



## Comparison of Diagnostic Accuracy of Acute Leukaemia by Flow Cytometry with Cytomorphology

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### Abstract

The objective of the study was to compare morphological and flow cytometry results of patients diagnosed with acute Leukaemia. This study was conducted for a period of 2 years prospectively between December 2019 to December 2021. A total of 61 Leukaemia patients attending the department of pathology GMC Jammu were identified. Cases were selected according to the bone marrow and peripheral blood study and were later analyzed by flow cytometry. 28 patients were diagnosed with ALL, 30 patients with AML, 1 was identified as Bi-phenotypic acute Leukaemia and 2 as unclassified. Concordance between morphology and flow cytometric studies for ALL, AML and BAL was found to be 80%, 89% and 50% respectively.

**Keywords:** Morphology, Flow cytometry and Acute Leukaemia

### Introduction

Acute Leukaemia is a heterogenous group of hematologic malignancies affecting blood and bone marrow cells. The disease follows mostly a poor prognosis but some specific types have better prognosis with specific therapeutic options. For years, morphology combined with special staining was the only method for diagnosis acute Leukaemia and based on these two parameters, Leukaemias were classified according to FAB classification. Based on this classification, it was unable to classify Leukaemias on the basis of underlying genetic causes. Therefore a more better system of classification was given by WHO that utilized morphology, genetic information, immunophenotyping, biologic and clinical features to define specific disease entity. Although, genotyping with molecular genetic techniques give a accurate detailed diagnosis, immuno-phenotyping by Flow cytometry gives an immediate prompt diagnosis providing help in accurate treatment.

This study was carried to see various types, subtypes, aberrant antigenic expression pattern of acute Leukaemia in patients (children as well as adults) in Jammu Region of J&K UT and also to compare the results between Flow cytometry and microscopic morphology.

**Materials and Methods:** The present study is a prospective study conducted from December 2019 to December 2021. A total of 61 patients of acute Leukaemias were included in the study with age ranging from 15 days to 60 years. Informed written consent was taken from all patients and their guardians. Blood and bone marrow reports were collected from department of Pathology (haematology section) GMC Jammu. Morphological features of all the cases were reviewed. The patients diagnosed with acute Leukaemias were further subjected to Immuno-phenotyping by Flow cytometer. Based on the cell morphology patients of acute Leukaemias were classified as having AML/ALL. Categorization was done based on the

French-American-British(FAB) system, according to which AML was further subdivided into 8 sub types (M<sub>0</sub> to M<sub>7</sub> ) and ALL into L<sub>1</sub> to L<sub>3</sub> . Immuno-phenotyping was done by Flow cytometry. 2 ml of blood or bone marrow aspirates were collected in a EDTA tube and immediately transported to the Lab for Immuno-phenotyping. The panel of monoclonal antibodies used in flow cytometry were-

For T-lymphocytes lineage- CyCD<sub>3</sub>, CD<sub>7</sub> and CD<sub>5</sub>

For B-lymphocytes lineage- CD<sub>10</sub>, CD<sub>19</sub> and CD<sub>79a</sub>.

For Myeloid subsets- CD<sub>13</sub>, CD<sub>33</sub>, CD<sub>117</sub>, Anti MPO, Glycophorin-A, CD<sub>14</sub>, CD<sub>64</sub>

Precursors- Tdt, HLA-DR and CD<sub>34</sub>

**Result:** The present study comprises of 61 patients suspected of Acute Leukaemias that attended the Department of Pathology GMC Jammu, during a

period of December 2019 to December 2021. The study comprises 61 patients with age ranging from 15 days to 60 years. 20 patients were below 14 years of age, initially the patient suspected of acute leukaemias were screened for bone marrow examination. On this basis they were classified into three groups- KLL, AML and Acute Leukaemia (not categorized). The final diagnosis was given after flow cytometry analysis. Based on the study the number of AML cases were 30, 28 were of ALL type and 3 were diagnosed as mixed phenotype.

Flow cytometry was particularly found useful in cases where morphology failed to give any diagnosis. By concordance, we mean similar results were seen between morphology and flow cytometry. Discordance was seen where flow cytometry was essential for lineage assessment.

**Table 1 :Comparison between Cyto-morphology and Flow cytometry reults:**

Cytomorphology	Number	Flow Cyto-metry	Number
AML	30	AML	26
		B-ALL	02
		T-ALL	0
		MPAL	01
		Undetermined	01
ALL	28	B-ALL	17
		T-ALL	08
		AML	02
		MPAL	01
		Undetermined	0
MPAL	01	B-ALL	01
Unclassified	02	B-ALL	01
		T-ALL	01

Unclassified- By cyto-morphology, presence of only Blast Cells not specified as Myloblast/Lymphoblast.

Undetermined- Blast count less than 20% in the sample.

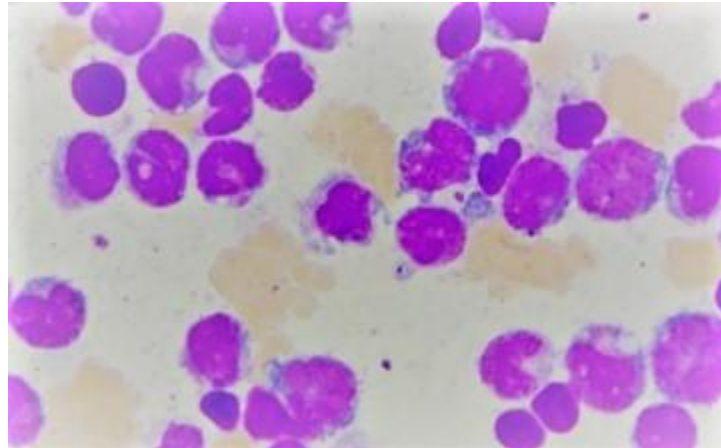
MPAL(mixed phenotype acute leukaemia).

**Table 2: Concordance and discordance between results of cytomorphology and flow cytometry**

	Number	Percentage
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Complete concordance	51	83.6
Partial Concordance	2	3.27
Discordance	8	13.11

**Figure 1:**



**Discussion:-** Acute leukaemias are a heterogeneous group of disorders characterized by the rapid expansion of a malignant clone of early hematopoietic progenitors that ultimately replace the normal bone marrow tissue resulting in marrow failure<sup>1,2</sup>

Prior to the invention of flow cytometry, we were completely dependent on 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<sup>3</sup>

On comparison morphology and flow cytometric diagnosis it was found that there was a complete concordance in 83.6% of cases, partial concordance in 3.27% of cases and discordance 13.11% of cases. Flow cytometry was particularly found useful in cases where morphology failed to give any diagnosis<sup>6</sup>.

By concordance we mean similar results were seen between morphology and flow cytometry. Discordance was seen where flow cytometry was essential for lineage assessment and partial concordance was considered when blast type could not be confirmed morphologically or in MPAL where additional information regarding the phenotype of blast lineage was found by flow cytometry<sup>4,5</sup>. Therefore, it is concluded that flow cytometric immunophenotyping could precisely delineate

different form of acute leukaemia and is especially important for confirming cytomorphology diagnosed acute lymphoblastic leukaemias.

Bone marrow smear yield >80% of blasts showing morphology of myeloblasts, promyelocytes, myelocytes, and many monoblasts with open chromatin and distinct nucleoli. Features suggestive of AML-M4 subtype. The patient was advised immunocytochemistry.

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