Original Article

Aberrant Expression of CD Markers on Flow Cytometric Analysis in Suspected Patients of Leukemias and Lymphomas: A Single-Centered Study

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ABSTRACT

Objective: To determine the frequencies and pattern of aberrant CD markers expression in Pakistani patients of leukemias and lymphomas.

Methodology: It was a cross-sectional study conducted from April to September 2020 at the Department of Hematology and Transfusion Medicine, Aga Khan University Hospital, Karachi. Peripheral blood/bone marrow samples from patients of suspected hematological malignancies subjected to flow cytometric evaluation were included. Samples were lysed, tested for viability, and analyzed through 5-colour flow cytometer (FC500). Data was entered in Statistical Package for the Social Sciences (SPSS) version 22.

Results: A total of 262 patients were enrolled. Male to female ratio in the study was determined to be 1.5:1. Further categorization showed acute leukemias comprised 75.78% of the total cases whereas chronic lymphoproliferative disorders were 22.26%. The overall presence of aberrant markers was 22.8% among all cases. The percentage of aberrant markers expression was higher in leukemic patients as compared to lymphoma patients.

Conclusion: Aberrant expression of CD markers is a common entity. Their possible presence should be considered and evaluated while determining the lineage for acute leukemias specifically according to the latest WHO criteria through flow cytometry.

Keywords: *Leukemias. Lymphomas. Flow cytometry.*

INTRODUCTION

Leukemias and lymphomas pose a major burden among all malignancies.[1] Their diagnosis relied upon morphology and cytochemical stains in the past.[2] Now after advancement in diagnostic tools, above mentioned malignancies are diagnosed by immunophenotyping, cytogenetic, and molecular genetics in addition to morphology and cytochemical examination of blast cells.[3] The paradigm shifted in 2008 after the revision of the WHO classification of tumors.[4]

Flow cytometry has become increasingly important in the field of hematology for clinical purpose.[5] It is an excellent tool for the diagnosis, monitoring, and evaluation of malignancies.[6] It does a commendable job by identification of all CD markers expressed by clonal cells. The presence or absence of aberrant markers may also be associated with poor or favorable prognosis.[7] This integrated evaluation utilizing flow cytometry and molecular studies is a basic requirement in diagnostic essentials. Flow cytometric analysis of peripheral blood as well as bone marrow aspirate samples has become a basic diagnostic tool for suspected patients of hematological malignancies since then.[6] Clonal cells in addition to lineage-specific markers, can also express markers of other lineages, so are called "aberrant" markers. Expression of aberrant markers brings about diagnostic difficulties and a sound knowledge about their pattern in our population can be helpful considering the application of targeted antibodies.[4]

Aberrant expression of CD markers has been a concern. A few ambiguities were laid down while the diagnosis was being established. It has been suggested to have prognostic values.[8] Pakistani data has not been widely studied for the presence of aberrant markers and further study on targeted therapies is also missing. The objective of the current study was to overview the frequency and pattern of expression of aberrant CD markers in suspected patients of leukemia and lymphoma on flow cytometric analysis. Aga Khan University Hospital receives a large number of samples across Pakistan. This study will help to predict the said pattern in our population at large as a first step.

METHODOLOGY

It was a cross-sectional study conducted from April to September 2020 at the Department of Hematology and Transfusion Medicine, Aga Khan University Hospital, Karachi. Peripheral blood/bone marrow samples from patients of suspected hematological malignancies subjected to flow cytometric evaluation were included. All patients of suspected leukemias and lymphomas

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whose samples were received from April to September 2020 at the Department of Hematology and Transfusion Medicine were enrolled in the study. Previously diagnosed patients, relapsed cases, and patients for minimal residual disease evaluation were excluded from the study. Samples from peripheral blood/bone marrow aspirates were lysed, tested for viability index, and incubated with monoclonal antibodies in 5-colour flow cytometer (FC500).

STATISTICAL ANALYSIS

The data was entered and analyzed by using Statistical Package for the Social Sciences (SPSS) version 22. Mean±standard deviation (SD) was given for normally distributed quantitative variables while the median and interquartile range was given for non-normally distributed quantitative variables. Qualitative variables were expressed in the form of frequencies and percentages.

RESULTS

A total of 262 patients were enrolled in the study. The mean age was found to be 38.8±21 years ranging from 8 months to 102 years. Seventeen percent of the study population was less than 15 years. Further stratification of patients in different age groups showed that a maximum number of patients (81/262) were between 15-35 years. There were 158(60.3%) males and 104(39.7%) female patients. Male to female ratio was 1.5:1. Majority of patients suffered from acute myeloid leukemia (AML) (35.55%) whereas the least number were diagnosed as suffering from T-cell lymphoproliferative disease (T-LPD) (0.39%) (Figure 1).

Categorization of the study population according to the type of malignancy showed acute leukemias [AML, B-cell acute lymphoblastic leukemia (B-ALL), T-cell acute lymphoblastic leukemia (T-ALL), acute promyelocytic leukaemia (APML), mixed phenotype acute leukemia (MPAL)] composed of 75.78% of the total cases, chronic lymphoproliferative disorders [chronic lymphocytic leukemia (CLL), B-cell lymphoproliferative disease (B-LPD), T-LPD] were 22.26% and hairy cell leukemia (HCL) & monoclonal B-cell lymphocytosis (MBL) were 1.17% and 0.78%, respectively.

The overall presence of aberrant markers was 22.8%. The percentage of aberrant markers expression was higher in leukemic patients as compared to lymphoma patients (Figure 2).

The commonest expression was seen for CD7 constituting 11.9% of the total expressed aberrant marker. Patients suffering from AML had the maximum aberrant expression whereas T-lymphoproliferative disorders did not show any aberrant markers. Table 1 shows the detailed expression of aberrant markers in all the diseases studied.

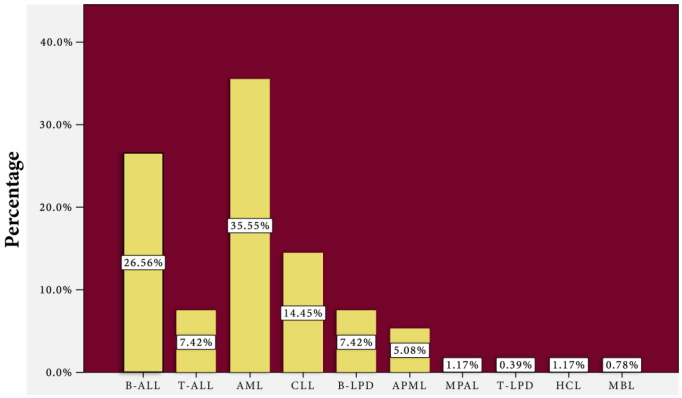

Figure 1: Disease Spectrum of the Study Population

Table 1: Pattern of Expression of Aberrant Markers in Patients of Leukemias and Lymphom as (n=262)

Sr. No.	Disease	Aberrant Markers (%)	CD3%	CD5%	CD7%	CD10%	CD19%	CD22%	CD79a%	CD13%	CD33%	MPO%
1	AML	14.12	0	8	70	5.4	16.2	0	0	NA	NA	NA
2	B-ALL	4.6	0	8.3	25	NA	8.3	NA	NA	0	58.3	0
3	T-ALL	3.43	NA	NA	NA	66	11.1	0	11.1	0	11.1	0
4	CLL	1.14	2.7	NA	5.5	NA	NA	NA	NA	0	0	0
5	B-LPD (MCL**, HCL, PCM***)	0.38	0	0	0	NA	NA	NA	NA	0	3.3	0
6	T-LPD	0	NA	NA	NA	0	0	0	0	0	0	0

**NA: Not applicable as aberrant maker for the disease*
***MCL: Mantle cell lymphoma*
***PCM: Plasma cell myeloma*

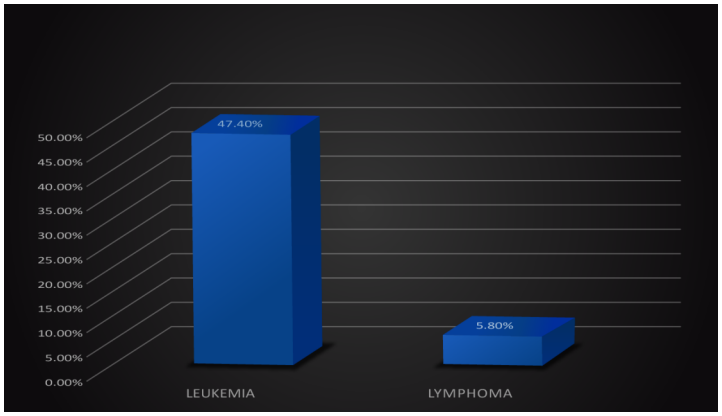

Figure 2: Expression of Aberrant Markers in Leukemias and Lymphomas

DISCUSSION

Flow cytometry is an excellent tool in addition to morphology and cytochemistry for reliable diagnosis of hematological malignancies.[9] It has a role both in early diagnosis and follow-up, with increasing importance in the detection of very small residual disease populations (minimal residual disease).[10] Due to financial constraints in our population, flow cytometry has not been extensively studied as yet for the expression of aberrant markers. In this study, we took a large number of patients (262) with a wide age range considering the occurrence of lymphoproliferative disorders. In the present study, the mean age of the patients was 38.8 years and there were 158(60.3%) males and 104(39.7%) females. Similar results were found in another study conducted by Shahni et al. They reported that the mean age of the patients was 32 years and male to female ratio as 1.5:1.[11]

Our results showed that acute leukemias were composed of 75.78% of the total cases whereas chronic lymphoproliferative disorders were 22.26%. Another study conducted in India reported that 66.8% of patients had acute leukemia and 33.2% suffered from chronic leukemia.[12]

In our study, the overall presence of aberrant markers was 22.8%. The percentage of aberrant markers expression was higher in leukemic patients as compared to lymphoma patients. The presence of aberrant markers showed both differences and similarities to available data. Comparable results were reported by Shahni et al., and Tipu et al. These studies reported aberrant marker expression in 21.2% and 19%, respectively.[11,13] Another study concluded that 20-24% of the study population expressed aberrant markers.[14] A Pakistani study revealed that 38% of the patients showed aberrant expression which is higher than the international and other local data.[15]

About 70% of AML patients under study having aberrant markers showed the presence of CD7. This was followed by CD19 and CD10. Comparable results were found in another study conducted in Lahore, Pakistan. They reported that the expression of CD7 aberrant marker is high in AML.[16] Another study conducted in Army Medical College, Rawalpindi found aberrant expression of CD7 and CD 19 in 26.4% and 1.1% of AML cases.[17]

In the current study, among lymphoid leukemias, CD33 was the commonest aberrant marker. In contrast, Shahni et al., and Tipu et al., reported CD13 & CD117, respectively as the commonest markers in lymphoid leukemias.[11,13] Another study reported CD33 and 13 as common aberrant markers.[14] Racial differences can be responsible for these differences.

Among the chronic lymphoproliferative disorders, chronic lymphocytic leukemia expressed CD7 as the most prevalent aberrant marker. Shahni et al. has reported CD11c in chronic lymphoid leukemia.[11] This entity has not been widely studied in the Pakistani population. Other B-lymphoproliferative disorders showed CD33 expression as an aberrant marker. On contrary, another study reported CD8 as the aberrant marker of B-lymphoproliferative disorders.[11] T-lymphoproliferative disorders have not been studied in all the other studies compared with our data. However, no aberrant expression was found in our study in T-LPD.

CONCLUSION

Aberrant expression of CD markers is a common entity. Their possible presence should be considered and evaluated while determining the lineage for acute leukemias specifically according to the latest WHO criteria through flow cytometry. Utilizing flow cytometry as a basic tool should always be considered in suspected cases of hematological malignancies. It provides an opportunity for the determination of aberrant markers which can be missed on immunohistochemistry only. Adequate knowledge of lineage-specific markers & cross-lineage expression is mandatory for diagnosis.

LIMITATIONS & RECOMMENDATIONS

The correlation of the expression of aberrant markers with molecular testing and impact on response to medication needs further study. The extensive population-based study may also help us in evaluating genetic makeup linked expressions in our population as well as organizing customized panels for patients reducing the financial impact on the healthcare system and forming a basis for further targeted therapies.

REFERENCES

1. Keykhaei M, Masinaei M, Mohammadi E, Azadnajafabad S, Rezaei N, Saeedi Moghaddam S, et al. A global, regional, and national survey on burden and quality of care index (QCI) of hematologic malignancies; global burden of disease systematic analysis 1990-2017. Exp Hematol Oncol. 2021; 10(1):11. doi:10.1186/s40164-021-00198-2.

2. Alahmari B, Alzahrani M, Al Shehry N, Tawfiq O, Alwasaidi T, Alhejazi A, et al. Management approach to acute myeloid leukemia leveraging the available resources in view of the latest evidence: consensus of the Saudi Society of Blood and Marrow Transplantation. JCO Glob Oncol. 2021; 7:1220-32. doi:10.1200/GO.20.00660.

3. Hamid GA, Akrabi MA. Aberrant antigen expression in patients with acute leukemia. EC Clin Med Case Rep. 2019; 2(3):53-60. Available from: https://www.researchgate.net/publication/333563047_Aberrant_Antigen_Expression_in_Patients_with_Acute_Leukemia.

4. Merino A, Boldu L, Ermens A. Acute myeloid leukaemia: how to combine multiple tools. Int J Lab Hematol. 2018; 40(Suppl 1):109-19. doi:10.1111/ijlh.12831.

5. Duetz C, Bachas C, Westers TM, van de Loosdrecht AA. Computational analysis of flow cytometry data in hematological malignancies: future clinical practice? Curr Opin Oncol. 2020; 32(2):162-9. doi:10.1097/CCO.0000000000000607.

6. Ortolani C. Flow cytometry of hematological malignancies. John Wiley & Sons. 2021. Available from: https://books.google.com/books?hl=en&lr=&id=o_chEAAAQBAJ&oi=fnd&pg=PA11&dq=Flow+Cytometry+Of+Hematological+Malignancies&ots=2savnBvL6n&sig=89inEmuu2inxnp36NRZHCpt4iWk#v=onepage&q=Flow%20Cytometry%20Of%20Hematological%20Malignancies&f=false.

7. Ikoma-Colturato MRV, Beltrame MP, Furtado FM, Pimenta G, da Costa ES, Azambuja AP, et al. Minimal residual disease assessment in acute lymphoblastic leukemia by 4-color flow cytometry: recommendations from the MRD Working Group of the Brazilian Society of Bone Marrow Transplantation. Hematol Transfus Cell Ther. 2021; 43(3):332-40. doi:10.1016/j.htct.2020.09.148.

8. Garg N, Kotru M, Kumar D, Pathak R, Sikka M. Correlation of expression of aberrant immunophenotypic markers in T-ALL with its morphology: a pilot study. J Lab Physicians. 2018; 10(4):410-3. doi:10.4103/JLP.JLP_35_18.

9. Arber DA, Borowitz MJ, Cessna M, Etzell J, Foucar K, Hasserjian RP, et al. Initial diagnostic workup of acute leukemia: guideline from the College of American Pathologists and the American Society of Hematology. Arch Pathol Lab Med. 2017; 141(10):1342-93. doi:10.5858/arpa.2016-0504-CP.

10. Keeney M, Hedley BD, Chin-Yee IH. Flow cytometry-recognizing unusual populations in leukemia and lymphoma diagnosis. Int J Lab Hematol. 2017; 39(Suppl 1):86-92. doi:10.1111/ijlh.12666.

11. Shahni A, Saud M, Siddiqui S, Mukry SN. Expression of aberrant antigens in hematological malignancies: a single center experience. Pak J Med Sci. 2018; 34(2):457-62. doi:10.12669/pjms.342.13996.

12. Singh G, Parmar P, Kataria SP, Singh S, Sen R. Spectrum of acute and chronic leukemia at a tertiary care hospital, Haryana, India. Int J Res Med Sci. 2016; 4(4):1115-8. doi:10.18203/2320-6012.ijrms20160792.

13. Tipu HN, Bashir MM, Altaf C, Noman M, Malik HS. Spectrum of acute leukemias and aberrant markers expression based on flow cytometry in a tertiary care centre. Pak Armed Forces Med J. 2018; 68(3):450-54. Available from: https://www.pafmj.org/index.php/PAFMJ/article/view/219/148.

14. 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.

15. Mazher N, Malik N, Imran A, Chughtai O, Chughtai AS. Aberrant expression of CD markers in acute leukemia. Ann Pak Inst Med Sci 2013; 9(2):99-102. Available from: https://apims.net/apims_old/Volumes/Vol9-2/ABERRANT%20EXPRESSION%20OF%20CD%20MARKERS%20IN%20ACUTE%20LEUKEMIA.pdf.

16. Raza H, Fatima M, Noor T, Umer S, Imran A, Malik NA. The frequency of aberrant CD7 antigen expression in acute myeloid leukaemia patients. Cureus. 2022; 14(2):e22309. doi:10.7759/cureus.22309.

17. Basharat M, Khan SA, Din NU, Ahmed D. Immunophenotypic characterisation of morphologically diagnosed cases of acute myeloid leukaemia (AML). Pak J Med Sci. 2019; 35(2):470-6. doi:10.12669/pjms.35.2.614.