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APPLICATION OF FLOW CYTOMETRY IN THE DIAGNOSIS OF ACUTE LEUKEMIA IN CHILDREN: KING HUSSIEN MEDICAL CENTER EXPERIENCE

***Bayan Al-Zghoul MD, Mohammad J. Maaita MD, Tania Ogeilat MD, Malak Al-Awamleh MT, Areen Al-Zgoul MD, Alaa Abu-Alkeshek MD, Mashael Khalayleh MD, Falah Faragat MD, Mothana Al-Nawaflh MD, Mohammad Masallha MD, Dima Al-Dabbas MD, Abdallah Shawabkeh MD and Hashim Shawabkeh MD**

KHMC Amman Jordan.

***Corresponding Author: Bayan Al-Zghoul MD**

KHMC Amman Jordan.

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ABSTRACT

Background: Acute leukemia is a group of clonal hematopoietic stem cell neoplasms. Flow cytometry plays a significant role in diagnosis of acute leukemia. **Aim**: To spot on the central role of flow cytometry in the diagnosis of acute leukemia. **Patients and Method**: We reviewed flow cytometry results of 130 patients during the period from January 2019 to December 2023. **Results**: 130 patients aged between 3 months to 14 years with a mean age of 7 years. Male to female ratio 1.4:1. Acute lymphoblastic leukemia of B-cell lineage (B-ALL) 87 patients (67%). Acute lymphoblastic leukemia of T-cell lineage (T-ALL) 18 patients (13.8%). Acute myeloid leukemia (AML) 25 patients (19.2%). **Conclusion**: Flow cytometry is a golden tool for the diagnosis of acute leukemia.

KEYWORDS: Flow cytometry, acute leukemia, diagnosis.

INTRODUCTION

Acute leukemia is a heterogeneous group of clonal hematopoietic stem cell neoplasm due to genetic mutation in the progenitor cells which leads to uncontrollable proliferation of these progenitor cells in the bone marrow, peripheral blood and other organs.[1,2,3] Acute leukemia is the commonest malignancy in pediatric patients, it accounts for about 30% of all malignancies diagnosed in childhood with acute lymphoblastic leukemia five times more common than acute myeloid leukemia.[4,5] Acute leukemia is diagnosed based on clinical presentation and laboratory investigations.[1]

Flow cytometry (FCM) is a powerful, fast, multi-parametric tool applied for the diagnosis of acute leukemia in pediatric patients.[5,6]

In this review, we study the application of flow cytometry in the diagnosis of acute leukemia in children at King Hussein Medical Center.

PATIENTS AND METHOD

This is a retrospective study which was carried out on 130 patients diagnosed as acute leukemia based on flow cytometry findings at king hussein medical center between January 2019 to December 2023.

Flow cytometry performed on bone marrow and peripheral blood samples. Bone marrow aspirate and peripheral blood fresh samples up to 72 hours are anticoagulated with EDTA at room temperature.

For surface antigen(Myeloid antigen: CD13, CD33, CD14, CD64, CD11b and CD11. Lymphoid antigen: CD2, CD3, CD4, CD5, CD7, CD8, CD19 and CD10. Primitive antigen: CD34 and CD117) Monoclonal fluorochrome-conjugated antibodies against cell surface were added to 50 ul of EDTA whole blood then incubated for at least 15 min in dark area.

The red blood cells were removed through a lysis process using a commercially available reagent then washed by cell wash supplied by the manufacturer.

For cytoplasmic antigen (Myeloid: MPO). Lymphoid: cytoplasmic CD79a, cytoplasmic CD3 and cytoplasmic CD22. Primitive antigen: TdT and CD1a) we proceed with the same steps as surface antigen then permeabilizing solution were added for 10 min next washing done and finally Monoclonal fluorochrome-conjugated antibodies against cytoplasmic antigen were added. All tubes were then analyzed using BD FACSCanto II.

We routinely run quality control for verification of our result and we use CD45 as a gating marker before applying samples.

We have been using BDFACS Canto II, a three-laser, eight-color flow cytometry for immunophenotyping of gated population (Blasts population).

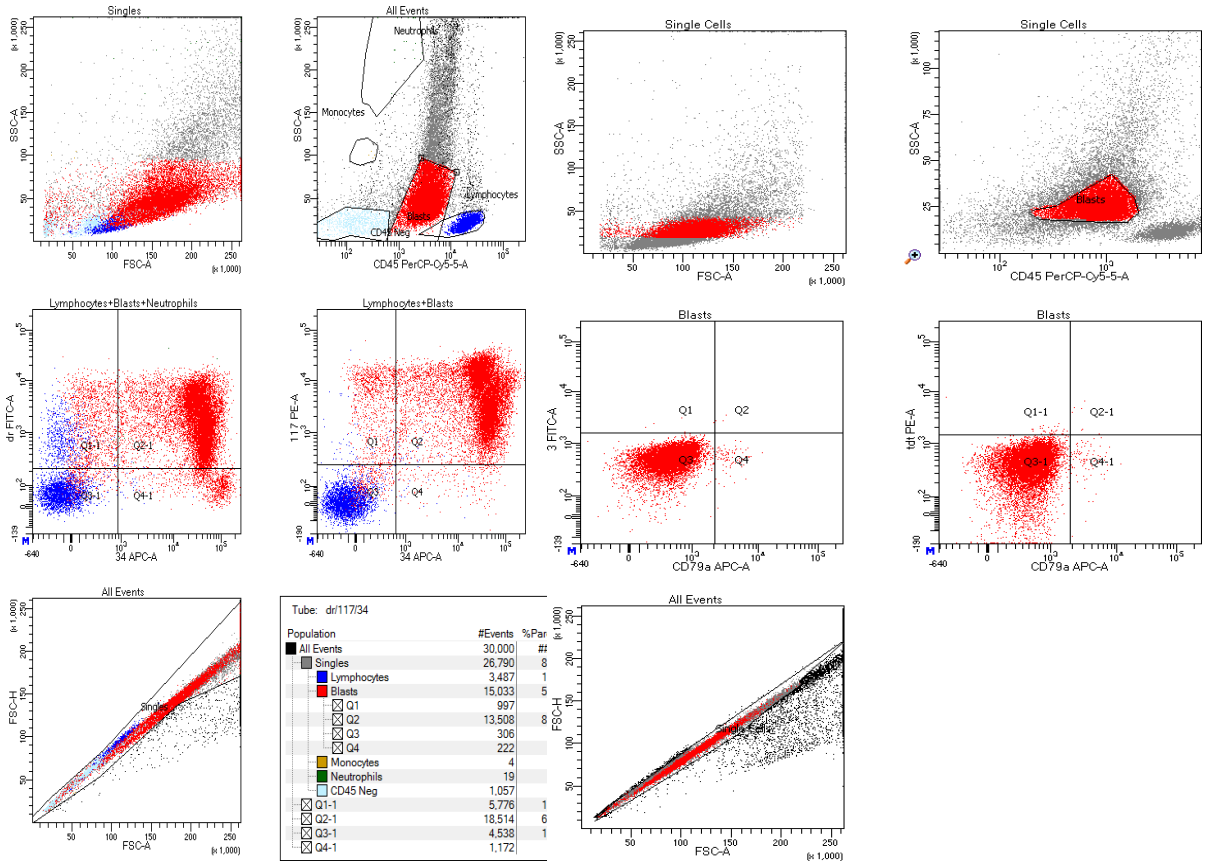
RESULTS

We reviewed the immunophenotype for 130 children diagnosed as acute leukemia. The age range is 3 months-14 years. The mean age is 7 years. Male to female ratio 1.4:1. Of these patients: b-cell lineage specific markers (Strong CD19 with ≥ 1 of the following strongly expressed: CD10, cytoplasmic CD22 and CD79a Or weak CD19 with ≥ 2 of the following strongly expressed: CD10, cytoplasmic CD22 and CD79a) expressed in 87 patients (67%) and diagnosed as Acute lymphoblastic leukemia of B-cell lineage (B-ALL), t-cell lineage specific marker (CD3) expressed in 18 patients (13.8%) and diagnosed as Acute lymphoblastic leukemia of T-cell lineage (T-ALL), myeloid specific markers (MPO or Monocytic differentiation, ≥ 2 of the following: CD64, CD14, CD11c) expressed in 25 patients (19.2%) and diagnosed as Acute myeloid leukemia (AML).

The review showed that the diagnosis of acute leukemia is made by using a complete panel containing a set of markers to evaluate sufficient antigens for lineage classification and immunophenotyping of hematopoietic neoplasm.

Table 1: Frequency of different types of acute leukemia in children at KHMC.

Acute leukemia	Number of patients	Percent %
B-ALL (Strong CD19 with ≥ 1 of the following strongly expressed: CD10, cytoplasmic CD22 and CD79a Or weak CD19 with ≥ 2 of the following strongly expressed: CD10, cytoplasmic CD22 and CD79a)	87	67
T-ALL (CD3)	18	13.8
AML (MPO or Monocytic differentiation, ≥ 2 of the following: CD64, CD14, CD11c)	25	19.2



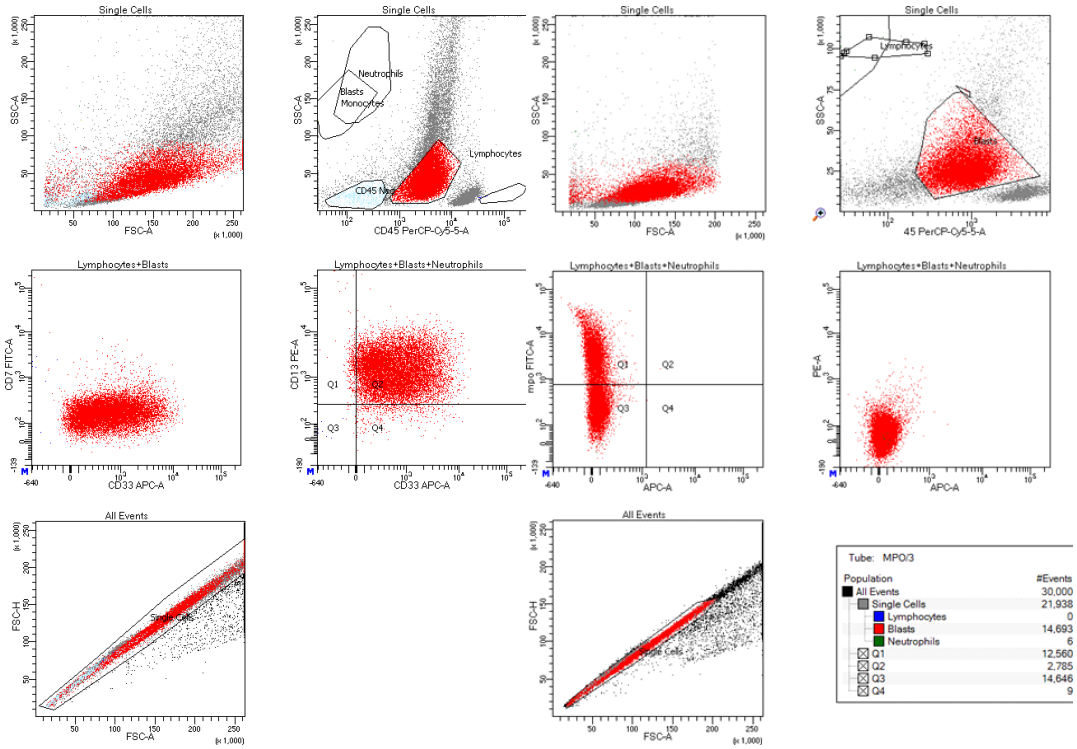
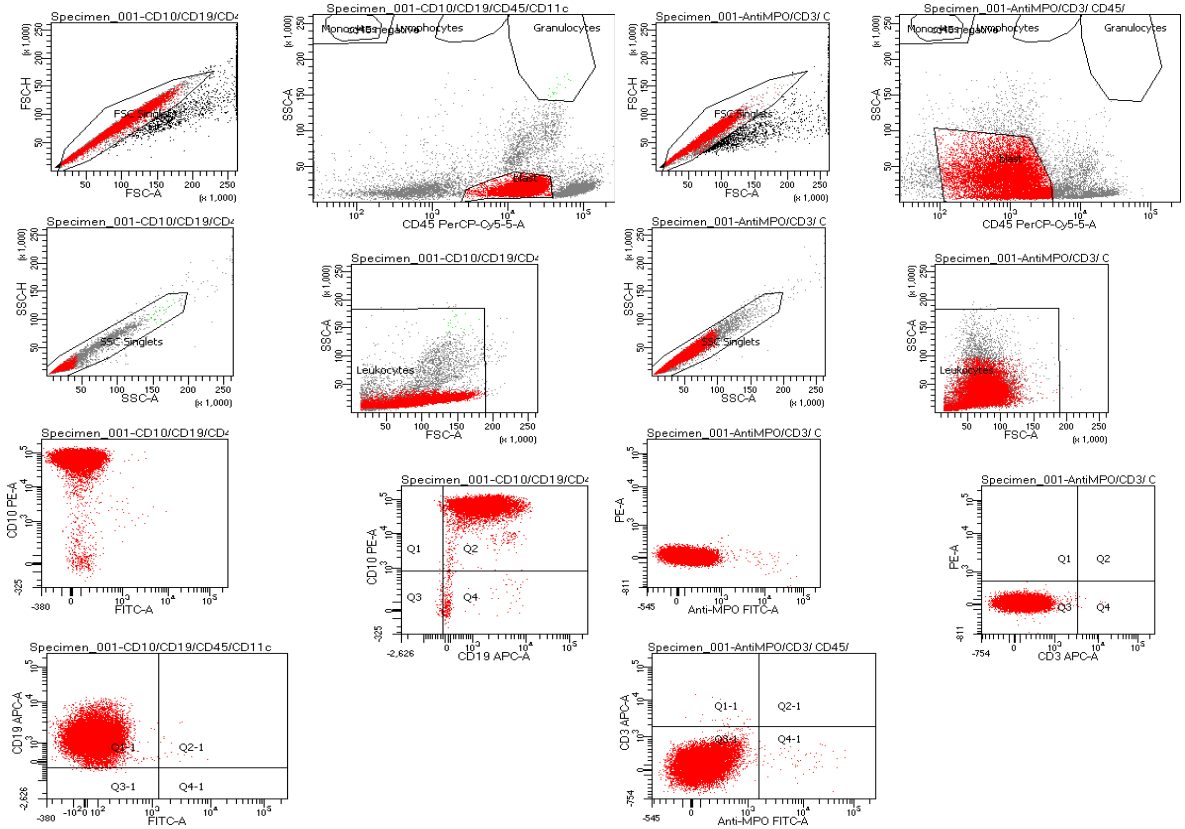


Figure 1: Acute myeloid leukemia.

Representative Flow cytometry dot plots of Acute myeloid leukemia showing side scatter, CD45, expression of CD34, CD117, HLA-DR, CD13, CD33, MPO.



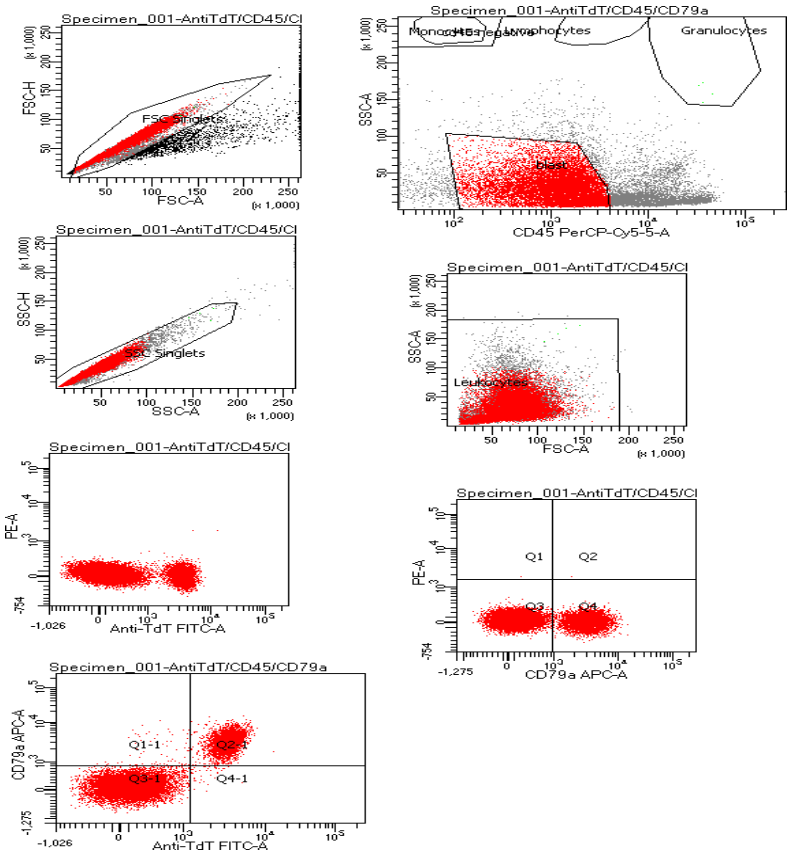
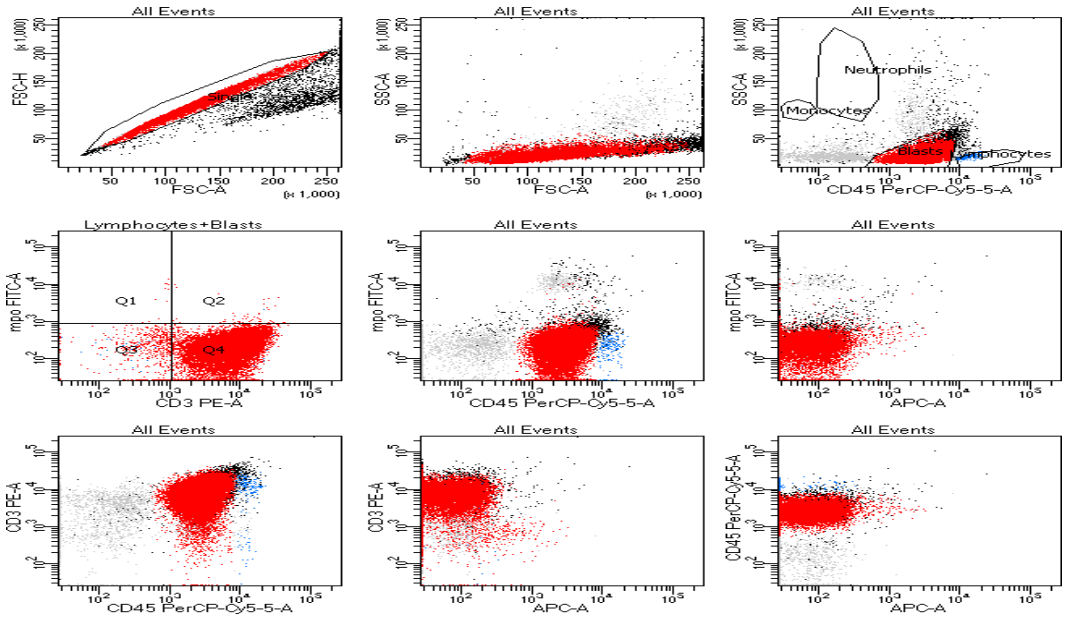
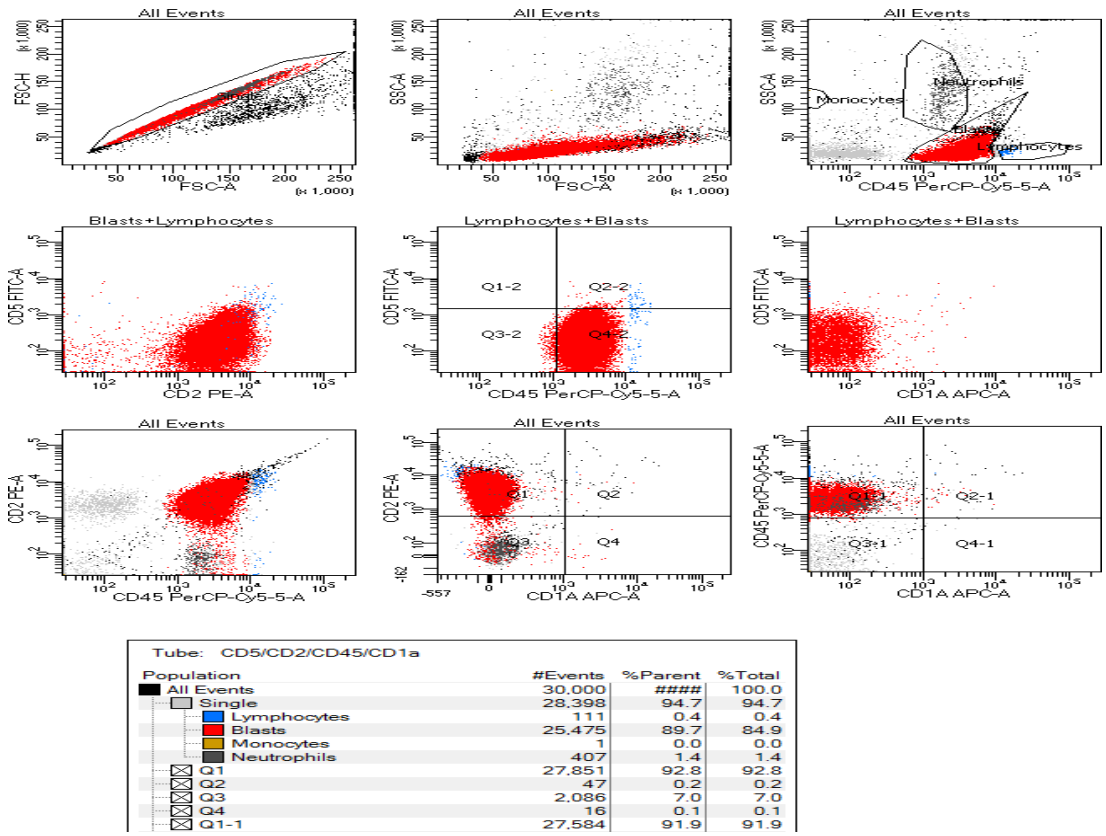


Figure 2: B- Cell Acute lymphoblastic leukemia.

Representative Flow cytometry dot plots of B-cell Acute showing side scatter, CD45, expression of CD10, CD19, anti-TdT, and CD79a.



Tube: cyCD3/MPO/CD45/sCD3			
Population	#Events	%Parent	%Total
All Events	30,000	####	100.0
Single	27,248	90.8	90.8
Lymphocytes	155	0.6	0.5
Blasts	24,751	90.8	82.5
Q1	12	0.0	0.0
Q2	51	0.2	0.2
Q3	551	2.2	1.8
Q4	24,137	97.5	80.5
Monocytes	0	0.0	0.0
Neutrophils	2	0.0	0.0



Tube: CD5/CD2/CD45/CD1a

Population	#Events	%Parent	%Total
All Events	30,000	####	100.0
Single	28,398	94.7	94.7
Lymphocytes	111	0.4	0.4
Blasts	25,475	89.7	84.9
Monocytes	1	0.0	0.0
Neutrophils	407	1.4	1.4
Q1	27,851	92.8	92.8
Q2	47	0.2	0.2
Q3	2,086	7.0	7.0
Q4	16	0.1	0.1
Q1-1	27,584	91.9	91.9

Figure 3: T-Cell Acute lymphoblastic leukemia.

Representative Flow cytometry dot plots of T-cell Acute leukemia showing side scatter, CD45, expression of CD3and CD2.

DISCUSSION

Acute leukemia is a malignancy with an expanded uncontrollable proliferation of blasts which replace the normal hematopoietic elements in the bone marrow and common involvement of peripheral blood. Lymph node, spleen and other organs.[1,7] Acute leukemia is the commonest malignancy in pediatric patients, it accounts for about 30% of all malignancies diagnosed in childhood, acute lymphoblastic leukemia accounts for up to 80% of childhood acute leukemias.[4,5.8] It is thought that acute lymphoblastic leukemia appears from the interaction of environmental factors and a pre-existing genetic susceptibility, the cause mostly is multifactorial: including infectious, immunologic and genetic factors.[3,4] Acute leukemia is diagnosed based on clinical presentation and laboratory investigations.[1] Presentations of acute leukemia are due to: 1. Bone marrow failure from expanded proliferation by blasts cells, preventing normal production of red blood cells, neutrophils and platelets so patients presents with symptoms of anemia, leukopenia and thrombocytopenia, also this expansion of the bone marrow cavity causes limping and bone pain. 2. Related to other tissue infiltration by blasts leading to hepatosplenomegaly and lymphadenopathy.[3,4] It is difficult to diagnose acute leukemia according to clinical history only. To confirm the diagnosis laboratory investigations like flow cytometry and genetic analysis is mandatory.[6]

Flow cytometry is an available, sensitive and rapid tool suitable for peripheral blood, bone marrow, cerebrospinal fluid, serous effusion and fine needle aspiration specimens analysis. Flow cytometry is a powerful and useful technology for diagnosis and lineage classification of acute leukemia.[1] Application of immunophenotyping is critically important in diagnosis of acute leukemia for demonstration of immunophenotypic abnormality, blast enumeration, lineage assignment and sub classification.These assays can be utilized as a crucial diagnostic method to confirm the clinical suspicion of acute leukemia.[6]

In this study we reviewed the records of 130 patients diagnosed as acute leukemia by using flow cytometry. (67%) 87 patients were diagnosed as Acute lymphoblastic leukemia of B-cell lineage (B-ALL) expressed b-cell lineage specific markers (Strong CD19 with ≥ 1 of the following strongly expressed: CD10, cytoplasmic CD22 and CD79a Or weak CD19 with ≥ 2 of the following strongly expressed: CD10, cytoplasmic CD22 and CD79a). (13.8%) 18 patients were diagnosed with Acute lymphoblastic leukemia of T-cell lineage (T-ALL) expressed t-cell lineage specific marker (CD3).(19.2%) 25 patients diagnosed as Acute myeloid leukemia (AML) expressed myeloid specific markers (MPO or Monocytic differentiation, ≥ 2 of the following: CD64, CD14, CD11c). This result is comparable to other study results carried out in 2007 by

M.Belson et al which reported that Acute lymphoblastic leukemia (ALL) occurs approximately five times more frequently than Acute myelogenous leukemia (AML) in childhood.[5] 75 patients were males (58%) and 55 (42%) were females. The age range is 3 months-14 years. The mean age is 7 years.

CONCLUSION

Flow cytometry plays a critically important role in the diagnosis of acute leukemia for lineage assignment and sub classification. Immunophenotyping can be utilized as a rapid, highly sensitive, cost-effective and crucial diagnostic method to confirm the clinical suspicion of acute leukemia.

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