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Utility of flow-cytometer in the diagnosis and classification of acute leukemia: A one year study at tertiary care hospital, Indore

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Abstract: Leukemia, the most common childhood cancer, is predominantly acute, with acute lymphoblastic leukemia (ALL) and acute myelogenous leukemia (AML) being the primary subtypes. The incidence rates for ALL and AML are 1.5 and 2.7 per 100,000 population, respectively. The objective of this study conducted at the Pathology department of M.G.M. Medical College in Indore was two-fold: firstly, to classify acute leukemia into ALL or AML using flow cytometry, and secondly, to assess the diagnostic value of specific markers (Anti-MPO, CD34, HLA-DR, CD45, CD79a, CD3) in acute leukemia diagnosis. The study involved evaluating cases through routine peripheral smear and subsequently analyzing relevant cases on a flow cytometer. The results revealed that 23 cases (77%) were diagnosed as ALL, while 7 cases (23%) were identified as AML. Among the ALL cases, 19 were classified as B-cell ALL (64%), whereas 4 were T-cell ALL (13%). In conclusion, the use of multicolor flow cytometry enables simultaneous assessment of multiple antigens, facilitating more precise categorization of cell populations. Furthermore, flow cytometry plays a crucial role in differentiating AML from ALL and its subtypes.

Keywords: Acute Leukemia; Acute Myeloid Leukemia; Acute Lymphoblastic Leukemia; Flowcytometry.

1. Introduction

Leukemia is a malignant neoplasm of hematopoietic stem cell characterized by diffuse replacement of bone marrow by neoplastic cells OR leukemia is a clonal malignant disorder characterized by proliferation of abnormal immature cells in bone marrow and peripheral blood with impaired production of normal blood cells [1]. Acute leukaemia - Acute leukemia can be diagnosed when blasts constitute 20% or more of the nucleated cells in a patientss peripheral blood [2]. Two major categories of acute leukemia are recognized: Acute Lymphoblastic Leukemia (ALL) subdivided into B- and T-cell precursor ALL and Acute Myeloid Leukemia (AML) characterized by an overproduction of immature myeloblasts or leukemic blasts [3].

Leukemia constitutes as the most common childhood cancer, more than 95% of which are acute [4]. The total age-adjusted incidence of leukemia, including both acute and chronic forms, is 9.6 per 100,000 population [5]. Whereas the overall incidence of acute lymphoblastic leukemia (ALL) is 1.5 per 100,000 and of acute myelogenous leukemia (AML) is 2.7 per 100,000 population [6]. Globally, the leukemia disease burden is higher among males than females. Mortality was also higher in males (4.2 per 100,000) than females (2.8 per 100,000) [7].

The diagnosis of acute leukemia requires the identification of an expanded population of hematopoietic progenitors having the morphologic appearance of blasts [8]. Flow cytometric immunophenotyping can assist in establishing a diagnosis of acute leukemia by more objectively [9]. It confirm both the presence of expanded hematopoietic progenitors and demonstrating immunophenotypic abnormalities on the progenitors that are different from the alterations found during typical bone marrow regeneration [9]. Various CD markers are used now a days some of them are listed in Table 1.

Table 1. Shows CD markers for different cell lineages

Different Cell Lineages	CD Markers
Stem cell/ Hematopoietic precursors	CD34, HLA-DR, Terminal deoxynucleotidyl transferase/TdT
Myeloid	cMPO, CD13, CD33, CD117, CD15
B lymphoid markers	CD19, CD10, CD20, CD22, cCD79a
T lymphoid markers	CD1a, CD2, CD4, CD8, CD3, CD5

Identification of the leukemia cell line, maturation stage, and detection of residual disease are all crucial aspects of leukemia cell flow cytometry. There are numerous diverse categories known to have predictable prognoses and need for treatments [10]. The relative survival rate for all ages and 5-year survival rate after diagnosis is about 29.5%.

2. Material and methods

This study was conducted in Department of Pathology, Mahatma Gandhi Memorial Medical College and M.Y. Hospital, Indore, Madhya Pradesh, India. Approval was obtained from the departmental scientific committee and the institutional ethical committee for the study. The study duration was one year. Minimum sample size for the study was 30 cases. In our study CD markers are used to identify the suspected cases of acute leukemia and to sub classify further with the help of flow cytometry. This test can just as readily be undertaken on whole blood as on cells separated on a density gradient. Venous blood samples must be taken using sterile tubes containing an EDTA salt as the anticoagulant. The use of other anticoagulants is not recommended. For the processing of sample, equipments required some of them are listed below:

- Flow cytometer - Navios type having 8 color two laser (red and blue).
- Sampling tubes and material necessary for sampling.
- Automatic pipettes with disposable tips for 20, 100 and 500 pl.
- Plastic haemolysis tubes.
- Calibration beads: Flow-Set Fluorospheres.
- Red cell lysis reagent with washing stage after lysis.
- Fixation reagent
- Isotypic control: A mixture of IgG2a-FITC and IgG1- PE, both from mouse.
- Colour Compensation adjustment reagent
- Control blood: IMMUNO-TROLTM Control Cells.
- Buffer
- Centrifuge
- Automatic agitator (Vortex type)

Suspected cases of acute leukemias on hematological findings were included and already diagnosed cases of acute leukemias on treatment or follow-up and Chronic Leukemias (i.e. CML, CLL) were excluded.

2.1. Storage and Stability

If you need to add numbers, use following

1. The conjugated liquid forms must be kept at between 2 and 8°C and protected from light before and after the vial has been opened.
2. Stability of closed vial: see expiry date on vial.
3. Stability of opened vial: the reagent is stable for 90 days.

2.2. Precautions

1. Do not use the reagent beyond the expiry date.
2. Do not freeze.
3. Let it come to room temperature (18 – 25°C) before use.
4. Minimize exposure to light as PE is sensitive to light.
5. Avoid microbial contamination of the reagents, or false results may occur.
6. Antibody solutions containing sodium azide(NaN3) should be handled with care. Do not take internally and avoid all contact with the skin, mucosa and eyes.

7. All blood samples must be considered as potentially infectious and must be handled with care (in particular: the wearing of protective gloves, gowns and goggles).
8. Never pipette by mouth and avoid all contact of the samples with the skin, mucosa and eyes.

3. Results

The distribution of acute leukemia cases based on age revealed that 13 cases were in the age group of 5-10 years, 9 cases were in children below 5 years, 6 cases were in the age group of 10-15 years, and the fewest cases (2) were observed in the age group of >15 years (Table 2).

Among the total 30 cases, 19 were classified as B-cell ALL, 7 as AML, and 4 as T-cell ALL (Table 3). Flow cytometric analysis further supported these findings (Table 4). The immunophenotyping results showed that 19 cases were identified as B-cell ALL, 7 cases as AML, and 4 cases as T-cell ALL (Table **??**).

The peripheral smear examination revealed blast cells with a high nucleus-to-cytoplasm ratio, clumped chromatin, indented nuclear membrane, inconspicuous nucleoli, and scant cytoplasm, consistent with acute leukemia (Figure 1). Additionally, the peripheral smear displayed blasts with a size 2 to 2.5 times larger than small mature lymphocytes, characterized by opened-up chromatin, irregular nuclear membrane, prominent 2 to 3 nucleoli, and scant to moderate cytoplasm, indicative of Acute Myeloid Leukemia (Figure 2). Immunophenotyping by flow cytometry in a case of B-cell ALL revealed positive HLADR, CD34, and CD79a expression, along with Anti-MPO (Figure 3).

Table 2. Age-Wise Distribution of Cases

Age Groups	**Total Number Of Acute Leukemia Cases**	**ALL Cases**	**AML Cases**
< 05 years	9	9	0
05 - 10 years	13	8	5
10 - 15 years	6	4	2
> 15years	2	2	0
TOTAL	30	23	7

Table 3. Distribution of cases as per immunophenotyping

Diagnosis	Total cases	CD34	CD45	HLA-DR	ANTI-MPO	CD79a	CD3
B-cell ALL	19	12	19	18	0	19	0
T-cell ALL	4	4	4	0	0	0	4
AML	7	6	7	6	7	0	0
Total	30						

Table 4. Flow cytometric findings

e Findings	Number of cases	Percentage
ALL(B-cell)	19	64
ALL(T-cell)	4	13
AML	7	23
Total	30	

Table 5. Distribution of Cases as Per Immunophenotyping

Diagnosis	Total cases	CD34	CD45	HLADR	ANTIMPO	CD79a	CD3
B-cell ALL	19	12	19	18	0	19	0
T-cell ALL	4	4	4	0	0	0	4
AML	7	6	7	6	7	0	0
Total	30						

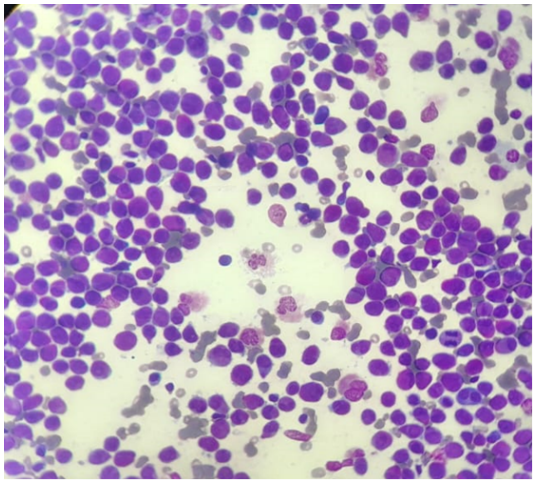

Figure 1. (100x view) Peripheral smear showing blast cells with high N/C ratio, clumped chromatin, indented nuclear membrane, inconspicuous nucleoli and scant amount of cytoplasm. Suggestive of acute Leukemia

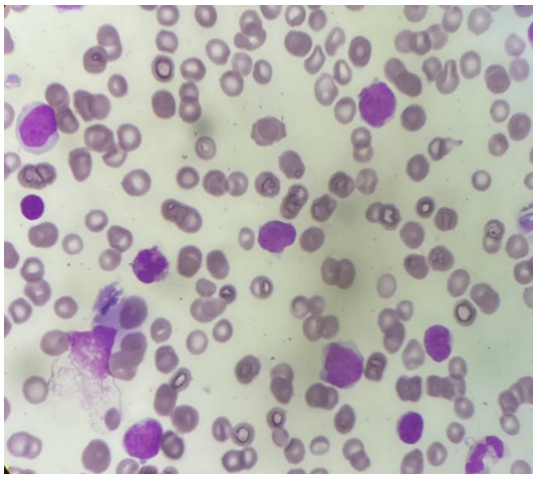

Figure 2. PS showing blast having size 2 to 2.5 times the size of small mature lymphocyte having opened up chromatin, irregular nuclear membrane, prominent 2 to 3 nucleoli and scant to moderate amount of cytoplasm suggestive of Acute Myeloid Leukemia

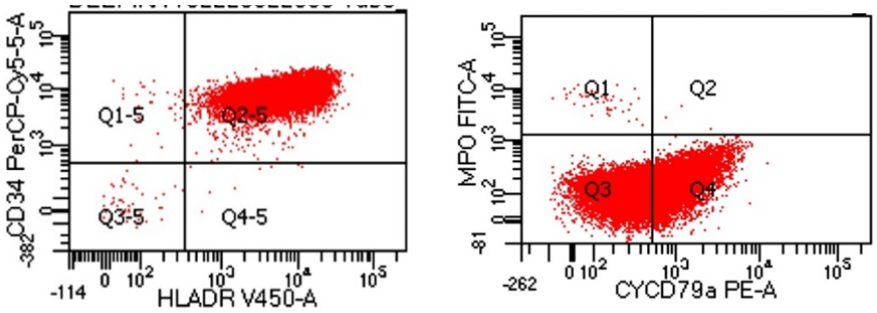

Figure 3. Diagrammatic representation of immunophenotyping by flow cytometry in a case of B-cell ALL: HLADR+, CD34+, CD79a+, Anti-MPO

4. Discussion

Multicolor flowcytometry it provides the opportunity to evaluate multiple antigens simultaneously hence making it possible to categorize various cell populations in a more precise manner. Flowcytometry also helps in differentiating AML from ALL and its subtypes. In our department this thesis work was carried out over

30 cases during 12 months duration. According to a related study, 38 (73%) of the 52 cases were male and 14 (27%) were female, with a male: female ratio of 2.7:1. These findings are like ours, as we have also discovered a male preponderance in our thesis investigation.

We have observed that among acute lymphocytic leukemia the most common type is B ALL than T ALL as out of 23 cases of ALL 19 were diagnosed as B ALL on flowcytometry remaining 4 as T ALL which is in concordance with the study of Dalia A et al done in 2011 where they have found that out of 51 cases of ALL 38 cases (74.5%) with B-ALL and 13 cases (25.5%) with T-ALL [10].

In one of the studies among the AL cases, 68.9% were classified as AML while 31.1% classified as ALL. The high percentage of AML may be due to the large number of adults involved in their study (65.8%) on the contrary in our study we have found more cases of ALL then AML i.e. 23 cases of ALL and 7 cases of AML this is justified as we have encountered more number of young adults and children's in our study [11].

Rogelio Paredes-Aguilera et all in his study discussed that cyCD79a [12–15] and cyCD22 [16–20], cyCD3 [18–23], and cyMPO [24] are the earliest identifiable, specific B, T, and myeloid markers, which are expressed in virtually all cases of B and T cell ALL and in all subtypes of AML, similar observation are found in other studies. This was in concordance with present thesis work as we have also chosen the above markers for diagnosis of acute leukemia cases.

5. Conclusion

At the end it was found that leukemia was the 15th most diagnosed cancer and11th leading cause of cancer mortality worldwide. Hence flow cytometer provides a direct assessment of the various surface antigen expression on hematolymphoid neoplasm which helps in rapid and early diagnosis of the disease and will in turn give the patients a better life and less side effects.

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Conflicts of Interest: "Authors declare no conflict of interests."

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