Bangladesh J Med Microbiol 2018; 12 (1): 10-14
Bangladesh Society of Medical Microbiologists

Original Article

Aberrant Antigen Expression in Children with Acute Leukemia A Flow *Cytometric* Analysis

Nusrat Jahan[1], Humayun Sattar[2], Shirin Tarafder[2], Chandan Kumar Roy[2], Irin Rahman[3], Fatima Tuj Johora[4]

[1]*Department of Pathology and Microbiology, University Dental College,* [2]*Department of Microbiology and Immunology Bangabandhu Sheikh Mujib Medical University,* [3]*Department of Microbiology, Universal Medical College,* [4]*Department of Microbiology, East West Medical college*

Abstract

The primary clinical tool for predicting the lineage potential of leukemic blasts is the characterization of protein expression by immunophenotyping. It is common for acute leukemias to aberrantly express protein markers more typically associated with other lineages. Therefore, this study was designed to assess the frequency of aberrant antigens expression in childhood acute leukemia. Peripheral blood and bone marrow samples were collected from 76 clinically suspected and morphologically diagnosed untreated cases of acute leukemia (children of 0-<18 years). Flow cytometry immunophenotyping was carried out with the help of Flow cytometer (BD FACSVerse). Total 9 (11.84%) cases showed aberrant antigens expression. Out of 9 aberrantly expressed cases, 2 (22.22%) AML cases showed aberrant lymphoid marker CD7 expression. Co-expression of myeloid markers CD13 and CD117 found in 2 (22.22%) B-ALL cases and 2 (22.22%) cases of B-ALL expressed myeloid marker CD117. Myeloid marker CD33 expressed by 2 (22.22%) case of B-ALL. Only 1(11.11%) T-ALL case co expressed myeloid markers CD33 and CD117. These findings may help to recognize patients with high risk group and low remission rate as aberrant antigen expression may represent a poor prognostic indicator. Further studies are needed to confirm the correlation between aberrant phenotypes with prognosis and therapeutic response of acute leukemia.

Key words: Flow cytometry, aberrant antigen expression, acute leukemia

Introduction:

Acute leukemia comprises a heterogeneous group of diseases characterized by rapid and uncontrolled clonal expansion of progenitor cells of haemopoietic system.[1] It is the most common childhood cancer[2] with a relative proportion varying from 25%-40%.[3] The immature progenitor cells are known as blast which are the characteristics of acute leukemia. According to the blast cell lineage acute leukemia is primarily divided into acute myeloblastic leukemia (AML) - with myeloid precursor and acute lymphoblastic leukemia (ALL) - with lymphoid precursor. In each type of acute leukemia, blast cells express characteristic pattern of molecules known as cluster of differentiation antigens (CD). These antigenic markers of hematopoietic differentiation are the most common leukemia biomarkers that characterize human leukocytes as well as other human cells such as endothelial and stromal cells. Blast cells from cases of acute leukemia do not exhibit the features of normal cellular differentiation and show unusual expression of CD markers. Classification of acute leukemia depends on expression of lineage specific markers on blast cells. In several cases of acute leukemia, blasts of one lineage do not exhibit the markers of normal differentiation but expressed unusual markers in which myeloid associated antigens expressed in lymphoblasts and lymphoid associated antigen expressed in myeloblasts. This phenomenon is called aberrant phenotypes.[4]

Immunophenotyping is a methodology used to detect cell surface and intracellular antigens using monoclonal antibodies conjugated with different fluorochromes. It plays a crucial role in diagnosis and classification of acute leukemia, predicting response to different treatment modalities, monitoring minimal residual disease, prognostic evaluation and predicting patients survival.[5] The World Health Organization (WHO) 2008 Classification of Hematopoietic neoplasms relies on the morphologic, immunophenotypic, cytogenetic, and molecular features for the diagnosis and sub-classification of acute leukemias.[6] Assignment of blast lineage is essential in the diagnosis of acute leukemia because the treatment of AML and ALL is different. Moreover, aberrant antigen expression can adversely influence the clinical response, remission rate and overall survival in patients with acute leukemia. Immunophenotyping of leukemic cells plays crucial role in identification of cell line, definition of maturation stage and finding possible aberrant antigens for individual treatment monitoring.

Correspondence:
Dr. Nusrat Jahan
Department of Pathology and Microbiology
University Dental College, Dhaka.
Mobile: 01828076555, E-mail: nusratkwmc@gmail.com

The expression of CD markers on leukocytes can be determined by flow cytometry. Recently, immunophenotyping by multi parameter flow cytometry has been developed to detect lineage characteristics of different subtypes of acute leukemia. This technique use to analyze multiple phenotypic and functional parameters simultaneously within a single cell or a population of cells.[7] It identifies cell markers by applying monoclonal antibodies against them. Applications of flow cytometric immunophenotyping mainly rely on the concept that even if neoplastic cells show a great similarity to normal hematopoietic precursors, they frequently display aberrant phenotypes that allow their specific identification and discrimination from normal cells, even when present at very low frequencies.[8] To a large extent such aberrant phenotypes would be a consequences of the genetic abnormalities shown by the neoplastic cells.[9, 10, 11] This subtype of acute leukemia is known to respond well to intensive therapy and should therefore be treated according to appropriate risk-based protocols.[12] This study was conducted to assess the frequency of aberrant antigen expression in children with acute leukemia.

Materials and Methods:

This cross sectional study was conducted in the Department of Microbiology and Immunology, Bangabandhu Sheikh Mujib Medical University (BSMMU) for a period of one year from March 2014 to February 2015. A total 76 clinically suspected and morphologically diagnosed untreated cases of acute leukemia(children of 0-<18 years) were enrolled in this study after taking written informed consent from the parents of the patients who were attending in the Department of Paediatric Haematology and Oncology (BSMMU) and DMCH (including both admitted and outpatient department OPD). 2 ml of peripheral blood and bone marrow samples were collected from the patients. Laboratory works were performed in the Department of Microbiology and Immunology (BSMMU).

Blood films and bone marrow smears were made and were stained with Leishman stain.

At first all the samples were examined by qualified hematologists examined under light microscope. Then samples were prepared for flow cytometry.

Samples of peripheral blood and bone marrow were collected in ethylene diamine tetraacetic acid (EDTA) for immunophenotyping. They were processed within twenty four hours of collection. For the immunophenotypic diagnosis of acute leukemias, various combinations of fluorochrome-conjugated monoclonal antibodies (MoAb) per tube were added to the samples. They were directed to antigens for T cells- [cytoplasmic (c) cCD3, CD5, CD7], [B cells- CD19, cCD79a], myeloid cells- [CD13, CD33, CD117 and cyto Myeloperoxidase (cMPO)], non-specific lineage pan-leukocytes (CD45) and precursor cells [CD34, TdT and human leukocyte antigen-DR (HLA-DR) and prognostic marker CD10]. Membrane and intracytoplasmic labeling was performed using 1 x 10^6 cells per tube. The blast gating strategy included using dot plots of CD45 expression versus intracellular complexity (side scatter angle, SSC) and also a second gate considering cell size (forward scatter angle, FSC) versus SSC. A total of 10,000 events were acquired in the target gate. Antigen was considered as positive if 20% or more of the blast cells reacted with a particular antibody.

BD FACSuite software application was used for data acquisition and analysis.

In order to ensure accuracy and precision of the test run, the instrument was calibrated daily by running controls before the start of the test or whenever new lots of the reagents were used. Controls were reported as passed or failed. It also verified reagents activity.

Statistical analysis:

All data after collection by data sheet were checked, edited analyzed by using computer based SPSS (Statistical Package of Social Science, version 20) software. Results were presented in the form of tables and figures. Descriptive analysis of all relevant variables was done by using proportion, central tendency and dispersion. P value was calculated by Chi square test. P value <0.05 was considered as minimum level of significance.

Results:

During this study period 76 morphologically diagnosed childhood acute leukemia cases were analyzed by flow cytometry (FCM) to find out the frequency of aberrant antigen expression in AML and ALL.

Lymphoid and myeloid cell subsets in various stages of maturation have distinct immunophenotypes and express lineage specific markers (Table I).

Distribution of acute leukemia cases which were diagnosed by FCM has been shown in Table II. Out of 76 acute leukemia cases, 68 (89.47%) cases were ALL of which, 55 (80.89%) cases were B-ALL and 13 (19.11%) cases were T-ALL. AML cases were only 08 (10.53%).

Table III shows aberrant antigen expression in 76 acute leukemia cases. Total 9 (11.84%) cases showed aberrant antigen expression. Out of 55 B-ALL cases, 6 (10.90%) cases of B-ALL and 1 (7.69%) T-ALL out of 13 cases showed aberrant antigen expression.

Only 2 (25.0%) cases out of 8 AML showed aberrant antigen expression.

Out of 9 aberrantly expressed cases, 2 (22.22%) AML cases showed aberrant CD7 expression. In ALL, co-expression of CD13 and CD117 found in 2 (22.22%) B-ALL cases and 2 (22.22%) cases of B-ALL expressed CD117. 2 (22.22%) case of B-ALL showed expression of CD33. Only 1(11.11%) T-ALL case co expressed CD33 and CD117 (Figure I).

Table I: Lineage specific markers

Myeloid markers	B cell markers	T cell markers
CD 13	CD19	cCD3
CD33	cCD79a	CD5
CD117		Cd7
c MPO		

Tble II: Distribution of acute leukemia cases (n=76)

Acute leukemia Type	No	%	Acute leukemia subtype	No	%
Acute Myeloblastic Leukemia (AML)	08	10.53	B-ALL	55	80.89
Acute Lymphoblastic Leukemia (ALL)	68	89.47	T-ALL	13	19.11
Total	76	100			

Note:
B-ALL: B lineage acute lymphoblastic leukemia.
T-ALL: T lineage acute lymphoblastic leukemia.

Table III: Frequency of aberrant phenotype in ALL and AML (n=76).

Acute leukemia cases	Aberrant phenotype		Total No. (%)
	Absent No. (%)	Present No. (%)	
B-ALL (n=55)	49 (89.09)	06 (10.90)	55 (72.37)
T-ALL (n=13)	12 (92.30)	01 (7.69)	13 (17.10)
AML (n=8)	06 (75.0)	02 (25.0)	08 (10.53)
Total (n=76)	67 (88.16)	09 (11.84)	76 (100)

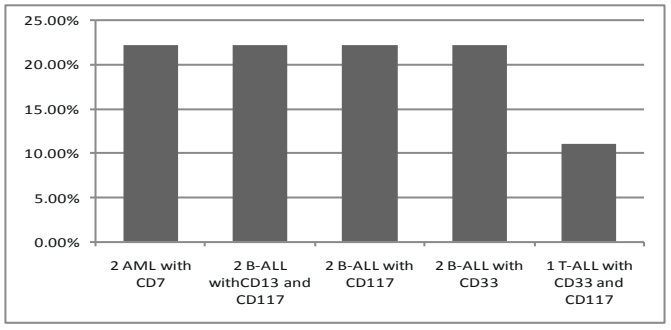

Fig I: Aberrantly expressed CD markers in ALL and AML.

Discussion

Acute leukemia is a neoplastic disorder that arises from the clonal expansion of immature lymphoid or myeloid progenitor cells that are blocked at a particular stage of differentiation.[13] It is the most common paediatric cancer accounting 25% of all childhood cancer.[14] Acute leukemia displays characteristic patterns of antigen expression, which facilitate their identification and proper classification. Early classification for acute leukemias was based only on cytomorphologycal and cytochemical investigations. Current classification system have incorporated immunophenotyping in order to achieve greater precision in delineating the haemopoietic lineage and differentiating stage of particular leukemias. Immunophenotyping refers to the determination of the expression of antigenic markers on/in the cell population of interest. Immunophenotyping of acute leukemia plays a crucial role to diagnose acute leukemia cases as well as to choose an appropriate treatment regime.

This study included 76 children (age ranging from 8 month to 17 years) with acute leukemia diagnosed by cytomorphology. Immunophenotyping was done by flow cytometry (FCM) from 76 peripheral blood and bone marrow samples.

In this study, 76 acute leukemia cases were diagnosed by flow cytometry of which AML were 8 (10.53%) and ALL were 68 (89.47%). Most studies revealed that in children, ALL accounts for 80%-85% and AML accounts for 15%-20%.[15,16] In current study, among 68 ALL cases, 55 (80.89%) cases were B-ALL and 13 (19.11%) cases were T-ALL. A study in Lahore, Pakistan showed, among children, B-ALL was 72% and T-ALL was 28%.[17] An Italian multicenter study revealed B-ALL in 85.8% of patients and T-ALL in 14.2%.[18] To determine the immunophenotypic profile of B-ALL CD19, cCD79a have been used along with CD34, CD10, HLA DR and TdT in this study. Out of 55 B- ALL cases, 6 (10.90%) cases showed aberrant antigen expression with myeloid antigen (My + ALL). In 2011, Atila et al[19] stated that myeloid antigens may be detected in 5-10% of childhood ALL and the most frequently encountered antigens are CD13 (6-16%) and CD33 (3-10%). Although some studies suggest that the presence of myeloid antigens is a poor prognostic factor in patients with ALL, others suggest no independent prognostic significance.[20,21] In this study co-expression of CD13 and CD117 found in 2 (22.22%) B-ALL cases and 2 (22.22%) cases of B-ALL expressed CD117. 2 (22.22%) case of B-ALL showed expression of CD33. No aberrant expression of T lineage antigen in B-ALL is seen in this study.

For the determination of T-ALL cCD3, CD5, CD7 along with CD34, CD10, HLA DR and TdT have been used in this study.

Out of 9 aberrantly expressed acute leukemia cases, 1 (11.11%) T-ALL case showed aberrant expression of CD33 and CD117

that is almost similar with the finding of a study carried out by Dalia et al[22] in 2011 who showed 7.69% aberrant expression of both CD33 and CD117.

To determine the immunophenotypic profile of AML CD13, CD33, CD117, cMPO have been used along with CD43 and HLA DR.

In this study, 2 (22.22%) cases of AML showed aberrant antigen expression with CD7 (Ly^+ AML). Most studies revealed CD7 is the most frequently seen in AML with aberrant expression.[23, 24] A study carried out by Lo Coco et al[25] in 1989 reported common association of CD7 in AML. Traweek et al[26] in 1993 reported the possibility of association of CD7 with blast immaturity in their study due to its association with M0, M1 and M5. A study carried out by El-Sissy et al[27] in 2006 suggested that CD7 expression in AML should be interpreted with cytogenetic as it may be associated with unfavorable cytogenetic. But another study by Drexler et al[28] in 1993 showed that there is no cytogenetic anomaly specific for Ly^+ AML; expression of lymphoid-associated antigens (with the exception of CD7) on AML blasts lacked prognostic, significance; $CD7^+$ AML appears to be a particular subset of malignant myeloid progenitors and suggested that, in general, Ly^+ AML may not represent a biologically distinct form of leukemia as these cases have similar clinical features and respond to therapy in a comparable manner.

Conclusion

In summary, we conclude that the incidence of aberrant antigens expression in acute leukemia was comparable with the other published international data and it may represent a poor prognostic indicator among this group of patients. This type of acute leukemia is a diagnostic and therapeutic challenge owing to its heterogeneity, overlapping features with other types of ALL and AML, and lineage plasticity. The aberrant phenotypes may help to assign patients with high risk group and low remission rate. Further studies are recommended to confirm the correlation of aberrant phenotypes in diagnosis, prognosis and therapeutic response of acute leukemia. Moreover, molecular cytogenetic studies are recommended to shed light on potential biomarkers that might play a role in pathogenesis of antigen aberrancy, prognostic stratification and possible targeted drug therapy.

References

1. McCulloch E A. Stem cells in normal and leuemic hemopoiesis (Henry Stratton Lecture, 1982). Blood 1983 Aug; 62 (1):1-13.
2. Poplack DG, Cassady JR and Pozzo PA. Leukaemias & lymphomas of childhood. In: Devita VT, Hellman S and Rosenberg SA (Eds). CANCER principles & practice of oncology, 2nd Ed; J.B. Lippincott company, Philadelphia 1985; p. 1591 –1622.
3. Arora RS, Eden TOB, Kapoor G. Epidemiology of childhood cancer in India. Indian journal of Cancer 2009; 46 (4): 264 – 273.
4. 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 Biomarkers 2015; 15 (4):501-505.
5. Jahedi M, Shamsasenjansti K, Sanaat Z. Aberrant Phenotype in Iranian Patients with Acute Myeloid Leukemia. Adv Pharm Bull 2014; 4 (1):43-47.
6. Hartmut D, Elihu H, Sergio A, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European Leukemia. Net Blood 2010: 115 (3).
7. Swerdlow S. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon, France: International Agency for Research on Cancer 2008.
8. Strobl H and Knapp W. Myeloid cell-associated lysosomal proteins as flow cytometry markers for leukocyte lineage classification. Journal of Biological Regulators & Homeostic Agents 2004; 18 (3-4): 335-9.
9. Campana D and Behm FG. Immunophenotyping of leukemia. Journal of Immunological Methods 2000; 243, 59-75.
10. Bahia DM, Yamamoto M, Chauffaille Mde L, et al. Aberrant phenotypes in acute myeloid leukemia: a high frequency and its clinical significance. Haematologica 2001 Aug; 86(8):801-6.
11. Wilkerson MJ, Dolce K, Koopman T, et al. Lineage differentiation of canine lymphoma/leukemias and aberrant expression of CD molecules. Vet Immunol Immunopathol 2005 Jul 15;106 (3-4):179-96.
12. Tong H, Wang Q, Lu C, et al. Immunophenotypic, cytogenetic, and clinical features of 207 cases of childhood acute lymphoblastic leukemia in china. J Pediatr Hematol Oncol 2011 Aug; 33(6):437-41.
13. Mazher N, Malik N, Imran A, Chughtai O, Chughtai A. Aberrant expression of CD markers in Acute Leukemia. Pakistan Institute of Medical Science 2013; 9 (2): 99-102.
14. Cartwright R.A. Epidemiology. In: Leukaemia. Whittaker J.A. (Eds). Blackwell Scientific Publications, Oxford 1992; pp. 3-33.
15. Arora RS, Eden TOB, Kapoor G. Epidemiology of childhood cancer in India. Indian journal of Cancer 2009; 46 (4): 264-273.
16. Parkin DM, Stiller CA and Draper GJ. The international incidence of childhood cancer. International Journal of Cancer 1988 ; 42: 511-520.

17. Samina N. Immunophenotypes in Acute Lymphoblastic Leukemia; association with demographic profile and clinical presentation. International Journal of pathology 2014; 12(1): 24-29.

18. Chiaretti S, Vitale A, Cazzaniga G, et al. Clinico-biological features of 5202 patients with acute lymphoblastic leukemia enrolled in the Italian AIEOP and GIMEMA protocols and stratified in age cohorts. Haematologica 2013 ; 98 (11): 1702-10.

19. Atila T, Fatih E, Ibrahim B, Mustafa K. Myeloid Antigen Positivity in Turkish Children With Acute Lymphoblastic Leukemia Lacks Influence on Prognosis. Asian Pacific Journal of Cancer Prevention 2010;11: 1823-1826.

20. Lauria F, Raspadori D, Martinelli G, et al. Increased expression of myeloid antigen markers in adults acute lymphoblastic leukemia patients: diagnosis and prognostic implications. British Journal of Haematology 1994; 87: 286-92.

21. Campana D and Behm FG. Immunophenotyping of leukemia. Journal of Immunological Methods 2000; 243, 59-75.

22. Dalia A, and Sherin M. 2012. Flowcytometric Immunophenotypic Profile of Acute Leukemia: Mansoura Experience. Indian journal of hematology and blood transfusion 2012; 28 (2): 89-96.

23. Elda P, Heliana T, Erika B, et al. Immunophenotypic characterization of acute leukemia at a public oncology reference center in Maranhão, northeastern Brazil. Sao Paulo Medical Journal 2011; 129(6).

24. Zheng J, Wang X and Hu Y. A correlation study of immunophenotypic, cytogenetic, and clinical features of 180 AML patients in China. Cytometry Part B Clinica Cytometry 2008; 74(1): 25.

25. Lo Coco F, De Rossi G, Pasqualetti D, et al. CD7 positive acute myeloid leukaemia: A subtype associated with cell immaturity. British Journal of Haematology 1989 ; 73: 480-5.

26. Traweek ST. Immunophenotypic analysis of acute leukemia. American Journal of Clinical Pathology 1993 ; 99: 504-12.

27. El-Sissy AH, El-Mashari MA, Bassuni WY, El-Swaayed AF. Aberrant lymphoid antigen expression in acute myeloid leukemia in Saudi Arabia. Journal of the Egyptian National Cancer Institute 2006 ;18: 244-9.

28. Drexler HG, Thiel E, Ludwig WD. Acute myeloid leukemias expressing lymphoid-associated antigens: Diagnostic incidence and prognostic significance. Leukemia 1993; 4: 489-98.