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Original Research Article

Comparative analysis of immunohistochemistry and flow cytometry in the diagnosis of acute leukaemia: a single centre study

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ABSTRACT

Background: Morphological evaluation and immunophenotyping are the major diagnostic modalities of acute leukaemia (AL). Although immunohistochemistry (IHC) and flow cytometry (FCM) are necessary for lineage assessment, but in many cases the use of these modalities alone might possess a diagnostic challenge. The study was aimed to analyse the diagnostic utility of IHC and FCM in the diagnosis of AL.
Methods: This cross-sectional hospital-based study was done for one year and included 55 cases. Following peripheral blood examination and bone marrow study, IHC and FCM analysis was done using CD34, anti-MPO, CD3 and CD20.
Results: There were 74.5% acute myeloid leukaemia (AML) and 25.5% acute lymphoblastic leukaemia (ALL) cases. By IHC, CD34 was positive in 94.5% cases, anti-MPO in 69.1%, CD3 in 3.6% and CD20 in 12.7% cases. But by FCM, CD34 was positive in 96.2% cases, anti-MPO in 61.5%, CD3 in 3.8% and CD20 in 19.3% cases. FCM could not be done for 3 cases as there was dry tap with pancytopenia and lineage assessment was done by IHC. On comparative analysis, CD34 was found to be better expressed by FCM. Anti-MPO and CD20 were better expressed by IHC and CD3 was equally expressed by both.
Conclusions: IHC is an easy and cost-effective technique which gives an accurate characterization of the lineage and subtype of AL, especially in cases where use of FCM is limited such as cases with dry tap and pancytopenia and in limited resource centers.

Keywords: AL, IHC, FCM

INTRODUCTION

Acute Leukaemia (AL) results when a normal hematopoietic stem cell acquires mutations and lead to clonal proliferation.[1] It is classified into AML and ALL, based on morphology and stem cell lineage.[2,3]

Morphological evaluation and immunophenotyping are major tools to diagnose AL.[4]Immunophenotyping is done by IHC or FCM.[5] FCM is used to subtype AL and to detect minimal residual disease.[6] IHC can be used for confirming the lineage in cases where use of FCM is limited. The diagnosis of AL entails an integrated approach of morphology, cytochemistry and immunophenotyping to determine the lineage, decide the choice of therapy and to assess the prognostic status of a patient. In our study, IHC could diagnose and subtype the cases where bone marrow aspirate was inadequate and FCM had to be done using peripheral blood and also in cases where FCM could not be done at all.

This study aims to analyze the diagnostic utility of IHC and FCM in diagnosis of AL.

METHODS

A hospital based cross sectional study was conducted for a duration of one year (August 2021 to July 2022) in the department of pathology and department of clinical haematology, including the patients attending the medicine and haematology OPD with clinical suspicion of AL and patients who have given consent for the study and excluding the patients of AL on chemotherapy and cases with inconclusive reports of bone marrow study.

Each case has been examined clinically and relevant history has been taken. Patients were examined for: pallor, fever, generalized weakness, bony tenderness. Petechiae, gum bleeding, hepatosplenomegaly, lymphadenopathy.

Peripheral venous blood samples were collected in EDTA anticoagulated vacutainers. Estimation of hemoglobin, Total leukocyte count, differential leukocyte count and platelet count were done using Sysmex XN-1000 automated cell counter. All the peripheral smears were stained in the clinical pathology laboratory using commercially prepared Leishman solution.

Under aseptic conditions, bone marrow aspiration was done for all patients using an 18G Salah needle. The smears were stained with Giemsa stain. 500 cell differential count was performed. Smears were analyzed for cellularity, myeloid: erythroid ratio, erythroid, myeloid, lymphoid and megakaryocytes, blast cells, atypical cells, mitosis, and parasites.

The bone marrow biopsy was done after aspiration using an 11G Jamshidi needle for adults and 15G needle for children. After proper fixation of the biopsy core in 10% Neutral Buffered Formalin for 6 hours, the specimen was subjected to decalcification with 5% nitric acid for a duration of 3-4 hours. Sections of 3 micron thickness were cut on a microtome at three levels: 25%, 50%, and 75% into the cross-sectional diameter of the core. The sections were stained with haematoxylin and the eosin stain.

In cases where the aspirate was a dry tap, touch imprints were made from the trephine biopsy prior to placing it in fixative. Imprints are made by gently touching the fresh unfixed core on the slide, then fixed and stained using Giemsa stain.

The panel of antibodies selected for the present study were CD 34 (for primitive hematopoietic cells), anti-MPO (for myeloid lineage), CD 3 (for T-cell lineage) and CD 20 (for B-cell lineage). IHC was performed on the representative blocks of paraffin embedded tissue. Three-micron thick sections were submitted for immunohistochemical staining. Antigen retrieval was done using HIER method in a microwave.

Samples used for Flow cytometric analysis were bone marrow aspirate and peripheral blood (in cases of dry tap, with total leukocyte count >10,000/UL**).** In cases of dry tap with pancytopenia on peripheral blood, FCM could not be done.

Panel of antibodies used were; Common leukocyte antigen: CD 45, immature cell antigens; CD34, and HLADR, myeloid/monocytic; CD33, CD117, MPO, CD13, CD14. T cell; CD3, CD7 and B cell; CD19, CD20.

Statistical analysis

The data collected was analysed using SPSS version 20. Microsoft word and excel have been used to generate graphs, tables. The significance of the comparative analysis between the results of IHC and FCM analysis were calculated using Chi-square test of significance by adopting the statistical software SPSS. Sensitivity and specificity was calculated using 2x2 tables and standard formula.

RESULTS

A total of 55 cases of AL were included under this study with an age range of 2 years to 75 years. Out of the 55 cases, there were 41 cases (74.5%) of AML and 14 cases (25.5%) of ALL. This distribution has been depicted in Table 1.

Table 1: Distribution of AL according to lineage, (n=55).

Type of AL	N	Percentage (%)
AML	41	74.5
ALL	14	25.5
Total	55	100

Among the AML cases, 7.3% (3 out of 41) cases were categorized as AML M0, 34.1% (14 out of 41) cases were categorized as AML M1, 56.1% (23 out of 41) cases were categorized as AML M2 and there was 1 (2.5%) case of APML. Among the ALL cases, there were 85.7% (12 out of 14) cases were categorized as B-ALL and 14.3% (2 out of 14) cases were categorized as T-ALL.

The mean total leukocyte count in AML was 55561.46±31440.23 (/µL) and in ALL was 85604±65976.96 (µL). The mean blast % in AML was 66.75±17.39 and in ALL was 70.9±17.45. The mean hemoglobin in AML was 6.52±1.90 and in ALL was 6.53±2.49. The mean platelet count in AML was 42395.12±36007.09 (µL) and in ALL was 37564.28±17907.35 (µL).

In the present study, bone marrow aspiration and Bone marrow biopsy were done for all the 55 cases. 70.9% of

the cases were diagnosed as AL in bone marrow aspiration and 16.4% cases were reported as suspicious for AL. Bone marrow aspirate of 12.7% (7 out of 55) cases came out to be dry tap, out of which 4 cases had high total leukocyte count and blast % on peripheral blood examination and 3 cases had pancytopenia. All the 55 (100%) cases were reported as AL on bone marrow biopsy.

IHC was done for all the 55 cases. CD 34 was expressed as positive in 94.5% (52 out of 55) cases, anti MPO was positive in 69.1% (38 out of 55) cases, CD 3 was detected in 3.6% (2 out of 55) cases and CD 20 was detected in 12.7% (7 out of 55) cases.

The 52 cases were evaluated by Flow cytometric analysis. FCM could not be done in 3 cases (2 cases of AML and 1 case of ALL) because bone marrow aspirate was a dry tap and peripheral blood could not be sent for analysis because of pancytopenia. The results of FCM analysis of the 52 cases are detailed in Table 6.

Results of comparative analysis pf IHC and FCM analysis: On comparative analysis of IHC and FCM evaluation, it was found that CD 34 was better detected by FCM than IHC. Anti-MPO was better detected by IHC than by FCM. CD 3 expression was equivalent for both IHC and FCM. Detection of CD 20 by IHC was found to be better than that by FCM.

The overall sensitivity of CD 34, anti-MPO, CD 3 and CD 20 were found to be 100%, 88.89%, 100% and 83.33% respectively.

Table 2: Distribution of AL subtypes according to FAB subtypes, (n=55).

Subtype of AL		N	Percentage (%)
AML, (n=41)	AML M0	3	7.3
	AML M1	14	34.1
	AML M2	23	56.1
	APML	1	2.5
ALL, (n=14)	B-ALL	12	85.7
	T-ALL	2	14.3
Total		55	100

Table 3: Distribution of mean hematological parameters in cases of AL, (n=55).

Parameters	Mean value in AML	Mean value in ALL
Total leukocyte count (µl)	55561.46± 31440.23	85604± 65976.96
Blast (%)	66.75±17.39	70.9±17.45
Hb (g/dl)	6.52±1.90	6.53±2.49
Platelet count (µl)	42395.12± 36007.09	37564.28± 17907.35

Table 4: Interpretation of bone marrow aspiration and bone marrow biopsy, (n=55).

Variables	Bone marrow aspiration	Bone marrow biopsy
Dry tap	7	-
Suspicious for AL	9	-
Diagnosed as AL	39	55

Table 5: Phenotypic profile of AL by IHC, (n=55).

IHC marker	AML, (n=41)				ALL, (n=14)		Total, (n=55)
	AML M0	AML M1	AML M2	APML	B-ALL	T-ALL	
CD 34 +ve	3/3	14/14	23/23	0/1	10/12	2/2	52/55
Anti-MPO +ve	0/3	14/14	23/23	1/1	0/12	0/2	38/55
CD 3 +ve	0/3	0/14	0/23	0/1	0/12	2/2	2/55
CD 20 +ve	0/3	0/14	0/23	0/1	7/12	0/2	7/55

Table 6: Phenotypic profile of AL by FCM, (n=52).

FCM marker	AML, (n=39)				ALL, (n=13)		Total, (n=52)
	AML M0	AML M1	AML M2	APML	B-ALL	T-ALL	
CD 34 +ve	3/3	13/13	22/22	0/1	10/11	2/2	50/52
Anti-MPO +ve	0/3	10/13	21/22	1/1	0/11	0/2	32/52
CD 3 +ve	0/3	0/13	0/22	0/1	0/11	2/2	2/52
CD 20 +ve	0/3	0/13	0/22	0/1	5/11	0/2	5/52

Table 7A: Comparison of CD 34 expression by IHC and FCM.

Variables	Positive expression by IHC	Positive expression by FCM
AML	38	38
ALL	11	12
Total	49	50

Table 7B: Comparison of antiMPO by IHC and FCM.

Variables	Positive expression by IHC	Positive expression by FCM
AML	36	32
ALL	0	0
Total	36	32

Table 7C: Comparison of CD 3 by IHC and FCM.

Variables	Positive expression by IHC	Positive expression by FCM
AML	0	0
ALL	2	2
Total	2	2

Table 7D: Comparison of CD 20 by IHC and FCM.

Variables	Positive expression by IHC	Positive expression by FCM
AML	0	0
ALL	6	5
Total	6	5

Case 1: A Case of AML M1 (Figure 1)

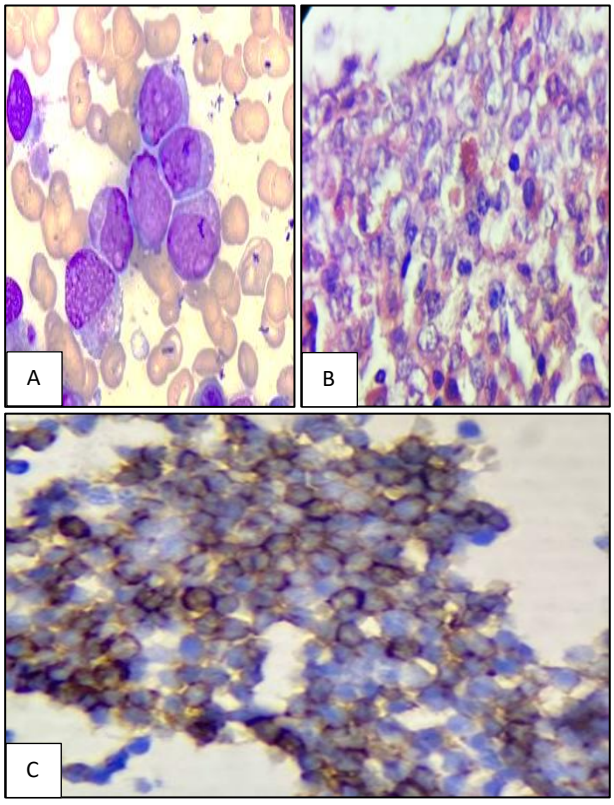

Figure 1 (A-C): Bone marrow aspirate showing presence of blasts, photomicrograph of bone marrow biopsy showing sheets of blasts replacing the normal hematopoietic marrow and photomicrograph of CD 34 membrane positivity by IHC.

Case 2: A case of AML M2 (Figure 2)

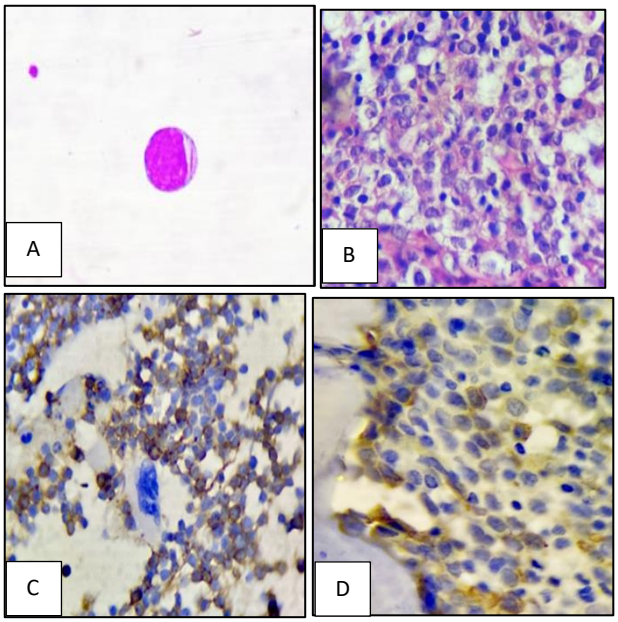

Figure 2 (A-D): Photomicrograph of peripheral blood smear showing blast with Auer rod. Hotomicrograph of bone marrow biopsy showing sheets of blasts along with presence of mature granulocytic cells. Photomicrograph of CD 34 membrane positivity by IHC. Photomicrograph of anti-MPO cytoplasmic positivity by IHC.

Case 3: A case of B-ALL (Figure 3)

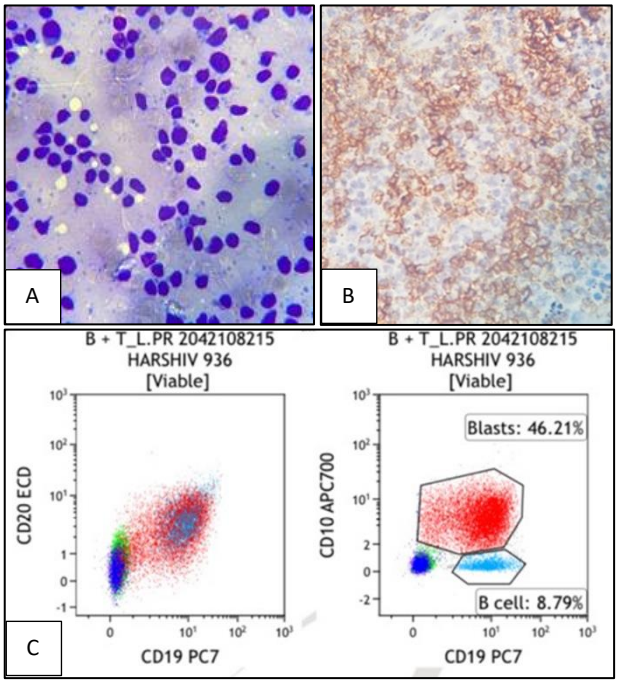

Figure 3 (A-C): Touch smear showing presence of blasts. Photomicrograph of CD 20 membrane positivity by IHC and CD 10, CD 19, CD 20 positivity on FCM.

DISCUSSION

Out of the 55 cases taken under this study, there were 41 cases (74.5%) of AML and 14 cases (25.5%) of ALL. The occurrence of AML was found to be more common in our study. This is consistent with the studies made by Vinsheth et al, Arun Jain et al (2015) and Shobana et al, the results of which show that the occurrence of AML is more common than ALL.[7-9]

The mean total leukocyte count in AML was 55561.46±31440.23 (µL) and in ALL was 85604±65976.96 (µL). The mean blast % in AML was 66.75±17.39 and in ALL was 70.9±17.45. The mean hemoglobin in AML was 6.52±1.90 and in ALL was 6.53±2.49. The mean platelet count in AML was 42395.12±36007.09 (µL) and in ALL was 37564.28±17907.35 (µL). These findings are similar with the results of the studies done by Harani et al, Wakui et al and Patel et al.[10-12] But there is variation in the values of the hematological parameters, which is because Total WBC count has a very wide range of presentation and also due to the difference in the study samples and nature of the studies.

Bone marrow aspiration and bone marrow biopsy were done for all the 55 cases. 70.9% of the cases were diagnosed as AL in bone marrow aspiration and 16.4% cases were reported as suspicious for AL. Bone marrow aspirate of 12.7% (7 out of 55) cases were dry tap, out of which 4 cases had high total leukocyte count on peripheral blood examination and 3 cases had pancytopenia. All the 55 (100%) cases were reported as AL on bone marrow biopsy. These findings were similar to the study of Rani et al who has also reported similar interpretation of the cases.[13] In the present study, all the 55 cases which were diagnosed and suspected as AL, including the cases of dry tap, could be subtyped by IHC. Rani et al in their study could subtype 81.9% of such similar cases having aparticulate aspirate with the help of IHC on bone marrow biopsy.[13]

In the present study, it has been found that CD 34 is better detected by FCM than IHC. This is in concordance with the study of Daniel Arber et al.[14] There is discordance with the study of Manaloor et al who included only AML cases in their study and found that CD 34 expression in cases of AML was equivalent by both FCM and IHC.[15] In the present study we have found similar results for the AML cases. The one case which has shown disparacy is a case of ALL. Anti-MPO was better detected by IHC than by FCM. This is consistent with the studies of Ahuja et al and Arber et al.[16,15] Subhaschandrabose et al have also mentioned that anti-MPO is best detected by IHC.[13]

CD 3 expression was equivalent by both IHC and FCM. This is consistent with the findings of Gwaiz et al and not with Daniel Arber et al.[17,15] Arber et al have mentioned that the difference in CD 3 positivity may be due to cytoplasmic expression of CD 3, instead of surface expression or may be due to immature cells expressing only the e subunit of the CD3. This subunit is not detected by monoclonal antibodies commonly used for FCM, but detected by polyclonal antibody used in IHC.[15] Detection of CD 20 by IHC was found to be better than that by FCM. This is in concordance with the study of Arber et al who has also found better expression of CD 20 by IHC.[15]

CONCLUSION

In conclusion, IHC can be used an adjunct in diagnosing and categorizing AL, especially in cases where use of FCM is limited. It is an easy and cost-effective technique which gives an accurate characterization of the lineage and subtype of AL, which will eventually help in deciding the treatment and aid in the benefit of the patient.

Limitations

Low number of study subjects and cross-sectional nature of the study may be a limitation. Due to non-availability of IHC markers and financial constraint, we could not categorise the M4, M5, M6 and M7 subtypes of AL.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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