

Use of Flow Cytometry Immunophenotyping for Diagnosis of Acute Leukemia at Moi Teaching and Referral Hospital, Eldoret, Kenya

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Abstract

The main objective of the study was to compare morphological and flow cytometric diagnosis in patients previously diagnosed with leukemia. The retrospective study was carried out at Moi Teaching and Referral Hospital and patient's data for the period of July 2013 and June 2014 was used. After Institutional Research Ethics approval was granted. Consecutive sampling was done and information was extracted from patient's files. Data for all who were previously diagnosed with leukemia through both morphology and immunophenotyping was recorded. The data was collected using a data collection form where socio-demographic data, morphological and flow cytometry results were recorded. The findings were based on 33 patients who underwent both flow cytometry and bone marrow morphology tests for diagnosis of leukemia between July 2013 and June 2014. The ages of the patients ranged from 3 to 76 years. The ratio of male to female was 1:1.1.

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Using the Bone marrow morphology, 17 patients had AML and 15 had ALL, one case was inconclusive There were five categories for the flow cytometry. They comprised of B-ALL-6 cases, T-ALL-13 cases, AML-10 cases, Biphynotypic-1 case and inconclusive-1case. There was concordance between the morphological and flow cytometry on 25 out of the 33 cases. As a conclusion we can say that at MTRH, Flow cytometry had a role to play to confirm a definite and a probable diagnosis of patients with acute leukemia.

Keywords: Leukemia; Cytomorphology; Flow cytometry; AML; B-ALL; T-ALL.

1. Introduction

The bone marrow (BM) is a complex tissue containing cells of multiple hematopoietic cell lineages in all stages of development. Flow cytometric immunophenotyping evaluates the frequencies of the various leukocyte (sub) populations in BM and blood that then helps in the diagnosis of leukemia's.

Historically, leukemia has been classified initially into four groups based on a combination of clinical presentation and morphologic appearance of malignant cells: Acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML, also named acute non lymphocytic leukemia ANLL), chronic myelogenous leukemia (CML) and chronic lymphocytic leukemia (CLL). Subsequent research investigations and technological advances in the last twenty years, have evaluated the morphologic, immunologic, growth regulation, cytogenetic, and molecular abnormalities in leukemic cells, further establishing that leukemia is actually a much more heterogeneous group of disorders than initially suggested. In a study by Sengar et al, 100 untreated patients with AL were studied using morpho-cytochemistry and immunophenotyping through FCM, it was concluded that FCM thus had a role to play in ALL patients to confirm a definite and a probable diagnosis, to define therapeutically and prognostically groups such as B and T lineage ALL and to distinguish AML – M0 from ALL. While morpho-cytochemistry provides a first-line investigation of great therapeutic value, and more so in AML, it needs to be supplemented by flow cytometry, particularly in ALL [1].

The use of flow cytometry provides an insight into differentiation pathways, maturation stages and abnormal features of these (sub) populations which are clinically relevant for the diagnosis of hematological malignancies. The presence and absence of antigens on or in the cell (sub) populations, are recognized by various monoclonal antibodies (MAb) which gives characteristic immunostaining defining the cell lineages thus helping in making the diagnosis of Leukemias [2,3].

The consideration of which Mab's for diagnosis is very important and a lot of researchers have addressed this problem. Morphological diagnosis alone for leukemia's cannot diagnose leukemia's correctly and cases can be missed out. At MTRH Flow Cytometric Immunophenotyping has been incorporated in the diagnosis and further characterization of acute leukemia in addition to morphological diagnosis. Leukemia is the 6th among the top ten oncological disorders in the Western region of Kenya (Eldoret Cancer Registry 2005-2009 data) with a total of 73 cases of acute leukemia. Among these leukemia's 5.3% are lymphoid, 5.3% myeloid and 1.4% were unspecified. The flow cytometry immunophenotyping helps to differentiate the unspecified leukemia's. In this study a four colour flow cytometric immunostaining combination was used to diagnose leukemia.

2. Materials and methods

2.1 Study Area

Moi Teaching and Referral Hospital hematology laboratory, AMPATH reference laboratories and Records department

2.2 Study Design

Retrospective study.

2.3 Study population

Files of patients diagnosed with leukemia

2.4 Sampling Procedure

File records and results for patients previously diagnosed to have leukemia on the basis of complete blood counts, peripheral blood films, bone marrow and flow cytometry findings were perused and data recorded in a data collection form. All files were sampled for the period of July 2013 to June 2014, those with complete records were included in the study. Those files with BMA morphological diagnosis but without flow results were not included in the study and vice versa.

2.5 Cytomorphological classification of Acute Leukemia

The original classification scheme proposed by the French-American-British (FAB) Cooperative Group divides AML into 7 subtypes (M0 to M7) and ALL into 3 subtypes (L1 to L3). More recently, an additional class AML M8 (acute basophilic leukemia) has been described. In this study the morphological diagnosis was put into only 2 categories i.e ALL and AML

2.6 Immunophenotyping

Equipment: Four colour computerized BD FACS caliber. The fluorochromes FITC, PE, PerCP, PerCP Cy5.5 and APC were used.

Samples: BMA (1-2mls) or Peripheral blood (4-7 mls) in EDTA tube ,processed within 24-48hrs.

The following panel of monoclonal antibodies were used depending on availability.

Pan Leukocyte antigen: CD 45 is a pan leukocyte marker

B- cell- CD10, CD19, CD20, CD79a

T cell; CD3, CD4, CD7, CD8, cyt CD3, Tdt

Myeloid; CD33, CD38, CD117, MPO.

Immature cell antigens; CD34, and HLADR

Erythroid marker: CD71

Some combinations used were as follows:

Tube 1:CD 45

Tube 2:CD19 and CD20

Tube 3: CD33 and CD5

Tube 4:HLADR and CD33

Tube 5:cyt CD79a

Tube 6: CD3 and CD4

Tube 7:cyt CD3

Tube 8: anti-MPO

3. Results

The findings that were used in this study were based on 33 samples for patients whose results showed both flow cytometry and morphological diagnosis between July 2013 and June 2014.

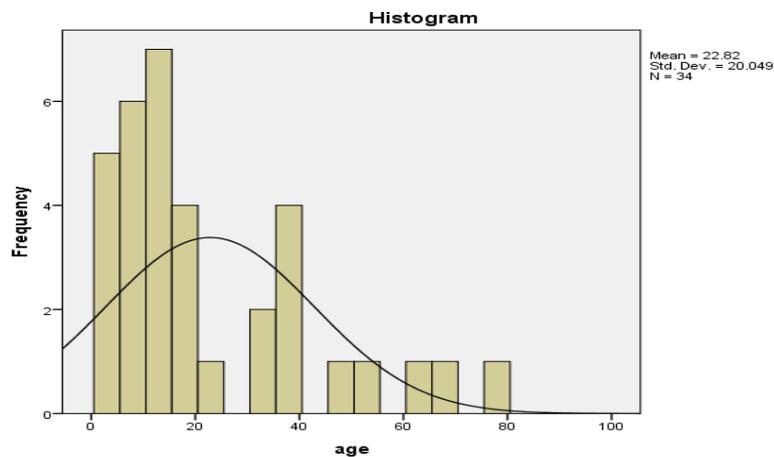


Figure1: Age distribution

The age of the patients ranged from 3 to 76 years. Females constituted 53% and the rest were males.

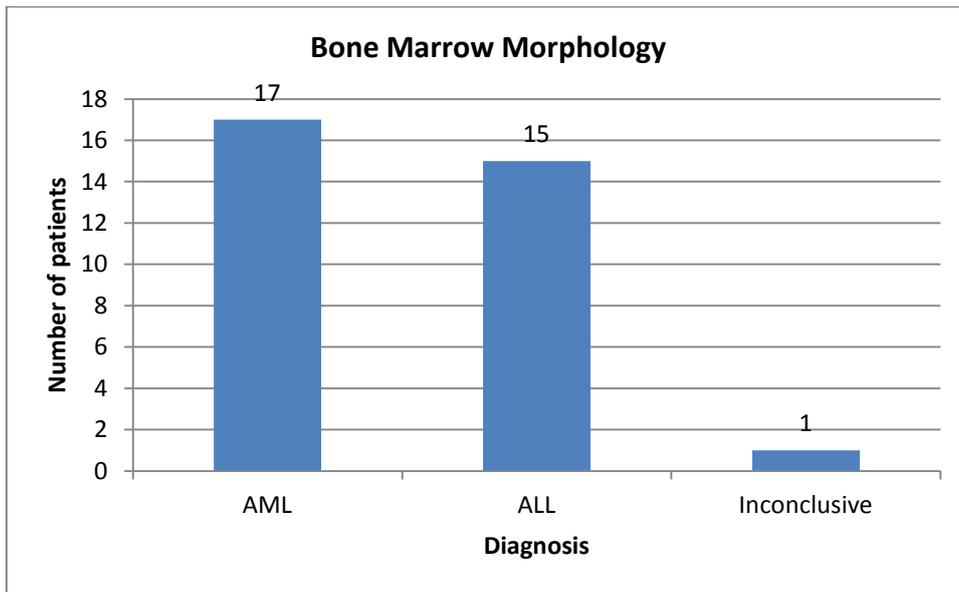


Figure 2: Bone Marrow Morphology diagnosis

Using the Bone marrow morphology, diagnoses were only classified conclusively as AML or ALL with only 1 having inconclusive results.

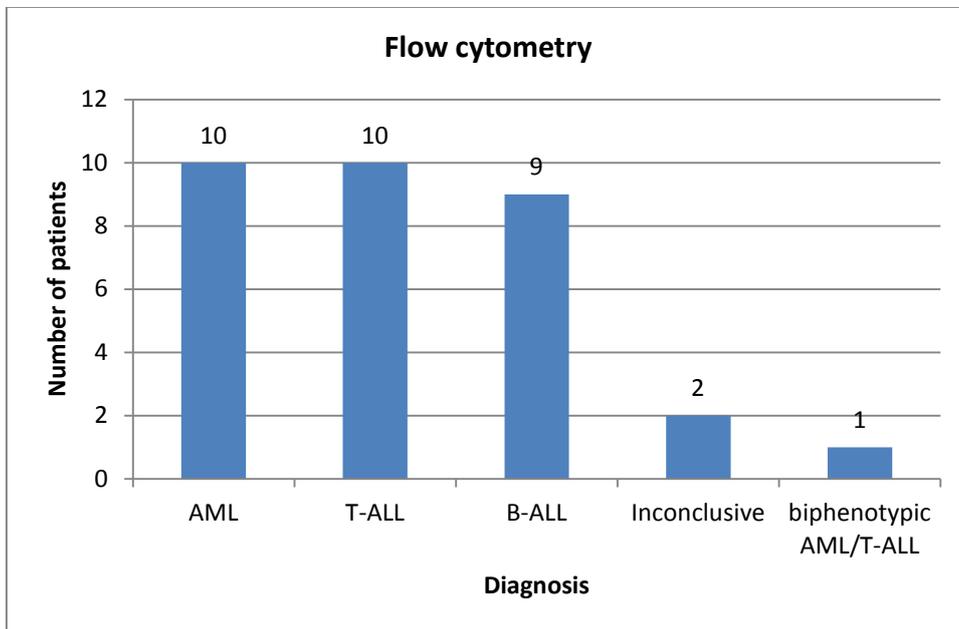


Figure 3: Flow Cytometry diagnosis

In Flow Cytometry the diagnoses were classified into 5 categories

Table 1: Comparison of flow Cytometry and bone marrow morphology tests using the morphology test as the Gold standard. Validity of the test (Cytometry) in diagnosing ALL

		Morphology Test (gold standard)		Total
		+ve	-ve	
Cytometry test	+ve	12	6	18
	-ve	3	11	14
Total		15	17	32

The ability of the Cytometry test to identify correctly those who *have* ALL (Sensitivity of cytometry test) was 80%.

The ability of the Cytometry test to identify correctly those who *do not have* ALL (Specificity of cytometry test) was 64.7%.

The probability of a person having the ALL on BMA and Cytometry test showing positive results is 66.7% likewise the probability of a person not having the ALL and Cytometry test showing so is 78.6%.

Table 2: Validity of the test (cytometry) in diagnosing AML

		Morphology Test (gold standard)		Total
		+ve	-ve	
Cytometry test	+ve	7	2	9
	-ve	10	13	23
Total		17	15	32

The ability of the Cytometry test to identify correctly those who *have* AML (Sensitivity of cytometry test) was 41.2%.

The ability of the Cytometry test to identify correctly those who *do not have* AML (Specificity of cytometry test) was 86.7%.

The probability of a person having the AML on BMA and flow Cytometry test showing positive results is 77.8% likewise the probability of a person not having the AML and Cytometry test showing so is 56.5%.

Table 3 shows the results of patients who had discrepancies between the flow cytometry and morphological diagnosis.

Table 3: Comparison of morphological and Flow cytometry diagnosis on disagreeing results

Serial number	Clinical data	Morphological diagnosis	Flow cytometry diagnosis
003	40yr M	AML M5	CD45(dim)-95%, CD33(95%), CD38(86%), HLADR(92%), CytCD3(95%), MPO-4% -Reported as T-ALL
004	60yr F	AML M5	CD 45(dim)-21%, cytCD7a-21% -Reported as B-ALL
006	12yr M	Inconclusive	CD45(dim)-66%, CD38(51%),CD33(50%) HLADR(43%) -Reported as AML
015	36yr,F	AML M2	CD45(bright)-30%, CD5(31%) -Inconclusive
017	7 yr M	ALL	CD45dim)-73%, CD5(61%), cyt CD3(34%), MPO(41%),CD71(74%) -Reported as T-ALL
025	24yr M	AML M1	CD45(dim)-82%, CD33(74%), HLADR(73%), cytCD3(93%) -Reported as T-ALL
032	17yr M	ALL	CD45 (bright)-28%, CD3(20%) -Inconclusive results
033	5yr M	AML(PBF),BMA Inadequate	CD45(bright)-54%,CD45(dim)-22% CD19(24%), CD3(54%), CD5(52%),HLADR-35%, CD79a(23%) -Reported as T-ALL

4. Discussions

We analyzed 33 patients with complete records, who had bone aspirate done for diagnosis of acute leukemia and both morphological diagnosis and flow cytometry had been performed on their samples. The data showed that the age of the patients ranged from 3 to 76 years with an average of 22±20 years the paediatric patients were 18 (50%) of the cases. The ratio of male to female was 1:1.1.

In a study by S et al 260 cases were analysed for the study as the diagnostic workup was complete in these cases. Sixty-two patients belonged to the pediatric age group while there were 198 adults. There were 187 males and 73 females. This differed from our study which paediatric patients constituted 50% and male to female ratio was almost 1.1[6].

Using the Bone marrow morphology, diagnoses were only classified conclusively as AML 17(51%) or ALL 15(45%) with only 1 having inconclusive results.

In Flow Cytometry the diagnoses were classified into 5 categories. B-ALL 9(27%), T-ALL 10 (30%), AML 10(30%), biphenotypic-1(3%) and 2 inconclusive cases.

In a study by Sengar et al, done on 100 untreated patients with Acute leukemia comparisons were done using morpho-cytochemistry and immunophenotyping through FCM. The findings showed 29 patients with acute myeloid leukemia (AML), 47 with B-acute lymphoblastic leukemia (ALL), 20 with T-acute lymphoblastic leukemia (ALL) and four with biphenotypic acute leukemia (BAL). Morpho-cytochemistry without FCM could provide definite diagnosis only in the AML cases. It failed to provide definite diagnosis in ALL patients. Over half (55%) of ALL patients were given the noncommittal label, AL. The remaining 45% patients were labeled a more definite, probable ALL [1].

This study differs from our study in that the patients with B-ALL were found to be almost double the number in comparison to those with T-ALL. In our study the number was 50/50. This study concluded that the flow cytometry was needed more for diagnosis of ALL

In this study results were concordant in 25 cases, whereas in 8 cases the results were discordant comparison was done between BMA morphology and immunophenotyping only. Morphology was used as the gold standard to compare the 2 tests. The ability of the Cytometry test to identify correctly those who have ALL (Sensitivity of cytometry test) was 80%. The ability of the Cytometry test to identify correctly those who have AML (Sensitivity of cytometry test) was 41.2%.

These findings were similar to a study by Beluakar et al whereby 50 cases of acute leukemia were analyzed and found concordance rate as high as 86% between morphologic/cytochemical diagnosis and flow cytometric diagnosis. Of these, complete concordance was seen in 58% of the cases and partial concordance was seen in 22% of the cases. Non-concordance was seen in only 4% of those cases. In remaining 16% of the cases FCA helped in sub classifying the acute leukemia where morphology and cytochemistry had failed to do so [11]. For the 8 discordant cases, flowcytometry able to make a final diagnosis in 2 cases, which lead to adaptation of treatment and finally complete remission. In the other 6 cases, the available markers were too limited to make a clear diagnosis. Especially in suspected biphenotypic cases, the flowcytometry could have been of extra value but was restricted by the available markers. There were also some inconclusive results because of too few events

5. Conclusions

Flow cytometric immunophenotyping was found to be especially useful in the correct identification and diagnosis of acute myeloid and lymphoblastic leukemia and should be developed in terms of training more personnel and validating the test for commercial use. This study shows that flow is complementary to morphological diagnosis. There were challenges especially where sample collection was not properly done or had few cells, this led to inconclusive results.

Competing interests

The author declares no competing interests.

Author contribution

The authors have read and agreed to the final version of this manuscript and have equally contributed to its content.

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