation of acute leukaemia. One is the French-American-British (FAB) classification system, which employs morphology and cytochemical staining to identify specific leukaemia types. The other is the World Health Organisation (WHO) system, which scrutinises classification information, cellular morphology, cytochemistry, immunophenotyping, cytogenetics, and clinical features to define and categorise disease entities of clinical significance.[3] WHO established ≥ 20% blasts in peripheral blood or bone marrow as cut-off to label acute leukaemia.[4]

WHO Classification of Haematolymphoid Tumours 5th edition dated 2022 are the most recent guidelines which may be incorporated in classifying acute leukaemias. Lineage attribution and maturational analysis rest on immunophenotyping and/or immunohistochemistry. Flow cytometry has significantly enhanced the precision of diagnoses by identifying cluster of differentiation (CD) markers on the surface or in the cytoplasm of leukaemic cells. The increased availability of a diverse array of multicolour monoclonal antibodies, enhanced characterisation of CD markers based on cell maturation stages, improved gating strategies, and the accessibility of multi-laser instruments have

collectively played a role in advancing the diagnosis, treatment, and monitoring of the disease.[5,6] Multi-parametric immunophenotyping analysis enables the identification of abnormal antigen expression and the assessment of heterogeneity and clonality among malignant cells in cases of acute leukaemia.[7,8]

Thorough laboratory assessment of individuals suspected of acute leukaemia is pivotal and has undergone substantial development through the integration of advanced laboratory methods. In addition to conventional approaches, such as cytomorphology, cytochemistry, immunophenotyping using multi-parameter flow cytometry or immunohistochemical staining, and molecular or cytogenetic studies, the growing significance of advanced molecular diagnostics, including next-generation sequencing (NGS) technology, is increasingly apparent in diagnosing and stratifying the risk of acute leukaemia.[9] Moreover, the emerging significance of these aberrant CD markers in monitoring measurable residual disease (MRD), potential therapeutic targets for immunotherapy, and prognostic significance has sparked a growing interest in elucidating their intricate roles in leukaemic progression and treatment response.[10,11]

Despite the accumulating evidence on the clinical relevance of aberrant CD markers, a comprehensive overview encompassing frequency of acute leukaemia and their subtypes in the local population along with aberrant CD markers remains scarce. An in-depth exploration of the prevalence of these markers in various subtypes of acute leukaemias is pivotal for advancing personalised therapeutic interventions and improving patient outcomes. The present study aims to address this gap by investigating the frequency of aberrant CD markers in various subtypes of acute leukaemias among various age brackets to equip clinicians, pathologists, and researchers with valuable insights that could potentially reshape the way to diagnose, classify, and treat acute leukaemia.

METHODOLOGY

This descriptive study was carried out at the Department of Immunology, Armed Forces Institute of Pathology, National University of Medical Sciences, Rawalpindi, Pakistan, over a period of 2 years, from November 2021 to October 2023 after formal approval by institutional review board (IRB/2216) and obtaining written informed consent from patients.

The inclusion criteria encompassed individuals of all ages and either gender who visited AFIP for the immunophenotyping of acute leukaemia. Exclusions comprised samples with haemolysis and patients with indeterminate test results.

Sample size (n) was calculated using OpenEpi sample size calculator (online) by keeping following assumptions; prevalence of aberrant CD marker expression in acute leukaemia 21.2%, confidence level 95%, margin of error ± 5%, adequate sample size came out to be 257, however, 875 (n) were included to cater design effect and better generalisation of results. Sampling technique was non-probability consecutive sampling.[12,13]

Total 1,115 (728 males and 387 females) peripheral blood, bone marrow, cerebrospinal fluid samples referred for immunophenotyping of acute leukaemia were included in the study. Specimens were collected in EDTA tubes for peripheral blood/bone marrow aspirate or plain tubes for cerebrospinal fluid (CSF) and underwent processing within a 12-hour timeframe. Monoclonal antibodies for cell staining were sourced from Becton-Dickinson (BD) Biosciences in San Jose, CA, USA. The primary antibody panel included CD3, CD5, and CD7 for T-lineage cell blasts; CD19, cCD79a for B lineage blasts; and CD13, CD33, CD117, myeloperoxidase (MPO) for myeloid lineage blasts. Additional antibodies covered CD45, CD10, CD14, CD34, and HLA-DR. Isotype control involved mouse anti-IgG1-FITC/IgG2-PE. When necessary, the primary panel extended to a secondary panel, introducing antibodies against cytoplasmic (cyt) CD3, CD4, CD8, CD2, terminal deoxynucleotidyl transferase (TdT), CD20, CD64, CD41, CD61, CD42a, CD42b, and glycophorin (CD235a). These monoclonal antibodies were labelled with fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP), phycoerythrin-cyanine7 (PE-Cy7), or pacific blue. The cell staining followed a standard lyse-wash procedure 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 chosen 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, labelling as positive if over 20% for surface and 10% for cytoplasmic CD marker positivity on leukaemic blast events exceeded the isotype control threshold; otherwise, they were deemed negative.

Data were recorded on a pre-designed proforma, and the percentages of abnormal cells along with the expression of analysed CD markers were recorded and processed using SPSS version 23.0. The data were subsequently scrutinised for the frequencies and percentages of AML subtypes (ranging from M0 to M7), ALL subtypes, encompassing both B and T lymphocytic ALL, mixed phenotypic acute leukaemia (MPAL), acute undifferentiated leukaemia (AUL), and the expression of aberrant CD markers in each leukaemia and age group. Flow cytometry dot plots were examined independently by two experienced observers who were kept unaware of each other's interpretations. In instances of any discrepancies, consensus was achieved through collaborative discussion. Qualitative variables (gender, diagnosis/positivity of CD markers) were expressed as frequency and percentages and quantitative variables (age) were expressed as mean ± SD. Chi-square test was used to compare positivity and aberrant expressions in male and female patients. The value of $p < 0.05$ was considered statistically significant.

RESULTS

Over a course of 2 years, a total of 1,115 samples (peripheral blood, bone marrow or cerebrospinal fluid) for immunophenotyping of acute leukaemia were received. This included 728 males (65.3%) and 387 females (34.7%) with mean age 28 ± 21 years, ranging from 1 week to 87 years. Out of total sample

received, 322/1115 (28.8%) were peripheral blood, 788/1115 (70.7%) were bone marrow aspirate, and only 5/1115 (0.44%) were cerebrospinal fluid samples. Among total tested for acute leukaemia by immunophenotyping, 875/1115 (78.5%) patients were diagnosed with acute leukaemia (p = 0.5712).

AML was the most commonly diagnosed leukaemia present in 408/875 (46.6%) patients followed by B-ALL and T-ALL found in 384/875 (43.8%) and 70/875 (8%) patients, respectively (Figure 1). However, MPAL and AUL were only diagnosed in 10/875 (1.14%) and 3/875 (0.34%) cases. Further analysis revealed AML-M2 as the most frequent subtype among AML accounting for 154/408 (37.7%) among AML cases and 17.6% of total leukaemia cases. Among B-ALL, common B-ALL accounted for 364/384 (94.8%) of cases and 41.6% of total leukaemia. CD3 + CD4-CD8- Double Negative (DN) T-ALL was the most frequent T-ALL accounting for 37/70 (52.85%) of T-ALL and 4.23% of overall acute leukaemia cases.

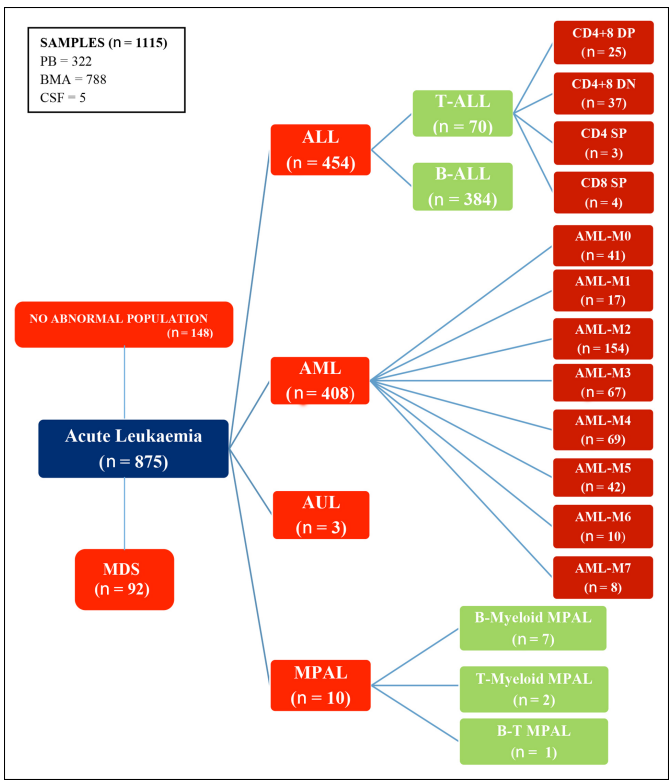
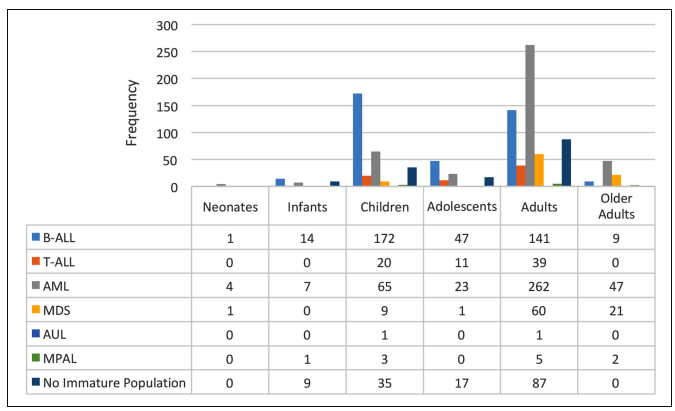


Figure 1: Frequency of acute leukaemias (n = 1,115).

Patients were distributed in age groups as per American Medical Association's age brackets, neonate (till 1 month), infant (1 month to 1 year), children (1 year to 12 years), adolescent (13 years to 17 years), adults (18 to 64 years), older adults (65 years and above) containing 5 (0.6%), 22 (2.5%), 261 (29.8%), 81 (9.25%), 448 (51.2%), and 58 (6.6%) of diagnosed acute leukaemia patients, respectively. Analysis revealed B-ALL was more common in children, whereas AML was relatively common in adults (Figure 2).

Out of 875 cases of acute leukaemia, 109 (12.5%) have expression of aberrant CD markers (p = 0.0628). Single aberrant expression was present in 90/875 (10.3%) and dual aberrant expression was present in 19/875 (2.17%) cases. Aberrant CD markers were expressed in 56/364 (15.4%) cases of common B-ALL and 14/37 (37.8%) cases of CD3+CD4-CD8- DN T-ALL. Among AML-M2 24/154 (15.6%) of cases express aberrant CD markers. Among common B-ALL, CD33 and CD13 were most commonly expressed aberrant CD markers present in 28/364 (7.7%) and 27/364 (7.4%) cases, respectively. However, among pro B-ALL, CD13+33 were most common aberrant expression detected in 2/20 (10%) cases. Among CD3+CD4-CD8-DN T-ALL aberrant expression of CD33 and CD13 was present in 7/37 (18.9%) and 5/37 (13.5%) cases. Among AML, AML-M2 most commonly express aberrant expression of CD7 and CD19 in 18/154 (11.68%) and 6/154 (3.89%) cases, respectively. Overall most common aberrant CD markers in B-ALL were CD13 and CD33 both present in 30/384 (7.8%) cases and CD117 present in 9/384 (2.34%) cases. Among T-ALL, aberrant CD33 and CD13 were present in 7/70 (10%) and 5 (7.14%) cases. In AML, aberrant CD7 and CD19 were present in 25 (6.13%) and 7 (1.7%) cases Table I.



	Neonates	Infants	Children	Adolescents	Adults	Older Adults
B-ALL	1	14	172	47	141	9
T-ALL	0	0	20	11	39	0
AML	4	7	65	23	262	47
MDS	1	0	9	1	60	21
AUL	0	0	1	0	1	0
MPAL	0	1	3	0	5	2
No Immature Population	0	9	35	17	87	0

Figure 2: Frequency of patients in different age groups (n = 1,115).

DISCUSSION

Flow cytometry has emerged as a powerful diagnostic tool in the realm of acute leukaemias. Its integration with traditional diagnostic methods, such as morphological assessment, cytochemistry, and cytogenetic analysis, has revolutionised the ability to decipher the complex landscape of these haematological malignancies. By meticulously characterising the type of blasts or abnormal cells and shedding light on their maturational stage, flow cytometry adds an invaluable layer of insight to leukaemia diagnosis. Moreover, the determination of distinct CD markers and their aberrancies have made substantial contributions to the refinement of leukaemia classification, both in terms of diagnosis and prognosis thus providing a more comprehensive understanding of the disease's heterogeneity.[14,15]

The present study demonstrated that AML is the most commonly diagnosed leukaemia present in 408/875 (46.6%) patients followed by B-ALL and T-ALL found in 384/875 (43.8%) and 70/875 (8%) patients, respectively. Findings by Tipu *et al.* and Ahmad *et al.* were consistent with findings of this study, they determined that most common leukaemia was AML M2.[12,16]

Table I: Subtypes of acute leukaemia and respective aberrant CD markers (n = 875).

Acute Leukaemia	Subtype	Aberrant expression and percentage	No. of aberrant CD markers	CD markers	Percentage of each aberrant CD marker
B-ALL (n = 384)	Pro B-ALL (n = 20)	4 (20%)	Single (2)	CD13 (1)	5%
				CD117 (1)	5%
			Double (2)	CD13 + 33 (2)	10%
	Common B-ALL (n = 364)	56 (15.4%)	Single (44)	CD13 (16)	4.39%
				CD33 (20)	5.5%
				CD117 (6)	1.6%
				CD5 (2)	0.5%
			Double (12)	CD13 + 33 (8)	2.2%
				CD13 + 117 (2)	0.5%
				CD13 + 7 (1)	0.27%
T-ALL (n = 70)	T-ALL (CD4-CD8-DN) (n = 37)	14 (37.8%)	Single (9)	CD13 (3)	8.1%
				CD19 (2)	5.4%
				CD33 (3)	8.1%
				CD117 (1)	2.7%
			Double (5)	CD19 + 33 (1)	2.7%
				CD19 + 117 (1)	2.7%
				CD33 + 117 (1)	2.7%
				CD13 + 33 (2)	5.4%
	T-ALL (CD4+CD8+DP) (n= 25)	-	-	-	-
	T-ALL (CD4+CD8-SP) (n = 3)	1 (33.33%)	Single (1)	cCD79a (1)	33.33%
	T-ALL (CD4-CD8+SP) (n = 4)	-	-	-	-
AML (n = 408)	AML-M0 (n = 41)	2 (4.87%)	Single (2)	CD7 (2)	4.87%
	AML-M1 (n = 17)	1 (5.88%)	Single (1)	CD7 (1)	5.88%
	AML-M2 (n = 154)	24 (15.6%)	Single (24)	CD7 (18)	11.68%
				CD19 (6)	3.89%
	AML-M3 (n = 67)	2 (3%)	Single (2)	CD7 (2)	3%
	AML-M4 (n = 69)	1 (1.44%)	Single (1)	CD19 (1)	1.44%
	AML-M5 (n = 42)	-	-	-	-
	AML-M6 (n = 10)	-	-	-	-
	AML-M7 (n = 8)	2 (25%)	Single (2)	CD7 (2)	25%
AUL (n = 3)	-	-	-	-	-
MPAL (n = 10)	B-Myeloid MPAL (n = 7)	1 (14.3%)	Single (1)	CD7 (1)	14.3%
	T-Myeloid MPAL (n = 2)	-	-	-	-
	T-B MPAL (n = 1)	1 (100%)	Single (1)	CD33 (1)	100%
Total Aberrant Expressions in Acute Leukaemias (n = 875)		Single Aberrant Expression 90 (10.3%) Double Aberrant Expression 19 (2.17%)		109 (12.5% of Acute Leukaemias)	

As per study conducted by Kareem *et al.* in Iraq, among 86 newly diagnosed acute leukaemia cases, the prevalence of aberrant phenotype was 40/86 (46.5%). The frequency of aberrant phenotype was 41% in AML, 48.8% in B-ALL, and 66.6% in T-ALL. Notably, common aberrant CD markers included CD22 and CD2 in AML, CD66c and CD13 in B-ALL, and CD13 and CD33 in T-ALL.[17] These findings were partially coherent with the present findings (p = 0.0628).

Khurram *et al.* studied 100 patients of acute leukaemia, of which 73% cases were of lymphoid and 27% cases were of myeloid lineage. Overall 26% cases showed expression of aberrant CD markers.[18] However, in the present study, 109/875 (12.5%) of acute leukaemia have expression of aberrant CD markers. This difference may be due to more extensive panels used and the limited sample size in another study.

Raza *et al.* in Lahore (Pakistan)-based study showed that out of 120 newly diagnosed patients with AML, the CD7 antigen was aberrantly expressed in 36 (30%) cases, with AML-M2 as most common subtype.[19] These findings were coherent with the present study in which AML-M2 accounts for 154/408 (37.7%) of AML cases. Among AML 25/408 (6.13%) cases expressed aberrant expression of CD7.

Samra *et al.* in their study determined that aberrant phenotypes were observed in 58.3% of AML (21 cases), 59.2% of B-ALL (36 cases), and 66.7% of T-ALL (6 cases). The most prevalent aberrant CD marker in AML was CD7 (33%) of cases, while CD117 was the most frequent aberrant marker in ALL (54% cases).[20] These findings were partially consistent with the present study in which CD7 was present in 25/408 (6.13%) cases of AML and CD117 was present in 12/354 (3.4%) of ALL.

In a study by Gupta *et al.* involving 38 T-ALL cases (27 males, 11 females), 55.3% (21/38) exhibited aberrant expression of one or more B cell antigens, while 39.5% (15/38) showed aberrant expression of one or more myeloid antigens. The predominant aberrant B cell antigen was CD79a, observed in 31.6% of cases (12/38), followed by CD10 in 28.9% (11/38), and CD19 in 2.6% (1/38). The most common aberrant myeloid antigen expressed was CD33 seen in 23.7% (9/38) followed by CD117 in 15.8% (6/38) and CD13 in 10.5% (4/38) cases.[21] As per present study aberrant expression of CD33, CD13, CD19, 117, and cCD79a were present in 7/70 (10%), 5/70 (7.14%), 4/70 (5.71%), 3/70 (4.28%), and 1/70 (1.42%) cases of T-ALL, respectively.

This study highlights the invaluable role of flow cytometry in enhancing the diagnosis and classification of acute leukaemias, emphasising the significance of aberrant CD markers.

The prevalence and variation in leukaemia subtypes and aberrant marker expression underscore the complexity of these malignancies and the need for tailored diagnostic and therapeutic approaches. The differences in findings among various studies can be attributed to the diverse antibody panels utilised, highlighting the importance of harmonising methodologies and sharing insights to advance understanding of these rare and complex diseases. Keeping in view resources, selected CD markers were used in the present study. Further studies need to be conducted to observe the relationship between aberrant expressions, prognosis, relapse, treatment response, cytogenetics, and genetic abnormalities in acute leukaemia.

CONCLUSION

AML, particularly M2 subtype, ranks as the most prevalent leukaemia, succeeded by B-ALL and T-ALL, respectively. In B-ALL, CD13, and CD33 are frequently aberrant, while in AML and T-ALL, CD7 and CD33 are common, respectively. This underscores the importance of considering these facts during multi-parameter flow cytometric immunophenotyping, to ensure accurate lineage assignment and appropriate panel extension. These aberrant CD markers can be targets of potential immunotherapy.

ETHICAL APPROVAL:

This study was approved by the institutional ethical review board (IERB) of the Armed Forces Institute of Pathology (AFIP) and an ethical approval letter (IRB/2216) dated 04 Aug 2021 was issued to the authors.

PATIENTS' CONSENT:

Informed and written consents were obtained from the patients to publish the data.

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

MZA: Conception, data acquisition, analysis, and drafting.
MAH: Acquisition and analysis of data.
MB, MH, MA, MOR: Critical revision.
All authors approved the final version of the manuscript for publication.

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