ScientificScholar®
Knowledge is power
Publisher of Scientific Journals

www.jhas-bhs.com

Journal of Hematology and Allied Sciences

Original Article

Aberrant antigenic expression in acute leukemia: Study from a tertiary care center in Southern India

Subhajit Hajra[1], Sathya Panduragan[2], Prabhu Manivannan[2], Rakhee Kar[2], Debdatta Basu[2]

[1]Department of Pathology and Laboratory Medicine, AIIMS, Rishikesh, Uttarakhand, India, [2]Department of Pathology, JIPMER, Gorimedu, Puducherry, India.

***Corresponding author:**
Subhajit Hajra,
Department of Pathology and Laboratory Medicine, AIIMS, Rishikesh, Uttarakhand, India.

subhajithajra@gmail.com

Received : 25 October 2021
Accepted : 19 May 2022
Published : 25 July 2022

DOI
10.25259/JHAS_26_2021

Quick Response Code:

ABSTRACT

Objectives: Aberrant antigenic expression in acute leukaemia is an expression of antigens which is not normally associated with acute leukaemia of that specific lineage and does not fulfil the criteria for diagnosis of Mixed phenotype acute leukaemia (MPAL). This aberrant immunophenotype can be used for the assessment of measurable residual disease (MRD) and sometimes may predict certain genetic events.

Material and Methods: This was a cross-sectional study conducted in a tertiary care centre in Southern India over two years to assess the type and frequency of aberrant antigen expression in acute leukaemia by multiparameter flow cytometry. All routine cases of acute leukaemia confirmed by flow cytometric immunophenotyping were enrolled.

Results: Among 244 acute leukemia cases, 84 (34%) B-ALL, 28 (11%) T-ALL, 124 (51%) AML and 8 (3%) MPAL were found. Aberrant antigenic expression was seen in 17 (20%) B-ALL, 5 (18%) T-ALL, 33 (27%) AML and 1(12.5%) MPAL. Most common aberrancy in AML was CD7(70.3%) followed by CD19 (13.5%) and CD79a (10.8%), in B-ALL was CD33(52.6%) followed by CD13 (10.5%) and CRLF2 (10.5%) and in T-ALL were CD33 and CD 79a (40% each).

Conclusion: B-ALL showed a greater heterogeneity in aberrant antigens compared to AML and T-ALL. The future implication of this study is that aberrant markers can help in monitoring measurable residual disease Some aberrant phenotypes may indicate the presence of a genetic event or may be of prognostic significance. Some aberrant antigens may also be used as therapeutic targets.

Keywords: Aberrant antigen, Acute leukaemia, Aberrant CD7, Mixed phenotype acute leukaemia (MPAL).

INTRODUCTION

Aberrant expression of antigen is an abnormal expression of antigens by blasts that are not expressed by a particular lineage (Myeloid, B-lymphoid, and T-lymphoid) and do not fulfill the WHO criteria for mixed phenotype acute leukemia (MPAL). This aberrant leukemia-associated immunophenotype (LAIP) can be used to monitor measurable residual disease (MRD).

Aberrant antigen in certain leukemias may indicate the presence of a genetic event or may affect prognosis. Acute promyelocytic leukemia (APL) with PML-RARA commonly expresses CD2 (classically in microgranular variant). Acute lymphoblastic leukemia (ALL) with KMT2A rearrangement showing aberrancy of CD9 and CD15. Ph+ALL shows aberrancy of myeloid-associated markers such as CD13 and CD33. CD9 over-expression is seen in ALL with t(1:19).[1]

Acute myeloid leukemia (AML) having CD123 has a worse prognosis.[2] AML with CD56 aberrancy also carries a poor prognosis, especially in AML (8:21) which usually is associated with a good prognosis.[3]

Sometimes there may be difficult to differentiate between leukemia with aberrant antigen expression or an MPAL. To avoid misdiagnosis, strict the World Health Organization (WHO) criteria should be followed. B-lineage markers (cCD79a, CD19, and PAX5) are commonly expressed in AML with t(8;21). Hence, it may be mistakenly diagnosed as B-myeloid MPAL, if the WHO criteria are not strictly followed. B-myeloid MPAL will express CD19 at a stronger level, and at least, some of the blasts will have positivity similar to normal B-cell, whereas in AML with t(8;21), the expression is dimmer. CD3 should only be considered positive when, at least, some fraction of the blasts show expression similar to mature T-lymphocytes, when a diagnosis of T-myeloid MPAL is considered.[4] Similarly, the expression of MPO also should be compared to normal granulocytes, especially in cases with blasts showing having a typical B-lymphoblastic immunophenotype. A small percentage (<10%) of CD19 positive B-lymphoblast may express MPO and should not be diagnosed as B-Myeloid MPAL.[4,5] There is a difference of opinion regarding ab CD7,[6] whether CD7 is truly a T-cell marker or not as it is expressed in some early precursors of non-T-lineage, especially early myeloid stem cells.[7]

This study was done to assess the type and frequency of aberrant antigen expression in acute leukemia.

MATERIAL AND METHODS

This was a cross-sectional study conducted in the department of pathology (Hematology section) of a tertiary care center in Southern India over 2 years (July 2018–July 2020). All routine cases of acute leukemia diagnosed based on morphology and confirmed by flow cytometric immunophenotyping were enrolled. Bone marrow aspirate and in a few cases peripheral blood sample in EDTA anticoagulant were used for flow cytometry (Navios-3 lasers, 12 parameters, Beckman Coulter Life Sciences, Indianapolis, IN, USA). Sample processing and instrument setup were done according to adapted EuroFlow guidelines.[8] The antibody panel used is given in [Table 1]. The acquisition was done for a minimum of 100,000 events per tube and cells of interest were selected using CD45/SSC gating strategy and analyzed by Kaluza software. An immunophenotypic marker was considered positive if, at least, 10% of cells showed surface and intracytoplasmic markers expression. CD56 and CD9 were not routinely used in our AML panel but used only in cases of suspected APL cases. All data have been presented using summary statistics.

Table 1: A panel of antibodies used in flow cytometry.

PANELS	FITC	PE	ECD	PC5.5	PC7	APC	APC700	APC750	PB	KO
OT TUBE	nTdT	cMPO	CD19	cCD79a	CD34	cCD3	CD45	CD7	sCD3	HLA-DR
MM TUBE	CD13	CD117	CD14	CD33	-	CD11c	CD45	CD64	CD11b	CD15
TUBE B1	CD81	-	CD123	CD19	CD34	CD10	CD20	CD38	CD9	CD45
TUBE B2	CD58	CD73	CD19	CD86	CD34	CD10	CD20	CD38	CD44	CD45
TUBE B3	CD13	CRLF2	CD19	CD33	CD34	CD11c	CD45	-	CD11b	CD15
TUBE T1	CD4	CD1a	CD8	CD5	CD34	CD3	CD56	CD7	CD2	CD45
TUBE T2	CD13	CD117		CD33		CD10	CD45			

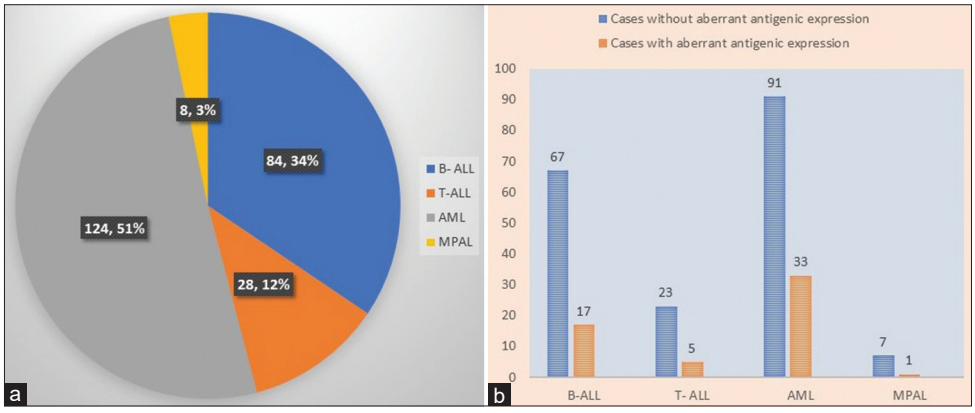

Figure 1: Distribution of acute leukemia cases during study (a). Cases with or without aberrant antigenic expression (b). B-ALL precursor B-cell acute lymphoblastic leukemia, T-ALL precursor T-cell acute lymphoblastic leukemia, AML acute myeloid leukemia, and MPAL mixed phenotype acute leukemia.

RESULTS

Among 244 cases of acute leukemia, 84 (34%) were B-ALL, 28 (11%) were T-ALL, 124 (51%) were AML, and 8 were (3%) MPAL. Aberrant antigenic expression was seen in 17 (20%) B-ALL, 5 (18%) T-ALL, 33 (27%) AML, and 1(12.5%) MPAL cases [Figure 1a and b]. The overall frequency of aberrancy was 23% (56/244). Cases meeting the criteria for early T-cell precursor ALL are not included since aberrant expression of stem cells and myeloid antigen is considered diagnostic criteria for such cases. Expression of TdT in AML was not considered aberrant. A few cases had more than one aberrant antigen expression (CD13/33 and CD11c/CRLF2 each in one B-ALL, CD19/CD79a (weak), and CD7/CD19/CD79a (weak) each in one AML, and CD9/CD56 in one APL).

The aberrant markers are seen and their frequency is summarized in [Table 2]. The aberrant markers in B-ALLs were CD33, CD13, CD117, CD5, CD7, CRLF2, CD11c, and CD15, among which CD33 was the most common. T-ALL had the aberrant expression of CD13, CD117, and CD79a. The aberrant markers in AML were CD7, CD19, and CD79a with the most common being CD7. One case of cytogenetically confirmed that APL showed aberrant expression of CD56 and CD9. However, CD56 is not routinely included in our AML panel. Hence, other AMLs which may have had aberrancy for CD56 could not be determined. Among eight MPAL cases,

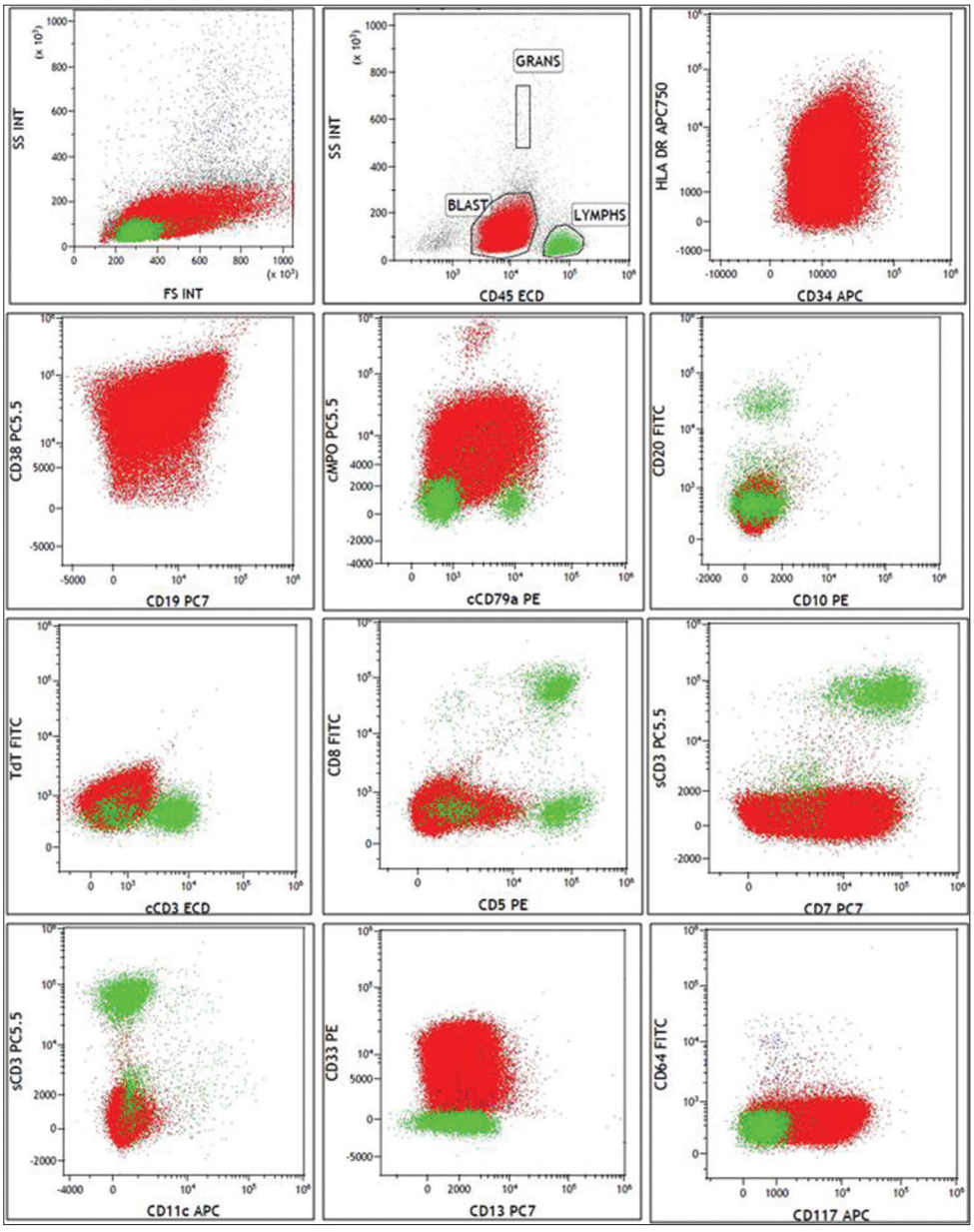

Figure 2: A case of mixed phenotype acute leukemia (B-myeloid) with aberrant CD7 expression.

Table 2: Distribution and frequency of different antigenic aberrancies.

Marker	CD33	CD13	CD117	CD19	CD79a	CD5	CD7	CRLF2	CD15	CD11c	CD9	CD56
B-ALL (*n*=19)	10 (52.6%)	2 (10.5%)	1 (5.3%)			1 (5.3%)	1 (5.3%)	2 (10.5%)	1 (5.3%)	1 (5.3%)		
T-ALL (*n*=5)	2 (40%)		1 (20%)		2 (40%)							
AML (*n*=37)				5 (13.5%)	4 (10.8%)		26 (70.3%)				1 (2.7%)	1 (2.7%)
MPAL (*n*=1)							1 (100%)					

one case of B-myeloid phenotype expressed aberrant CD7 [Figure 2].

DISCUSSION

Aberrant antigenic expression in acute leukemia shows the plasticity of leukemic clones; in some cases, leukemic blasts expressing more than one lineage-specific antigen are categorized as MPAL according to the WHO criteria and treated differently. Aberrant antigens detected by a larger panel of antibodies can help in predicting the genotype of leukemia as most of the aberrant markers associated with specific genotypes of acute leukemia are increasingly being defined.

In our study, aberrant antigenic expression was noted in 27% of AMLs, among which the majority (70.3%) expressed CD7. Excluding CD7, B-lineage markers CD19 (13.5%), and CD79a (10.8%) were other aberrant markers. However, a smaller number of markers were used in our acute leukemia panel for myeloid lineage. We used an extensive panel for lymphoid markers and 20% of B-ALL and 18% of T-ALL cases showed aberrancy with CD33 (52.6% and 40%, respectively) being the most common aberrant marker.

A study done by Drexler *et al*. showed that only 10% of AMLs showed aberrancies of CD1, CD2, CD3, CD5, CD8, CD10, CD19, CD20, CD21, and CD22. They found CD4 and CD7 expression in 24% and 15% of AML cases, and their expression correlated with AML with monocytic differentiation, although CD4 is expressed in monocytes and CD7 can be expressed in some early myeloblasts.[9] In a study by Momani *et al*. on 368 acute leukemias, 23% AML and 29% of ALL showed aberrant antigen expression with the most common aberrancy being CD7 and CD33, respectively, similar to our study. On follow-up, 70% of ALL and 53% of AML which were not in remission had aberrant phenotypes.[10]

CONCLUSION

This study shows that aberrant antigenic expression is common in acute leukemias (23%). AML (27%) more commonly expressed aberrant antigen compared to B-ALL (20%) and T-ALL (18%). The most common aberrancy in AML was CD7 followed by CD19 and CD79a, in B-ALL was CD33 followed by CD13 and CRLF2, and in T-ALL was CD33 and CD 79a. B-ALL showed more heterogeneity in antigenic aberrancies compared to AML and T-ALL.

The expression of certain aberrancy can help to predict possible genetic abnormality. AML with t(8;21) is known to show aberrancy of pan B-cell markers, commonly CD19 and PAX5, and on rare occasions cCD79a. Therefore, adherence to strict WHO criteria is needed to prevent these cases from being misdiagnosed as B-myeloid MPAL.[11] CD2 aberrancy is seen in AML with Inv(16) and acute promyelocytic leukemia.[12]

Expression of myeloid-associated markers (CD13 and CD33) and CD25 is commonly observed with B-ALL with t(9;22).[13] CD15 expression is noted in MLL-rearranged B-ALL.[13]

The limitation of the study was that the correlation of aberrant immunophenotypic markers with molecular/cytogenetic subtypes of acute leukemia has not been studied. The future implication of this study is that aberrant markers such as Leukemia associated immunophenotype (LAIP) can help in disease monitoring and detecting Measurable residual disease (MRD), since, nowadays, it is being consistently used as a part of risk stratification and determining treatment intensity. Some of the aberrant antigens can be therapeutic targets as more and more monoclonal antibodies are being developed and help in disease clearance.

Ethical standards

This study is based on data compilation and analysis of routine diagnostic reported patient data. No direct patient interaction or additional sampling was needed.

Declaration of patient consent

Patient's consent not required as patients identity is not disclosed or compromised.

LIST OF ALL ABBREVIATIONS

APL: Acute promyelocytic leukemia
MPAL: Mixed phenotypic acute leukemia

ALL: Acute lymphoblastic leukemia
AML- Acute myeloid leukemia
WHO: World Health Organization
MPO: Myeloperoxidase
SSC: Side scatter
TdT: Terminal deoxynucleotidyl transferase
CD: Cluster differentiation
LAIP: Leukemia-Associated Immunophenotype
MRD: Measurable Residual Disease

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Hrusák O, Porwit-MacDonald A. Antigen expression patterns reflecting genotype of acute leukemias. Leukemia 2002;16:1233-58.
2. Bras AE, de Haas V, van Stigt A, Jongen-Lavrencic M, Beverloo HB, Te Marvelde JG, *et al*. CD123 expression levels in 846 acute leukemia patients based on standardized immunophenotyping. Cytometry B Clin Cytom 2019;96:134-42.
3. Iriyama N, Hatta Y, Takeuchi J, Ogawa Y, Ohtake S, Sakura T, *et al*. CD56 expression is an independent prognostic factor for relapse in acute myeloid leukemia with t(8;21). Leuk Res 2013;37:1021-6.
4. Borowitz MJ, Béné MC, Harris NL, Porwit A, Matutes E, Arber DA. Acute leukemias of ambiguous lineage. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, *et al*., editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: IARC Lyon; 2017. p. 180-2.
5. Arber DA, Snyder DS, Fine M, Dagis A, Niland J, Slovak ML. Myeloperoxidase immunoreactivity in adult acute lymphoblastic leukemia. Am J Clin Pathol 2001;116:25-33.
6. Mahmoud MS, Abd Elhafeez HA, Abd Elmouez SM. Verification of aberrant expression of CD7 in acute myeloid leukemia. J Curr Med Res Pract 2020;5:133-40.
7. Kriegsmann K, Löffler H, Eckstein V, Schulz R, Kräker S, Braun U, *et al*. CD7 is expressed on a subset of normal CD34-positive myeloid precursors. Eur J Haematol 2018;101:318-25.
8. Van Dongen JJ, Lhermitte L, Böttcher S, Almeida J, van der Velden VH, Flores-Montero J, *et al*., EuroFlow Consortium (EU-FP6, LSHB-CT-2006-018708). EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia 2012;26:1908-75.
9. Drexler HG, Thiel E, Ludwig WD. Acute myeloid leukemias expressing lymphoid-associated antigens: Diagnostic incidence and prognostic significance. Leukemia 1993;7:489-98.
10. Momani A, Abbasi A, Alsokhni H, Habahbeh L, KhasawnehR, Kamal N. Aberrant antigen expression in patients with acute leukemias; experience of King Hussein Medical Center in Jordan. J R Med Serv 2016;23:59-67.
11. Hurwitz CA, Raimondi SC, Head D, Krance R, Mirro J Jr., Kalwinsky DK, *et al*. Distinctive immunophenotypic features of t(8;21)(q22;q22) acute myeloblastic leukemia in children. Blood 1992;80:3182-8.
12. Exner M, Thalhammer R, Kapiotis S, Mitterbauer G, Knöbl P, Haas OA, *et al*. The "typical" immunophenotype of acute promyelocytic leukemia (APL-M3): Does it prove true for the M3-variant? Cytometry 2000;42:106-9.
13. Paietta E, Racevskis J, Neuberg D, Rowe JM, Goldstone AH, Wiernik PH. Expression of CD25 (interleukin-2 receptor alpha chain) in adult acute lymphoblastic leukemia predicts for the presence of BCR/ABL fusion transcripts: Results of a preliminary laboratory analysis of ECOG/MRC Intergroup Study E2993. Eastern Cooperative Oncology Group/Medical Research Council. Leukemia 1997;11:1887-90.

How to cite this article: Hajra S, Panduragan S, Manivannan P, Kar R, Basu D. Aberrant antigenic expression in acute leukemia: Study from a tertiary care center in Southern India. J Hematol Allied Sci 2022;2:10-4.