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Diagnostic Accuracy of Acute Leukaemia by Flow Cytometry In Comparison To Morphological Diagnosis – A Study

Dr. Ravi Murmu[1], Dr. R. K. Srivastava[2], Dr. Saurav Banerjee[3], Dr. Sunil Kumar Mahto[4], Dr. Anu Singh[5]
Junior resident[1], Professor[2], Tutor[3], Asst. Professor[4], Junior Resident[5]
Department of Pathology, Rajendra Institute of Medical Sciences, Ranchi, India.

Abstract: *The objective of this study was to compare morphological and flow cytometric results of patients previously diagnosed with acute leukaemias. This study was conducted for a period between May 2013 to November 2015. The study was done in 51 acute leukaemia patients attending the department of pathology, RIMS, Ranchi. Cases were selected according to the bone marrow and peripheral blood study and were later analysed by flow cytometry studies. 22 patients were diagnosed ALL, 27 as AML and remaining 2 as acute biphenotypic leukaemia. Concordance between morphology and Flow cytometry studies for ALL, AML and BAL was found to be 84%, 89% and 50% respectively.*

Keywords: *Morphology, Flow cytometry, Acute Leukaemia*

I. Introduction

With the need of subclassification of leukaemias and their refinement in treatment, diagnosis of leukaemias have 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 flow cytometry gives an immediate prompt diagnosis providing help in accurate treatment.

Historically, leukemia has been classified initially into four groups based on a combination of clinical presentation and morphologic appearance of malignant cells: Acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML, also named acute non lymphocytic leukemia ANLL), chronic myelogenous leukemia (CML) and chronic lymphocytic leukemia (CLL).

Morphological diagnosis for leukemias may sometimes be not correlating with flow cytometry diagnosis which in the present scenario is ultimate. Flow cytometer is a technique that provides with the details of the cell characteristics as well as the pathway of cell differentiation. This provides with an accurate information of the stage of cell maturation. In this technique the cells are coated with monoclonal antibodies that recognises the antigens present in the cells and give characteristic immunostaining thus defining their cell lineages and helping in the correct diagnosis of leukaemia.

II. Materials And Methods

Study Area – Department of Pathology, RIMS, Ranchi.

Study Population - All the patients who were diagnosed to have acute leukaemias on bone marrow aspirate/peripheral blood smear examination.

Sampling Procedure – All the patients who clinically presented with features of acute leukaemia without any prior chemotherapy were investigated for complete blood count. The suspicious patients were further scrutinized for bone marrow evaluation. The patients diagnosed with acute leukaemias were further subjected to immunophenotyping by Flow cytometer. A detailed history, physical examination and socioeconomic background were recorded at the time of selection of the patients Cytomorphological classification of acute leukaemias – After the patients were diagnosed as acute leukemias, they were further divided into 2 categories according to their cell morphology that is AML and ALL. Categorisation was done based on the French-American-British(FAB) system according to which AML was subdivided into 8 subtypes(M0 to M7) and ALL into 3 subtypes(L1 to L3).Immunophenotyping – Immunophenotyping was done by flow cytometry. 2 ml of blood or bone marrow aspirates were collected. After maintaining quality control such as checking the pressure and vacuum gauges, checking the optical alignment, fluorescence standardization and testing the integrity of antibody, the samples were studied for expression of specific markers.

The panel of monoclonal antibodies used in flow cytometry were :-
For T lymphocytes lineage : cyCD3, CD7, CD5, Tdt
For B lymphocytes lineage : CD10, CD19, CD79a, HLA-DR
For Myeloid subsets : CD13,CD33, CD117, anti MPO, Glyophorin A, CD14, CD64
Non lineage restricted : CD34
Glycophorin A was used in cases in suspected acute erythroid leukaemia (M6).

Table 1. 6 Colour Acute Leukaemia Panel (Basic) in present study

Flurochromes	Tube 1	Tube 2	Tube 3 (cytoplasmic)	Tube 4	Tube 5 (blank)
FITC	CD 7	CD 5	MPO	---------	---------
PE	CD 117	CD 13	CD 79a	CD 64	---------
PCP C5.5	CD 34	CD 34	CD 34	CD 34	---------
PC 7	CD 19	HLA-DR	Cy CD 3	---------	---------
APC	CD 10	CD 33	Tdt	CD 14	---------
APC H7	CD 45	CD 45	CD 45	CD 45	---------

III. Result And Analysis

The present study comprises of 51 cases suspected of acute leukaemias that attended the Department of Pathology, RIMS, Ranchi during a period of May 2013 to December 2015. The study comprises of 34 males and 17 females patients. The age of patients ranged from 15 days to 60 years. 15 patients were below 14 years of age. Initially the patients suspected of acute leukaemias were screened for bone marrow examination. On this basis they were classified into 3 groups – ALL, AML and Acute Leukaemia (not categorized). The final diagnosis was given after flow cytometric analysis.

Based on the study, the number of AML cases were 27, ALL cases were 22 and 2 were diagnosed as mixed phenotype.

Among ALL cases, 22 were diagnosed as B-Cell ALL and the remaining 5 as T-Cell ALL. B-Cell ALL were further subclassified into Pro-B (2 cases), Common-B (12 cases), Pre-B(2 cases), and Mature-B (1 case). 18 cases were CALLA positive.

Table 2. Table showing immunophenotyping f ALL group :-

Type of ALL	CD34	HLA-DR	CD10	CD19	CyCD79a	Tdt	CD7	CD5	CyCD3
Pro-B	2/2	2/2	2/2	2/2	2/2	2/2	-	-	-
Common B	11/12	12/12	12/12	12/12	12/12	11/12	-	-	-
Pre-B	1/2	2/2	2/2	2/2	2/2	0/2	-	-	-
Mature B	0/1	1/1	0/1	1/1	1/1	0/1	-	-	-
T-Cell ALL	4/5	5/5	2/5	0/5	0/5	4/5	4/5	5/5	5/5

Table 3. Table showing immunophenotyping of AML group :-

Type of AML	CD34	HLA-DR	CD117	CD13	CD33	MPO	CD14	CD64
M0	5/5	5/5	5/5	5/5	5/5	0/5	0/5	0/5
M1/M2	14/14	14/14	14/14	14/14	14/14	12/14	0/14	0/14
M3	0/1	0/1	0/1	1/1	1/1	1/1	0/1	0/1
M4/M5	4/6	6/6	3/6	6/6	6/6	6/6	6/6	6/6
M6	-	-	-	-	-	-	-	-
M7	0/1	0/1	0/1	1/1	1/1	0/1	0/1	0/1

2 cases were diagnosed as biphenotypic acute leukaemia as the blasts were positive for both ALL and AML markers.

Table 4. Table showing immunophenotyping in biphenotypic leukaemia :-

	CD34	HLA-DR	CD117	CD13	CD33	MPO	CD14	CD64	CD10	CD19	CD79a
Case 1	+	+	-	-	-	-	+	+	+	+	-
Case 2	+	+	-	+	+	+	-	-	+	+	+

Table 5. Table showing correlation between Morphological Diagnosis and Flow cytometry

Type of Acute Leukaemia	Morphological diagnosis	Flow Cytometric diagnosis	Final Diagnosis	Concordance
ALL	26	22	22	84%
AML	24	27	27	89%
BAL	1	2	2	50%

IV. Discussion

Prior to the invent of flow cytometry, we were completely dependent upon morphology and special stains. Present study intends to find out what percentage of cases were said to be misdiagnosed before the use of flow cytometry. On comparison of Morphology and Flow Cytometric diagnosis, it was found that there was a complete concordance in 81% of cases, partial concordance in 3% of cases and non-concordance in 13% of cases between both modalities. Flow cytometry was particularly found useful in cases where morphology failed to give any diagnosis. There were 5 cases of M zero which were difficult to differentiate between AML and ALL, were confirmed by flowcytometry. Flow cytometry also proved to be very useful in those 2 cases of biphenotypic acute leukaemias, the accurate diagnosis which were likely to be missed. Furthermore it helped in subtyping the disease which helped clinicians to appropriately treat the patients in time.

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