# Role of Immunohistochemistry in Diagnosis and Subtyping of Acute Leukemia using Selected IHC Markers in a Resource Limited Setting

**Suchita Pant[1], R. K Misra[2]**


## ABSTRACT

**Introduction:** In resource limited settings where genetic studies and flow cytometry were not routinely done or available, morphology is primarily used for diagnosis and subtyping of acute leukemias with use of ancillary tests like cytochemistry and immunohistochemistry in selected cases for a more accurate and definitive diagnosis. This study was done to assess the role of immunohistochemistry in diagnosis and subtyping of acute leukemias in resource poor setting using selected IHC markers and to find diagnostic accuracy of morphologic diagnosis.

**Material and methods:** The study was done on 45 selected cases of acute leukemia. Immunohistochemistry was done on all cases for typing and confirmation of diagnosis after morphological examination of peripheral blood smears and all three bone marrow preparations.A limited panel of IHC markers were used including Anti MPO, Anti CD3, Anti CD20, Anti CD22, Anti TdT, Anti CD117, Anti CD15 and Anti CD68(KP1) for immunohistochemistry.

**Results:** The accuracy of morphological diagnosis in cases of AML and ALL in the study was 84.09% and 82.22% respectively. By intercalating immunohistochemical studies with morphological examination almost all cases of acute leukemia can be diagnosed and subclassified, and diagnostic accuracy is increased as compared to morphological examination alone.

**Conclusion**: Though morphology remains the gold standard for paraffin embedded bone marrow trephines,immunohistochemical staining has became an integral part of diagnostic workup in cases of hematologic malignancies in limited resource settings.


**Keywords:** Immunohistochemistry, Acute Leukemia, Trephine Section

## INTRODUCTION

Hematological malignancies affect people of all age groups. The diagnosis and classification of acute leukemia is evolving continuously. The FAB classification was based initially on the morphological features of the blast cells and cytochemical studies,and later included immunophenotyping as well.

As immunophenotyping became more available, attempts to classify acute leukemia based on their immunophenotypic features met with variable acceptance. The application of immunohistochemistry (IHC) to diagnose bone marrow specimens is a relatively new practice.[1,2] Recent advances have significantly improved paraffin-section immunohistochemistry (IHC). These include antigen retrieval techniques, automated staining devices, and commercial antibody production.[3,4] In cases where immunohistochemistry is required and where antigen expression has to be evaluated in spatial context, bone marrow trephine sections have a major role.[5]

Detailed immunophenotyping of blasts can be done with lineage specific and maturation markers. Although no one marker is pathognomonic for one malignancy a well chosen panel of antibodies can efficiently aid in the diagnosis and subtyping of acute leukemias.

In resource limited settings like ours, where genetic studies and flow cytometry were not routinely done or available, morphology is primarily used for diagnosis and subtyping of acute leukemias with use of ancillary tests like cytochemistry and immunohistochemistry in limited cases for a more accurate and definitive diagnosis.

Meticulous morphologic examination combined with immunohistochemical approach represents a useful laboratory tool for classifying various hematologic malignancies.[6]

This study was conducted to assess the role of immunohistochemistry in diagnosis and subtyping of acute leukemias in resource poor setting using limited IHC markers and to find the diagnostic accuracy by morphological examination alone in cases of acute leukemias.

## MATERIAL AND METHODS

The present study was conducted on a total of 45 selected patients of acute leukemias attending the out patient department and indoor department of Nehru Chikitsalaya, B.R.D Medical College, Gorakhpur. Only those cases in which bone marrow examination was done by using all the three methods of bone marrow aspiration [BMA], bone marrow imprint [BMI] and bone marrow biopsy [BMB] were included in the study.

Detailed clinical examination was performed on each patient with particular emphasis on hematological examination.

[1]Assistant Professor, Department of Pathology, Government Medical College.Haldwani, [2]Professor, Department of Pathology, B.R.D Medical College, Gorakhpur, India

**Corresponding author:** Dr. Suchita Pant, G.B. Pant Marg, Civil lines, Bhotia Parao, Haldwani. Uttarakhand, India

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Blood tests were performed on every patient. Total leucocyte count and platelet count was done by automated cell counter as well as manually. Differential cell count was done by visual method after staining the blood film with Leishman and May Grunwald Giemsa (MGG).

Bone marrow aspiration and biopsy were performed from posterior superior iliac spine. The biopsy imprints were made by gently touching and rolling the core on slides, which were then fixed and stained as bone marrow aspiration smears. This was performed before placing the specimen in AZF (acetic acid, zinc, formalin) fixative for histological processing.

All the aspirate and touch imprints were routinely stained by Leishman and May Grunwald Giemsa, while trephine biopsy sections were stained by routine Haematoxylin and Eosin after bone marrow processing.Immunohistochemistry was then applied on all cases of acute leukemias using a selected panel of IHC markers after morphological examination of bone marrow aspiration smears [BMA], touch imprints [BMI] and trephine sections [BMB].

Bone marrow processing was done as per the Hammersmith Protocol.[5] In this the bone marrow core biopsy specimens are fixed in AZF fixative for 20-24 hrs and then the biopsy specimens are decalcified in Gooding and Stewart's decalcification fluid (10% formic acid and 5% formaldehyde) for 6 hrs before being processed and embedded in paraffin wax, with procedures similar as for other specimens.Antigen retrieval was done using microwave. The panel of antibodies used in our study includes: Anti MPO (polyclonal), Anti TdT(TdT 88), Anti CD 20 (L-26), Anti CD22 (FPC-1) Anti CD3 (PS1), Anti CD68 (KP1), Anti CD15 (BRA4F1), Anti CD 117 (YRI 45) of Biogenex, U.S.A. A positive control is used for every antibody to eliminate the possibilities of wrong interpretation. Accuracy, sensitivity and specificity of morphological diagnosis alone in cases of acute leukemias were calculated using Diagnostic test evaluation calculator of MEDCALC statistical software.

## RESULTS

Out of the total 45 cases of acute leukemias, 20 cases were diagnosed as acute myeloid leukemia(AML) and 25 cases were diagnosed as acute lymphoblastic leukemia(ALL) on the basis of morphological examination of peripheral blood smears and bone marrow.

The patients varied between 2 years to 70 years of age with a slight male preponderance.Pallor, fever and generalized weakness were the most common presenting symptoms. There was a bimodal distribution of cases and most cases were seen in first and seventh decade of life.

Immunohistochemistry was applied for confirmation of diagnosis and further typing on 45 cases of acute leukemias. After immunohistochemical studies on 45 cases of acute leukemias initial diagnosis was confirmed in 37 cases, whereas there was a revised diagnosis in 8 cases of our study.

After immunohistochemistry total number of cases changed as-AML [n=21]; ALL[n=23]; Biphenotypic [n=01].[1]

**Immunophenotypic profile of acute myeloid leukemia (AML) in trephine sections (Table 1.)**

**MPO**: MPO was one of the most commonly expressed myeloid antibody.The intensity and proportion of positive leukemic cells reflected the degree of differentiation of these cells and was most prominent in 2 cases of M3 [Figure.1].

**CD 68(KP1):** KP1 was positive in 20 of 21 cases of AML classified as M0 through M6 and reacted with immature granulocytes and monocytes.[Figure. 2]

**CD 15:** CD 15 was positive in 8 of 21 cases of AML. All 4 cases of M0 and 1 case of M6 were negative.

**CD117:** CD 117 was positive in 19 of 21 cases of AML classified as M0 to M6.

**TdT:** TdT was variably positive in 3 cases of M0.

**Immunophenotypic profile of acute lymphoblastic leukemia (ALL) in trephine sections (Table-2)**

**TdT**: TdT nuclear positivity was seen in all cases of ALL(17 B-ALL and 6 T-ALL) in trephine sections.

**CD3:** CD 3 cytoplasmic positivity was seen in all 6 cases of T-ALL [Fig. 3&4] and in none of B-ALL cases.

**CD20:** CD20 positivity was seen at the cytoplasmic membrane level in 8 of 17 cases of B-ALL and in none of T-ALL cases.

**CD22:** Cytoplasmic CD22 positivity was seen in all cases of B-ALL.

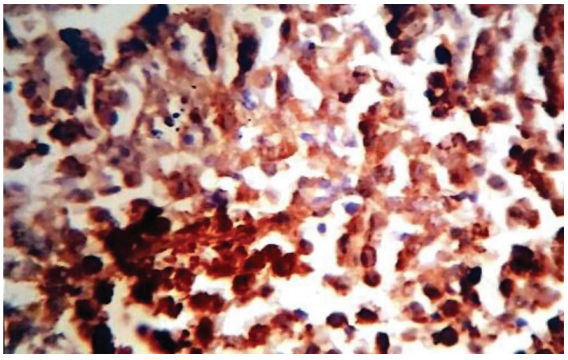

**Figure-1:** Trephine section of a case of diagnosed as AML-M3 shows strong cytoplasmic positivity [x400]

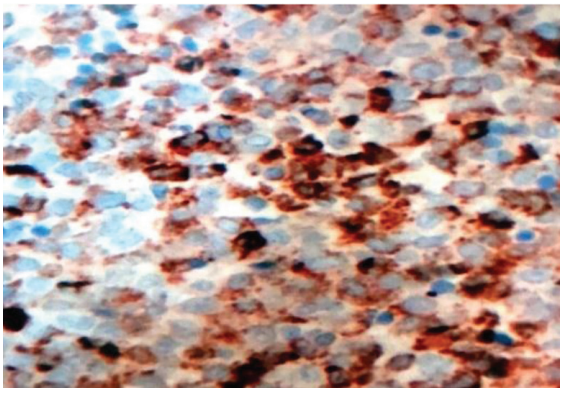

**Figure-2:** Trephine section of a case diagnosed as AML-M5, shows strong CD68 positivity of monocytic cells [x400]

| AML Subtypes | MPO | TdT | CD 68(KP1) | CD 15 | CD 117 |
|---|---|---|---|---|---|
| M0 | 0/4 | 3/4 | 3/4 | 0/4 | 4/4 |
| M1 | 3/4 | 2/4 | 4/4 | ¼ | 4/4 |
| M2 | 6/6 | 1/6 | 6/6 | 4/6 | 5/6 |
| M3 | 2/2 | 0/2 | 2/2 | ½ | 2/2 |
| M4 | 1/1 | 0/1 | 1/1 | 1/1 | 1/1 |
| M5 | 1/3 | 0/3 | 3/3 | 1/3 | 2/3 |
| M6 | 1/1 | 0/1 | 1/1 | 0/1 | 1/1 |

**Table-1:** Immunophenotypic profile of acute myeloid leukemia(AML) in trephine sections:

| | TdT | CD 3 | CD 20 | CD 22 |
|---|---|---|---|---|
| B-ALL | 17/17 | 0/17 | 8/17 | 17/17 |
| T-ALL | 6/6 | 6/6 | 0/6 | 0/6 |

**Table-2:** Immunophenotypic profile of acute lymphoblastic leukemia(ALL) in trephine sections

| IHC based final diagnosis | No. of cases | Percentage |
|---|---|---|
| Confirmatory for morphology based diagnosis | 37(out of total 45 cases with definitive or suggestive diagnosis on morphology alone) | 82.22% |
| Revised Diagnosis/Diagnosis Changed | 08(out of total 45 cases with definitive or suggestive diagnosis on morphology alone) | 17.77% |
| Total cases | 45 | |

**Table-3:** Evaluation of the morphological diagnosis of cases on the basis of IHC staining

| | |
|---|---|
| Sensitivity | 80.95% |
| Specificity | 86.96% |
| Positive predictive value(PPV) | 85% |
| Negative predictive value(NPV) | 83.33% |
| Accuracy | 84.09% |

**Table-4:** Morphological diagnosis in cases of AML

| | |
|---|---|
| Sensitivity | 86.96% |
| Specificity | 77.27% |
| Positive predictive value(PPV) | 80.00% |
| Negative predictive value(NPV) | 85.00% |
| Accuracy | 82.22% |

**Table-5:** Morphological diagnosis in cases of ALL

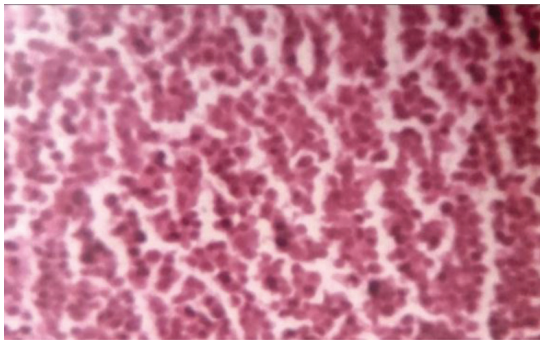

**Figure-3:** Trephine section of a case diagnosed as ALL,shows complete replacement by immature blast cells. [H&Ex400]

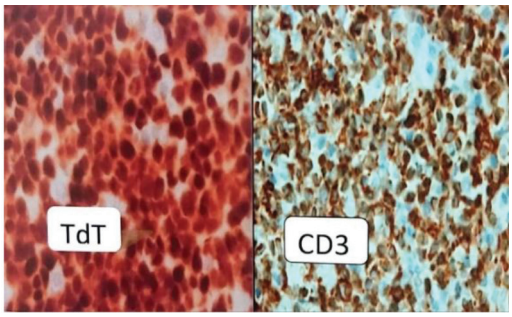


**Figure-4:** (same case) Sections shows strong nuclear TdT and cytoplasmic CD3 positivity [x400]

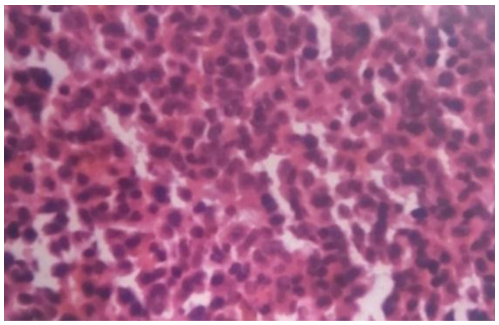

**Figure-5:** Trephine section of a case diagnosed as AML-M2,shows blasts with abundant cytoplasm along with mature forms [H&Ex400]

**Immunophenotypic profile of Biphenotypic leukemia(T/Myeloid) in trephine sections**

One case of Biphenotypic leukemia showed positivity for TdT, CD117, CD3 and MPO. Thus confirming the diagnosis of Biphenotypic leukemia(T/Myeloid).

In 37 (82.22%) out of total 45 cases of acute leukemias, immunohistochemistry confirmed the definitive or suggestive diagnosis given after morphological examination, whereas final diagnosis changed in 8 cases(17.77%) [Table 3.]

The sensitivity and specificity of morphological diagnosis in cases of AML in our study was 80.95% and 86.96% respectively,whereas accuracy was 84.09%[Table 4.]

The sensitivity and specificity of morphological diagnosis in cases of ALL in our study was 86.96% and 77.27%

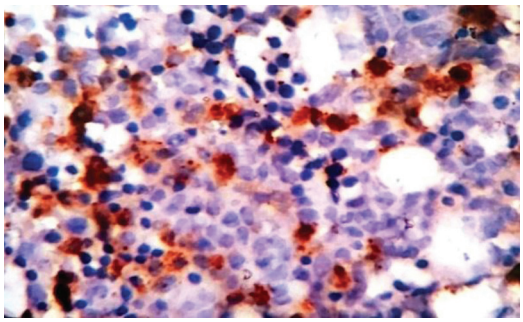

**Figure-6:** (Same case) Trephine section shows scattered CD 15 positivity. [x400]

respectively,whereas accuracy was 82.22%[Table 5]

By intercalating immunohistochemical studies with morphological examination almost all cases of acute leukemia can be diagnosed and subclassified and diagnostic accuracy is increased as compared to morphological examination alone.

## DISCUSSION

This study was conducted to study the role of immunohistochemistry in diagnosis and subtyping of acute leukemias in limited resource setting. After morphological examination of peripheral blood smears, bone marrow aspirate (BMA), bone marrow imprint (BMI) and bone marrow biopsy (BMB), immunohistochemistry was applied on 45 cases of acute leukemias which included 20 cases of AML and 25 cases of ALL on the basis of morphology.

We followed Hammersmith protocol[5] for bone marrow processing where specimens of bone marrow trephine are fixed in acetic acid-zinc-formalin(AZF) fixative and decalcified in Gooding and Stewart's decalcification fluid (10% formic acid and 5% formaldehyde). This approach which was followed by conventional processing and paraffin embedding was chosen because of optimal preservation of cytological details and antigen.

This study included only cases that had been diagnosed on the basis of morphological examination. After immunohistochemical studies on 45 cases of acute leukemia initial diagnosis was confirmed in 37 cases as had also been observed in study by Toth et al[7] who studied 45 cases of ALL without prior immunophenotyping, and where original diagnosis was confirmed in 38 out of 45 cases.

In our study we observed that immunophenotyping enables a reliable differentiation of AML from ALL in all cases as had also been observed by Pileri et al.[8] According to them bone marrow biopsy may be regarded as a reliable tool for acute leukemia diagnosis.

In our study 25 cases diagnosed as acute lymphoblastic leukemia (ALL) on the basis of morphological examination when subjected to immunohistochemistry (IHC), initial diagnosis was confirmed in 20 cases(80%), whereas there was a revised diagnosis in 5 cases.. It was concluded that immunohistochemical investigation of routinely processed bone marrow biopsy specimens enables reliable detection of ALL subtypes.[7]

20 cases were diagnosed as acute myeloid leukemia (AML) on the basis of morphology [Figure.5]. After immunophenotyping initial diagnosis was confirmed in 17(85%) cases and 3 cases were rediagnosed as ALL (2 B-ALL, 1 T-ALL)

Arber DA et al[2] also established the diagnostic utility of paraffin section immunohistochemistry in lineage determination of acute leukemia.

Like Chuang and Li[9] and others[10], we also found a limited panel of antibody to be very useful for the classification of acute leukemias.

MPO has been established as a specific marker for myeloid cells in paraffin sections.[11] In our study MPO was positive from M1-M6, whereas all cases of M0 were found to be negative, this is in contrast to the study by Pileri.[8] Chuang and Li[9] found MPO to be positive in 84% of cases,whereas cases of M0 were negative for MPO in their series also. Manaloor et al[12] also found MPO negativity in 7 out of 8 cases of M0 by immunohistologic examination.

Kurec and associates[13] found that the anti-CD68 antibody(KP1) could differentiate between ALL and AML but could not distinguish between AML subtypes. Arber and Jenkins[2] reported similar findings.

CD117 was positive in 90% cases of AML in our study.CD 117 strongly favors a myeloid lineage because it is not seen in B-ALL and is reported very rarely in T-ALL.[14]

TdT is positive in 20% cases of AML cases and most TdT-positive AML cases were AML-M0.[6] We also found TdT positivity in cases of AML-M0.

CD 15 is normally expressed in maturing neutrophils, monocytes and NK cells.This is a marker of granulocytic differentiation.In our study we found CD 15 to be lacking in M0,whereas there was variable expression in 38% cases of myeloid leukemias from M1-M6 [Figure.6] as had also been observed by Rousselet MC et al.[15]

We found anti-CD3 to be the most reliable and sensitive T-cell marker as had also been observed in other studies.[9,15]

In our study it was observed that TdT positivity was regularly detected at the nuclear level in the neoplastic cells of acute lymphoblastic leukemias (ALL), which was also seen in other studies.[8]

CD20 expression was observed in 8/17(47%) B-ALL cases in our study, whereas it is expressed in 14/39(36%) cases of B-ALL in study by Pileri et al.[8]

CD22 expression was seen in all cases of B-ALL in our study,which was similar to other studies.[16]

One case which was initially diagnosed as ALL on the basis of morphological examination was found to be immunoreactive for TdT, MPO, CD3 and CD117. This was then diagnosed as Biphenotypic (T/Myeloid) leukemia. This corresponds to study by others[2,8], according to whom immunophenotyping enables the recognition of hybrid forms.

The results of our present study are similar to earlier studies.[2,7,8,12,13] We observed that immunophenotyping of acute leukemias (1) is easily feasible in routine sections; (2) has practical relevance by allowing clear-cut distinction between myeloid and lymphoid leukemias; (3) permits the

recognition of hybrid forms; (4) enables the differentiation of T-and B-ALLs (5) facilitates the subclassification of AMLs, at times in association with morphology.

## CONCLUSION

Immunohistochemistry is a powerful auxillary technique for the diagnosis of hematologic disorders in bone marrow trephine biopsies.An accurate characterization and primary classification of hematolymphoid neoplasms is possible in the majority of cases and although conventional morphology remains the gold standard for paraffin embedded bone marrow trephines,immunohistochemical staining has became an integral part of diagnostic workup.[17]

Therefore immunohistochemistry can be applied for a more accurate and definitive diagnosis in cases of hematologic malignancies in resource limited setting.

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