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Flow cytometry diagnosis of acute leukemia and comparison of cytomorphological diagnosis with Flow cytometry diagnosis

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Abstract
Introduction: Clinical, morphological, immunophenotypic, genetic and molecular characteristics are the most important diagnostic modalities in established diagnosis of acute leukemia. Immuno phenotyping flow cytometry has become a proven valuable tool for precise characterization of acute leukemia. This study was done to see various types of acute leukemia and aberrant antigen expression pattern in acute leukemia in patients and also to compare the results between microscopic morphology and flow cytometry.
Materials and Methods: Total number of acute leukemia diagnosed was 103 and of all the age groups. All the new cases reported which were showing atypical cells in peripheral smear and treated cases to find out the minimal residual disease (MRD) were included in this study.
Results: When the immune phenotyping of the acute lymphocytic leukemia patterns was done, most of the B-ALL showed positivity for CD79 alpha and HLA-DR followed by other markers and most of the T-ALL showed positivity for CD3 and CD7 markers followed by other markers. When the immune phenotyping patterns of acute myeloid leukemia was done, almost all the cases showed positive for CD13 and varying degrees of positivity with other markers.
Conclusion: The flow cytometry immune phenotyping is a powerful tool for accurate diagnosis of acute leukemias and also help in identifying myeloid or lymphoid lineage of these leukemias and has great prognostic and therapeutic implications.

Keywords: Acute leukemia, atypical cells, blast, myeloid, lymphoblast, flow cytometry, immunophenotyping, minimal residual disease

Introduction
Acute leukemia is a rapidly progressing clonal hematopoietic disorder which affects the bone marrow and is characterized by the production of immature cells (blasts) in bone marrow and peripheral blood. Depending upon the morphology of the immature cells, immunological and cytogenetic findings, clinical features and molecular irregularities findings, Acute Leukemia is divided into two major types: acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) where myeloblasts and lymphoblasts are dominant respectively [1]. AML is further classifiable into eight subtypes (M0–M7) based on the cell type of origin and its grade/stage of maturity, while ALL is classified into three subtypes (L1, L2, and L3) [2, 3]. Acute Myeloid Leukemia (AML) in adults accounts for 80-90% of cases of acute leukemias**.** Acute Lymphoid Leukemia (ALL), though common in childhood comprises 12% of all leukemias. Incidence rises again in the sixth decade but this age peak is absent in developing countries. Leukemias have varied presentations around the world and it requires a multimodal approach for diagnosis and treatment. In the past, hallmark for the diagnosis of acute leukemias was morphology and cytochemistry and the accuracy of the diagnosis was 80 % [4]. In the present era, the ability of immunophenotyping to identify myeloid versus lymphoid differentiation increased to 98% [5]. Flow cytometry immunophenotyping is a strong and mighty technological tool that is used to recognize antigens present on the cell membrane of the immature cells [6].The identification of these antigens on leukemic cells not only helpful in classifying and sub-classifying the leukemia grade and stage but also helps in deciding the treatment protocols for the for the patients [7]. Flow cytometry also helps in estimating the prognosis of acute leukemia patients and explore for applicable markers to detect minimal residual disease.

Aim of the study

This study was done to see various types of acute leukemia and aberrant antigen expression pattern in acute leukemia in patients and also to compare the results between microscopic morphology and Flow cytometry.

Materials and Methods

This is a record based study and the study duration was for one year, between July 2019 and June 2020 at Meherbai Tata Memorial Hospital (MTMH), Jamshedpur, and Jharkhand, India and in collaboration with Onquest Labs. Total number of acute leukemia diagnosed was 103 and of all the age groups. Written informed consent was taken from the patients was taken. Blood and bone marrow samples were collected and processed for interpretation. All slides were reviewed by the experienced pathologist and diagnosis was established by the interpretation of the morphology of the cells. All the new cases reported which were showing atypical cells in peripheral smear and treated cases to find out the minimal residual disease (MRD) were included in this study. Patients who did not want to be the part of this study were excluded.

Sample collection: - The sample preferred for the study was bone marrow aspirate, in some cases where the bone marrow aspirate was not possible, peripheral blood was used. Approximately 2-2.5ml of bone marrow aspirate was collected from 96 patients, 06 patients peripheral blood was used for the study and in 01 patient ascitic fluid was used for the morphology and flow cytometry. Bone marrow procedure was done by the pathologist and slides were made for cytomorphological analysis. Bone marrow aspiration procedure was done from the posterior superior iliac spine after ensuring strict asepsis and necessary precautions. Approximately 2 ml of peripheral blood from 06 patients were collected mostly from ante-cubital vein with aseptic precautions. All the samples were collected in EDTA tubes.

Bone marrow aspirates or particulate matter and peripheral blood smears were made from the blood sample were spread onto the slides and these slides were air dried and stained with Wright-Giemsa stain or May-Grundwald Geimsa (MGG) stain. Special stains like Myeloperoxidase were done routinely in all cases. Other cytochemical stains like periodic acid-Schiff (PAS); non-specific esterase and specific esterase were performed as and when required based on the morphological details of the leukemic blasts. Immunophenotyping data was correlated with cytogenetics / molecular genetics data wherever available.

Immunophenotyping/Flow cytometry-Flowcytometric immunophenotyping is useful in diagnosing the lineage in acute leukemia by the expression of lineage specific CD markers (Cluster of Differentiation). Flow cytometry Instrument and software used in this study was BD FACS Canto II/BD FACS DIVA. CD45, the common leukocyte antigen was used to gate the population of blasts or the atypical cells and expression of the other lineage specific markers are analyzed on the gated population. The common antibodies used were- 1) T-cell markers CD3, CD5, Cyt CD3, CD7, CD8, CD16, CD56, 2) B- cell markers CD19,CD10,SIgM,CD20,CD22,

CD23, CD25.CD 34, CD103, CD123, FMC7, ZAP 70 (Zeta-chain-associated protein kinase 70), Kappa, Lambda, 3) Myeloid lineage markers- CD13, CD14, CD16, CD11b, CD33, CD64, MPO (Myeloperoxidases), 4) Other supportive precursor markers- CD45, CD117, CD36,CD38.CD56, CD79 alpha, HLADR, terminal deoxynucleotidyl transferase/TdT. For minimal residual disease panel (MRD) markers used were CD10, CD19, CD20, CD34, CD38, CD45, CD58, CD73, CD123, and CD13/CD33. Out of the 103 cases included in this study, 16 cases were minimal residual disease (MRD).Flow cytometry immunophenotyping helps in diagnosing bi-lineage and tri-lineage acute leukemia by identifying more than one lineage markers. In acute myeloid leukemia with features of myelodysplastic syndrome (MDS), along with the morphological findings, the dysgranulopoiesis is noted on Flow cytometry immunophenotyping as alter maturation patterns. The diagnostic criteria of the acute leukemia in this study comprised of cytomorphological features with percentage of blasts (> 20%), cytochemical staining and immunophenotyping.

FISH analysis (where applicable) was performed for confirmation of translocations using dual color dual fusion probes for BCR-ABL1 (Zytovision, Bremerhaven, Germany), t(8;21) (Metasystems, Germany) and t(15;17) (Abbot Vysis, Illinois, U.S.A.), locus-specific dual color break apart probes for MLL and inv(16) (Abbot Vysis Illinois, U.S.A.) and locus-specific dual color extra signal ETV6/RUNX1 probe (Abbot Vysis Illinois, U.S.A.) according to manufacturer's instructions following standard techniques.

Results

In this study, a total of 103 patients were included, males were 65 and 38 females. Maximum number of cases was in the age group 41-50 years, followed by 11-20 years group and the least was seen in 71-80 years group. Mean age in this study is 33.66 years. The age group wise number and percentage is tabulated in Table 1. Fever was the most common complaint by the patients (82 cases-79.61%), followed by generalized weakness (44 cases-42.72%). Pallor was the most common sign observed in all the patients with few patients showing associated findings such as splenomegaly 31 cases (30.10 %,) hepatomegaly 12 cases (11.65%) and lymphadenopathy 19 cases (18.44%). Anemia (hemoglobin median value 4.8 grams/dL) was the most common laboratory finding followed by thrombocytopenia (median platelet count was 33,000/cu.mm). Leukocytosis was seen in 68 cases (66%), normal leukocyte count was shown by 28 cases (27.20%) and 7 cases (6.80 %) showed leucopenia.

Table 1: Age wise distribution of the cases

Age Group	Number Of Cases	Percentage
< 10 YEARS	14	13.65
11-20 YEARS	18	17.50
21-30 YEARS	15	14.60
31-40 YEARS	16	15.35
41-50 YEARS	19	18.45
51-60 YEARS	12	11.65
61-70 YEARS	06	05.85
71-80 YEARS	03	02.95
Total	103	100

Peripheral Blood Smear findings (PBS) and Bone Marrow (BM) diagnoses are tabulated in Table 2 and showed a wide range of leukemia diagnoses. Atypical cells or blasts ranged

from 2% to 30 % in peripheral smear, 20-80 % in bone marrow and ascitic fluid. Median peripheral smear blast percentage in our study is 56.31 % (58 cases) in ALL and 25.24% (26 cases) in AML. In bone marrow it is 82.52% (85 cases) in ALL and 56.31% (58 cases) in AML.

Table 2: Age wise distribution of the cases

Diagnosis	Number of Cases	Percentage
Acute Leukemia	80	77.67
Non-Hodgkin's / Chronic Lymphocytic Leukemia (CLL)	06	05.84
Leukocytosis with Atypical Cells	04	03.88
Pancytopenia with Atypical cells	03	02.91
Leukopenia with Atypical Cells	02	01.94
Chronic Myeloid Leukemia in Blast Crisis (CML)	02	01.94
Multiple Myeloma	02	01.94
Anemia with Renal Failure	01	00.97
Hairy Cell Leukemia	01	00.97
Suspected Myelodysplastic Syndrome (MDS)	01	00.97
Hodgkin's Lymphoma	01	00.97
Total	103	100

All the samples i.e., bone marrow aspirates (96 samples), peripheral blood (6 samples) and ascitic fluid sample (1) were subjected to the Flow cytometry and the diagnoses of the same are tabulated in Table 3

Table 3: Flow cytometry immunophenotyping diagnoses of the cases

Flow cytometry Diagnosis	Number of Cases	Percentage
Acute Lymphoblastic Leukemia (ALL)	41	39.81
Acute Myeloid Leukemia (AML)	34	33.01
Multiple Myeloma (MM)	06	05.82
Chronic Lymphocytic Leukemia (CLL)	08	07.76
Chronic Myeloid Leukemia (CML)	06	05.83
Non-Hodgkin's Lymphoma (NHL)	05	04.86
Erythroid Hyperplasia	02	01.94
Pro B-ALL	01	00.97
Total	103	100

In this study, when the immunophenotyping of the acute lymphocytic leukemia patterns was done, most of the B-ALL showed positivity for CD79 alpha and HLA-DR followed by other markers and most of the T-ALL showed positivity for CD3 and CD7 markers followed by other markers which are tabulated in Table 4. Similarly, when the immunophenotyping patterns of acute myeloid leukemia was done, almost all the cases showed positive for CD13 and varying degrees of positivity with other markers which are tabulated in the Table 5.

Table 4: Immunophenotyping of patients with B-ALL and T-ALL (n=41)

Immunophenotyping Marker	B-ALL (number -percentage) n=41 (B-ALL = 29)	T-ALL(number-percentage) n=41 (T-ALL = 12)
CD79alpha	29 – 100 %	02—16.66 %
HLA-DR	29 – 100 %	11—91.66 %
CD34/CD33	24—82.75%	12—100 %
TdT	18—62.06%	09 – 75.00%
CD19	28—96.55%	02—16.66 %
CD10	28—96.55%	10 – 83.33%
CD20	15—51.73%	--
CD22	13—44.83%	--
CD103	06—20.68%	--
CD123	09—31.03%	--
FMC7	03—10.35%	--
ZAP70	04—13.80%	--
CD3	--	12 –100 %
CD5	--	12 –100 %
CD7	--	12 –100%
CD16	--	11—91.66%
CD56	--	11—91.66%
CD23	--	10—83.33%
CD25	--	08—66.66%

Table 5: Immunophenotyping of Acute myeloid leukemia patients (n=34)

Immunophenotyping Marker	Number of cases	Percentage
CD13	34	100
CD33	30	88.25
CD117	34	100
CyMPO	34	100
CD34	30	88.25
HLA-DR	28	82.35
CD14	09	26.48
CD15	08	23.53
CD64	19	55.89

In this study, apart from AML and ALL, immunophenotyping also diagnosed 1) multiple myeloma (06 cases) with the panel CD19, CD20, CD27, CD38, CD45, CD81, CD56, CD117, Cy Kappa, Cy Lambda,CD19/CD138 (CD38)/CD45, 2) Chronic lymphocytic leukemia (CLL)-08 cases with the panel CD5, CD19, CD20, CD23, CD43 and CD200, 3) Chronic myeloid leukemia (CML)- 06 cases with CD34 and CD7 panel, 4) Non-Hodgkin's Lymphoma (NHL)-05 cases with the panel CD19,CD22 and CD200, 5) Erythroid hyperplasia -02cases with the panel CD71, CD235alpha,CD36 and CD117. Out of 103 cases, 16 cases were minimal residual disease (MRD) and among them 12 cases showed < 0.1% of abnormal cells, which were not significant clinically.

When concordance of the cytomorphological and immunophenotyping diagnosis was done, it was observed that 93.75 % of the cytomorphological diagnosis is concordance with the immunophenotyping Flow cytometry diagnosis and remaining is in partial concordance.

FISH analysis (where applicable) was performed for confirmation of translocations using dual color dual fusion probes for BCR-ABL1, t (8; 21) and t (15; 17).

Acute leukemias are the diversified group of hematological malignancies that exhibit different cytomorphological, cytochemical features and assessment of these features helps in accurate diagnosis, treatment and establishing the prognosis of the cases. Immunophenotyping helps in better diagnosis of the cases of acute leukemias. These are characterized by excess proliferation of the hemopoietic progenitor cells and ultimately replace the physiological bone marrow tissue leading to the bone marrow failure. Well timed intervention in the form or chemotherapy or radiotherapy would minimize the morbidity and complications in the patients especially in the initial cases [8, 9]. In immunophenotyping Flow cytometry large number of cells can be studied accurately in a short span of time giving accurate diagnosis.

In this study, maximum number of cases was in the age group 41-50 years, followed by 11-20 years group and the least was seen in 71-80 years group which is consistent with the findings of the author Ratnamala *et al.* the maximum number of cases were reported in the age group of 41-50 years followed by 51-60years age group [10]. The mean age in this study is 33.66 years whereas Ratnamala *et al.* study showed the mean age as 41 years, this study is also consistent with the findings of the Ratnamala *et al.* in terms of male preponderance [10].

Pallor was the most common sign observed in all the patients (103 cases-100%) with few patients showing associated findings such as splenomegaly 31 cases (30.10 %,) hepatomegaly 12 cases (11.65%) and lymphadenopathy 19 cases (18.44%). Anemia (hemoglobin median value 4.8 grams/dL) was the most common laboratory finding (103 cases-100%) followed by thrombocytopenia (median platelet count was 33,000/cu.mm- (86 cases - 83.50%). This study was compared with the study done by Ratnamala *et al.* where they have observed that pallor was seen 79% cases, splenomegaly in 30 % cases and lymphadenopathy in 28% cases. In other study done by Nwannadi *et al.* where fatigue (82.2%) was the most common symptom, followed by fever (78.5%), weight loss (54.6%), lymph node enlargement (53.4%), bone pain (31.9%), and bleeding (10.4%) [11]. Anemia was seen in 98 % of the cases and thrombocytopenia in 93 % of the cases. Leukocytosis was seen in 68 cases (66%), normal leukocyte count was shown by 28 cases (27.20%) and 7 cases (6.80 %) showed leucopenia whereas 49% of the patients had leukocytosis at presentation, 32% had leukopenia while 19% presented with a normal total count in the study done by Ratnamala *et al.* [10]. Median peripheral smear blast percentage in our study is 56.31 % (58 cases) in ALL and 25.24% (26 cases) in AML. In bone marrow it is 82.52% (85 cases) in ALL and 56.31% (58 cases) in AML. In a study by Rathee *et al*., median blast percentage was 45% in AML and 38% in ALL [12]. Ghosh *et al*., demonstrated mean values and range for peripheral blood blasts in AML as 41.4% (5-77%) and bone marrow blasts as 57.6% (34-96%) [13]. In the present study, diagnosis of acute leukemia was made in 84 cases (81.55%) on peripheral smear study, this diagnosis is slightly higher than Rabizadeh *et al.* and Ratnamala *et al.* where they have reported acute leukemia on peripheral smear in 75% and 62 % cases respectively [14, 10].

In the present study, B-ALL cases expressed positivity with CD79alpha, HLA-DR,CD33,CD34,TdT,CD19,CD10 with varying degrees of positivity ranging from 62.% to 100% and T-ALL cases expressed positivity with CD20, 22, 103, 123, FMC7, ZAP70 with varying degrees of positivity ranging from 16.66% to 100%. These markers showed high sensitivity in this study. In the study conducted by Gupta N *et al*., immaturity markers like HLA-DR, TdT, and CD34 were expressed in 97.4%, 97% and 81.3% of cases respectively which is consistent with the findings of this study [15]. According to Shrestha S *et al.* and Rothe G *et al*., an ALL of the B-lymphocyte lineage is assumed if CD22 or CD79a expression is found either cytoplasmic or on the cell surface with the expression of CD19 and HLA-DR [4, 16]. In the study done by Asif Rashed *et al*., TdT, a nonspecific immaturity marker of lymphoid series, was expressed in 47.6% of B-ALL which is almost similar to this study done by Mukda E *et al.* where it was expressed in 51.1% [17, 18].

In this study, all 103 cases of T-ALL expressed CD3, CD5, CD7 (100% expression), CD16 and CD56 expression was seen in 91.66% and CD23 expression seen in 83.33% and CD25 expression was noted in 66.66% cases. According to Gupta N *et al*., T-lineage ALL is diagnosed based on the presence of cytoplasmic CD3 in leukemic blasts [15]. Shrestha S *et al.* and Rothe G *et al.* also stated that an ALL of the T-lymphocyte lineage is assumed if CD3 expression is found either cytoplasmically or on the cell surface with the simultaneous expression of CD7. T-lineage ALL subtypes can be defined based on the surface expression of CD1a, CD2, CD3, CD4 and CD8 [4, 16]. Studies by Rashed *et al*., Mukda *et al.* and other studies also show that the expression of cyCD3 in 100% cases of T-ALL was

consistent with the findings of these studies. CyCD3 is the most sensitive and specific marker for T-ALL. Expression of CD7 was 90% (9/10) among the T-ALL cases which is within the range found in these studies (100%, 92.3%, and 85.7% respectively). Expression of CD5 was found in 80% (8/10) of the T-ALL cases which is nearly similar to these study results (84.6% and 86% respectively) [17, 18, 19, 20].

In a study done by Rawstron AC *et al.*, it was CD5, CD19, CD20, CD200, CD23 are the required markers for establishing diagnosis of chronic lymphocytic leukemia (CLL) which is consistent with the finding of this study where positivity of the above markers was more than 85% [21]. Multiple Myeloma (MM) is a Plasma Cell (PC) neoplasm and is marked by the agglomeration of malignant PCs (MMCs) within the bone marrow (BM). MMCs express several markers aberrantly, compared to normal plasma cells, and a combination of several markers is necessary to delineate optimally MMCs from N-PCs. The antigens used for detecting normal plasma cells and malignant plasma cells include CD19, CD56, CD20, CD117, CD28, CD33, CD27, CD81, CD31, CD39, CD40 and CD44 [21]. In the present study multiple myeloma diagnosed with the panel of markers CD19, CD20, CD27,CD38, CD45, CD81, CD56, CD117, Cy Kappa, Cy Lambda,CD19/CD138 (CD38)/CD45 which is in tandem with the finding of Rawstron *et al.* [21].

Out of 103 cases, 16 cases were minimal residual disease (MRD) and among them 12 cases showed < 0.1% of abnormal cells, which were not significant clinically and the same panel of markers were used. These findings are also consistent with the findings of the other authors [17-21].

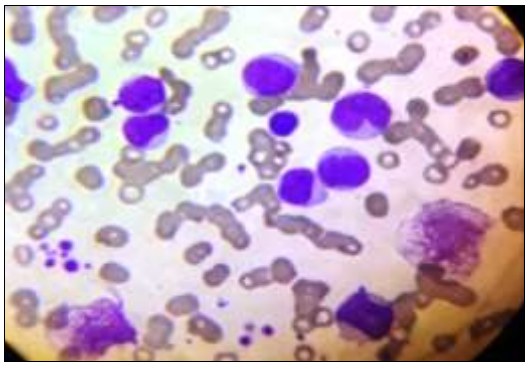

Fig 1: Photomicrograph of the peripheral smear under 100 X view showing microcytic hypochromic red blood cells (Blue Arrow), Blasts (Black Arrow) and platelets in tiny clumps (Orange Arrow).

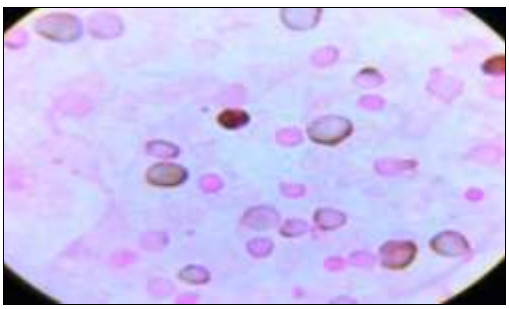

Fig 2: Photomicrograph showing blasts with myeloperoxidase (MPO) staining in the cytoplasm (White Arrows).

Prognosis

In acute myeloid leukemia (AML), positivity or expression of CD7, CD9, CD11b, CD13, CD14, CD33, CD34, CD56, TdT are associated with bad prognosis [22-28]. In a study done by author Venditti A *et al*., expression of CD34 and HLA-DR reported to have an independent predictor of non-success to achieve complete remission (CR) [29]. Another study done by Chang SHF *et al*. reported that blasts expressing panmyeloid markers: myeloperoxidase (MPO), CD13, CD33, CDw65 and CD117 have more favorable prognosis in cases with [30].

Poor prognosis is associated with CD20 expression in adult precursor B-lineage ALL [31]. Bad prognosis and high chances of relapse is connected with bright CD45 expression of B and T cell ALL [32].

Follow-up and Minimal Residual Disease (MRD)

Minimal Residual Disease (MRD) is defined as leukemic population unnoticeable by cytomorphological methods (microscopy). Alternatively, MRD is a word used when there is evidence of leukemic cells remain in the bone marrow but there are insufficient cells to be detected by routine morphological examination, but can be detected by immunophenotyping Flow cytometry, cytogenetics or molecular genetics. Identification of MRD is very important because it can predict early relapse and will help in risk stratification in acute leukemia.

Conclusion

The uniqueness of Flow cytometry allows the detection of 1 leukemic cell among 10,000 normal cells (0.01%). The most common differential of neoplastic blasts is hematogones and regenerating blasts, which can be differentiated on flowcytometric immunophenotyping. In this study ALL was the most common among the acute leukemia, further it was subclassified into B-ALL and T-ALL. Flowcytometric immunophenotyping directly correlates with prognosis and in an era of novel agents may help in development of monoclonal antibodies to the tumor antigens. In addition to that, Flow cytometry is the main stay of evaluating minimal residual disease, particularly in cases without any specific molecular signature.

Limitations

More number of the studies can be done involving large population of leukemia cases. Sub-classification of myeloid and lymphoid by flow cytometry gives better diagnosis, better treatment and better prognosis which are not done in this study. Correlation with the Fluorescent in-situ hybridization (FISH) for chromosomal translocation will additionally help in diagnosis.

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References

1. Petrov I, Suntsova M, Mutorova O *et al*. Molecular pathway activation features of pediatric acute myeloid leukemia (AML) and acute lymphoblast leukemia (ALL) cells. Aging (Albany NY) 2016;8:2936-2947.
2. Bennett JM, Catovsky D, Daniel MT *et al*. Proposals for the classification of the acute leukaemias. French-American-British (FAB) co-operative group. Br J Haematol 1976;33:451-458.
3. Davey FR, Castella A, Lauenstein K *et al*. Prognostic significance of the revised French-American-British classification for acute lymphocytic leukaemia. Clin

Lab Haematol 1983;5:343-351.
4. Shrestha S, Shrestha J, Pun C.B1, Pathak T, Bastola S, Bhatta R. Immunophenotypic study of acute leukemia by flow cytometry at BPKMCH. Journal of Pathology of Nepal 2013;3:345-350.
5. Weir EG, Borowitz MJ. Flow cytometry in the diagnosis of acute leukemia. Seminars in hematology 2001;38:124-128.
6. Xu B, Hu C, Miao XD *et al*. Immunophenotyping of 106 adult patients with acute leukemia by flow cytometry. Di Yi Jun Yi Da Xue Xue Bao 2003;23:1043-1046.
7. Khoury H, Dalal BI, Nevill TJ *et al*. Acute myelogenous leukemia with t(8; 21)–identification of a specific immunophenotype. Leuk Lymphoma 2003;44:1713-1718.
8. Pulte D, Jansen L, Gondos A *et al*. Survival of Adults with Acute Lymphoblastic Leukemia in Germany and the United States. PLoS ONE 2014;9(1):e85554.
9. Venkateswaran SP, Jojo A, Unni M. A Clinicopathological Correlation of Acute Leukaemias in relation to Immunophenotyping and Cytogenetics. International Journal of Collaborative Research on Internal Medicine & Public Health 2012;4(10):1713-1737.
10. Ratnamala Choudhury, Sreevalli A, Chaitanya H, Balakrishnan Cecil R. Ross. Clinical and laboratory profile of acute leukemia with special reference to flow cytometry. Medica Innovatica 2017;6(2):10-19.
11. Nwannadi O, Alao G, Bazuaye M, Nwagu Borke M. Clinical and Laboratory Characteristics of Patients with Leukaemia in South-Nigeria. The Internet Journal of Oncology 2009,7(2).
12. Radha Rathee, Minakshi Vashist, Ashok Kumar *et al*. Incidence of acute and chronic forms of leukemia in Haryana. International Journal of Pharmacy and Pharmaceutical Sciences 2014;6(2):323-325.
13. Ghosh S, Shinde SC, Kumaran GS, Sapre RS, Dhond SR, Badrinath Y *et al*. Haematologic and immunophenotypic profile of acute myeloid leukemia: an experience of Tata Memorial Hospital. Indian J Cancer 2003;40(2):71-76.
14. Rabizadeh *et al*. Acute leukemia detection rate by automated blood count parameters and peripheral smear review. Int J Lab Hematol 2015;37(1):44-49.
15. Gupta N, Pawar R, Banerjee S, Brahma S, Rath A, Shewale S *et al*. Spectrum and immunophenotypic profile of acute leukemia: a tertiary center flow cytometry experience. Mediterr J Hematol Infect Dis 2019;11(1):e2019017. DOI: http://dx.doi.org/10.4084/MJHID.2019.017.
16. Rothe G, Schmitz G. European Working Group on Clinical Cell Analysis (EWGCCA). Consensus Document on Leukemia Immunophenotyping. Leukemia 1996;10:877-95.
17. Asif Rashed, Shirin Tarafder, Humayun Sattar, Sadia Hossain. Flow Cytometric Immunophenotyping of Acute Leukaemia in Adult and Its Comparison with Cytomorphology. Int J Med Res Prof 2018;4(5):157-63.
18. Mukda E, Pintaraks K, Komvilaisak P, Wiangnon S. Cytochemistry and Multi-color Flow Cytometric Immunophenotype for Diagnosis of Childhood Acute Leukemia. J Hematol Transfus Med 2011;21:23-31.
19. Gujral S, Badrinath Y, Kumar A *et al*. Immunophenotypic Profile of Acute Leukemia: Critical Analysis and Insights Gained at a Tertiary Care Center in India. Cytometry B Clin Cytom 2009;76(3):199-205. doi: 10.1002/cyto.b.20451.
20. Paredes-Aguilera R, Romero-Guzman L, Lopez-Santiago N, Burbano-Ceron L, Camacho-Del Monte O, Nieto-Martinez S. Flow cytometric analysis of cell-surface and intracellular antigens in the diagnosis of acute leukemia. Am. J. Hematol 2001;68:69-74.
21. Rawstron AC, Kreuzer K-A, Soosapilla A, Spacek M, Stehlikova O, Gambell P *et al*. Reproducible Diagnosis of Chronic Lymphocytic Leukemia by Flow Cytometry: An European Research Initiative on CLL (ERIC) & European Society for Clinical Cell Analysis (ESCCA) Harmonisation Project. Cytometry Part B 2018;94B:121-128.
22. Webber BA, Cushing MM, Li S. Prognostic significance of flow cytometricimmunophenotyping in acute myeloid leukemia. Int. J Clin Exp Pathol 2008;1:124-133.
23. Lee E, Yang J, Leavitt RD, Testa JR, Civin CI, Forrest A *et al*. The significance of CD34 and TdT determinations in patients with untreated de novo acute myeloid leukemia. Leukemia 1992;6:1203-1209.
24. Del Poeta G, Stasi R, Venditti A, Cox C, Aronica G, Masi M *et al*. CD7 expression in acute myeloid leukemia. Leuk Lymphoma 1995;17:111-119.
25. Graf M, Reif S, Kroll T, Hecht K, Nuessler V, Schmetzer H. Expression of MAC-1 (CD11b) in acute myeloid leukemia (AML) is associated with an unfavorable prognosis. Am J Hematol 2006;81:227-235.
26. Kita K, Miwa H, Nakase K, Kawakami K, Kobayashi T, Shirakawa S *et al*. Clinical importance of CD7 expression in acute myelocytic leukemia. The Japan Cooperative Group of Leukemia/Lymphoma. Blood 1993;81:2399-2405.
27. Raspadori D, Damiani D, Michieli M, Stocchi R, Gentili S, Gozzetti A *et al*. CD56 and PGP expression in acute myeloid leukemia: impact on clinical outcome. Haematologica 2002;87:1135-1140.
28. Saxena A, Sheridan DP, Card RT, McPeek AM, Mewdell CC, Skinnider LF. Biologic and clinical significance of CD7 expression in acute myeloid leukemia. Am J Hematol 1998;58:278-284.
29. Venditti A, Del Poeta G, Buccisano F, Tamburini A, Cox-Froncillo MC, Aronica G *et al*. Prognostic relevance of the expression of Tdt and CD7 in 335 cases of acute myeloid leukemia. Leukemia 1998;12:1056-1063.
30. Chang HSF, Yi QL, Patterson B, Brien B, Minden MD. Prognostic relevance of immuno-phenotyping in 379 patients with acute myeloid leukemia. Leuk Res 2004;28:43-48.
31. Xu F, Yin CX, Wang CL, Jiang XJ, Jiang L, Wang ZX *et al*. Immunophenotypes and immune markers associated with acute promyelocytic leukemia prognosis. Dis Markers 2014, 421906.
32. Thomas DA, O'Brien S, Jorgensen JL, Cortes J, Faderl S, Garcia-Manero G *et al*. Prognostic significance of CD20 expression in adults with de novo precursor B-lineage acute lymphoblastic leukemia. Blood 2009;113:6330-6337.