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Immunophenotypic Profile in Adult Patients with Acute Leukemia Association with Clinical Feature: Fluorescence Cytometry Quantitative Analysis

Walaa Fikry Mohammed Elbossaty*

Department of Chemistry, Biochemistry Division, Faculty of Science, Damietta University, Damietta, Egypt

Abstract

Antigen surface markers represent as the new prognostic tool for detection of acute leukemia. To investigate the prevalence expression of lymphoid and myeloid antigen lineage in acute leukemias. This study included 100 acute leukemias patients. Specimens were selected from consecutive patients who had sufficient material available. Among the 100 patients in which a detailed history, hematological, clinical and immunophenotyping analysis were performed. This study was showed distribution of immunophenotyping characters between studied AML and ALL cases. The most abundant immunophenotyping features in acute myeloid leukemia were cMPO, CD33, CD117, CD13, CD14 and CD64, while the most abundant immunophenotyping features in acute lymphoblastic leukemia were CD19, CD79a, TdT, CD20, CD10 and CD34. cMPO which act as independent prognostic factor for AML, CD10 and TdT can used as independent prognostic factor for differentiate between ALL and AML.

Introduction

Most leukemias fall into one of two general groups: Myeloid leukemia (about 60% of the cases) and Lymphocytic leukemia (about 40%). People also classify leukemias according to whether they are acute (55%) or chronic (45%). In acute leukemias, the malignant cells, or blasts, are immature cells that are incapable of performing their immune system functions. The onset of acute leukemias is rapid (weeks), and in most cases fatal unless the disease is treated quickly. All constitutes of two main immunologic types: B cell and T cell while AML constitutes of main immunologic types: granulocyte cell and monocytic cell [1].

Each type has its own immunophenotyping characters, but in there were especial cases may have the both myeloid and lymphoid this known as biphenotypic acute leukemia (BAL). The immunological methods for discriminating between normal and leukemic cells rely on the fact that leukemic cells may express some antigens absent in, or rarely expressed by, their normal counterparts [2].

Immunophenotyping of hematological malignancies is typically performed by flow cytometry with gating to identify the leukemic population and then study by fluorescence the antigen profile of these cells [3].

The "cluster of differentiation" (CD) antigens were discovered on leukocytes using monoclonal antibodies that "cluster" for reaction with particular cells. Today, antibodies that react with CD19 or CD20 are typically used instead of surface immunoglobulin (sIg) as an indicator of B-cell lineage, although intensity of sIg is still used to define subsets of monoclonal peripheral B-cell populations. Pan-T-cell-specific antibodies that react with CD2 or CD3 have replaced the cumbersome sheep erythrocyte- rosettes methodology to identify T lymphocytes. CD5 is the target of T65 (monoclonal antibody T101), which is generally a pan-T-cell marker, but is rarely coexpressed with B-cell markers on some lymphocytes [4].

Flow cytometric immunophenotyping (FCI) plays an important role in the diagnosis of patients who have acute leukemia. The morphologic characteristics of lymphoblasts may be indistinguishable from those of the myeloblasts in many cases of acute myeloid leukemia (AML), and treatment protocols for ALL and AML differ significantly. Because FCI enables rapid (within hours), typically unambiguous characterization of leukemic blasts with respect to lineage, this modality has become a routine component of the diagnostic work-up of patients who have suspected acute leukemia.

Moreover, complete immunophenotypic characterization of the lymphoblasts at diagnosis may predict associated cytogenetic and molecular abnormalities and serve as a reference point for the detection of residual disease after therapy. Immunophenotyping improves both accuracy and reproducibility of acute leukemia classification and is considered particularly useful for identifying poorly differentiated subtypes of acute leukemia, acute myeloid leukemia (AML) with lymphoid marker expression and acute lymphatic leukemia (ALL) with myeloid marker expression. Immunological studies of leukemic blasts have become critical also for identifying Biphenotypic and bilineal acute leukemias [5].

Materials and Methods

This study was carried out on 100 patients with acute leukemias at initial diagnosis. Diagnosis and classification of the acute leukemias cases were based on the morphology and the cytochemistry according to FAB classifications and the presence of maturation and differentiation antigens that were determined by routine immunophenotyping criteria of WHO 2008 (Dohner et al. 2010).

Informed consent was obtained from each patient. Regarding the FAB classification, 50 AML patients were categorized to have

*Corresponding author: Walaa Fikry Mohammed Elbossaty, Department of Chemistry, Biochemistry Division, Faculty of Science, Damietta University, Damietta, Box 34517, Egypt, Tel: 020573761683; E-mail: walaafikry1985@gmail.com

undifferentiated leukemia (M0 and M1, $n=2, 6$), 15 patients with immature Granulocytic leukemia (M2, $n=7$; M3, $n=8$), 20 patients with monocytic leukemia (M4, $n=10$; M5, $n=10$), 5 patients were diagnosed with M6 and two patients were diagnosed with M7. The median age was 42.5 years (range 25-65 years). The male/female ratio was 1.4:1. Also, 50 ALL patients were categorized to have14 patients were diagnosed with L1, have30 patients were diagnosed with L2 and 6 patients with L3. The median age was 38.4 years (range 20-67 years). The male/female ratio was 1.9:1. All patients were previously untreated and entered this study at the time of initial diagnosis.

All patients subjected to detailed history taking and clinical examinations. Hematological parameters were detected by fully automated 5 part cell counter (SYSMEX XT 2000-I). Peripheral smear examination was done to find out the presence of leukemic blasts. Bone marrow aspiration was performed from right iliac crest under strict asepsis with 5 ml of 2% xylocaine infiltration anesthesia. Smears were analyzed by staining with Leishman stain and Myeloperoxidase (MPO).

The FAB criteria were also used to diagnose and sub classify acute leukemias morphologically. Flowcytometric analysis was performed on a FACS calibur flow cytometer (Becton Dickinson, CA, USA) and Cell Quest Pro software was used to acquire and analyze the data. It was done using either bone marrow sample or peripheral blood.

Surface markers used for B cells were CD10, CD19, CD20, CD22; for T cells were CD2, CD5, CD7, for myeloid cells CD13, CD33, CD64, CD117. Other markers like CD34, HLA-DR were also tested as per recommendations. Additional cytoplasmic markers i.e. specific for B cells cCD79a, for T cells cCD3 and for myeloid cells c Anti-MPO was taken.

Flow Cytometric Analysis

EDTA-BM samples were used to establish the blast phenotype in every single acute leukemia cases, the following were performed as described elsewhere (Hiddemann et al. 1986). Reagents and 12 mm × 75 mm Falcon TM capped polystyrene test tubes were provided by Becton-Dickinson Bioscience in United States. For each tube, we added 100 μL (about 10^6 cells/ml) well-mixed EDTA anticoagulated BM sample, and 10 μL or 20 μL of 3-color direct fluorescent-labeled Abs which had to be fit together according to the detection protocol and the different fluorescence, into the bottom.

Samples were incubated for 20 min at room temperature (20°C to 25°C) and then added with 2 ml 1 × lysing solution, and incubated for 10 min. After that, tubes were centrifuged at 400 g for 5 min, supernatant aspirated, and 2 ml of PBS was added to wash the cells two times. For the detection of intracellular antigens, permeabilizing solution was added and incubated. Then they were added with MPO, cCD79a, and TdT and incubated.

After that the PBS resuspended cells were ready for the FACS Calibur™ flow cytometer (BD Bio). From each tube, we caught 10^4 cells for following analysis with Cell Quest and Paint-A-Gate software. Abnormal populations were recognized by CD45/SSC gating, which was the base of calculating the positive rate of leukemia-related antigens expressed on the abnormal cells. They regarded no less than (>20%) of cell expression in a tube as positive expression, unless TdT and MPO (>10%) [6].

FACS Analysis

All samples were analyzed on a FACScan flow cytometer equipped with an argon ion laser with a wavelength of 488 nm using the Cell Quest software (Becton Dickinson). Fluorescence intensity was measured with FL1 and FL2 detectors and amplifiers set on a logarithmic scale (Rothe et al. 1996). The cytometer was set up using standard operating procedures and the quality control was performed using the Calibrate Beads Kit (Becton Dickinson).

The mean channel number of the fluorescence intensity (MFI) determined for each population of beads was plotted versus the corresponding antibody binding capacity (ABC) values, and a best fit line was calculated by linear regression using the Quick Cal software (Becton Dickinson). The gating procedure was based on CD45 staining and sideward light scatter (SSC) and the area containing the lymphoid cells and most of the B-progenitors were selected (Syrjala et al. 1994).

Statistical Analysis

Data were statistically described in terms of mean ± SD. Comparison of quantitative parametric variables between the two studied groups was performed by the Student's'-test. The Mann-Whitney U-test was applied to determine whether the distribution of the variables differed significantly ($P<0.05$) between groups [7].

Fisher's exact test was performed in this study. The association between different parameters and the expression of cluster of differentiation markers were tested by regression analysis and the Spearman rank correlation coefficient was used to determine the probability of correlation between the two variables.

A probability value (P-value) less than 0.05 was considered statistically significant. All statistical calculations were performed using SPSS version 22 (SPSS Inc., Chicago, Illinois, USA).

Results

Results of the present study were presented in Tables 1-8 and Figures 1-16.

Patients' characteristics

This study included 100 patients diagnosed as 50 cases AML and 50 case ALL, 62% of all cases were male. Among cases with ALL, 66% were male while cases with AML, 58% were male. ALL was major in young age when compared with AML. As shown in Table 1; Figures 1 and 2).

Fatigue and weight loss was the most common symptom (92%) followed by fever (90%), pallor (86%), bleeding manifestations (84%) were associated with AML. splenomegaly and Lymphadenopathy were the most common sign (58%) in ALL, as shown in the Table 2.

Median hemoglobin values for AML and ALL were 8.41 and 9.09 g/dL, respectively. Median values of total Leucocytes count for AML and ALL were $50.4 \times 10^3/mm^3$ and $65.50 \times 10^3/mm^3$, respectively. Median values of platelet count were $60.2 \times 10^3/mm^3$ and $96.2 \times 10^3/mm^3$ respectively. Blast cells were present in peripheral smear in 45% of AML cases versus 45.5% of ALL cases, while Blast cells were present in bone marrow smear in 65.16% of AML cases versus 73.2% of ALL cases.

	AML (n=50)	ALL (n=50)	*P*
Age (years)	42.50 ± 12.76	38.40 ± 13.45	0.168
Males	29 (58)	33 (66)	0.441
Females	21 (42)	17 (34)	

Note: p: comparison between ALL versus AML; AML: Acute myeloid leukemia; ALL: Acute lymphoblastic leukemia. Age is expressed as mean ± SD; sex is expressed as frequency (percentage)

Table 1: Age and gender distribution in different studied groups.

No significant association was found by comparing the hematological parameters in between cases with AML and those with ALL (Table 3).

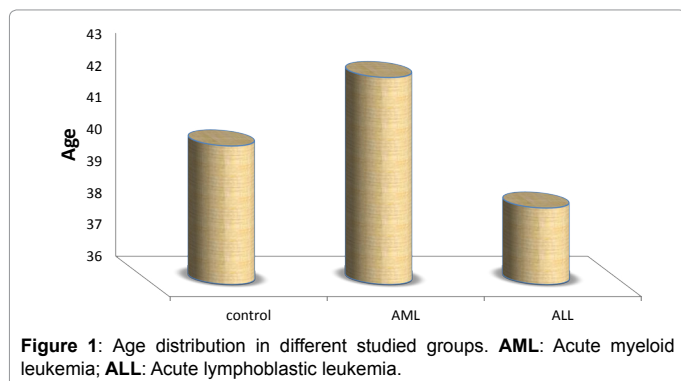Among the 50 cases of AML in this study, 2 were of the M0, 6 M1, 7 M2, 8 M3, 10M4, 10 M5, 5 M6 and the remaining 2 were of the M7.

Among the 50 cases of ALL in this study, 14 were of the ALL-L1, 30 ALL-L2, and 6 ALL-L3 (Table 4; Figures 3 and 4).

Immunophenotyping of studied AML and ALL cases

The most abundant immunophenotyping features in acute Lymphoblastic leukemia (ALL) were CD19, CD79a, CD20, CD10, Td T, and CD34. The most abundant clusters of differentiation in acute myeloid leukemia (AML) were CD33, CD34, CD36, CD64, CD117, CD79a, cMPO and HLA-DR (Figure 5).

Immunophenotyping of studied cases

Distribution of immunophenotyping characters between studied AML and ALL cases. Among the 50 cases of AML in this study, c Anti MPO was expressed only in 100% of AML cases.

Expression of CD2 was expressed in 12% of AML cases while in 38% in ALL cases. CD3 was expressed only in 38% from ALL cases. CD4 was expressed only in 14% of AML cases. Expression of CD5 was expressed in 22% of AML cases while expressed in 30% of ALL cases. Expression of CD7 was expressed in 18% of AML cases and was expressed in 30% of ALL cases.

CD19 was expressed in 6%, 80% of AML, ALL cases respectively. Expression of CD10 in 58% in ALL cases respectively.CD14 was expressed in 40% in AML cases. Of CD20 was expressed in 2% in cases of AML while in 58% of ALL cases. Expression of CD22 in 6%, 32% in cases of AML and ALL respectively.CD8 expression only in 38 % of ALL cases.

Expression of CD13 in 78%, 8% in cases of AML and ALL respectively. CD36 was expressed in 14% in both of AML and ALL cases. CD79a was expressed in 10%, 64% in cases of AML and ALL respectively. CD64 were expressed only in 40% in cases of AML. TdT was expressed only in 58% in cases of ALL. Expression of CD117 was observed only in 82% of AML cases. HLADR was expressed in 36%, 14% in cases of AML and ALL respectively.

CD33 was expressed only in 98% in cases of AML. Expression of CD34 was observed in 54%, 44% in cases of AML and ALL respectively. The most abundant immunophenotyping features in acute myeloid leukemia were cMPO, CD33, CD117, CD13, CD14 and CD64, while the most abundant immunophenotyping features in acute lymphoblastic leukemia were CD19, CD79a, TdT, CD20, CD10 and CD34 (Table 5).

Immunophenotypes characteristics

By Immunophenotypes characteristics we can classify acute lymphoblastic leukemia and acute myeloid leukemia in to sub classes

Figure 1: Age distribution in different studied groups. **AML**: Acute myeloid leukemia; **ALL**: Acute lymphoblastic leukemia.
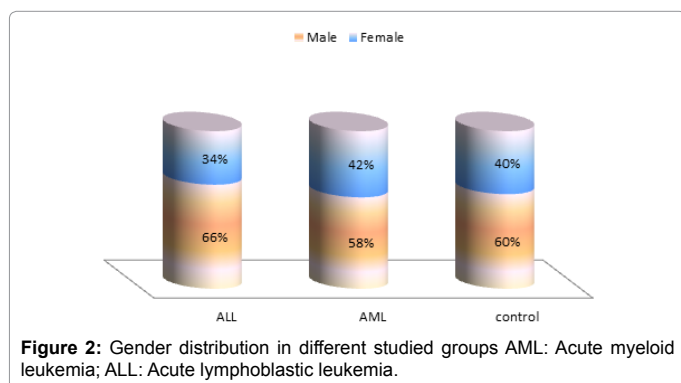

Figure 2: Gender distribution in different studied groups AML: Acute myeloid leukemia; ALL: Acute lymphoblastic leukemia.

Clinical data	AML (n=50)		ALL (n=50)		P
	No	%	No	%	
Fever/infection	45	90	39	78	0.120
Fatigue	46	92	40	80.0	0.164
Weight loss	46	92	40	80.0	0.164
Pallor	43	86	37	74	0.160
Bleeding tendency	42	84	35	70.0	0.138
Splenomegaly	27	54	29	58	0.816
Hepatomegaly	30	60	27	54	0.559
Lymphadenopathy	24	48	29	58	0.453

Note: p: comparison between ALL versus AML; AML: Acute myeloid leukemia; ALL: Acute lymphoblastic leukemia. Age is expressed as mean ± SD; sex is expressed as frequency (percentage).

Table 2: Clinical data in acute leukemia patients.

Hematological data	AML (n=50)		ALL (n=50)		P
	Mean ± SD	Range	Mean ± SD	Range	
Total leucocytic count (× 10⁹/L)	50.4 ± 45.3	1.4-213	65.5 ± 49.8	3.7-225	0.223▲
Platelets (× 10⁹/L)	60.2 ± 23.4	2-249	96.2 ± 34.2	4-307	0.842▼
Red Blood cells count (× 10⁹/L)	3.18 ± 0.98	1.6-5.79	3.31 ± 0.73	1.62-4.12	0.454▲
Hemoglobin concentration (g/dL)	8.41 ± 2.8	4.5-17.3	9.09 ± 2.07	4.8-12.7	0.143▲
HCT (%)	26.94 ± 9.3	7.48-54.2	27.37 ± 5.91	16-37.1	0.787▲
MCV(FL)	85.69 ± 11.56	39.6-104	83.67 ± 6.66	75.1-98.5	0.287▼
MCH (Pg)	26.82 ± 4.54	8.3-34	26.8 ± 2.62	22-32.5	0.978▼
Peripheral blasts (%)	45 ± 10.98	16-85	45.50 ± 11.32	16-89	0.815
Bone marrow blasts (%)	65.16 ± 28.28	25-95	73.2 ± 24.58	23-90	0.452

Note: P: comparison between ALL versus AML; Mean ± SD: Mean ± Standard Deviation

Table 3: Comparison between hematological data at diagnosis of different studied groups.

according to the expression of clusters of differentiation which present on the surface of the cells. From our results, we conclude that, Cluster of differentiation classify in to three main groups, and can used as prognostic marker for differentiation between acute lymphoblastic leukemia and acute myeloid leukemia, not only but also in differentiation between their subclasses (Table 6 and Figure 6).

Group 1: Myeloid Markers which include: CD33, CD117, cMPO, CD14, CD64, CD34.

Group 2: B cell Markers which include: CD10, CD19, CD20, CD22, CD79a, and TdT.

Group 3: T cell Markers which include: CD2, CD3, CD4, CD5, and CD7.

Immunophenotyping classification of studied ALL cases

Among the 50 cases of ALL in this study, 42 were of the B-ALL, 8 were T-ALL (Table 7 and Figure 6).

Correlation between immunophenotypes and clinical features of acute leukemia

The correlation between immunophenotypes and clinical features

	FAB	No	%
AML (n=50)	M0	2	4
	M1	6	12
	M2	7	14
	M3	8	16
	M4	10	20
	M5	10	20
	M6	5	10
	M7	2	4
ALL (n=50)	L1	14	28
	L2	30	60
	L3	6	12

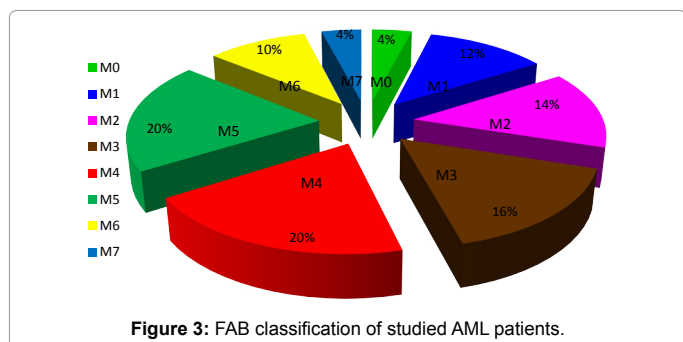**Table 4:** FAB classification of studied cases.

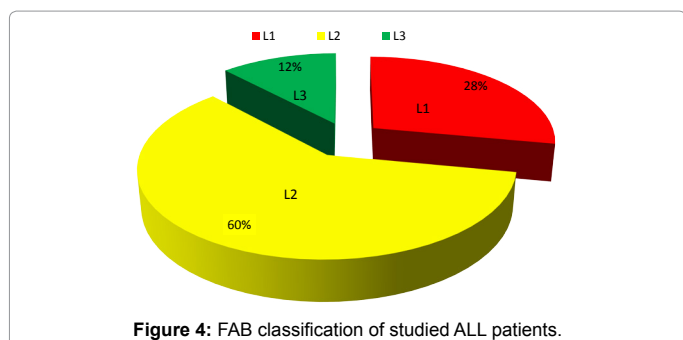**Figure 3:** FAB classification of studied AML patients.

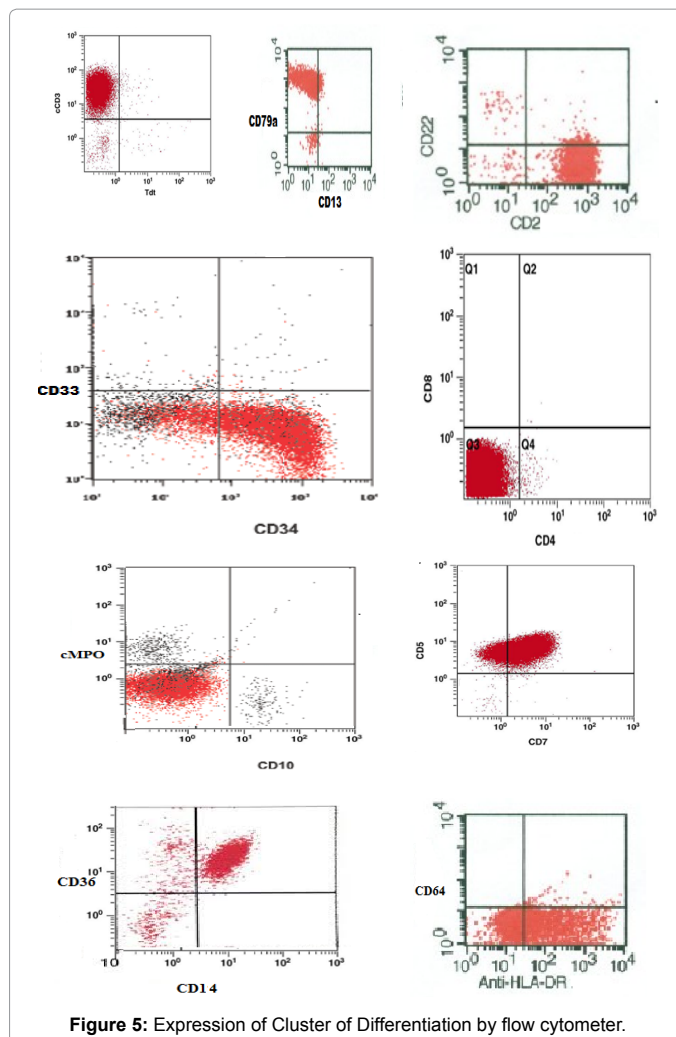**Figure 4:** FAB classification of studied ALL patients.

Figure 5: Expression of Cluster of Differentiation by flow cytometer.

Immunophenotyping	AML (n=50)		ALL (n=50)	
	No	%	No	%
cMPO	50	100	0	0
CD3	0	0	19	38
CD2	6	12	19	38
CD4	7	14	0	0
CD5	11	22	18	36
CD7	9	18	15	30
CD19	3	6	40	80
CD10	0	0	29	58
CD20	1	2	29	58
CD22	3	6	16	32
CD8	0	0	19	38
CD13	39	78	4	8
CD36	7	14	7	14
CD79a	5	10	32	64
CD14	20	40	0	0
CD64	20	40	0	0
TdT	0	0	29	58
CD117	41	82	0	0
HLADR	18	36	7	14
CD33	49	98	0	0
CD34	27	54	22	44

Table 5: Immunophenotyping of studied cases.

Lineage Specific Markers	Acute myeloid leukemia								Acute lymphoblastic leukemia		
	M0	M1	M2	M3	M4	M5	M6	M7	ALL-L1	ALL-L2	ALL-L3
Myeloid Markers											
CD13	50%	83%	57%	100%	100%	50%	100%	50%	0	13%	0
CD33	50%	100%	67%	75%	83%	50%	50%	50%	0	0	0
CD117	100%	83%	33%	75%	100%	100%	100%	50%	0	0	0
Anti-cMPO	100%	100%	100%	100%	100%	100%	100%	100%	0	0	0
CD14	0	0	0	0	100%	100%	0	0	0	0	0
CD64	0	0	71%	75%	50%	40%	0	0	0	0	0
CD34	100%	67%	71%	0	50%	70%	20%	100%	0	67%	22%
B-Cell Markers											
CD10	0	0	0	0	0	0	0	0	25%	78%	100%
CD19	0	50%	0	0	0	0	0	0	0	93%	25%
CD20	0	17%	0	0	0	0	0	0	0	50%	67%
CD22	0	0	43%	0	0	0	0	0	0	53%	0
CD79a	0	0	0	0	20%	30%	0	0%	21%	83%	44%
TdT	0	0	0	0	0	0	0	0	21%	57%	100%
T-Cell Markers											
CD2	0	17%	0	50%	0	0	0	0	25%	11%	0
CD3	0	0	0	0	0	0	0	0	100%	17%	0
CD4	0	0	0	0	30%	40%	0	0	0	0	0
CD5	0	0	0	0%	50%	60%	0	0	87.6%	23%	0
CD7	0	0	0	50%	50%	0	0	0	50%	27%	0

Table 6: Immunophenotypes characteristics in acute leukemia.
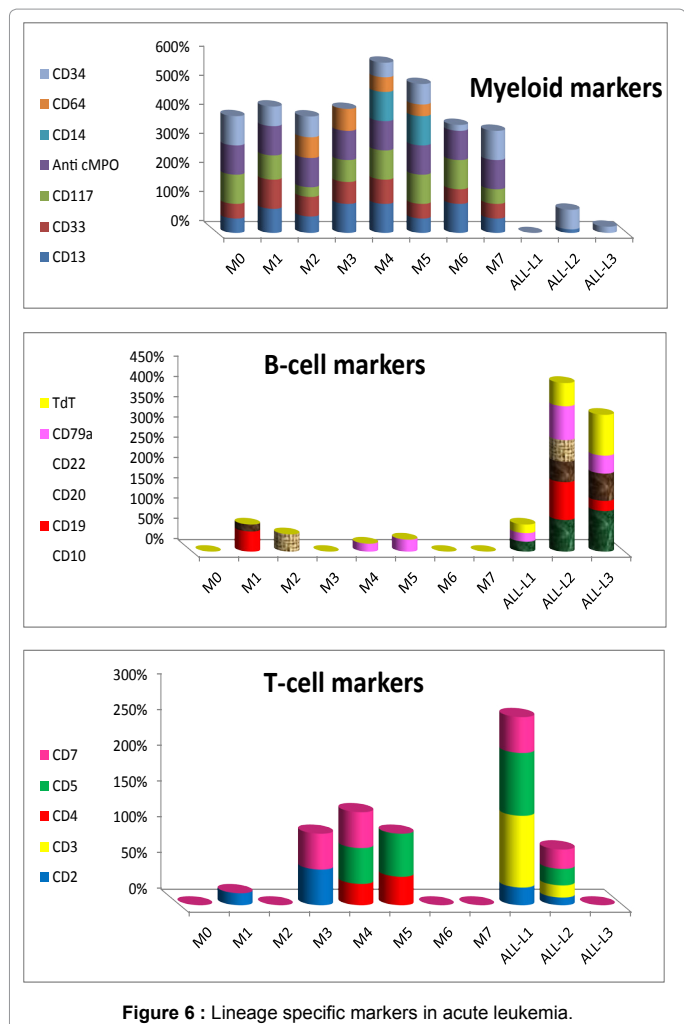
Figure 6 : Lineage specific markers in acute leukemia.

ALL	No	%
B-ALL	42	84
T-ALL	8	16

Table 7: Immunophenotping classification of studied ALL cases.

of acute leukemia. Among 100 cases of acute leukemia there were significant correlations between CD5/CD7/CD14CD33/CD34, CD13/CD14/CD64, CD4/CD34/CD117, CD4/CD34, CD13 and age, TLC, HB, RBCs respectively. c Anti MPO which act as independent prognostic factor for AML. CD10 and TdT can use as independent prognostic factor for ALL (Table 8 and Figures 8 to 16).

Fever, Bleeding, Hepatomegaly, splenomegaly, lymphadenopathy, peripheral blood and bone marrow blasts, liver functions and kidney functions were not statistically significant associated with progenitor cell markers expression (data not shown) [8].

Discussion

Acute leukemias are heterogeneous tumors of diverse clonal origin, cytogenetic abnormality, clinical presentation, biologic behavior, and response to treatment [9].

Human acute leukemias are broadly defined as having myeloid or lymphoid differentiation according to the expression of surface and/or cytoplasmic antigens associated with their normal myeloid or B/T-lymphoid counterparts [10]. During the past two decades, flow cytometry-based immunophenotyping has impacted the diagnosis and management of acute leukemia immensely. Mainly due to significant advances in laser and computer technologies, several hundred MoAbs to a variety of antigens expressed by hematopoietic cells can be produced cost effectively. Moreover, distinct flourochrome conjugated with MoAbs have become available [11].

In this study, we analyzed the immunophenotype of 100 cases of acute leukemias, and we were investigated the correlation between different immunophenotyping and clinical features. There was higher number of male patients in the present study (62%) as compared to

CD	Age		Hb		RBCS		WBCs		PLT		LDH	
	P	R	P	r	P	r	P	r	P	r	P	r
CD1a	0.116	0.7	0.425	-0.405	0.652	-0.236	0.516	-0.334	0.887	-0.0753	0.861	0.215
CD2	0.698	0.0875	0.256	0.259	0.672	0.100	0.29	0.2329	0.387	-0.193	0.242	-0.365
CD3	0.253	0.223	0.787	-0.052	0.701	-0.074	0.653	-0.086	0.201	-0.244	0.929	0.022
CD4	0.14	0.414	0.005**	-0.280	0.0003***	-0.356	0.179	-0.38	0.587	-0.158	0.178	-0.437
CD5	0.019*	-0.199	0.352	0.248	0.494	0.178	0.255	0.292	0.151	-0.363	0.345	-0.357
CD7	0.013*	-0.163	0.634	0.12	0.488	0.180	0.595	0.13	0.092	-0.396	0.968	-0.0128
CD8	0.429	0.465	0.141	-0.858	0.828	0.135	0.706	-0.232	0.680	-0.253	0.656	-0.343
CD10	0.220	0.324	0.958	-0.0095	0.691	0.074	0.194	-0.231	0.532	0.112	0.309	0.227
CD13	0.129	0.265	0.559	0.103	0.231	0.217	0.231	-0.408	0.057	-0.377	0.580	-0.127
CD14	<0.0001***	0.451	0.557	-0.143	0.950	-0.016	0.0085	-0.404	0.554	-0.144	0.312	-0.318
CD19	0.203	0.227	0.971	0.0069	0.858	0.034	0.274	-0.202	0.337	0.178	0.287	0.243
CD20	0.01	0.31	0.530	-0.17	0.93	-0.026	0.179	-0.35	0.093	0.43	0.38	0.36
CD	Age		Hb		RBCS		WBCs		PLT		LDH	
	P	R	P	r	P	r	P	r	P	r	P	r
CD22	0.512	0.199	0.131	-0.440	0.594	-0.163	0.450	-0.229	0.321	0.298	0.361	0.457
CD33	<0.0001***	0.522	0.074	-0.330	0.444	-0.14	0.111	-0.291	0.068	-0.33	0.725	-0.088
CD34	<0.0001***	0.447	<0.0001***	-0.834	<0.0001***	-0.404	0.363	0.155	0.925	0.0162	0.321	-0.221
CD36	0.271	0.303	0.157	-0.280	0.443	0.233	0.586	0.152	0.324	0.273	0.129	0.513
CD64	0.080	0.389	0.79	-0.061	0.444	0.186	0.37	-0.456	0.134	-0.337	0.711	0.113
CD79	0.445	0.186	0.859	-0.043	0.360	0.222	0.375	-0.215	0.211	0.300	0.196	-0.421
CD117	0.428	0.145	0.0517	-0.386	0.164	-0.26	0.889	0.0255	0.056	-0.341	0.504	-0.150
HLADR	0.378	0.166	0.097	-0.314	0.204	-0.238	0.532	-0.118	0.809	-0.045	0.171	0.422
TdT	0.394	0.196	0.286	-0.194	0.927	0.021	.456	.105	0.896	-0.030	0.252	0.284
cMPO	0.333	0.176	0.231	0.245	0.730	-0.065	0.449	-0.138	0.070	-0.323	0.171	0.422

Note: WBCs: White Blood Cell Count; Hb: Hemoglobin Concentration; PLT: Platelets; RBCs: Red Blood Cells; LDH: Lactate Dehydrogenase; CD: Cluster of Differentiation

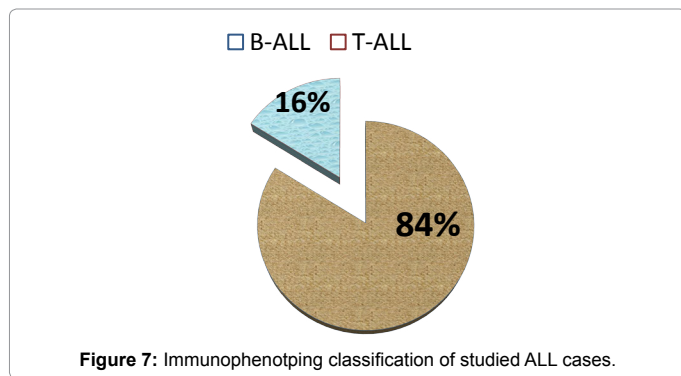**Table 8:** Correlation of immunophenotypes to clinical features of acute leukemia.

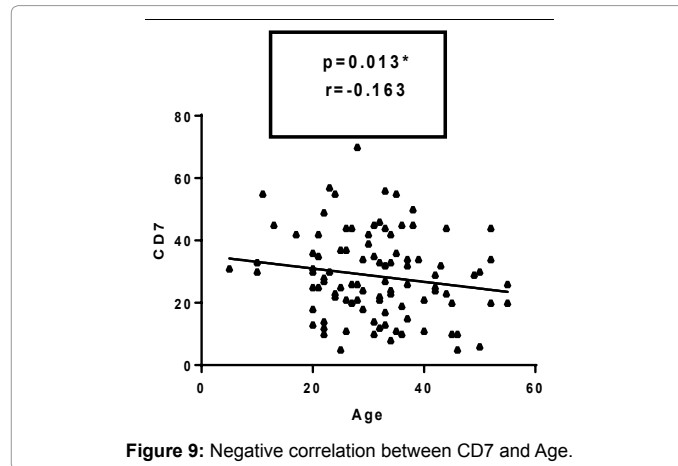**Figure 7:** Immunophenotping classification of studied ALL cases.

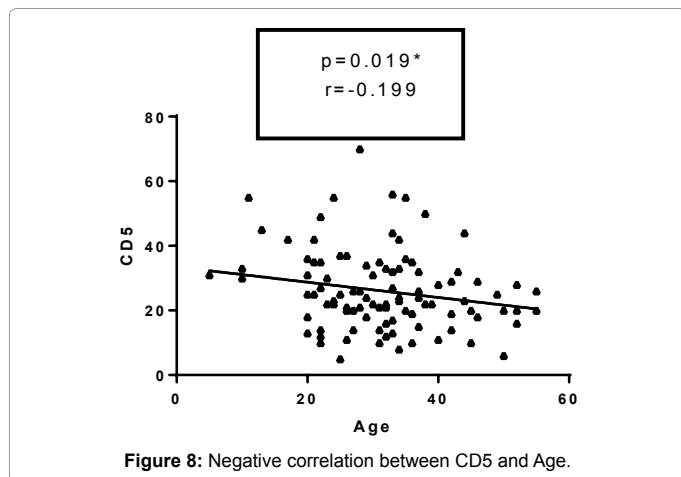**Figure 9:** Negative correlation between CD7 and Age.

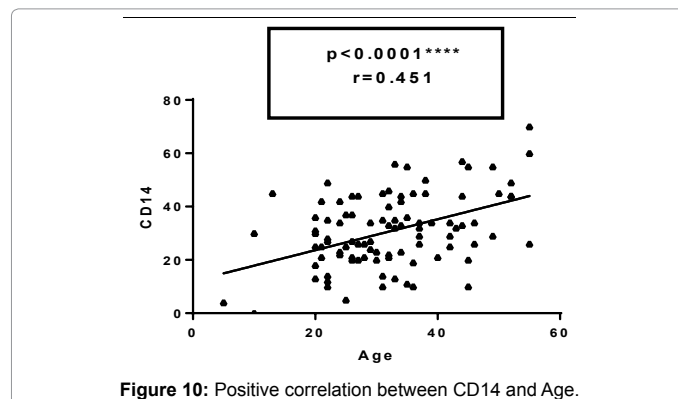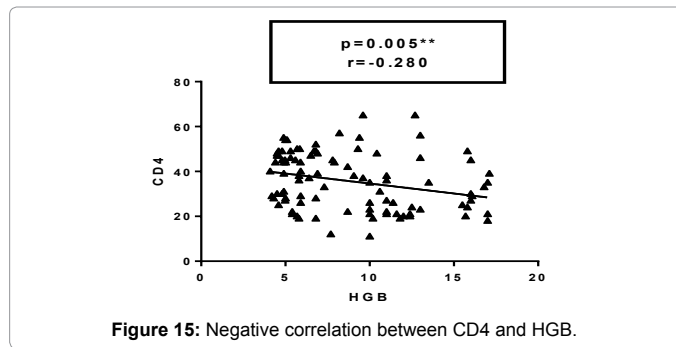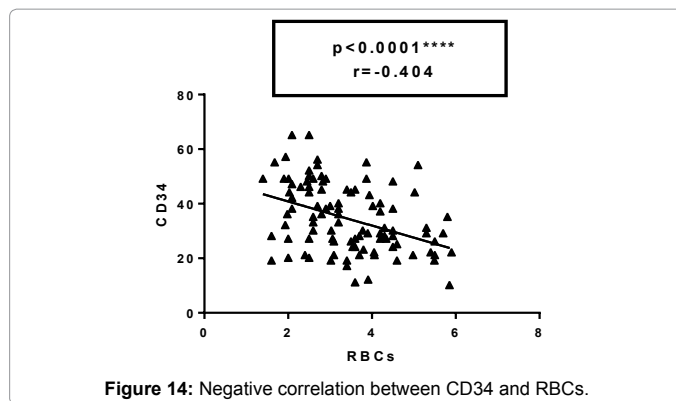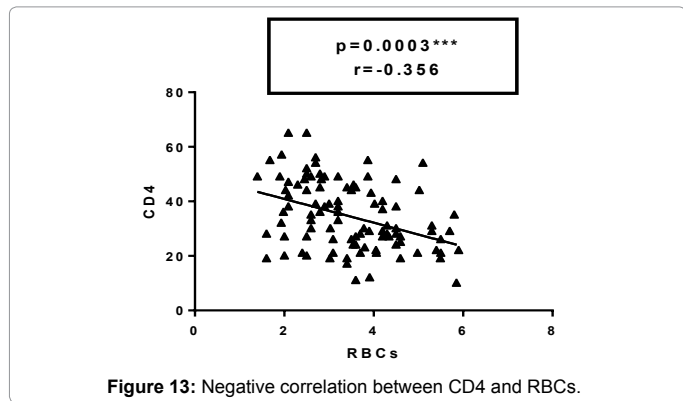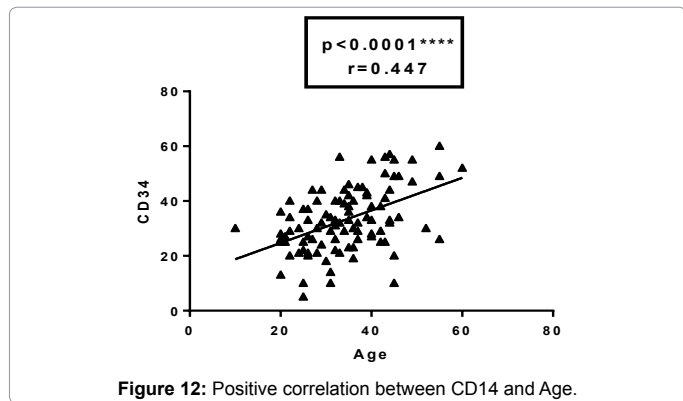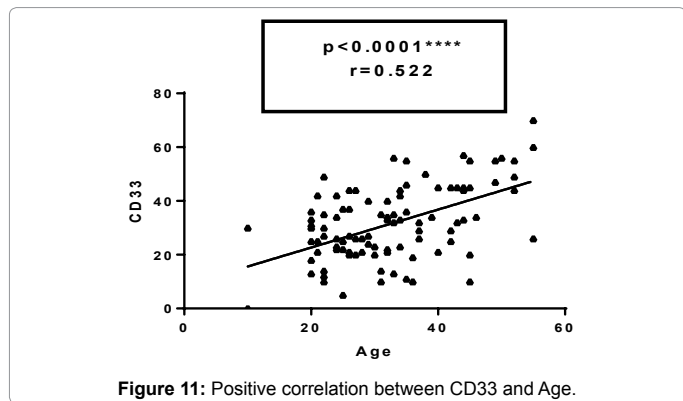**Figure 8:** Negative correlation between CD5 and Age.

Figure 10: Positive correlation between CD14 and Age.

previous studies, where male gender ranged from 59% to 63% [12] incidence of all was common in young, while incidence of AML was common in adult [8].

In patients with acute lymphocytic leukemia (ALL) or acute myeloid leukemia (AML), the Clinical presentation of acute leukemias is very vague and variable which makes it difficult to diagnose, including fatigue, fever or bleeding. However, certain patients with acute leukemia present with clinical signs of hyperleukocytosis [13].

In the present study, fatigue (92%) fever (90%) and pallor (86%) were found to be the most common presenting complaints of AML patients versus fatigue (80%) fever (78%) and pallor (74%) in ALL patients, compared to the study done by Poplack study's [14], in which cases with pallor and fever were 43% and 61% respectively.

Fever was present at diagnosis in approximately 40% to 50% of

Figure 11: Positive correlation between CD33 and Age.

Figure 12: Positive correlation between CD14 and Age.

Figure 13: Negative correlation between CD4 and RBCs.

Figure 14: Negative correlation between CD34 and RBCs.

Figure 15: Negative correlation between CD4 and HGB.

Figure 16: Negative correlation between CD34 and HGB.
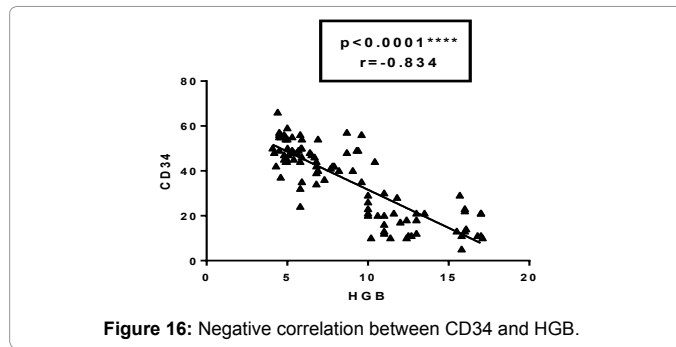
the patients, anorexia and weight reduction are also common, and percentages of 25% being reported [15].

In ALL patients, hepatomegaly was seen in 54% patients, splenomegaly in 54% patients versus in AML patients, hepatomegaly was seen in 60% patients, splenomegaly in 54% patients, especially in those with a monocytic or monoblastic morphologic subtype. These findings are consistent with the notion those patients in our part of world present to hospitals when the disease has reached an advance stage. Splenomegaly and hepatomegaly might be caused by infiltration of leukemic cells are seen in some cases of leukemia [16].

According to, Sharma and Kark, [17] also assessed the Acute leukemia patients with leucocytosis are more prone to develop abnormal liver function and splenomegaly. Approximately one half of the patients presented at diagnosis with lymphadenopathy, splenomegaly and hepatomegaly (Hoelzer, 2000).

In ALL patients, lymphadenopathy was seen in 58% patients, while in AML patients it was seen in 48% patients. This is consistent with previous studies. These findings can be explained by the mechanism of leukemia as a maturation block and/or suppression of erythroid and

megakaryocytic cells by increased production of blast cells resulting in decreased/defective production of normal leucocytes/neutrophils (resulting in fever), erythrocytes (resulting in anemia/pallor) and platelets (resulting in bleeding). In the present study, it was found that the mean value of white blood cells count was significantly increased in both ALL and AML, while no statistically significant differences in white blood cell between ALL and AML.

According to, Hendrik et al. [18] also found that high WBC count predicts an adverse outcome among AML patients with favorable or intermediate cytogenetic risk. There was no statistically difference between ALL and AML due to there are increase the level of white blood cell in acute leukemia patients in both ALL and AML, although the WBC count may typically be higher in ALL than in AML cases [19].

The leukemic process itself could also contribute to platelet function abnormalities, in this study the level of platelets count statistically significant decrease in acute leukemia cases, this agree with Saba et al study's [20] in which a statistically significant lower platelets count was found in patients than controls. This lower platelets count may be from the expansion of immature blast cell in the bone marrow resulting in bone marrow failure. In this study, it was found that the mean value of red blood cell count was significantly decreased in acute myeloid leukemia patients and acute lymphoblastic leukemia patients. This agree with [21] who decided that lower levels red blood cells were found in acute leukemia when compared with healthy control. Also, there were significant decrease in values of hemoglobin, hematocrit and mean corpuscular volume in acute leukemia (date not shown).

There were no significant differences in the mean values of red blood cells (RBCs), blood hemoglobin levels between ALL and AML.

In this study, higher level of both peripheral blast (PB) and bone marrow (BM) blast were found in both acute myeloid leukemia and acute lymphoblastic leukemia also the level of blasts cell in AML greater than in ALL. These results were in agreement with [22] who reported that The French-American-British (FAB) classification requires a blast percentage of at least 30% in bone marrow (BM) or peripheral blood (PB) for the diagnosis of acute leukemia. In a small proportion of patients with acute leukemia known as 'peripheral leukemia,' however, there is no diagnostic increase in BM blast percentage; the diagnosis is based on the presence of at least 20% PB blasts [23].

ALL is rare in adults and is commonly characterized by L1 or L2 morphology (FAB classification) [24]. TdT expression, lack of surface light chains, and variable genetic alterations are also common [24] B lymphoblastic leukemia (B-ALL) is a neoplasm of hematopoietic precursor cells committed to the B-cell lineage [25]. Immunophenotypic analysis is an essential component of the diagnostic work-up of acute leukemias (AL), and flow cytometry (FC) is the preferred method of analysis (Xin, 2014).

Acute lymphoblastic leukemia (ALL)

The separation of ALL into B- and T-cell types is clinically important, with both therapeutic and prognostic implications [26]. The cases of ALL in this study were therefore divided into three subtypes, one based on the FAB classification into ALL-L1, ALL-L2 and ALL-L3 and the other based primarily on immunophenotypic findings.

B-ALL cells are characterized by the expression of various B-cell lineage associated antigens [27]. In the vast majority of cases, leukemic B-cells express CD19, cytoplasm (cyt) CD79a, cyt CD22, and PAX5, while expression of CD20 is variable [28]. A diagnosis of ALL of B-cell lineage requires the use of a panel of B-cell markers, commonly including CD19, CD20, and CD22 [29]. However, some of these markers are known to be not entirely B-cell specific.

In this study CD19 and CD20 were expressed in 6% and 2% of cases of AML, respectively. On other study showed that CD19 and CD20 were expressed in 9.8% and 17.0% of cases of AML, respectively [30].

In addition, CD20 is expressed only in a small subset of precursor B cells [31]. Expression of cyt IgM and immaturity antigens (e.g. TdT, CD34, and/or CD10) can be variable, often corresponding to the stage of maturation of leukemic cells. At times, the immunophenotypes are discordant or asynchronous, deviating from the normal stages of B-cell development seen in haematogones [32]. An example of such asynchrony is the expression of immaturity markers along with markers of more mature B-cells (e.g. CD20).

In this study the majority (89%) of B-cell ALL's expressed CD19. CD20 is B cell differentiation antigen with variable expression B acute lymphoblastic leukemia [33]. In our study expression of CD20 was highly in ALL-L2 more than in ALL-L1. As has been previously reported, CD20 was more common in ALL-L2 than in ALL-L1 cases [32] the expression of T cell infrequently detected in this type of leukemia [33].

CD22 is found on the surface of mature B cells and to a lesser extent on some immature B cells. In this study 56% of B-ALL expresses CD22 which closed to [34] which reported that expression of CD22 on B-precursor ALL cells varies from 60% to 85% in adult cohorts and up to 90% in children. Burkitt leukemia/lymphoma is now considered a mature B-cell neoplasm.

In addition, most of cases were HLA-DR positive. HLA-DR is expressed on B lymphocytes, monocytes, macrophages, activated T lymphocytes, activated NK lymphocytes, and human progenitor cells [35]. It is also present on thymic epithelium, B-lymphocyte-dependent areas of spleen and lymph node, and B-cell lymphomas [36].

In this study the majority of ALL cases expressed CD10, which is a marker of long standing and well known prognostic value [37]. On the other hand; CD10 negativity has been associated with a poor prognosis [38]. In this study the most expression antigens are CD10 and dTd, these results coincide with Bryce Higa study which reported that the most mature stage of blasts of precursor B ALL with L3 morphology express CD10 and dTd [39]. Aberrant expression of T-cell antigens in B-ALL, in our study, the most commonly expressed T-cell antigens were CD2, CD5, and CD7. Several smaller case series and case reports have demonstrated CD2, CD5, and CD7 expression in B-ALL [40].

Based on our study, aberrant expression of T-cell antigens in B-ALL may be a useful marker to identify patients at increased risk for relapse and for harboring prognostically poor cytogenetic abnormalities. This in agreement with [41], who reported that there was correlation between T-cell antigen and clinical outcome.

CD79a is a highly lineage-specific marker of B lymphoid cells and plays an important role in the diagnosis of acute leukemia [42]. In this study 33% of B-ALL cases express CD79a. CD79a has been considered a specific and sensitive B-cell marker and has been used extensively for immunophenotyping of lymphoid malignant neoplasms [41]. Our findings in the present study are in keeping with the concept that CD79a is a reliable marker for ALL of B-cell lineage, since 64% of ALL cases in our cohort (n=32) were positive for CD79a. In addition, CD79a was not expressed in all cases of AML about 10% from AML cases

expressed CD79a (n=5). Taken together, CD79a positivity detected by flow cytometry is a useful means to confirm ALL and to exclude AML [43].

The phenotyping heterogeneity of T-cell neoplasms is well documented and covers the range of phenotypes expressed during thymic differentiation [44]. Most cases will express more than one T-lineage marker Aberrant deletion of one or more pan T-cell antigens is common in this disease, however, and maybe a helpful diagnostic finding. T early phenotype: leukemic cells express cCD3 as well as any other pan T marker (CD7, CD5, or CD2), The threshold of positivity was considered 20% for all markers except for CD10, CD34 and TdT, ALL the 5 cases of T-ALL showed deletion of one or more of the 4 Pan T-cell antigens used (CD2, CD3, CD5 and CD7) [45].

CD3 was the pan-T-cell antigen most often expressed by 100% of T-ALL cases in our study. The CD3 antigen is expressed on 61% to 85% of normal peripheral blood lymphocytes 65% to 85% of thymocytes and on Purkinje cells in the cerebellum [44].CD7 is also the antigen that is most commonly deleted in these acute leukemias [45].

Traweek [46], showed in their series that CD7 was the most often expressed pan-T-cell antigen.

The HLA-DR antigen is not expressed in T-cell ALL which is another phenotypic feature that distinguishes this acute leukemia from B-cell ALL [47]. None of the cases of T-cell ALL in this study were HLA-DR positive thus concurring with other studies in literature. CD10 has been reported in 20% to 30% of T-cell ALL's, [47]. This frequency is approximately similar to that seen in this study in which the concentration of CD10 in T-ALL is 25%.

No myeloid antigen expression was seen in T-Cell ALL of our series; though this has been reported in literature [48]. Expression of myeloid antigens may be a potential source of confusion considering the occasional presence of CD2 and CD7 in AML [26].

Acute myeloid leukemia (AML)

In this study, we analyzed the immunophenotype of 50 cases of Acute Myeloid leukemia. cMPO, CD33, CD13, CD117 and CD64 were the most commonly expressed myeloid antigens. These results in agreement [26]. CD34 is expressed on the surface of immature hematopoietic normal progenitor cells that compromise 1% to 2% of the cells [49]. It is not lineage restricted and thus not useful for distinguishing AML from ALL. In addition, CD34 is involved in cellular adhesion and mediates resistance to apoptosis [49]. CD34 AML blast cells are even more resistant to programmed cell death with increased percentages of CD34 cells [50].

In this study cases of acute myeloid leukemia express CD34 and HLA-DR but these are lost by the AML-M3 and AML-M6 subtypes, this in agreement with [51] who reported that 54% of our cases were positive for CD34. These data were consistent with other studies using the same technique who detected 65% expression of CD34 [52]. This was also in agreement with Petrovici et al. and Legrand et al. [52,53], who stated that the expression of CD34 was 57% and 68% respectively.

Mona and Sherin [50] reported that CD34 was expressed in 61% of the patients with AML.

In our study, CD34 expression was highest in M0-M7 FAB subtypes; however, this was not statistically significant. This was consistent with other studies [52]. Petrovici et al. who detected no correlation between CD34 expression and FAB subtypes. In this study

showed CD2 expression, though positivity for this T cell marker has been well documented in AML-M3, this was also in agreement with [26] who detected CD2 expression in AML-M3. Initially thought to be a T-cell antigen, CD7 is now known to be present in AML.CD7 expression appears to be associated with blast cell immaturity [54,55] because the percentage of positive cases was greatest in AML-M1, AML-M5 and AML-M7.

In this study CD7 present in AML cases with high value in AML-M1 and AML-M3 subtypes. Traweek found greatest expression of CD7 in AML-M0, AML-M1 and AML-M5a [46].

B-cell antigen expression was almost exclusively CD19, and was predominantly seen in AML-M1. Kita et al. showed an association between CD19 expression and AML-M2, and also has been linked with a specific cytogenetic abnormality, the t (8; 21) translocation [45]. CD19 has been associated previously with AML-M5, but one case was detected in our study in AML-M1 subtype. Zheng et al. [6], who studied 180 cases of AML and showed that CD7 was the most commonly expressed followed by CD2, CD19 and CD22. 2 out of 6 cases of AML-M4 in our study expressed CD14 and 2 out 4 cases of the AML-M5 cases showed CD14 expression. FCI was an insensitive method to determine the percentage of monocytes [56]. In addition, previous study showed that in AMLs with a monocytic component (i.e. AMML and AMoL), CD14 was an insensitive marker in confirming monocytic cells [57]. The CD14 clone (M0P9) used in the present study and previous study detects monocytes/ macrophages, and to our knowledge, differential expression at various stages of monocytic differentiation has not been analyzed with this particular epitope (Kaplow et al. 1963). However, we would like to acknowledge that in a study by Yang et al. [57], a 4-color flow cytometry technique with the MO2 and MY4 epitope of CD14 was able to identify all stages of monocytic differentiation (i.e. monoblasts, promonocytes, and mature monocytes) because they appear on mature monocytes after the promonocytes stage and at the early promonocytes stage, respectively [58]. This finding supports the study by Krasinskas et al. showing that CD14 is only moderately specific for monocytic differentiation. On the other hand, Traweek [46], in his study of 207 AML cases found expression of CD14 in 6% of AML-M2 cases and 1 case of AML-M3. Upto 25% of M1 and M2 AML's have been previously reported to express this antigen, Roberts et al. [59] but CD14 expression in AML-M3 is apparently rare [46]. Acute Promyelocytic leukemia (AML-M3) is an unusual and distinctive disease clinically, morphologically, immunophenotypically and even genotypically [51]. As previously reported, the majority of cases of AML express HLA-DR [60] the clear exception to this rule is AML-M3, in which only rare cases are reported to be positive [61].

In this study, 0 out of 4 cases of AML-M3 expressed HLA-DR. Tarik et al. [32] found that HLA-DR expression was lacking in 88.9% (16/18) of APL and 9.5% (4/42) of non-M3 AML cases (p<0.0001). Whereas 0 case showed CD34 expression.

CD13 is a myeloid-associated antigen which is found to be expressed by macrophages, monocytes, granulocytes, endothelial, epithelial, stem cells and precursor cells [41]. In this study CD13 express in 50% of AML cases.

Myeloperoxidase (MPO) is the hallmark enzyme of the myeloid lineage [61,62]. MPO can be detected by cytochemical staining, immunohistochemistry, or flow cytometry [61]. 100% of AML cases express MPO. The highest value in AML-M7 (100%).

Leong et al. [63] found that 23/25 (92%) of AML showed concordance results for both anti-MPO. The diagnosis of AML is easy

if more than 3% of blast cells are confirmed to be cytochemical MPO positive [64]. A few studies have previously shown the prognostic significance of MPO in AML.

Our study generates immunophenotypic profiles that may help to better define the range of antibody reactivity for many types of acute leukemia and act as potential prognostic significance markers for detection and differentiation of acute leukemia cases.

Conclusion

Our data confirmed that acute leukemias classified in too many groups each one has different immunophenotyping features, so, we can use specific system during diagnosis acute leukemias with its different types. Also, we found that, CD3 act as independent prognostic factor for detection of T-ALL, CD10 and TdT act as independent prognostic factors for detection of B-ALL. Moreover, c anti MPO act as specific marker for AML. But for detection monocytic myeloid leukemia CD14 and CD64 act as independent diagnostic markers, while for differentiation granulocytic myeloid leukemia from other types we can used CD13 and CD33 as diagnostic markers.

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