

## A STUDY OF CLINICO-HEMATOLOGICAL PROFILE IN ACUTE LEUKEMIA WITH CYTOCHEMICAL CORRELATION

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### Abstract

**Background:** Acute leukemia is characterized by mutation in hematopoietic stem cells that block differentiation and accumulation of more than 20% of leukemic blast cells in the bone marrow. Diagnosing the type and sub types of leukemia is very important as the therapy, prognosis and survival rate changes with each type and sub type. Aim of this study in acute leukemia is to study the clinicopathological profile, to distinguish acute lymphoid leukemia from acute myeloid leukemia and to sub classify acute myeloid leukemia using cytochemical stain. **Materials and Methods:** A total of 129 cases were included in the study. For all cases, detailed clinical history obtained. Complete blood count, peripheral smear study, bone marrow examinations, cytochemical stains were done and analysed for diagnosis. Flow cytometry was done when needed. **Result:** Among the 129 cases studied, AML was more common than ALL. The most common leukemia in children was acute lymphoblastic leukemia (59.45%) and in adults, acute myeloid leukemia (82.22 %). Males slightly predominate in both types of leukemia. Fatigue was the most common physical symptom. Pallor was the most common physical sign followed by splenomegaly, hepatomegaly and lymphadenopathy. Anaemia was the most common hematological abnormality followed by thrombocytopenia and leukocytosis. In subtyping of acute lymphoblastic leukemia, ALL-L2 was more common and in AML, M2 was the most common subtype. In the present study, morphology alone showed a diagnostic accuracy of 59.59% in ALL and 80% in AML. Morphology combined with cytochemical stains showed an accuracy of 78.37% in ALL and 87.77% in AML. Only 13.17% of cases required immunophenotyping for classification and subtyping of acute leukemia cases. **Conclusion:** Morphology combined with cytochemical stains helped in accurate typing of acute leukemia. But flow cytometry was required for ambiguous cases.

## INTRODUCTION

### Acute Leukemia Definition

Acute leukemias are heterogenous group of malignancies that result from malignant transformation of immature hematopoietic cells followed by clonal proliferation and accumulation of transformed cells. They are characterized by aberrant differentiation and maturation of malignant cells, with maturation arrest and accumulation of more than 20% of leukemic blast cells in the bone marrow.<sup>[1]</sup>

Laboratory diagnosis of acute leukemia is based on morphology in peripheral blood smear and bone marrow, cytochemistry, cytogenetic analysis and immunophenotyping. The cytochemical stains employed in acute leukemia are Myeloperoxidase,

Sudan Black B, Non Specific Esterase and Periodic Acid Schiff. The main aim of cytochemical studies in acute leukemia is to diagnose and classify acute leukemia and to subclassify acute myeloid leukemia. Morphology and cytochemistry together diagnoses 80-90% of acute leukemia.

With recent advances in molecular biology and treatment modalities, it is essential to subtype the leukemia to institute a specific chemotherapy and to assess the prognosis

### Cytochemical Stains

Cytochemical stains are performed on peripheral blood films, bone marrow aspirates and also touch preparations from bone marrow biopsies.

With the advent of flow cytometry and other ancillary studies, the cytochemical stains use is decreased.

### Sudan Black B

It stains intracellular lipid and phospholipids. It gives positive staining in granulocytic cell, weak staining in monocytes and no staining of lymphocytes.

It even stains older blood or bone marrow smears in which MPO cannot be performed.

### Myeloperoxidase

It is located in primary granules of neutrophils and secondary granules of eosinophils. It acts by Oxidation of 3-amino-9-ethylcarbazole or 4-chloro-1-naphthol substrate in the cell to form a brown coloured precipitate.

Monocytic lysosomal granules are faintly positive. MPO helps to differentiate a myelogenous or monocytic leukemia from acute lymphoblastic leukemia.

Blasts in AML show granular positivity in subtypes M1, M2, M3 and M4, while ALL blasts are negative for Myeloperoxidase.

### Periodic Acid Schiff

It oxidizes 1-2 glycol groups to produce stable dialdehydes which gives a red reaction product when exposed to Schiff's reagent. PAS stain detects intracellular glycogen and neutral mucopolysaccharides.

Lymphoblast show variable PAS block positivity granules. It shows block like positivity in clear cytoplasmic background which is seen in blast of ALL. In ERYTHROLEUKEMIA, there is intense diffuse cytoplasmic positivity.

### Flow Cytometry

It employs a fluid stream to carry cells through a counter. It evaluate multiple parameters of individual cells by measuring the characteristics of light they scatter or the photons they emit through light source.

Nowadays, FCM is a common ancillary test used when hematology malignancy is suspected. It allows rapid and accurate analysis of lymphoma and leukemia and T-cell subsets. In acute leukemia, FCM provide important prognostic information and detection of minimal residual disease.

T-CELL markers	CD2, cCD3, CD4, CD5, CD7, CD8 and CD1a
B-CELL markers	CD10, CD19, CD20, CD22 and cCD79a
MYELOID CELL markers	CD13, CD33, CD117, CD15 and cMPO
MONOCYTE markers	CD14, CD64 and CD11b, CD11c / LYSSYME
Non-specific lineage pan-leucocyte	CD45
Stem cell / hematopoietic precursor	CD34, HLA-DR
Natural killer cells	CD56

## MATERIALS AND METHODS

The study sample included 129 cases of Acute Leukemia. Newly diagnosed cases of acute leukemia irrespective of age and gender were

included and already diagnosed cases under treatment were excluded.

A detailed history and physical examination findings pertaining to leukemia was obtained in each case. Blood samples collected in 2ml of K2EDTA vacutainer were used for complete haemogram and peripheral smear preparation. Measurement of hemoglobin, total leucocyte count and platelet count were done using sysmex analyzer and cross checked by peripheral smear examination.

Peripheral smears were stained by Leishman's stain. Bone marrow smears were stained by Giemsa and Leishman stain. Smears were examined for blast cell morphology.

Cytochemical features of the blast cells were analysed using cytochemical stains such as Myeloperoxidase, Sudan Black B and Periodic Acid Schiff stain.

## RESULTS

Incidence of acute leukemia was 1.15 % (129) of the peripheral smear received. Out of 129 cases, 37 cases were acute lymphoblastic leukemia, 90 cases were acute myeloid leukemia, 1 case was acute undifferentiated leukemia and 1 case was biphenotypic leukemia.

### Age distribution

In the present study on acute leukemia, the mean age group affected was around 35 years, with minimum age being 2 years and maximum age being 70 years of age.

19.7% of acute leukemia cases occurred in children, 15.7% of acute leukemia cases occurred in adolescents between 13-20 years. 64.6% of acute leukemia cases occurred in adults between 20-70 years.

### Gender distribution

Out of 129 cases studied, 66 cases (52%) were male and 61 cases (48%) were female. Males are more commonly affected than females.

### Leukemia distribution

The most common leukemia was acute myeloid leukemia (70.9%), followed by acute lymphoblastic leukemia (29.1%).

ALL was more common in children, accounting for 45.94% of the cases followed by adolescents accounting for 32.43% of cases and 21.62% in adults.

AML was common in adults, accounting for 82.22 % . In adolescents and children, it accounted for 8.88 % of cases. In ALL, 54.05% cases are male and in AML, 51.11% of cases are Male. Both AML and ALL are common in males.

### Clinical symptoms

Fatigue was the most common symptom in both all (88.88%) and AML (77.52%), followed by fever.

There was no statistically significant difference in clinical symptoms between AML and ALL.

### Clinical signs

Signs	ALL	AML
Pallor	77.77%	87.2%
Splenomegaly	36.11%	43.82%
Hepatomegaly	33.33%	30.33%
Lymphadenopathy	22.22%	26.46%
Mediastinal mass	5.55%	1.12%

Pallor was the most common sign in both ALL (77.77%) and AML (87.2%), followed by splenomegaly, hepatomegaly, lymphadenopathy and mediastinal mass. There was no statistically significant difference between clinical signs of AML and ALL.

### Hemoglobin level in leukemia types

In ALL and AML, anemia was more common. Further, most of the ALL and AML cases presented with severe anaemia.

### WBC level in leukemia types

24.32% of ALL cases and 13.33% of AML cases showed hyperleukocytosis. Majority of cases presented with WBC count between 12,000 and 50,000 per cu.mm – 40.5% of ALL and 64.4% of AML cases

### Platelet level in leukemia types

Mild thrombocytopenia (37.83%) was more common in ALL and severe thrombocytopenia was more common in AML (38.88%). Severe thrombocytopenia was more common in AML (38.88%) than ALL (29.72%).

### Blast in leukemia types

In ALL, all cases showed more than 20% blast in peripheral smear. In AML, 94.4% of cases showed more than 20% blast in peripheral smear.

### ALL distribution among subtypes

In ALL, L2 subtype (41.66%) was more common than L1 subtype (27.7%).

In adults, L2 subtype (40%) was more common and in children L1 subtype (80%) was more common.

### AML distribution among sub types

M2(46.67%) was more common among AML subtypes followed by M3, M1, M4, M5 and M6.

### AML subtype distribution among age groups:

AML	Age		
	0-12 years	13-20 years	20-90 years
M0	0(0.0%)	0(0.0%)	0(0.0%)
M1	1(5.3%)	2(10.5%)	18(84.2%)
M2	4(9.5%)	4(9.5%)	34(81.0%)
M3	1(4.5%)	2(9.1%)	19(86.4%)
M4	0(0.0%)	0(0.0%)	2(100.0%)
M5	2(100.0%)	0(0.0%)	0(0.0%)
M6	0(0.0%)	0(0.0%)	1(100.0%)
M7	0(0.0%)	0(0.0%)	0(0.0%)

### Cytochemical Stains

The SBB staining was positive in 75 cases out of 90 AML cases (83.33%). The percentage of sensitivity, specificity and accuracy in SBB are 83.33%, 100% and 88.18% respectively.

The MPO staining was positive in 76 cases out of 90 AML cases (84.44%). The percentage of sensitivity, specificity and accuracy in MPO are 84.44%, 100% and 88.97% respectively.

Out of 37 ALL cases, PAS was positive in 22 cases (59.46%).

### Comparison of morphology with cytochemistry in ALL and AML

59.59% cases of ALL were diagnosed by morphology alone. With morphology and cytochemistry, 78.37% of cases of ALL were diagnosed.

80% of AML cases were diagnosed by morphology alone. 87.77% of cases of AML were diagnosed by morphology with cytochemistry.

## DISCUSSION

The present study was based on 129 cases of acute leukemia from Coimbatore Medical College and Hospital between January 2020 to June 2021.

Relevant clinical history was obtained and examination done in each case. Routine blood counts, peripheral smear study, bone marrow examinations and cytochemical stains were done for all cases. Flow cytometry was done where cytochemistry was inconclusive.

Out of these 129 cases, 37 cases were ALL, 90 cases were AML, 1 case of Acute undifferentiated leukemia and 1 of biphenotypic leukemia.

Apart from these, 6 cases were diagnosed as evolving phase of leukemia and 2 cases as subleukemic leukemia.

In the present study, the incidence of leukemia in Coimbatore was 1.15% which is comparable with Indian studies quoting an incidence of 0.34%-1.5%.

The most common type of acute leukemia in the present study was AML (70.07%) followed by ALL (28.34%) which was similar with a study of Alpana Choudhary et al,<sup>[3]</sup> (72%), Fuzail Ahmad et al (65%) and Ratnamala choudhury et al (68%).<sup>[4,5]</sup>

In Adults, ALL-L2 subtype was more common accounting for 40% similar to the study of Loffler H et al (43%).<sup>[6]</sup>

But the study conducted by Chouhdary A et al,<sup>[3]</sup> showed that in ALL- L1 (56.52%) subtype was more common than ALL-L2 subtype.

In our study, AML-M2 subtype (46.67%) was the commonest subtype, followed by AML-M3(24.44%) similar to the study of Kulshreshtha et al (43%), Belurkar et al (48%), and Gupta et al (52%).<sup>[7-9]</sup>

### Age Incidence

Among children, ALL was more common than AML. 59.45% of ALL and 18.91% of AML cases was seen in children, which is similar to the study of Neglia JP et al and Ribera JM et al.<sup>[10,11]</sup>

In AML, 82.22% case were observed among adults, which is similar with the study of Sajid Hussain Shah et al (79%) and Kinney Marsha C et al (80%).<sup>[12,13]</sup>

### Sex

In the present study out of 129 cases, 52.75% were males and 47.28% were females. Males slightly

predominate, which was similar to the studies conducted by Shahab et al and Nwaandi et al.<sup>[14,15]</sup> Male: female ratio is 1.09:1 in our study.

#### **Presenting Symptoms**

In ALL and AML, the most common presentation in our study was fatigue followed by fever, bleeding, bony pain and weight loss.

Fatigue was the most common symptoms in study done by Nwannadi et al.<sup>[15]</sup> But Shahab et al,<sup>[14]</sup> and Ratnamala Choudhury et al,<sup>[5]</sup> showed that fever was the most common symptom followed by fatigue.

#### **Clinical Features**

In our studies, Pallor was the most common physical signs in acute leukemia followed by splenomegaly, hepatomegaly, lymphadenopathy and mediastinal mass, similar to Nwannadi et al,<sup>[15]</sup> and Ratnamala et al.<sup>[5]</sup>

Shome et al,<sup>[16]</sup> and Mathur et al,<sup>[17]</sup> studies showed that hepatomegaly was more common than splenomegaly.

Incidence of generalized lymphadenopathy in acute leukemia, our study had 24% similar to Shome et al,<sup>[16]</sup> (25%) and Mathur et al (31%).<sup>[17]</sup> A study done by Advani et al,<sup>[18]</sup> showed lymphadenopathy in only 4%.

In our study, organomegaly was slightly more common in AML than ALL but in a study done by Shahab et al,<sup>[14]</sup> organomegaly was common in ALL than AML.

#### **Laboratory Features**

In our study, anaemia was the most common hematological abnormality constituting 93%, followed by thrombocytopenia (91%) and leukocytosis (84%) which has also been illustrated by Ratnamala et al,<sup>[5]</sup> (92%) and Manisha B et al (85%).<sup>[19]</sup>

But, a study done by Preethi et al,<sup>[20]</sup> showed that thrombocytopenia was the most common hematological abnormality followed by anaemia and leukocytosis.

#### **Peripheral Smear**

In the present study, a blast count of less than 20% in PS was seen in 3.87% of acute leukemia cases. All these cases were AML constituting 5.55% of AML cases.

Blast count of 20-90% was noted in 62.16% of ALL, while in AML, it was 77.77%.

More than 90% blast cells in peripheral smear were seen in 37.83% of ALL cases and 16.66% of AML cases.

94% of cases of acute leukemia showed more than 20% of blast in peripheral smear in our study which was similar to the study done by Rabizadeh et al.<sup>[21]</sup>

Bone marrow is required for confirmation and also typing of acute leukemia because in our study 5.55% of case had a blast count <20% which was confirmed by bone marrow examination.

#### **Morphology**

In our study, 16.66% of cases of AML had Auer rods.

But in a study done by Ritter J et al,<sup>[22]</sup> 50% of AML cases had Auer rods.

In our study, by morphology alone we were able to diagnose 59.59% of ALL cases and 80% of AML cases.

#### **Cytochemical Stains**

##### **Sudan Black B**

Out of 90 cases studied by Sudan Black B, 83.33% of cases were positive and 16.66% of cases were negative.

The sensitivity, specificity and accuracy of SBB in diagnosing AML cases are 83.33%, 100% and 88.18% respectively which was similar to studies conducted by A AM Deghady et al,<sup>[23]</sup> and Fuzail Ahmad et al.<sup>[4]</sup>

##### **Myeloperoxidase**

Out of 90 cases studied by MPO, 84.44% of cases were positive and 15.55% of cases were negative.

In our study, the sensitivity, specificity and accuracy of MPO in diagnosing AML was 84.44%, 100% and 88.97% respectively as comparable with the study conducted by Fuzail Ahmad et al,<sup>[4]</sup> and Estey et al.<sup>[24]</sup>

As per studies conducted by Yang O et al, MPO positivity of >3% confirms myeloid lineage which is helpful in distinguishing AML from ALL, however AML M0, AML M5 and AML M7 may be negative for which flow cytometry is essential.

##### **Periodic Acid Schiff**

Out of 37 ALL cases studied by PAS, 59.46% of cases were positive and 40.54% of cases were negative.

The sensitivity, specificity and accuracy of PAS in our study was 59.45%, 98.88% and 87.40% respectively which is similar to a study done by Rajinikant Ahirwar et al.<sup>[25]</sup>

Alphana choudhary et al,<sup>[3]</sup> studies concluded that almost all cases of ALL showed PAS positivity.

Belurkar et al,<sup>[8]</sup> Fuzail Ahmad et al,<sup>[4]</sup> and Gupta et al,<sup>[9]</sup> studies also showed that in less than 60% of ALL cases, PAS stain was helpful to diagnose ALL.

##### **Immunophenotyping**

In cases with non-committal morphology and cytochemical stain especially in adult population, Flow cytometry is helpful to characterize and also subclassify acute leukemia.

According to Bennett JM et al,<sup>[26]</sup> surface markers in AML are most appropriate for evaluation of morphologically and cytochemically atypical or suspected hybrid leukemia and also undifferentiated leukemia.

In the present study only 17 cases (13.17%) required flow cytometry in which cytochemical stain such as SBB, MPO and PAS were negative. Among them 11 cases were positive for lymphoid markers and turned out to be ALL and 4 cases were positive for myeloid markers and turned out to be AML.

In the present study, CD markers positive in ALL are CD 10, CD 19 and CD34 suggesting precursor B cell ALL. CD 33 and CD 14 are positive in AML cases in which MPO and SBB are negative.

##### **Rare Cases**

1. 4 year old male came with complaints of mass in chest wall. Peripheral smear showed microcytic



hypochromic anaemia with few reactive/atypical lymphocytes and bone marrow aspiration study was suggested. Bone marrow revealed 25% blastoid cells. SBB, MPO and PAS were negative in bone marrow smears. Flow cytometry was done and diagnosed as T-cell lymphoblastic leukemia.

2. 10 years old child came with complaints of fatigue and abdominal pain. Peripheral smear showed 60% of blast cell. Cytochemical stains such as SBB, MPO and PAS were negative. Immunophenotyping was done and it was revealed as Biphenotypic leukemia in which both lymphoid and myeloid markers were positive.
3. 38 years old male, came with complaints of fatigue and bleeding and presented with anaemia and hepatosplenomegaly. In peripheral smear, more than 60% were feblasts. SBB, MPO and PAS were negative. Immunophenotype was done and showed CD34 positive and all other lineage markers were negative and was diagnosed as acute undifferentiated leukemia.
4. 50 year old male came with complaints of breathlessness and abdomen pain and presented with mild hepatomegaly. Peripheral smear showed macrocytic anaemia with nucleated red blood cells- 38/100 WBCs. Bone marrow aspiration showed Myelodysplastic syndrome with evolving phase of erythroid leukemia. SBB, MPO and PAS were negative. Immunophenotyping was done and showed CD36 positivity and was negative for all other lineage markers. Hence was diagnosed as Pure Erythroid Leukemia (AML-M6).

## CONCLUSION

Acute leukemia being a heterogenous group of malignancies, vary in clinical, morphological, immunological, molecular features and also in prognosis and specific therapy.

Diagnosing Acute leukemia entails a step wise process. First we need to distinguish it from other hematological malignancies and reactive disorders. Secondly we need to differentiate between acute myeloid leukemia and acute lymphoblastic leukemia. Third facet was subclassification of acute myeloid leukemia and acute lymphoblastic leukemia for treatment and prognostic purpose.

Morphological analysis of the blast cell helped out in differentiating between acute myeloid leukemia and acute lymphoblastic leukemia in most of the cases. Cytochemical stain provides additional information to distinguish between the two distinct acute leukemia entities.

With both morphology and cytochemistry, most of the acute leukemia cases were designated as either AML or ALL. So the best method to differentiate between myeloblast and lymphoblast was combined use of MPO, SBB and PAS.

Even though morphology and cytochemistry are the gold standard methods for diagnosing acute leukemia, use of recent technique like flow cytometry and cytogenetics are essential for difficult cases, but they are costly. So, in a developing country like India, where immunophenotyping is not available for poor patients, cytochemical stains are simple, cheap, cost efficient, economic, handy, easy to do and also reliable method to provide an additional information in identification.

In conclusion, morphology along with cytochemistry improved the classification of acute leukemia but flow cytometry is of prime importance where morphology and cytochemistry are inconclusive.

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