Polish Annals of Medicine

Journal homepage: https://www.paom.pl

Research paper

Immunophenotypic aberrancy in acute leukemia – a retrospective single institute study

Ehsan Asghari[1], *Seyedeh Niayesh Radfar*[1], *Pardis Nematollahi*[2]

[1] *Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*
[2] *Pathology Department, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

ARTICLE INFO

Article history
Received 4 March 2020
Accepted 16 October 2020
Available online 13 May 2021

Keywords
Acute myeloid leukemia
Acute lymphoblastic leukemia
Aberrant antigen expression
Fow cytometry

Doi
https://doi.org/10.29089/2020.20.00155

ABSTRACT

Introduction: In 2016, WHO gave a comprehensive classification system that included morphology, immunophenotyping, molecular and genetic findings. Flow cytometric immunophenotyping is the backbone of WHO classification, helping to make a more accurate and faster diagnosis.

Aim: This study has been conducted to find immunophenotyping aberrancy of acute leukemia (AL) and its association with gender and age group.

Material and methods: This is a descriptive retrospective study of 1012 AL patients diagnosed between January 2011 and January 2019, including all new patients of all ages and both sexes, who had available immunophenotyping data.

Results and discussion: The most common aberrant antigen on acute myeloid leukemia (AML) was CD7, on precursor B lymphoblastic leukemia (B-ALL) was CD33 and on precursor T lymphoblastic leukemia (T-ALL) was CD13. There was no association between sex/age and antigen aberrancy except a significant increase in CD34 loss on pre B acute lymphoblastic leukemia (pre B-ALL) and HLA--DR expression on acute promyelocytic leukemia (APL) in males compared to female patients and an increase of CD19 expression on non-APL-AML and an elevation of CD34 loss on T-ALL in adult compared to pediatric patients.
Not only detection of aberrant expression of CD markers in leukemic cells helps to estimate molecular abnormalities and has a prognostic effect, but also a specification of neoplastic cell markers at the time of diagnosis is essential for monitoring patients after treatment.

Conclusions: Although in this study, no association between sex/age and aberrancy of antigens was detected (except in certain AL subtypes), AL specific immunophenotype is essential for minimal residual disease detection.

Corresponding author: Pardis Nematollahi, Pathology Department, Faculty of Medicine, Isfahan University pf Medical Sciences, HezarJerib St-Isfahan-Iran 81746-73461.
E-mail address: pardis.nematolahy@med.mui.ac.ir.

1. INTRODUCTION

Neoplasms of hematopoietic cells are one of the most frequent malignancies. The United States Cancer Institute reports the incidence as high as 4.3 cases in 100 000 people annually. Acute leukemia (AL) comprises a heterogeneous group of neoplasms characterized by progressive clonal expansion of progenitor cells in the bone marrow. AL divides into acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). ALL is the most frequent neoplasm in children. AML is the most frequent AL in adults.[1] In acute hematopoietic malignancies, cancerous cells are growing fast and autonomously and acquire various clinical, morphological, immunological, and molecular characteristics.[2] Clonal proliferation of neoplastic cells in AML causes tissue infiltration, mostly in the marrow, in which hematopoietic precursors get replaced by tumoral cells. The tissue infiltration process happens less commonly in extramedullary tissue, identified as granulocytic sarcoma.[3] ALL is caused by progressive genetic evolutions in progenitor cells and divides into precursor T lymphoblastic leukemia (T-ALL) and precursor B lymphoblastic leukemia (B-ALL).[4] In recent years, immunophenotyping alongside morphology, cytogenetic, and molecular data have become essential for diagnosis and patient monitoring.[5] Flow cytometry is a valuable tool in the diagnosis of the disease and has a critical role in the understanding of the developmental stage and minimal residual disease. Available multicolor flow cytometry, various fluorochromes, and new analytical strategies increase the efficacy of flow cytometry in minimal residual disease detection.[6] The amount of residual disease detected by multicolor flow cytometry is the most important prognostic factor in AL disease. Most of the neoplastic cells, especially in AML, express aberrant antigens, which are different from the normal equivalent cells. Recognition of these antigens at the presentation of leukemia is essential for the detection of residual neoplastic cells by flow cytometry in the monitoring of the patient after induction therapy or during maintenance therapy.[7] Moreover, some morphologic and molecular subtypes of AL show characteristic antigen aberrancy and distinctive immunophenotype which have prognostic or even therapeutic effects.[8] Understanding of these associations can help save time and money, especially in resource-poor countries. In order to detect AL specific immunophenotyping, leukemic cell analysis should be performed, using a comprehensive panel of monoclonal antibodies by multicolor flow cytometry.[9] Due to the high prevalence of leukemia in some parts of the country[10] and the probability of geographical and environmental effects on phenotype and behavior of leukemia, we decided to evaluate the relative frequency of AL immunophenotyping, including antigen aberrancy and its correlation with age and gender.

2. AIM

This study has been conducted to find immunophenotyping aberrancy of AL and its association with gender and age groups.

3. MATERIAL AND METHODS

Included patients were AL cases with available demographic and immunophenotyping data. Patient's information retrieved from the hematopathology archive of Omid hospital affiliated to Isfahan University of Medical Sciences, Isfahan, Iran, during 8 years (between January 2011 and January 2019). In total, 1012 AL patients were evaluated, including AML, B-ALL, T-ALL, and ambiguous lineage acute leukemia (ALAL).

Immunophenotyping was performed by CyFlow Space (Sysmex, Germany) flow cytometry on the four-color assay by the following backbone panel containing six tubes, using a 20% cut-off for observation of positive and negative antigen expression:

CD34 (FITC)/CD117 (PE)/CD33 (APC)/CD45 (PerCP), CD14 (FITC)/CD64 (PE)/HLA-DR (APC)/CD45 (PerCP), CD10 (FITC)/CD19 (PE)/CD20 (APC)/CD45 (PerCP), CD2 (FITC)/CD7 (PE)/CD5 (APC)/CD45 (PerCP), CD3 (FITC)/CD4 (PE)/CD8 (APC)/CD45 (PerCP), CD13 (FITC)/CD19 (PE)/CD7 (APC)/CD45 (PerCP)

AML divides into acute promyelocytic leukemia (APL) and non-APL-AML. This classification is based on the morphology, immunophenotyping, and molecular cytogenetic results. According to immunophenotyping data, B-ALL has three subtypes: pro B-ALL, common B-ALL, and pre B-ALL. ALAL subclassifies to mixed phenotype AL (MPAL) and acute undifferentiated leukemia (AUL).

Patients include two age groups: (1) up to 15 years, (2) older than 15 years. Frequent aberrant markers include the expression of CD7, CD19 or loss of CD34, HLA-DR in non-APL-AML, expression of CD13, CD33, CD117, or loss of CD34 in all types of ALL, and expression of CD34, HLA-DR in APL. Demographic and immunophenotyping markers filled in the IBM SPSS v. 21 software sheet and analyzed with χ^2 and Fisher's exact and Mann–Whitney U tests to check the relationship between gender and age with aberrant marker expression. The $P < 0.05$ was considered to be statistically significant.

4. RESULTS

We evaluated a total of 1012 AL patients in a period of 8 years. Age range was from 6 months to 92 years. The mean age in AML was 48.9 ± 22.2 years and in ALL was 17.4 ± 20.1 years ($P < 0.001$). Table 1 shows the total number of leukemia cases and their distribution, according to leukemia subtypes, gender, and age groups.

Table 2 presents aberrant markers observed and their distribution and association with gender.

The relationship between gender and aberrant CD markers is not significant (all $P > 0.05$) except loss of CD34 in pre-B-ALL patients ($P < 0.001$) and the expression of HLA-DR in APL patients ($P < 0.03$), which were significantly more frequent in males than in females. Table 3 shows aberrant markers, their distribution, and their association with age. The relationship between age groups and

Table 1. Total number of leukemia cases and their distribution.

Leukemia subtypes	Gender		Age		Total, *n*(%)
	male, *n*(%)	female, *n*(%)	adult, *n*(%)	pediatric, *n*(%)	
Non APL AML	306(30.2)	186(18.3)	446(44)	46(4.5)	492(48.6)
Pro BALL	25(2.5)	25(2.5)	25(2.5)	25(2.5)	50(4.9)
Common B-ALL	112(11)	83(8.2)	58(5.7)	137(13.6)	195(19.3)
Pre B-ALL	78(7.8)	77(7.6)	45(4.5)	110(10.8)	155(15.3)
T-ALL	47(4.6)	18(1.8)	32(3.2)	33(3.3)	65(6.5)
APL	28(2.8)	19(1.9)	44(4.3)	3(0.3)	47(4.6)
AUL	5(0.5)	1(0.1)	6(0.6)	0(0)	6(0.6)
MPAL	1(0.1)	1(0.1)	2(0.2)	0(0)	2 (0.2)
Total	602(59.5)	410(40.5)	658(65)	354(35)	1012(100)

Table 2. Expression of aberrant markers, their distribution and association with gender.

Leukemia	Marker	Marker expression	Male, *n*(%)	Female, *n*(%)	χ^2 Fisher exact test	*P* value
Non APL AML	CD19	expression	17(5.5)	13(6.9)	0.41	0.51
	CD7	expression	62(20.2)	42(22.5)	0.34	0.54
	CD34	non expression	157(51.3)	97(52.1)	0.03	0.85
	HLA-DR	non expression	70(22.8)	43(23.1)	0.004	0.95
Pro B-ALL	CD13	expression	2(8)	1(4)	0.34	0.50
	CD33	expression	6(24)	4(16)	0.50	0.36
	CD117	expression	0(0)	0(0)	—	—
	CD34	non expression	7(28)	10(40)	0.80	0.37
Common B-ALL	CD13	expression	5(4.4)	2(2.4)	0.62	0.42
	CD33	expression	15(13.3)	11(13.2)	0.009	0.92
	CD117	expression	1(0.8)	1(1.2)	0.03	0.68
	CD34	non expression	40(35.7)	34(40.9)	0.37	0.32
Pre B-ALL	CD13	expression	1(1.2)	2(2.5)	0.35	0.52
	CD33	expression	6(7.6)	5(6.4)	0.08	0.77
	CD117	expression	1(1.2)	0(0)	0.99	0.31
	CD34	non expression	38(48.7)	16(20.7)	13.32	<0.001
T-ALL	CD13	expression	2(4.2)	1(5.5)	0.05	0.62
	CD33	expression	1(2.1)	1(5.5)	0.51	0.48
	CD117	expression	2(4.2)	0(0)	0.79	0.37
	CD34	non expression	32(68)	10(55.5)	0.89	0.34
APL	CD34	expression	1(3.5)	2(10.5)	0.91	0.35
	HLA-DR	expression	11(39.2)	2(10.5)	4.67	0.03

aberrant CD markers is not significant (all $P > 0.05$) except in CD19 aberrancy in non-APL-AML patients ($P < 0.001$) and loss of CD34 in T-ALL patients ($P < 0.02$), which were significantly more frequent in adults than in pediatric cases.

CD7 was the most common aberrancy on non-APL-AML and CD33 on ALL. Almost 51% of non-APL-AML patients did not express CD34, and 23% did not express HLA-DR. Approximately 36% of B-ALL and 64% of T-ALL cases did not express CD34. HLA-DR was expressed in 27% of APL cases.

5. DISCUSSION

Flow cytometry is an invaluable tool in AL diagnosis. It helps to determine not only the type of blasts but also their aberrant antigen expression. The immunophenotypic profile also provides strong prognostic and predictive information. Recognition of ALs specific phenotype is essential for monitoring patients after treatment.[11] In this study, non-APL-AML was the most prevalent leukemia followed by early pre-B-ALL. Tipu HN et al. studied 151 AL patients. They reported acute mye-

Table 3. Expression of aberrant markers, their distribution and association with age groups.

Leukemia	Marker	Marker expression	Age group		Chi-square Fisher exact test	*P* value
			up to 15 years old, *n*(%)	more than 15 years old, *n*(%)		
Non APL AML	CD19	expression	7(15.2)	23(5.1)	7.37	0.001
	CD7	expression	12(26)	92(20.6)	0.47	0.38
	CD34	non expression	26(56.5)	228(51.1)	0.48	0.48
	HLA-DR	non expression	13(28.2)	100(22.4)	0.80	0.37
Pro B-ALL	CD13	expression	2(8)	1(4)	0.35	0.50
	CD33	expression	4(16)	6(24)	0.50	0.48
	CD117	expression	0(0)	0(0)	—	—
	CD34	non expression	7(28)	10(40)	0.80	0.37
Common B-ALL	CD13	expression	4(2.8)	3(5)	0.57	0.35
	CD33	expression	14(10.1)	12(20.6)	3.74	0.05
	CD117	expression	1(0.7)	1(1.7)	0.38	0.53
	CD34	non expression	50(36.2)	24(41.3)	0.34	0.55
Pre B-ALL	CD13	expression	1(0.9)	2(4.4)	2.10	0.20
	CD33	expression	6(5.4)	5(11.1)	1.55	0.21
	CD117	expression	0(0)	1(2.2)	2.46	0.29
	CD34	non expression	39(35.4)	15(33.3)	0.06	0.80
TALL	CD13	expression	2(6)	1(3.1)	0.31	0.51
	CD33	expression	1(3)	1(3.1)	00.0	1.00
	CD117	expression	1(3)	1(3.1)	00.0	0.98
	CD34	non expression	17(51.5)	25(78.1)	5.03	0.02
APL	CD34	expression	1(33.3)	2(4.5)	3.89	0.18
	HLA-DR	expression	0(0)	13(29.5)	1.22	0.36

locytic leukemia subtype M2 (AML M2) as the most common AL in adults while in pediatric patients, pre-B-ALL was the most frequent.[12] In the comprehensive panel which we used for all AL patients, several common aberrancies analyzed in this study: aberrant expression of CD7, CD19 and loss of CD34, HLA-DR on non-APL-AML, aberrant CD13, CD33, CD117 and loss of CD34 on ALL, and an aberrant expression of CD34 and HLA-DR in APL patients. CD33 was the most frequently expressed cross lineage antigen in B-ALL (12%) followed by CD13 (3%). CD7 was the most frequently expressed cross lineage antigen, expressed in non-APL-AML (21%) followed by CD19 (6%). Almost 51% of non-APL-AML cases did not express CD34, and 23% did not express HLA-DR. About 36% of B-ALL and 64% of T-ALL cases did not express CD34. HLA-DR was expressed on 27% of APL cases. About 11% of T-ALL showed myeloid antigens aberrancy. Seegmiller et al. worked on immunophenotypic aberrancies on 200 cases of ALL. Their result showed that about 86% of B-ALL patients expressed aberrant myeloid antigens and 9% aberrant T-cell antigens.[13] The percent of lymphoid antigen expression on AML is reported in a range 30%–88% in different studies.[14] Tipu HN et al. showed at least 21.2% of AL express cross lineage antigens of CD7, CD13, CD33.[12] In a study done by Khurram et al., 26% of AL patients expressed cross lineage antigens of CD2, CD4, CD7, CD13, CD14, CD19, CD117, and CD11c[15] while Koju et al. results showed 37,6% antigen aberrancy in AL but they did not determine the specific CD markers.[16] In another study by Sarma et al., 60% of AL patients expressed cross lineage antigens, CD7 was the most common aberrant antigen in AML, and CD117 the most common aberrant antigen in ALL[17]. Their results are not in conjunction with our study. This may be due to differences in the cut off value for the aberrancy of a particular antigen, the number of antigens studied, the analyzed signal size of flow cytometry, and differences in the instruments and clones of antibodies used in flow cytometry. One of the limitations in this study is that we used four color flow cytometry and 20% cut-off for observation of positive and negative antigen expression, which may decrease the accuracy of findings, compared to higher color flow cytometry methods.

6. CONCLUSIONS

Detection of immunophenotype specific AL which defined as immunophenotype alteration in neoplastic cells in comparison with normal blasts is essential for monitoring of AL. These aberrancies may correlate with age or gender in some populations, in this study, there was no association found between sex/age and aberrancy of antigens except in certain AL subtypes.

Conflict of interest
Authors declare no conflict of interest.

Funding
Fund of the study provided by vice-chancellery for research and technology,Isfahan University of Medical Sciences (research code 398785).

Ethics
Protocol of the study was approved by ethic committee, vice-chancellery for research and technology, Isfahan University of Medical Sciences (IR.MUI.MED.REC.1398.552).

Acknowledgement
We thank our colleagues from pathology ward at Omid hospital, Isfahan, Iran who provided insight and expertise that greatly assisted the research, although they may not agree with all of the interpretations/conclusions of this paper.

References
1 Deschler B, Lübbert M. Acute myeloid leukemia: Epidemiology and etiology. In: Faderl SH, Kantarjian HM, eds. *Acute Leukemias*. Berlin: Springer; 2008:47–56. https://doi.org/10.1007/978-3-540-72304-2.

2 Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: Rationale and important changes. *Blood*. 2009;114(5):937–951. https://doi.org/10.1182/blood-2009-03-209262.

3 Misiukiewicz-Poć M, Poniatowska-Broniek G, Gizelbach-Żochowska K, et al. Isolated primary myeloid sarcoma of small intestine–A case report and review of the literature. *Pol Ann Med*. 2012;19(2):122–128. https://doi.org/10.1016/j.poamed.2012.06.002.

4 Wartenberg D, Groves FD, Adelman AS. Acute lymphoblastic leukemia: epidemiology and etiology. In: In: Faderl SH, Kantarjian HM, eds. *Acute Leukemias*. Berlin: Springer; 2008:77–93. https://doi.org/10.1182/blood-2009-03-209262.

5 Béné M, Nebe T, Bettelheim P, et al. Immunophenotyping of acute leukemia and lymphoproliferative disorders: a consensus proposal of the European LeukemiaNet Work Package 10. *Leukemia*. 2011;25(4):567–574. https://doi.org/10.1038/leu.2010.312.

6 Harakati MS, Al-Momen AM, Ajarim DS, et al. Adult acute myeloblastic leukemia: experience at King Khalid University Hospital. *Ann Saudi Med*. 1998;18(3):221–225. https://doi.org/10.5144/0256-4947.1998.221.

7 Saxena R, Anand H. Flow cytometry in acute leukemia. *Indian J Hematol Blood Transfus*. 2008;24(4):146–150. https://doi.org/10.1007/s12288-008-0037-4.

8 Bain BJ, Béné MC. Morphological and immunophenotypic clues to the WHO categories of acute myeloid leukaemia. *Acta Haematol*. 2019;141(4):232–244. https://doi.org/10.1159/000496097.

9 Albano F, Mestice A, Pannunzio A, et al. The biological characteristics of CD34+ CD2+ adult acute promyelocytic leukemia and the CD34 CD2 hypergranular (M3) and microgranular (M3v) phenotypes. *Haematologica*. 2006;91(3):311–316.

10 Kermani I. Immunophenotyping of acute leukemia in northwestern Iran. *Iran J Med Sci*. 2002;27(3):136–138.

11 Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. *Blood*. 2008;111(8):3941–3967. https://doi.org/10.1182/blood-2007-11-120535.

12 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 centre. *PAFMJ*. 2018;68(3):450–454.

13 Seegmiller AC, Kroft SH, Karandikar NJ, McKenna RW. Characterization of immunophenotypic aberrancies in 200 cases of B acute lymphoblastic leukemia. *Am J Clin Pathol*. 2009;132(6):940–949. https://doi.org/10.1309/ajcp8g5rmtwuemuu.

14 Sharma M, Varma N, Sachdeva MUS, Bose P, Varma S. Clinical and hematological correlates of aberrant immunophenotypic profiles in adult and pediatric acute myeloid leukemia at presentation. *J Cancer Res Ther*. 2020;16(1):105. https://doi.org/10.4103/jcrt.jcrt_770_17.

15 Khurram MM, Jafri SA, Mannan A. Frequency of aberrant expression of CD markers in cases of acute leukemia. *Med J Islamic World Acad Sci*. 2010;109(395):1–6.

16 Koju S, Sachdeva MUS, Bose P, Varma N. Spectrum of acute leukemias diagnosed on flow cytometry: Analysis from tertiary care centre from North India. *Clin Chem Lab Med*. 2015;1(1):12–15. https://doi.org/10.3126/acclm.v1i1.12308.

17 Sarma A, Hazarika M, Das D, et al. Expression of aberrant CD markers in acute leukemia: A study of 100 cases with immunophenotyping by multiparameter flowcytometry. *Cancer Biomark*. 2015;15(4):501–505. https://doi.org/10.3233/cbm-150482.