ORIGINAL RESEARCH PAPER

Volume-7 | Issue-1 | January-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

STUDY OF IMMUNOPHENOTYPIC CHARACTERISTICS OF ACUTE LEUKAEMIA. A HOSPITAL BASED STUDY.

Immunohematology

Dr Bibhash Gogoi Assistant Professor, Department of Pathology, Jorhat Medical College, Jorhat, Assam.

Dr Nitu Mani Khakhlari* Associate Professor, Department of Pathology, Assam Medical College, Dibrugarh, Assam. *Corresponding Author

ABSTRACT

Introduction: Acute leukemias (AL) are a group of neoplastic disorders characterized by proliferation and accumulation of immature hematopoietic cells in peripheral blood (PB) and/or bone marrow (BM). This group of malignancies has varying clinical, morphologic, immunologic and molecular characteristics. AL displays characteristic patterns of antigen expression, which facilitate their identification and proper classification. AL are classified according to their commitment to either the myeloid or lymphoid lineage. Immunophenotyping has become very useful and reliable tool to diagnose and subtype acute leukemia precisely into different subtypes. Immunophenotyping can identify various intracellular and extracellular cell lineage specific markers and categorizes AL into Acute myeloid leukemia (AML), B cell acute lymphoid leukemia (B-ALL), T cell acute lymphoid leukemia (T-ALL) and Mixed Phenotypic Acute Leukemia (MPAL). Our study aimed to analyze Acute leukaemia immunophenotypically.

Methods: We prospectively investigated the phenotype of blast cells from 183 cases of acute leukemia patients using a large panel of monoclonal antibodies by multiparametric flowcytometry.

Results: 183 cases of acute leukemia were analyzed using multiparametric flowcytometry. Out of which 115 cases (62.84%) were Acute Myeloid Leukemia (AML), 49 cases (26.77%) were B cell Acute Lymphocytic Leukemia (B-ALL), 8 cases (4.37%) were T cell Acute Lymphocytic Leukemia (T-ALL) and 11 cases (6.01%) were Mixed Phenotypic Acute Leukemia (MPAL). 43/115 (37.4%) of AML cases, 16/49 (32.7%) of B-ALL cases and 6/8 (75.0%) of T-ALL cases demonstrated aberrant phenotype in our study. 38.26%, 6.08% and 1.7% cases of AML showed further lineage specific differentiation to monocytic, megakaryocytic and erythroid respectively. Most of the cases of megakaryocytic leukemia were from the paediatric age group.

Discussion: In view of the pitfalls in French American British (FAB) classification which was based on morphology, the World Health Organisation (WHO) classification emphasized the importance of immunophenotyping and defined the myeloid and lymphoid malignancies by the antigenic features of the neoplastic cells. Flowcytometry plays a major role in ALL patients to define therapeutically and prognostic groups such as B and T lineage ALL and to distinguish AML–M0 from ALL. Further, flowcytometry aid in diagnosing AML cases as well as defining different subgroups in AML. Thus immunophenotyping by flowcytometry not only helps in categorizing Acute leukemia but gives an immediate prompt diagnosis and providing help in accurate treatment.

KEYWORDS

Acute leukemia, Immunophenotyping, Flowcytometry.

INTRODUCTION:

Acute leukemia (AL) is a clonal hematopoietic stem cells disorder characterized by increase in immature cells (≥20%) in peripheral blood (PB) and/or bone marrow (BM).[1] It is divided into Acute Myeloid Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL) on the basis of morphological, cytochemical and antigenic characteristics. Further sub classification of AML and ALL has been done on the basis of morphology, cytochemistry, immunophenotyping, cytogenetics and molecular studies.[2]

French-American-British (FAB) and recent WHO classifications require help of immunophenotyping in diagnosing and differentiating the acute leukemia into acute myeloid leukemia (AML), B cell acute lymphoid leukemia (B-ALL), T cell acute lymphoid leukemia (T-ALL) and Mixed Phenotypic Acute Leukemia (MPAL). Especially, the diagnosis of FAB types: AML-M0 (undifferentiated), AML-M5 (monocytic), AML-M6 (erythroid) and AML-M7 (megakaryocytic).[3-4]

Multiparameter flowcytometry is the preferred method of immunophenotypic analysis in Acute Leukemia for its ability to analyse large numbers of cells in a relatively short period of time with simultaneous recording of information about several antigens for each individual cell. Evaluation of expression patterns of several antigens, both membrane and cytoplasmic, is necessary for lineage assignment, to detect mixed phenotype acute leukaemia, to detect aberrant phenotypes and allowing for follow-up of minimal residual disease.[5]

MATERIALS and METHODS:

The study was carried out in the DBT Health Care Flowcytometry Laboratory, Department of Pathology, Assam Medical College, Assam, India for a period of four years from June, 2012 to May, 2016. A total number of 183 cases of acute leukemia were included in the study, which were subjected to routine haematological investigations and cytochemistry followed by multiparametric flowcytometry. Diagnosis of acute leukemia was made on routinely stained bone marrow aspiration and blood smears. Immunophenotyping was carried out on bone marrow or peripheral blood.

Complete blood count was done by using Sysmex XS-800i and peripheral blood film stained by Giemsa stain to find the presence of blast cells. A total of 500 cells of WBC were counted and blasts cells over 20% are regarded as acute leukemias. Then whole blood or aspirate samples were prepared by cell Stain-Lyse-Wash method for immunoflourescence staining with different antibodies which were conjugated with fluorochromes (i.e APC H7, PE cy7, FITC, PE, APC and PerCPcy5.5). Cell washing was done with phosphate buffer saline (PBS) (NaH2PO4.2H2O, Na2HPO4 and NaCl). When whole blood is added to the monoclonal antibody reagent, the fluorochrome labeled antibodies in the reagent bind specifically to leucocyte surface antigens. The stained samples were then treated with FACS Lysing solution (NH4CL) which lyses erythrocytes under gentle hypotonic conditions while preserving the leucocytes.

The permeablizing solution containing 15% formaldehyde and 50% diethylene glycol and proprietary permeablizing agent used for intracellular staining of antigens such as MPO, CD79a, CD3 Cytoplasmic and Tdt. Data acquisition and analysis were performed on a FACS Canto 2 flow cytometer (Becton Dickinson, San José, USA) using BD FACS Diva software. Identification of blast cells was performed using side scatter (SSC) versus CD 45 intensity and SSC versus forward scatter (FSC) parameter dot plots. The percentage of gated abnormal population expressing a particular CD marker was analyzed whether expression was positive or negative (> 20% for surface antigen and >10% for cytoplasmic antigen).

Acute leukemia were classified into Acute Myeloid Leukemia (AML), B cell Acute Lymphocytic Leukemia (B-ALL), T cell Acute Lymphocytic Leukemia (T-ALL) and Mixed Phenotypic Acute Leukemia (MPAL).

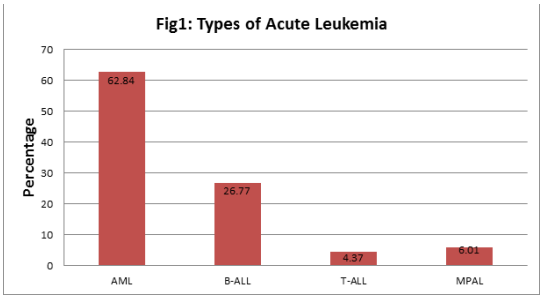
RESULTS:

The study comprises of 113 males and 70 females patients. The age of patients ranged from 2 days to 60 years. Acute leukemia has been classified into AML, B-ALL, T-ALL and MPAL by flowcytometric

immunophenotyping. In the present study, 183 cases of acute leukemia were studied. Out of which, 115 cases (62.84%) of AML, 49 cases (26.77%) of B-ALL, 8 cases (4.37%) of T-ALL and 11 cases (6.01%) of MPAL were found as shown in Table 1 and Figure 1.

Table 1. Types of Acute Leukaemia by Flowcytometric Immnunophenotyping.

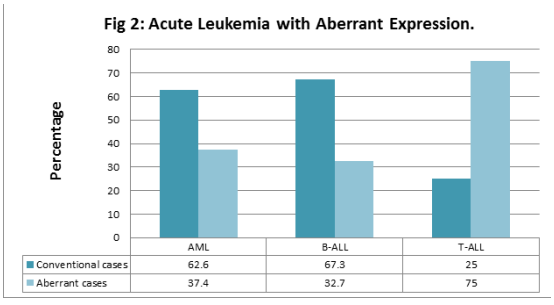
Types	No. of cases (%)
AML	115 (62.84)
B-ALL	49(26.77)
T-ALL	8(4.37)
MPAL	11(6.01)



In our study, 43/115 (37.4%) of AML cases, 16/49 (32.7%) of B-ALL cases and 6/8 (75.0%) of T-ALL cases demonstrated aberrant phenotype in our study as shown in the Table 2 and Figure 2.

Table 2. Acute Leukemia showing Aberrant expression.

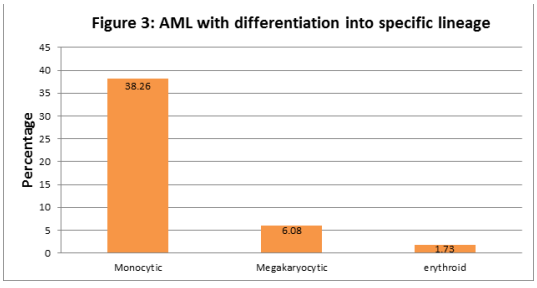
Types	No. of Cases	Conventional cases (%)	Aberrant Cases (%)
AML	115	72(62.6)	43(37.4)
B ALL	49	33(67.3)	16(32.7)
T ALL	8	2(25.0)	6(75.0)



In the present study, 38.26%, 6.08% and 1.7% cases of AML showed further lineage specific differentiation to monocytic, megakaryocytic and erythroid respectively as shown in the Table 3 and Figure 3. Most of the cases of megakaryocytic leukemia were from the paediatric age group.

Table 3. AML with further differentiation into specific lineage

Types	No. of cases (%)
AML with monocytic differentiation	44 (38.26)
AML with megakaryocytic differentiation	7(6.08)
AML with erythroid differentiation	2(1.73)



DISCUSSION:

With the need of sub-classification of leukaemias and their refinement in treatment, diagnosis of leukaemias has become increasingly complicated. For years morphology combined with special staining was the only method for diagnosing acute leukemias. This was used to classify leukaemias according to FAB classification. However this type of classifications had certain limitations and difficulty in reproducibility. They were unable to classify leukaemias on the basis of underlying genetic causes. This called for a more better system of classification which was given by WHO that utilised morphology, genetic information, immunophenotyping, biologic and clinical features to define specific disease entity. Although genotyping with molecular genetic techniques gives an accurate detailed diagnosis, immunophenotyping by flowcytometry gives an immediate prompt diagnosis and helps in accurate treatment.[6]

Multiparameter flowcytometry is an invaluable tool in the diagnosis of AL. It is a technique that provides with the details of the cell characteristics as well as the pathway of cell differentiation. This provides with accurate information of the stage of cell maturation. In this technique the cells are coated with monoclonal antibodies that recognise the antigens present in the cells and give characteristic immunostaining thus defining their cell lineages and helping in the correct diagnosis of leukaemia. Several advances in flowcytometry, including availability of an expanded range of antibodies and fluorochromes, improved gating strategies, and multiparameter analytic techniques, have all dramatically improved our ability to identify different cell populations and recognized phenotypic aberrancies, even when present in a small proportion of the cells analyzed.[7]

Our present study found AML (62.84%), B-ALL (26.77%), T-ALL (4.37%), MPAL (6.01%) which similar to Dalia A et al[8] AML (68.9%), B-ALL (23.1%), T-ALL (7.9%), MPAL (0%) and to Ashish G et al[9] AML (51.8%), B-ALL (35.6%), T-ALL (5.6%), MPAL (6.8%).

Of 115 cases of AML, all cases (100%) expressed CD45 and HLA-DR antigen was present in the majority of AML cases. CD13 was the myeloid lineage antigen most commonly present, followed in frequency by CD33. Of 49 cases of B-ALL, CD19 was the most common antigen found while in T-ALL, CD3 was the most common antigen expressed.

Though CD7 is a T-cell antigen, it is seen to express aberrantly in many cases of AML. In our present study too, CD7 was the most common aberrantly expressed antigen in AML while CD13 was the most common aberrantly expressed antigen in both the types of ALL.

CONCLUSION:

The motivation to institute immunophenotypic studies on acute leukemia was fuelled initially by the desire to improve the characterization of this heterogeneous group of neoplasms. French-American-British criteria and WHO classification now require phenotypic studies for subsets of AML and are also an essential component in the diagnosis of ALL. Furthermore, the existence of mixed-lineage leukemia is essentially dependent on phenotypic studies. In addition to identifying cell lineage in acute leukemia, certain antigenic markers have been associated with patient outcome, and will undoubtedly play a bigger role in prognostication as more cases are studied and clinically significant parameters become clearer. Finally, immunophenotyping can play a role in the evaluation of treatment efficacy, by monitoring blast-associated markers such as CD34, and may also prove to be an important tool in assessing minimal residual disease.

ACKNOWLEDGEMENT:

We acknowledge Department of Biotechnology, Govt. of India. We further acknowledge Miss Rashmi Roy, JRF; Mr Sautom Roy, Data entry operator; Mr Pranjal Saikia and Mr Gautam Saikia, Technician; Mrs Labonya Gogoi, Lab attendant.

Funding: Department of Biotechnology, Government of India.

Conflict of interest: None.

Ethical approval: Ethical approval was given by the institutional ethical committee for conducting the present study.

REFERENCES:

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Stein H., Thiele J, Vardiman JW. (Eds.): WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. IARC Press, Lyon 2008.
2. Jaffe ES, Harris NL, Stein H, Vardiman JW. World Health Organization Classification of

Tumours: Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues, IARC Press, Lyon 2001.
3. Vardiman JW, Harris L, Brunning RD. The world health organization (WHO) classification of the myeloid neoplasms. Blood 2002; 100(7):2292-2302.
4. 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.
5. Zahid Kaleem, Eric Crawford, M. Hanif Pathan, Leah Jasper, Michael A. Covinsky, Lawrence R. Johnson, and Glenda White (2003) Flow Cytometric Analysis of Acute Leukemias. Archives of Pathology & Laboratory Medicine:127(1):42-48.
6. Sengar M, Rai AK, Saxena A, Singh A, Raina V, Seth T. Acute leukemia: Diagnosis improved by flowcytometry in addition to morphology. Asia Pac J Clin Oncol. 2009 Mar 1; 5(1):55–65.
7. Jolanta W, Joanna Kopec S (2008) Standared immunophenotyping of leukemia cells in acute myeloid leukemia (AML). Central Euro J Immunol 33(1):24–32
8. Dalia A. Salem • Sherin M. Abd El-Aziz. Flowcytometric Immunophenotypic Profile of Acute Leukemia: Mansoura Experience. Indian J Hematol Blood Transfusion (Apr-June 2012) 28(2):89–96.
9. Ashish Gupta, Abhijit Pal, Silas Supragya Nelson. Immunophenotyping in Acute Leukemia: A Clinical Study. International Journal of Scientific Study. (August 2015) 3(5):129-136.
10. Lo Coco F, Dc Rossi G, Pasqualetti D. CD7 positive acute myeloid leukemia: A subtype associated with cell immaturity. Br J Haematol 1989;73:480-485.