DOI: 10.7860/JCDR/2023/59906.17470

Original Article

Pathology Section

Classification of Acute Leukaemia based on Blast Morphology, Cytochemistry and Flow Cytometry

P JUBIT PAULOSE[1], J LATHA FATHIMA[2]

ABSTRACT

Introduction: Acute leukaemias are a heterogeneous group of malignancies due to the abnormal proliferation of immature cells. The classification of acute leukaemia has been transformed over a period. French American British's (FAB) classification of acute leukaemia is based on morphology and cytochemistry. Later, the World Health Organisation (WHO) classification stressed immunophenotyping to be done in the classification of acute leukaemia. Immunophenotyping is a powerful tool for classification and also for treatment and prognosis.

Aim: To study the morphology of blasts in the peripheral smear and bone marrow cytochemistry and to compare with immunophenotyping in the classification of acute leukaemia.

Materials and Methods: A prospective and cross-sectional study was undertaken in the Department of Transfusion Medicine and Immunohematology, at St. Johns National Academy of Health Sciences a Tertiary Care Hospital in Bengaluru, India, over a period of one year from November 2018 to October 2019. The morphology of the blast was studied in peripheral blood and bone marrow aspirate stained by Leishman stain. Cytochemistry in peripheral smear and bone marrow with Sudan Black B and Periodic Acid-Schiff (PAS) stain was done in all acute leukaemia. The flow cytometry samples were processed within 24 hours of collection of the samples with a panel of markers including myeloid and lymphoid lineage and precursor markers. The results of morphology were confirmed with flow cytometry and final reports were released. Microsoft excel was used to enter data.

Results: A total of 112 cases of acute leukaemia were studied and classified based on morphology, cytochemistry and immunophenotyping of blasts. A total of 46 cases of Acute Myeloid Leukaemia (AML) and 63 cases of Acute Lymphoblastic Leukaemia (ALL). Two cases were found with Chronic Myeloid Leukaemia (CML) in blast crisis, one with myeloid and another with lymphoid blast crisis. One biphenotypic leukaemia was noted. Sudan Black was positive in 35 (76%) of AML and PAS was positive in 28 (44%) of ALL. The most common AML subtype was AML-M2 and the most common ALL subtype was B cell ALL. The most common symptom was fever and the sign was pallor.

Conclusion: The present study showed that morphological diagnosis is important, based on which the cytochemistry and flow cytometry is performed for diagnosis. In cases of the diagnostic dilemma of acute leukaemia with morphology and cytochemistry, immunophenotyping supports diagnosing and classifying acute leukaemia.

Keywords: Acute lymphoblastic leukaemia, Acute myeloblastic leukaemia, Blast crisis

INTRODUCTION

Acute leukaemias are a heterogeneous group of malignancies due to the abnormal proliferation of immature cells arising from uncommitted or partially committed stem cells. Therefore, the retained capacity of the stem cells to differentiate and maturate forms the basis of classification [1]. These blasts proliferate in the bone marrow and lymphoid tissues and finally migrate to the peripheral blood. The clinical course varies from days to weeks or months and years depending on the type of leukaemia. The classification of acute leukaemia has been transformed over a period. French American British's (FAB) classification of acute leukaemia is based on morphology and cytochemistry. Later, the World Health Organisation (WHO) classification stressed immunophenotyping to be done in the classification of acute leukaemia [2]. Immunophenotyping is a powerful tool for classification and also for treatment and prognosis. Immunophenotyping is done by flow cytometric analysis with the peripheral or bone marrow samples and immunohistochemistry is done on bone marrow and tissue biopsy. For immunophenotyping flow cytometry is a preferred method as a large number of cells can be analysed in a short period of time. A panel of the Cluster of Differentiation (CD) markers is used in the diagnosis of leukaemia. Recent advances in flow cytometry, the availability of a varied range of antibodies and fluorochromes and improved gating strategies have been a major role in the identification of aberrant clones and minimal residual diseases [3].

According to WHO (2017) (revised 4th edition), the definition of Acute Myeloid Leukaemia (AML) is the presence of 20% or more of blasts in the peripheral blood and or bone marrow and 25% in the case of Acute Lymphoblastic Leukaemia (ALL). The classification of acute leukaemia into AML and ALL helps to decide the treatment and prognosis of individual patients [4]. The incidence of acute myeloblastic leukaemia varies with age and accounts for 80% of acute leukaemia in adults. Acute lymphoblastic leukaemia usually occurs in children [4].

Hence, this study is undertaken to compare the morphology of blasts in the peripheral smear and bone marrow aspirate with cytochemistry and immunophenotyping in the classification of acute leukaemia.

MATERIALS AND METHODS

A prospective and cross-sectional study was undertaken in the Department of Transfusion Medicine and Immunohematology, at St. Johns National Academy of Health Sciences a Tertiary Care Hospital in Bengaluru, India, over a period of one year from November 2018 to October 2019. Due approval was obtained from the Institutional Ethic Committee (IEC no.369/2018).

Inclusion and Exclusion criteria: Peripheral blood smears with the diagnosis of acute leukaemia followed by bone marrow with a request for flow cytometry were included in the study. Reference smears and slides with acute leukaemia were excluded from the study.

Study Procedure

A total number of 894 bone marrow samples were received of which 112 patients were diagnosed with acute leukaemia.

Morphology of the blast: This was studied in peripheral blood and bone marrow aspirate stained by Leishman stain. Differential count with the blast, other precursors, and mature cells was performed in peripheral smear and bone marrow aspirate.

Cytochemistry: In this, the peripheral smear and bone marrow with Sudan Black B (SBB) and Periodic Acid-Schiff (PAS) stain was done in all acute leukaemia. Non specific esterase with fluoride inhibition was done in cases with monocyte differentiation. Bone marrow biopsy stained by Haematoxylin and Eosin stain (H&E).

Flow cytometry: The flow cytometry samples were processed within 24 hours of collection. Ethylenediaminetetraacetic Acid (EDTA) anticoagulated peripheral blood or bone marrow samples were used for flow cytometry. Flow cytometry was done in bone marrow aspirate samples in 107 cases and five with peripheral blood samples. BD FACSCanto™ II instrument was used to perform flow cytometry. All monoclonal antibodies were obtained from BD Biosciences. A minimum of 1000,000 events were acquired. The cells were gated using low-side scatter and dim CD45 positivity. Flow cytometry was done with the panel of CD markers, including myeloid, monocytic, megakaryocytic, B, and T lymphoid lineages. The markers used were shown in [Table/Fig-1].

Precursor markers	Myeloid markers	Monocytic markers	Megakaryocytic markers	B lymphoid markers	T lymphoid markers
CD34, TdT, HLA-DR	MPO, CD13, CD33, CD117	CD14, CD64	CD41, CD61	CD19, CD10, CD20, CD79a	Cytoplasmic and surface CD3, CD5, CD7

[Table/Fig-1]: CD markers used in the acute leukaemia.
MPO: Myeloperoxidase; TdT: Terminal deoxynucleotidyl transferase

Clinical history and details were obtained from medical record folders. Presenting complaints of patients like fever, fatigue, and anorexia, and clinical examination findings like pallor, jaundice splenomegaly, lymphadenopathy and hepatomegaly were recorded.

STATISTICAL ANALYSIS

Microsoft excel was used to enter data. Data was expressed as percentages and frequencies.

RESULTS

During the one year study period, a total number of 894 bone marrow samples were received of which 112 patients were diagnosed with acute leukaemia. The male:female ratio was 1.6:1. The predominant age group was in range 31-40 years. Age and gender distribution are shown in [Table/Fig-2].

Age (in years)	Gender		AML	ALL	Biphenotypic acute leukaemia	CML in blast crisis	Total
	Male	Female					
0-5	14	9	3	20			23
6-10	5	5	2	8			10
11-18	6	5	2	9			11
19-30	14	3	8	8		1	17
31-40	19	5	15	7	1	1	24
41-50	3	6	3	6			9
51-60	3	5	5	3			8
61-70	2	3	4	1			5
>70	3	2	4	1			5
Total	69	43	46	63	1	2	112

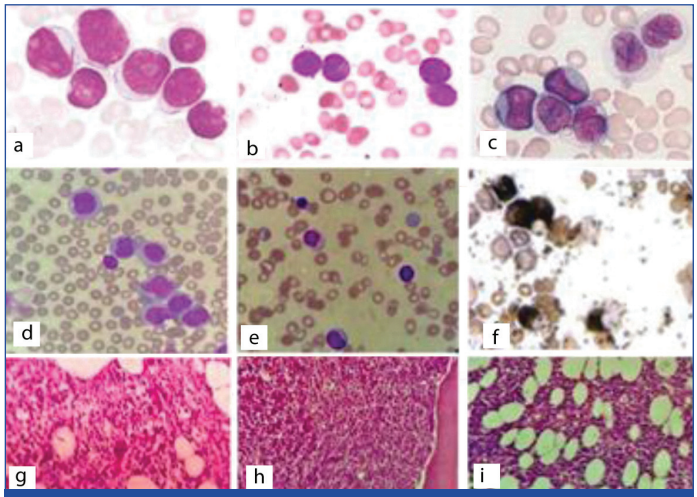
[Table/Fig-2]: Showing age and gender distribution in acute leukaemia.

There were 46 cases of AML, 63 of ALL, two cases with CML in blast crisis and one with biphenotypic leukaemia based on morphology and cytochemistry.

Acute myeloblastic leukaemia: The AML was classified according to FAB classification and AML-M2 25 (54%) cases was predominantly found followed by AML-M3 13 (28%) cases [Table/Fig-3]. Among the 46 cases of AML, 35 (76%) were positive for SBB and 11 (24%) were negative. Nonspecific Esterase (NSE) with fluoride inhibition was done in AML with monocytic differentiation and found to be positive in AML-M5. The morphology of the blast is shown in [Table/Fig-4].

Acute myeloblastic leukaemia	n (%)
AML-M0	Nil
AML-M1	4 (9%)
AML-M2	25 (54%)
AML-M3	13 (28%)
AML-M4	2 (4%)
AML-M5	1 (2%)
AML-M6	1 (2%)
AML-M7	Nil
Total	46 (100%)

[Table/Fig-3]: Showing types of Acute Myeloblastic Leukaemia (AML) according to French American British (FAB) classification.
FAB: French American British; N=112

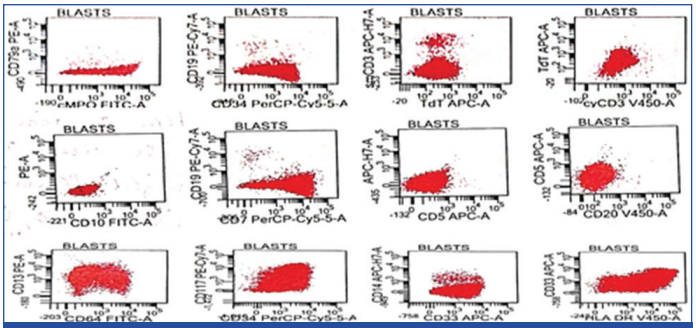


[Table/Fig-4]: Showing morphology of blast in AML. (a) Myeloblast with auer rod (Leishman stain, 100x); (b) Hypergranular promyelocyte (Leishman stain, 100x); (c) Bilobed promyelocyte (Leishman stain, 100x); (d) Monoblast (Leishman stain, 100x); (e) Myeloblast with normoblast (Leishman stain, 100x); (f) Sudan black stain showing the positivity in myeloblast (100x); (g) Bone marrow biopsy of acute promyelocytic leukaemia (H&E stain, 40x); (4) Bone marrow biopsy of acute myeloblastic leukaemia (H&E stain, 40x); (i) Bone marrow biopsy of acute monoblastic leukaemia (H&E stain, 40x).

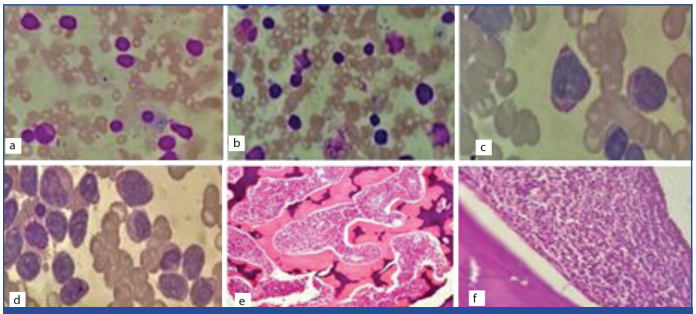
The morphological diagnosis was confirmed by immunophenotyping with flow cytometry. Flow cytometric analysis showed CD34 expression in 27 (59%) cases of AML. CD13 and CD33 were expressed in 35, CD117 was expressed in 28, HLA-DR expression in 31 (67%). Among the 13 cases of Acute Promyelocytic Leukaemia (APML) HLA-DR was negative in 10 (77%) and positive in 3 (23%). AML with monocytic differentiation showed CD14 and CD64 expression. Aberrant expression of CD7 was found in 10 (22%) cases of AML. CD19 was expressed in 1 (2%) case of AML. The flow cytometry image of AML is shown in [Table/Fig-5].

In 63 cases of ALL, only 28 (44%) were positive for PAS and 35 (56%) were negative for PAS. The morphology of the blast is shown in [Table/Fig-6].

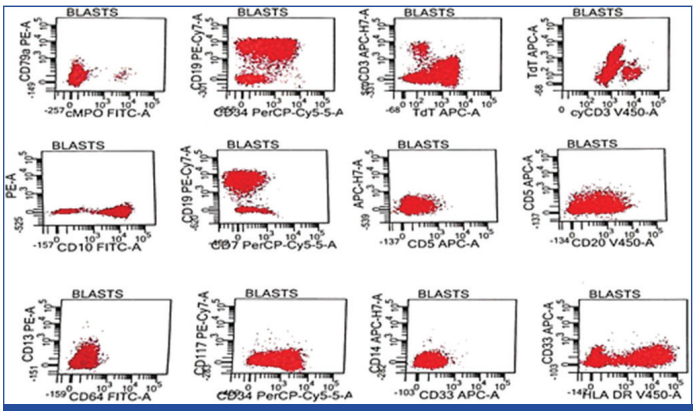
Immunophenotyping showed 53 (84%) were B lymphoblastic leukaemia and 10 (16%) were T lymphoblastic leukaemia. B lymphoblastic leukaemia expressed the lineage marker CD19 in all 53 (100%) cases, CD10 in 44 (83%) cases, CD20 in 27 cases, and CD79a in 24 cases. Precursor marker CD34 was expressed in 47 cases and Terminal Deoxynucleotidyl Transferase (TdT) in 33 of cases. Aberrant expression CD13 was found in five cases, CD7 in three cases and CD5 in one case [Table/Fig-7].



[Table/Fig-5]: Flow cytometric analysis of AML showing CD34, MPO, CD13, CD33, CD117, HLA -DR, CD 64 positivity.

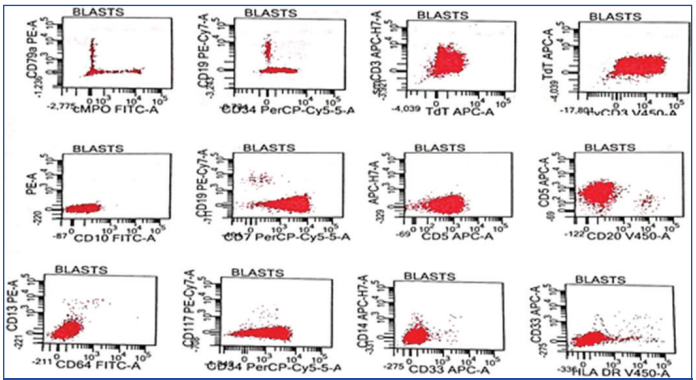


[Table/Fig-6]: Showing morphology of blast in ALL. (a) Lymphoblast in peripheral smear (Leishman stain, 100x); (b) Lymphoblast in bone marrow (Leishman stain, 100x); (c) Granular positivity of lymphoblast (Periodic Schiff stain, 100x); (d) Block and granular positivity of lymphoblast (Periodic Schiff stain, 100x); (e) Bone marrow biopsy of ALL (H&E stain, 10x); (f) Bone marrow biopsy of ALL (H&E stain, 40x).



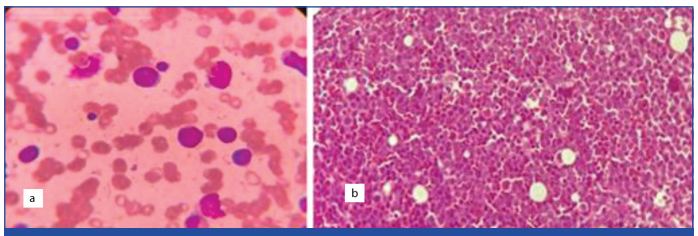
[Table/Fig-7]: Flow cytometric analysis of B-acute lymphoblastic leukaemia showing CD34, CD19, TdT, CD 10, CD20 and HLA-DR positivity.

The T-cell Acute Lymphoblastic Leukemia (T-ALL) expressed cytoplasmic and surface CD3 in all 10 (100%) cases, CD5 in 8 (80%) cases, and CD7 in 8 (80%) cases. Aberrant expression of CD13 in one case and CD33 in one more case was noted. The flow cytometric analysis of T-ALL is shown in [Table/Fig-8].

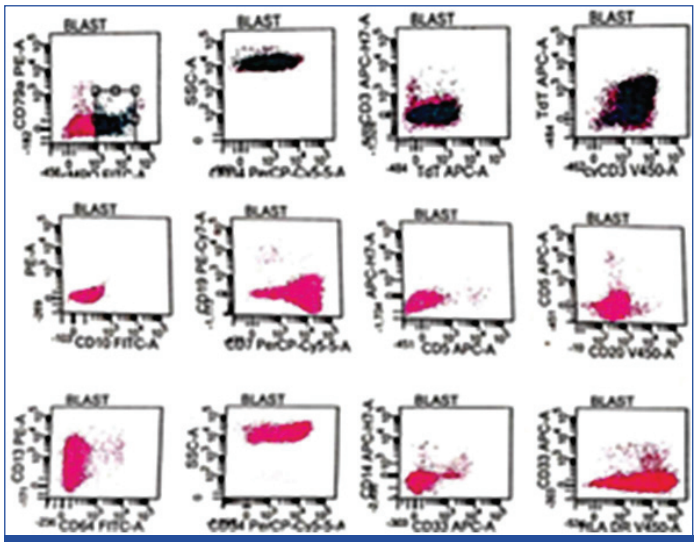


[Table/Fig-8]: Flow cytometric analysis of T acute lymphoblastic leukaemia showing cytoplasmic and surface CD3, CD5 and CD7 positive.

Biphenotypic acute leukaemia: A 34-year-old male patient was diagnosed with biphenotypic acute leukaemia. A 91% of blasts were seen in the bone marrow smears in which few of them were positive for SBB. Flow cytometric analysis showed positivity for both myeloid and T lymphoid markers. It was positive for MPO, CD13, CD117 (myeloid markers), cytoplasmic CD3, CD7 (T-lymphoid markers), and CD34, HLA-DR (precursor markers) was dim positive. It was negative for CD19, CD10, CD20, CD79a, CD14, CD64, CD33, and CD5. The morphology of the blasts and the flow cytometry are shown in [Table/Fig-9,10].

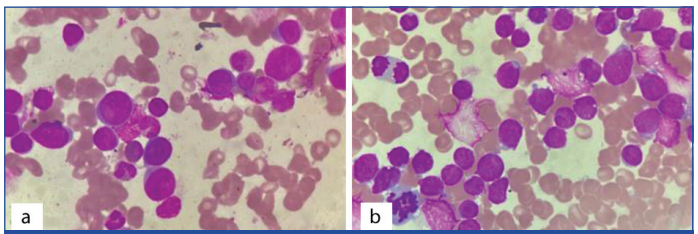


[Table/Fig-9]: Morphology of biphenotypic leukaemia. (a) Biphenotypic leukaemia peripheral smear (Leishman stain, 100x) (b) Biphenotypic leukaemia bone marrow biopsy (H&E, 40x).



[Table/Fig-10]: Shows flow cytometric analysis of biphenotypic leukaemia. The blasts are positive for CD34, MPO, CD13, HLA-DR, TdT, cytoplasmic CD3 and CD7.

Chronic myeloid leukaemia blast crisis: Two cases were diagnosed as CML in the blast crisis. One was a 37-year-old female patient in myeloid blast crisis with positivity for CD34, HLA-DR, MPO, CD13, CD33 and CD117. Another was a 28-year-old male patient in lymphoid blast crisis with cytoplasmic and surface CD3, CD34, CD5, and CD7 positivity with aberrant CD33. The morphology of CML in blast crisis is shown in [Table/Fig-11].



[Table/Fig-11]: (a) Chronic Myeloid Leukaemia (CML) in myeloid blast crisis-Bone marrow aspirate (Leishman stain, 100x); (b) Chronic myeloid leukaemia in lymphoid blast crisis-Bone marrow aspirate (Leishman stain, 100x).

The morphology, cytochemistry and immunophenotyping characteristics of the blasts are tabulated as shown in [Table/Fig-12].

Fever was the most common symptom followed by fatigue. Pallor was the most common sign (54.46%) followed by splenomegaly (16.07%), cervical lymphadenopathy (14.28%), hepatomegaly (13.39%) and jaundice in 1.78% of the total cases. Clinical features are shown in [Table/Fig-13]. Anaemia was seen in 105 (94%) cases and thrombocytopenia in 102 (91%) cases. Leukopenia in 35 (31%), leukocytosis in 54 (48%) cases, and normal white blood cell count in 23 (21%) cases were found.

Morphology of the blast (number)	SBB	PAS	Flow cytometry					Final diagnosis
			Precursor markers	Myeloid markers	B lymphoid markers	T lymphoid markers	Aberrant marker	
AML (28)	Positive-21	Negative	CD34, HLA-DR	CD13, CD33, MPO	Negative	Negative	CD7 -in 7 cases	AML
APML (13)	Positive-13	Negative	HLA-DR (dim+in 2 cases)	CD13, CD33, MPO	Negative	Negative	CD7-in 2 cases	APML (4 cases-PML-RARA positive)
AML with monoblasts (3)	Negative	Negative	CD34, HLA-DR	CD13, CD33, MPO and CD14, CD64	Negative	Negative	CD7 in 1 case	AML with monocytic differentiation
AML with increased megakaryocytes (1)	Negative	Negative	CD34, HLA-DR	CD13, CD33, MPO CD41, CD61	Negative	Negative		AML
AML with normoblasts (1) (AML-M6 by morphology)	Negative	Negative	CD34, HLA-DR	CD13, CD33, MPO	Negative	Negative		AML
AML (1)	Positive	Negative	CD34, HLA-DR	CD13, CD33, MPO, CD117	Negative	Cytoplasmic CD3 and CD7		Biphenotypic leukaemia
CML in blastic phase (2)	Negative	Negative	CD34, HLA-DR	CD13, CD33, MPO, CD117	Negative			CML in myeloid blast crisis
CML in blastic phase (2)	Negative	Negative	CD34, HLA-DR	Negative	Negative	Cytoplasmic CD3, CD5 and CD7	CD33	CML in lymphoid blast crisis
Acute lymphoblastic leukaemia	Negative	Positive-26	CD34, HLA-DR, TdT	Negative	CD19, CD10, CD79a, CD20	Negative	CD5, CD7, CD13, CD33	B ALL
Acute lymphoblastic leukaemia	Negative	Negative-27	CD34, HLA-DR, TdT	Negative	CD19, CD10, CD79a, CD20	Negative		B ALL
Acute lymphoblastic leukaemia	Negative	Positive-2	CD34, HLA-DR, TdT	Negative	Negative	Cytoplasmic CD3, CD5 and CD7	CD13	T ALL
Acute lymphoblastic leukaemia	Negative	Negative-8	CD34, HLA-DR, TdT	Negative	Negative	Cytoplasmic CD3, CD5 and CD7	CD33	T ALL

[Table/Fig-12]: Showing the comparison of morphology, cytochemistry and immunophenotyping in acute leukaemia.
ALL; B ALL; T ALL; CML; APML; PML-RARA: Promyelocytic leukemia/retinoic acid receptor alpha; MPO: Myeloperoxidase

Acute leukaemia	Number (n)	Percentage (%)
Symptoms		
Fever	95	84.82
Fatigue	6	5.35
Anorexia	3	2.67
Joint swelling	3	2.67
Gum bleeding	2	1.78
Signs		
Pallor	61	54.46
Splenomegaly	18	16.07
Lymphadenopathy	16	14.28
Hepatomegaly	15	13.39
Jaundice	2	1.78

[Table/Fig-13]: Showing signs and symptoms of acute leukemia.

DISCUSSION

Acute leukaemias are a group of heterogeneous malignancies which are rapidly progressive and ultimately replace the normal bone marrow. Early diagnosis and classification are crucial for treatment [5]. The current study is done to classify acute leukaemia based on morphology and cytochemistry and to compare it with immunophenotyping by flow cytometry. In a total of 112 cases studied, 46 were classified as AML, and 63 as ALL. Two cases with CML one in myeloid blast crisis and the other in lymphoid blast crisis was found. One biphenotypic leukaemia was noted. The predominant age group affected was between 31-40 years as compared with a study done by Alwan AF et al., [6].

Anaemia is one of the presenting features in acute leukaemia which is found in 105 (94%) of the patients and thrombocytopenia in 102 (91%) of cases. This observation is comparable with a study done by Ghosh S et al., in which 82% of patients presented with pallor and weakness [7]. In the morphological subtype, AML-M2 was more common followed by AML-M3 (APML). Studies done by Ghosh S et al., and Advani SH et al., show similar observations [7,8].

B cell ALL was the most common ALL subtype (84.13%). The predominant age group was children. CD10 positivity was seen in patients. Aberrant expressions of CD13, CD33, CD7 are seen. The proportion of T cell ALL in this study was (15.87%). A similar study done by Rajkumar NN and Vijay RH, showed B ALL (74.94%) was more than T ALL (20%) [9].

In the present study, one case was diagnosed as biphenotypic acute leukaemia based on immunophenotyping by flow cytometry. Cytochemical staining, in this case, showed positivity on SBB and negative on PAS, therefore was given a preliminary diagnosis of AML. Flow cytometric analysis showed more than 90% blasts in the gating. These blasts expressed myeloid markers MPO, CD33, CD117 and CD13 along with T cell lineage markers cytoplasmic CD3 and CD7. CD34 was showing bright positivity. TdT was dim positive. Other markers CD19, CD10, CD20, CD79a, CD14 and CD64 were negative. This was diagnosed as biphenotypic leukaemia, since it had a single population of blast cells that expressed both the myeloid lineage markers and T lymphoid lineage markers. Lee HG et al., study showed biphenotypic leukaemia is rare and accounted for 2-5% of acute leukaemia [10]. A study done by Charles NJ and Boyer DF, showed the mixed phenotype acute leukaemias have a male preponderance with worse prognosis when compared to patients with AML or ALL [11].

Two cases were diagnosed as CML in the blast crisis. One patient was a 37-year-old female patient with a myeloid blast crisis, had 58% blasts in the bone marrow, and expressed CD34, MPO, CD13, CD33, CD117 and HLA-DR positivity. The other patient was a 28-year-old male with lymphoid blast crisis, who had 72% blasts in the bone marrow with CD34, cytoplasmic CD3, surface CD3, CD5 and CD7 positivity. In both cases SBB and PAS were negative. Cedric reported de novo presentation of CML in myeloid blast crisis. Cytogenetic abnormality proved it to be CML and flow cytometry

showed bright positivity for CD33, CD13 and CD117, proving it to be in myeloid blast crisis [12].

Sudan Black B was done in all cases of acute leukaemia. A 35 (76%) of AML cases were SBB positive, and negative in 11 (24%) cases. PAS was done in all the ALL cases in that only 28 (44%) cases showed positive results and 35 (56%) cases were negative. All the cytochemistry findings were compared with flow cytometry. In a few cases, it was difficult; to differentiate between myeloblast and lymphoblast only with morphological features, which requires additional testing with cytochemistry. In these cases, the cytochemical stains are of great help in recognising the type of blasts, especially when there is asynchronism between nuclear and cytoplasmic maturation. Morphology aided with cytochemistry rendered diagnosis in 56% (35 AML and 28 ALL) of cases. Flow cytometric analysis is important in cases of AML where the blasts do not show auer rods or cytoplasmic granules and cytochemistry for SBB and NSE are negative. It can be AML-M0, AML-M7 [13]. Murmu R et al., studied 50 cases of acute leukaemia with 27 cases of AML and concordant 89% with flow cytometry. There were five cases where it was difficult to differentiate between AML and ALL, in which flow cytometry played a major role in diagnosing as AML [14]. A study done by Biren P et al., proved the same [15]. If AML is suspected in morphology and if flowcytometric analysis reveals Myeloperoxidase (MPO) to be negative, it is necessary to do immunohistochemistry in the bone marrow biopsy to prove MPO positivity in AML [16].

Immunophenotyping has become an important diagnostic tool in establishing the diagnosis and classification of acute leukaemias. It is useful in the early detection of minimal residual disease and is also reported to have prognostic value. In this study, we have found that MPO and CD13 was the myeloid marker that was most commonly present in all the AML subtypes. CD33 was the next commonly expressed antigen followed by CD34, HLA-DR, CD117, CD14, and CD64. In this study, CD19 was the B cell ALL marker present in 52 cases and followed by CD20, CD10, CD79a, CD34, and TdT. Cytoplasmic CD3 was the marker for T cell ALL and it was present in all 10 cases. Followed by surface CD3, CD5, CD7, CD34 and TdT, a study done by Shailendra J et al., showed that AML cases diagnosed by morphology and cytochemistry were confirmed by flow cytometry [17].

Limitation(s)

The study was done in 2018-19 (one year), when the molecular studies for acute leukaemia were expensive and many were not available in-house, therefore FAB classification was done and further confirmed by immunophenotyping by flow cytometry.

CONCLUSION(S)

Acute leukaemias are classified into AML and ALL by morphology and cytochemistry in most of cases. But in some cases when morphological dilemma between AML, M0 and ALL or biphenotypic leukaemia, immunophenotyping by flow cytometry plays a crucial role in the diagnosis and for the classification of ALL into B ALL and T ALL.

The present study showed that morphological diagnosis is important, based on which the cytochemistry and flow cytometry is done for a definitive diagnosis. In a few cases, where there was a diagnostic dilemma, cytochemistry and flow cytometry helped in the diagnosis of leukaemia.

REFERENCES

[1] Greer PJ, Foerster J, Lukens JN, Rodgers GM, Parakeva F, Glader B. Wintrobes Clinical Hematology.11th edition, Philadelphia: Lippincott Williams & Wilkins; 2004.
[2] Belurkar S, Mantravadi H, Manohar C, Kurien A. Correlation of morphologic and cytochemical diagnosis with flowcytometric analysis in acute leukaemia. J Cancer Res Ther. 2013;9(1):71-79.
[3] Casasnovas RO, Slimane FK, Garand R, Faure GC, Campos L, Deneys V, et al. Immunological classification of acute myeloblastic leukaemias: Relevance to patient outcome. Leukaemia. 2003;17:515-27.
[4] Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th edition). IARC: Lyon 2017.
[5] Canaani J, Beohou E, Labopin M, Socié G, Huynh A, Volin L, et al. Impact of FAB classification on predicting outcome in acute myeloid leukaemia, not otherwise specified, patients undergoing allogeneic stem cell transplantation in CR1: An analysis of 1690 patients from the acute leukaemia working party of EBMT. Am J Hematol. 2017;92:344-50.
[6] Alwan AF, Alrahal NK, Alnuaimi MA. A one-year anticipated blood transfusion requirement among patients with acute leukaemia undergoing chemotherapy in the National Centre for Hematology in Baghdad. Iragi J Hematol. 2019;8:25-28.
[7] Ghosh S, Shinde SC, Kumaran GS, Sapre RS, Dhond SR, Badrinath Y, et al. Haematologic and immunophenotypic profile of acute myeloid leukaemia: An experience of Tata Memorial Hospital. Indian J Cancer. 2003;40(2):71-76.
[8] Advani SH, Rao DN, Kutty PM, Gopal R, Nair CN. Acute non-lymphoblastic leukaemia in Indian population FAB classification and response to therapy. Indian J Cancer. 1983;20(3):131-36.
[9] Rajkumar NN, Vijay RH. Immunological subtypes of acute lymphoblastic leukaemia- beyond morphology: Experience from Kidwai State Cancer Institute, Bengaluru, India. J Assoc Physicians India. 2017;65(7):14-17.
[10] Lee HG, Baek HJ, Kim HS, Park SM, Hwang TJ, Kook H. Biphenotypic acute leukaemia or acute leukaemia of ambiguous lineage in childhood: Clinical characteristics and outcome. Blood Res. 2019;54(1):63-73.
[11] Charles NJ, Boyer DF. Mixed- Phenotype Acute Leukaemia. Arch Pathol Lab Med. 2017;141(11):1462-68.
[12] Cedric P, Roch H. Chronic Myelogenous leukaemia in primary blast crisis rather than de novo BCR-ABL1-positive acute myeloid leukaemia. Clinical Case Report. 2017;5(6):757-60.
[13] Gujral S. Role of flow cytometry I diagnosis of Hematolymphoid malignancies. 4th conference on evidence-based management of cancer in India. 2006.
[14] Murmu R, Srivastava RK, Banerjee S, Sunil KM, Anu S. Diagnostic accuracy of acute leukaemia by flow cytometry in comparison to morphological diagnosis-A study. IOSR Journal of Dental and Medical Science. 2016;15:52-54.
[15] Biren P, Kumar A, Trivedi P, Dheval J, Nisha K, Siddharth T. Correlation between morphology and special stains with flow cytometry in the diagnosis of acute leukaemia. Sch J App Med. 2017;5:2047-51.
[16] Ahuja A, Tyagi S, Seth T, Pati HP, Gahlot G, Tripathi P, et al. Comparison of immunohistochemistry, cytochemistry, and flow cytometry in AML for myeloperoxidase detection. Indian J Hematol Blood Transfus. 2018;34(2):233-39.
[17] Shailendra J, Nitin YS, Purnima K, Jayshree T, Kumbhalkar DT. Correlation of cytomorphology with flowcytometric immunophenotyping of acute myeloid leukaemia in tertiary care hospital. International Journal of Contemporary Medical Research. 2019;6(2):B19-B22.

PARTICULARS OF CONTRIBUTORS:
1. Laboratory Incharge, Department of Laboratory, St. Sebastian Hospital, Kannur, Kerala, India.
2. Assistant Professor, Department of Transfusion Medicine and Immunohematology, St. Johns National Institute of Health Sciences, Bangalore, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. J Latha Fathima,
Assistant Professor, Department of Transfusion Medicine and Immunohematology,
St. Johns National Institute of Health Sciences, Bangalore, Karnataka, India.
E-mail: lathafathimashaju@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Sep 01, 2022
- Manual Googling: Nov 10, 2022
- iThenticate Software: Nov 17, 2022 (11%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Aug 28, 2022**
Date of Peer Review: **Sep 15, 2022**
Date of Acceptance: **Nov 18, 2022**
Date of Publishing: **Feb 01, 2023**