Original article

Expression of Aberrant Antigens CD7 and CD19 in Adult Acute Myeloid Leukemia by Flow Cytometry.

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ABSTRACT

Background: Flow cytometric immunophenotyping (FCI) is an indispensable tool for quantitative and qualitative evaluation of antigen expression of hematopoietic cells. From a diagnostic and therapeutic point of view, distinction between acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) and for diagnosis and definition of particular AML subtypes. Aberrant antigen expression (i.e. expression of an antigen which is inappropriate for a lineage) is rarely seen in normal bone marrow cells and their incidence is varied between various studies.

Objectives : To explore the existence of the aberrant lymphoid antigens (CD7) and (CD19) in adult acute myeloid leukemia patients and their distribution among French-American-British (FAB) classification subtypes and correlate them with hematological parameters including red cell count (RBC) , hemoglobin, white cell count (WBC) , platelets count, blast cell percent and hematological response to the induction chemotherapy.

Patients and Methods: EDTA anticoagulated peripheral blood (PB) sample of 2.5 ml and /or bone marrow (BM) aspirate samples of 0.5 ml from 25 cases of de novo AML including 14 male and 11 female with mean of age of 37.56±18.07 were included from June to November 2012.Hematological parameters including RBC, hemoglobin, WBC, platelets count, blast cell percent were obtained from the case file of the patients where they were done by automated device (Cell-DYN, RUBY list) and diagnosed by cytomorphology by Leishman stain and cytochemistry by Sudan Black B and PAS stain and AML cases were classified according to the FAB criteria. Aberrant lymphoid antigens, (CD7) and (CD19) expression was explored by CyFlow© multiparametric flow cytometry at diagnosis. The patients were evaluated after three weeks of one cycle of chemotherapy for complete remission.

Results: CD7, CD19 and co-expression of CD7 and CD 19 were expressed in 40%, 16% and 12% of AML patients respectively. Statistically significant associations were found between aberrant CD7 expressions and age, low WBC, and early FAB AML (M1, M2) subtypes. Complete remission was achieved in 19 out of 25 patients (76%) with standard chemotherapy whereas six patients did not achieved complete remission; three of them had aberrant CD7 expression (two of them died during induction therapy) and the other one had poor response to induction therapy.CD7 was detected in 7 patients; 6 of them were male. There is no statistically significant association between aberrant CD7 expression and hepatosplenomegaly and treatment response to one cycle of chemotherapy (P value > 0.05).Also there is no relation between CD7 expression and percent of blast cells.

Conclusions:

1. The incidence of aberrant expression of CD7, CD19 and both CD7, and CD19 were 40%, 16%, and12% respectively.
2. CD7 was detected mainly in males whereas CD19 was distributed among males and females.
3. Total WBC count and malignant cell percent were lower in patients harboring aberrant expression compare to those without aberrant expression.
4. CD7 was mainly detected in early FAB classification (M1 and M2).
5. Six out of twenty five AML patients had no response to standard therapeutic regimen; three of them were harboring CD7 and no one had CD19 alone, thus we may propose that CD7 was associated with poor response to induction therapy.

Introduction

Acute myeloid leukemia (AML) represents a group of hematopoietic neoplasms derived from the bone marrow precursors of myeloid lineage. The neoplastic process is the result of clonal proliferation of an aberrant, committed stem cell at the level of CFU-S or later stages of differentiation leading to the accumulation of immature forms without, or with limited, maturation [1].

Flowcytometric immunophenotyping (FCI) had become an indispensable tool for quantitative and qualitative evaluation of antigen expression of hematopoietic cells. From a diagnostic and therapeutic point of view, distinction between AML and acute lymphoblastic leukemia (ALL) is extremely important and Flow Cytometry (FCM) is very instrumental in this matter [2-4].

Neoplastic cells frequently show nonrandom expression of antigens in a manner that deviates from the tightly regulated patterns of antigen expression seen in normal maturation. This is the basic principle that allows for the detection of hematopoietic neoplasia by immunophenotyping [5].

Abnormal antigenic expression in acute leukemia can be grouped into four basic categories [2, 5]:

- Abnormally increased or decreased levels of expression (intensities) of antigens normally expressed by cell type or lineage at a particular stage of maturation, including the complete loss of normal antigens in some instances.
- Asynchronous antigen expression (deviations of the normal differentiation and maturation pathway; i.e., expression of antigens normally expressed by the cell type or lineage but at an inappropriate time during maturation).
- Abnormally homogeneous expression of one or more antigens by a population that normally exhibits more heterogeneous expression.
- Gain of antigens not normally expressed by cell type or lineage (include expression on myeloid blasts of markers usually not present on cells of

that particular lineage, e.g. lymphoid markers, such as CD7, CD19 and CD56).

Occurrence of these aberrant phenotype has been reported in both ALL and AML with varying frequencies. In AML, aberrant lymphocyte phenotype in AML (Ly+ AML) has been reported in up to 48% cases. The most frequent lymphoid antigens in AML that have been reported include, CD7 (T-cell marker) and CD19 (B-cell marker) [6].

Immunophenotypic aberrancies also have been explored to predict treatment outcome in AML as they are useful for MRD detection and quantification with the aim of providing prognostic information [2, 3].

Materials and Methods

2.1 Patients:

This cohort study was conducted on twenty five adult AML patients, including 14 male and 11 female, their mean age was 37.56 ± 18.07(mean ± SD) and 14 of them were male and 11 were female from June to November 2012.

Those patients were admitted to the Hematology Department of Baghdad Teaching Hospital. The Patients 'peripheral blood (PB) and bone marrow aspiration (BMA) samples and their staining's were analyzed in the teaching laboratories of the Medical City in Baghdad. Flowcytometry was done at Al-Rawabi Private Laboratory in Baghdad.

For each patient a questionnaire form was done, hematological parameters including hemoglobin (HB) , packed cell volume (PCV) , WBC count, and blast cells per cent and platelet count were obtained from the case file of the patients where they were done by automated device (Cell-DYN, RUBY list).

2.2 Inclusion Criteria

Criteria for the inclusion of the patients:

1. The patients were randomly collected in relation to sex
2. All AML patients were above 15 years old.
3. All AML patients were newly diagnosed, de novo and they were not receiving any chemotherapy before the time of collecting blood samples and secondary AML cases were excluded from the study.
4. Patients with AML were classified according to the FAB classification criteria (from M0 to M7) are usually diagnosed by cytomorphology [7, 8]. However, cases of AML FAB M0 and M7 categories were diagnosed by Flowcytometry in the Central Medical Laboratories in Sulymania City.

2.3 Therapy and Follow-up

All patients were evaluated for complete remission achievement three weeks after one cycle of chemotherapy. Complete remission (CR) was defined by Cheson et al (< 5% bone marrow blast cells of normal cellularity and restoration of normal peripheral blood values of at least 1500/µL neutrophils and 100,000/µL platelets) [9] .

For remission induction , all patients received therapeutic regimens routinely used for AML (i.e. the standard protocol of '3+7' of Doxorubicin 30 mg/m^2 on days 1-3 or daunorubicin 60 mg/m^2 + Cytosine Arabinoside 100 mg/m^2 on days 1-7) . AML-M3 patients received different regimen (ATRA 45 mg/m^2 daily until complete remission plus Doxorubicin 30 mg/m2 for 4 doses) [10] .

For all patients with AML, PB and BM aspirate samples were repeated 2 weeks after completion of chemotherapy to assess response or CR, for AML M3 patients bone marrow repeated only after recovery of hematological parameters [11].

Two patients experience early death during early therapy induction.

2.4 Laboratory Tests

2.4.1 Blood sampling

A total venous blood sample of 2.5 ml and /or bone marrow aspirate sample of 0.5 ml were obtained from each patient included in this study by venipuncture from antecubital fossa or bone marrow aspirate from posterior superior iliac crest under aseptic technique respectively, and the samples were collected in EDTA tubes.

Blood sample from suspected patient was examined for complete blood indices in the teaching laboratory department of the Medical City, a blood film was made by taking a drop of blood sample spreads it on a clean dry slide, and staining it by Leishmen, Sudan black B and PAS stain; the slides were examined by a specialist in the teaching laboratory department of Medical City.

Accordingly sample of peripheral blood or bone marrow aspirate were obtained from AML confirmed cases (by FAB cytomorphology or Flowcytometry) for flowcytometry study to investigate the expression of aberrant surface marker antigens CD7, CD19 (both or one of them).

Three weeks after the patients had received induction chemotherapy PB samples was withdrawn from the patients to assess the patients' response to therapy by cytomorphology and complete blood count, for AML M3 patients bone marrow repeated only after recovery of hematological parameters [11].

2.4.2 Staining:

2.5 Flowcytometry Immunophenotyping

In this study immunophenotyping for aberrant lymphoid antigens CD7, CD19 were investigated in those AML patients by using four –color Cyflow® Cube 6 flow cytometry device (PartecCyflow®, Germany) in AL Rawabi private laboratory.

2.5.1 Reagents and Assay Procedure

Cy Lyse® stands for an erythrocyte lysing reagent kit with a complete preservation of the surface proteins and particularly no loss of cells.

2.5.2 Determination of the Aberrant Phenotype

Identification of blast cells was performed using forward scatter (FSC) versus side scatter (SSC) parameters. Antigen expression was considered to be positive when the percentage of positive blast cells was equal or greater than 20%. Similarly, aberrant phenotypes were defined when at least 20% of the blast cells expressed that particular phenotype [12]. (**Figures1-3**)

2.6 Statistical Analysis

Statistical analysis was carried out using SPSS version 18. Categorical variables were presented as frequencies and percentages. Continuous variables were presented as means with their 95% confidence interval (CI).

Independent (t-test) was used to find mean differences between two variables. One way analysis of variance (ANOVA) was used to find the mean differences among more than two variables. Pearson's chi square (X2) test was used to find the association between dependent and independent variables. A p-value of < 0.05 was considered as statistically significant.

Results

This study included 25 adult patients with de Novo acute myeloid leukemia diagnosed cytomorphologically by Leishmen stain and cytochemichally by Sudan black B and PAS stain on peripheral blood (PB) and bone marrow (BM) aspirate smears.

Immunophenotyping was done by Flowcytometry to detect the aberrant expression of CD7 and CD19 in adult acute myeloid leukaemia patients.

3.1. Clinical parameters

3.1.1 Age Groups

The mean age of all patients included in this study was 37± 18.07* SD, with a median of 33 years old and a range of 15 to 70 years old. Figure 4 Showed that more than half of AML patients (52%) were in the age group of 21-40 years.

3.1.2 Gender

Regarding the gender of patients; AML was observed more in male (14 male (56%)) than female (11 female (44%)) with a male to female ratio of 1.3:1.

Regarding the relation of gender to the age groups; the per cent of male patients in the most common age group (21-40 years) (57.1%) was more than the percent of female (45.4%) **table 1**.

3.1.3 Clinical Features

Clinical features of all AML patients included in the study were shown in Figure 2 which revealed that the two most frequent signs and symptoms for all patients included in this study were easy fatigability and fever, followed by hepatosplenomegaly whereas lymphadenopathy and nausea with vomiting were the least frequent.

3.1.4 Distribution of AML Subtypes according to FAB Classification

In current study 7 out of 25 patients were of M1 sub type, 5 patients were of M2 subtype, 3 patients were of M5, M3 and M0, and 2 patients were of M4 and 1 patient for each M6, M7. Most patients with aberrant antigens expression were of M1 FAB classification. Figure 6

3.2. Aberrant Antigen Expression

The AML patients in this study were divided into two groups according to the presence or absence of aberrant expression of CD7 and CD19 marker by Flowcytometry.

The first group composed of 11 patients who present with aberrant expression. The second group composed of 14 patients with negative expression.

Figure 6 showed that the CD7 was the most predominant aberrant marker, 7 out of 11 patients (63.6%) had only CD7 marker and 3 out of 11 patients (27.3%) had CD7 with CD19; Thus 10 out of 11 patients (90.9%) expressed CD7.CD19 was expressed as a sole marker in one out of 11patients (9%) and 3 patients having CD19 with CD7.Thus the total expression of CD19 was in 4 cases

3.2.1Relationship between the aberrant antigens expression and the Clinical parameters.

Age

The first group compose of 11 patients who present with aberrant antigen expression had mean age of 33.45 ±17.16 years (mean ± SD), seven of them who had CD7 had mean age of 35.85 ±12.18 years (mean ± SD). The age of one patient with CD19 was 67 years and the three patients with both CD7 and CD19 had mean age of 16.66 ± 4.72 years (mean ± SD).

The second group composed of 14 patients who had no aberrant antigen expression and their mean age of 40.78 ± 18.72 years (mean ± SD). **Table 2** showed that there was a significant association between age and aberrant CD7, CD19 expression (P value < 0.05).

Gender

Table 3 showed that there was no significant association between gender and aberrant CD7 and CD19 expression (P value > 0.05). However 6 out of 7 patients with CD7 + were male.

Hepatosplenomegaly

Table 4 showed that there was no significant association between hepatosplenomegaly and aberrant CD7 and CD19 expression (P value > 0.05)

3.2.2. Relationship between the aberrant antigens expression and the haematological parameters.

Table 5 showed that the mean WBC count and the malignant cells percent of patients without aberrant expression were significantly higher than those of patients with aberrant expression (p value < 0.05).

Table 3.6 showed that there was a significant relationship between mean of WBC count and the

type of aberrant marker expression, so that the lowest mean WBC count was detected in patients with CD 7+, whereas the highest WBC count was detected in those harbouring both markers, however there was no significant relationship with other haematological parameters i.e. malignant cell percent, RBC, HB and platelet count .Table 3.6.

3.2.3 Distribution of Aberrant expression of CD7 and CD19 markers according to FAB classification

By relating the FAB classification to the expression of aberrant markers 4 out of 10 patients of CD7+ were M2, 3 out of 10 were M1, 2 out of 10 were M5 and 1 out of 10 was M3. The 4 patients with CD19+ distributed equally on M0, M1, M2 and M5 subtypes. The 3 patients harbouring CD7 and CD19 present in M1, M2 and M5. Table 7

3.2.4. The Distribution of Aberrant Groups in Relation to FAB Classification

CD7 was detected in the 4 patients with M2 subtype; 3 of them showed CD7 alone and one patient showed CD7 with CD19. CD7 was detected in 3 patients with M1 subtype; 2 of them showed CD7 alone and one showed CD7 with CD19. In M5 sub type two patients had CD7 and one of them had CD19 also. Only one patient with M0 subtype showed CD19 expression.

3.2.6. The relation of expression of aberrant marker to AML induction therapy response.

Complete remission after medical therapy was achieved in 19 patients (76%) with standard chemotherapy. Six patients had no response to standard therapeutic regimen. Figure 3.5 showed that 3 out of 11 with the aberrant expression did not respond to treatment (two patients with CD7 had passed early in study during receiving induction therapy and they were included with the patients who have no response) and 3 out of 14 patients with no aberrant expression have no response; there was no significant relationship between response and not response to treatment and the expression of aberrant Antigen.

Figure 6 Show the distribution of aberrant antigens expression according to responsive and non-responsive to treatment after 3 weeks at the end of induction chemotherapy. 8 patients with aberrant antigen had responded to treatment induction therapy. 5 of them had CD7, one patient had CD19 and 2 patients had both CD7 and CD19.

Three patients who had aberrant expression had fail to respond to induction therapy; of them 2 patients had CD7 and one patient had both CD7 and CD19. There was no statistically significant association between aberrant antigen expression and treatment response to one cycle of chemotherapy (P value>0.05).

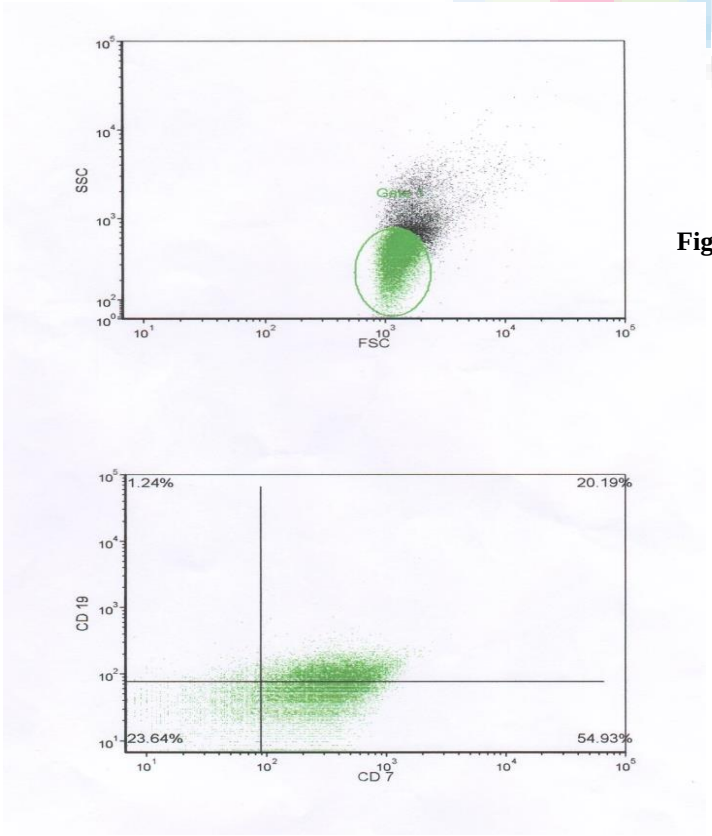

Figure 1: Showed patient with aberrant co-expression of CD7and CD19. The FSC vs SSC plot was used to gate on the blast cell population. This is the older technique to gate on blast cells. The newer technique is by using CD45 vs SSC. However, using the older technique of FSC vs SSC is still useful especially in patients with high blast cell percentage.

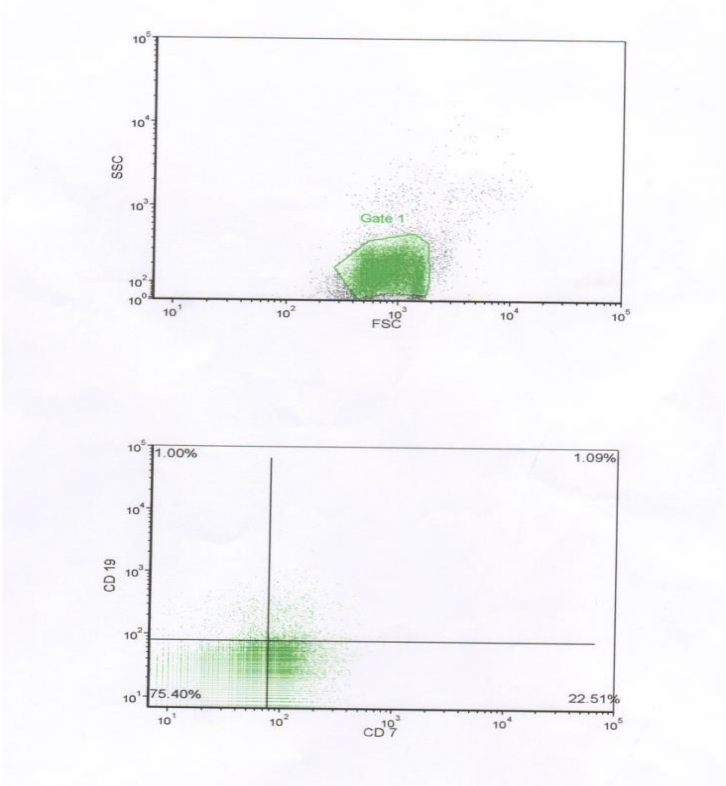

Figure 2 Showed patient with aberrant expression of CD7

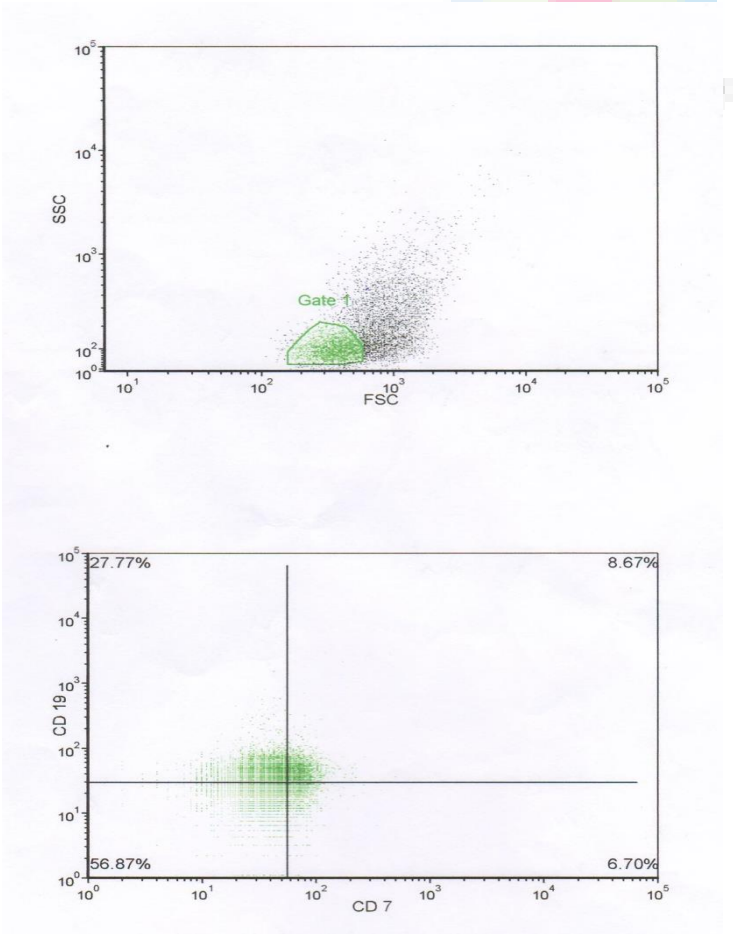

Figure 3 Showed patient with aberrant expression of CD19

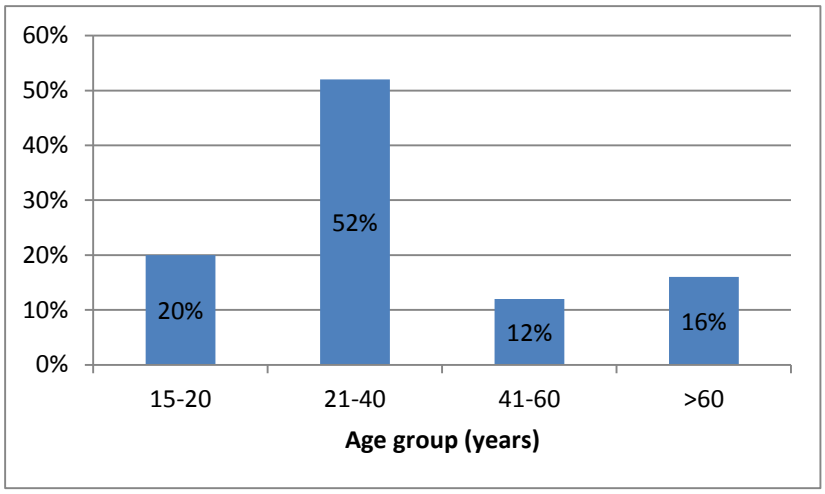

Figure 4 Distribution of the patients according to the age groups.

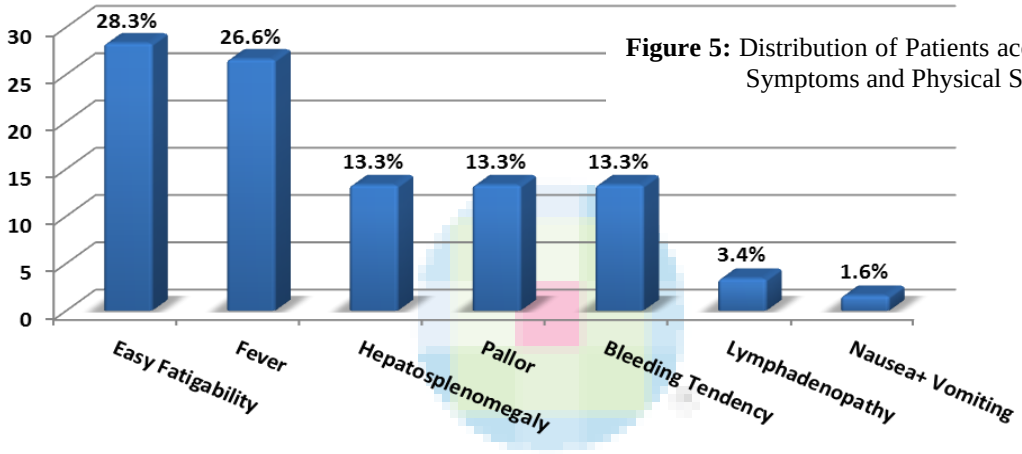

Figure 5: Distribution of Patients according to Symptoms and Physical Signs.

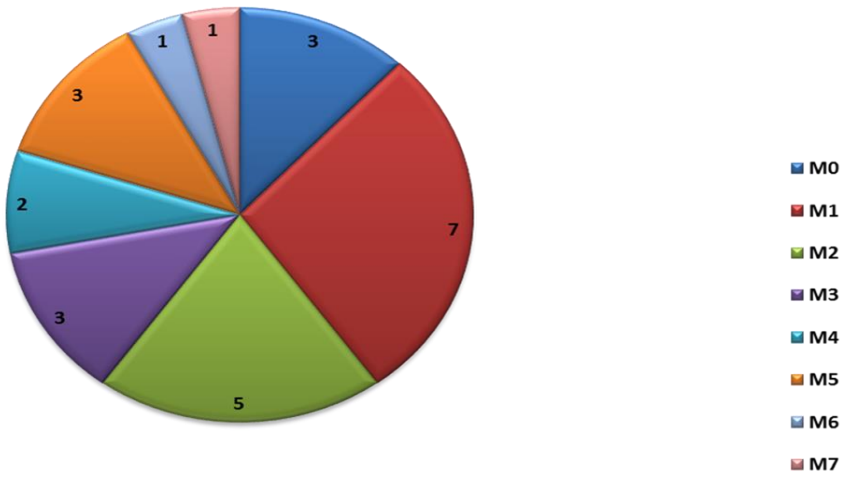

Figure 6: Distribution of patients according to FAB classification.

Note: all numerical values in this figure mean number of patients.

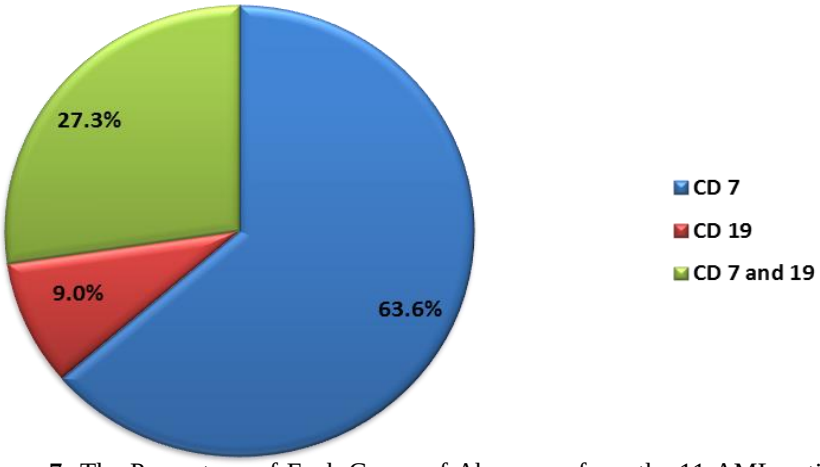

Figure 7: The Percentage of Each Group of Aberrancy from the 11 AML patients having aberrant antigen expression.
Note: all numerical values in figure 3.4 mean percent of patients.

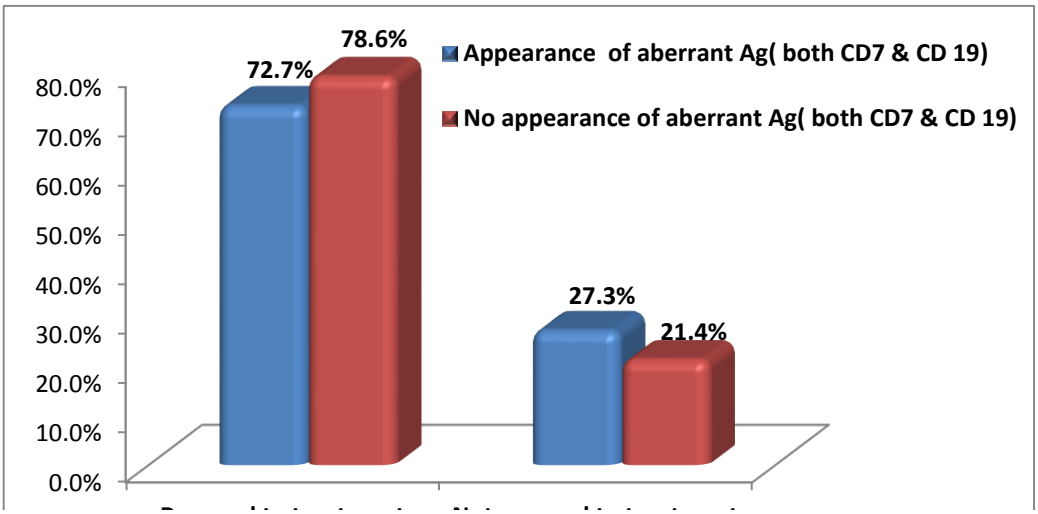

Figure 8: Distribution of aberrant antigens expression of CD7 and CD19 in relation to response and non-response to treatment after 3 weeks of end of induction chemotherapy.

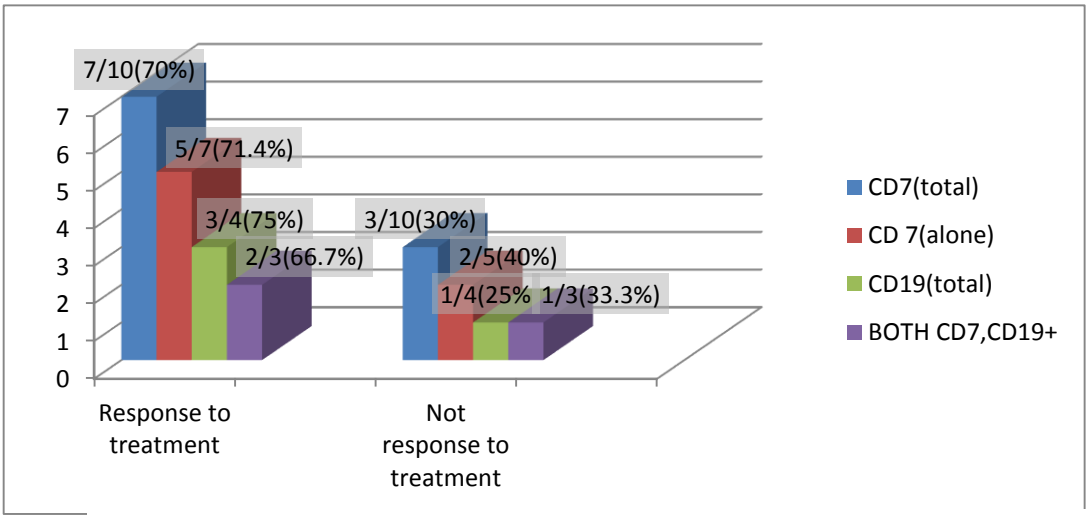

Figure 9: The Distribution of type Aberrant Expression Antigens in Relation to the Responsive and Non Responsive Patients.

Table 1: The Frequency of Gender in Relation to Age Group

Age groups	Gender		Total
	Male	Female	
15- 20 years	1 (7.1%)	4 (36.4%)	5 (20.0%)
21-40 years	8 (57.1%)	5 (45.5%)	13(52.0%)
41-60 years	2 (14.3%)	1 (9.1%)	3 (12.0%)
> 60 years	3 (21.4%)	1 (9.1%)	4 (16.0%)
Total	**14 (100.0%)**	**11 (100.0%)**	**25 (100.0%)**

Table 2: Relationship between age and aberrant antigen expression

Variable	CD marker	No.	Median (year)	Mean (year)	S.D	F test	P value
Age	CD7	7	32	35.85	12.18	8.599	**0.01***
	CD 19	1	67	67.00	------		
	CD 7, 19	3	15	16.66	4.72		
	Total of positive	11	27	33.45	17.16	2.13	0.34
	Not aberrant Ag	14	40	40.78	18.72		

Table 3: Correlation between gender and aberrant antigen expression

Gender		CD EXPRESSION			Total	Not aberrant
		CD 7	CD 19	CD 7, 19		
male	Count	6	1	1	8	6
	% of Total	54.5%	9.1%	9.1%	72.7%	42.9%
female	Count	1	0	2	3	8
	% of Total	9.1%	.0%	18.2%	27.3%	57.1%
Total	Count	7	1	3	11	14
	% of Total	63.6%	9.1%	27.3%	100.0%	100%

Table 4 Correlation between Hepatosplenomegaly and Aberrant Antigen Expression

HEPATOSPLENOMEGALY		CD expression			Total	Not aberrant Ag
		CD 7	CD 19	CD 7, 19		
Splenomegaly	Count	2	1	0	3	3
	% of Total	18.2%	9.1%	.0%	27.3%	21.4%
Hepatomegaly	Count	5	0	3	8	4
	% of Total	45.5%	.0%	27.3%	72.7%	28.6%
No hepatosplenomegaly	Count	0	0	0	0	7
	% of Total	0%	0%	0%	0%	50%
Total	Count	7	1	3	11	14
	% of Total	63.6%	9.1%	27.3%	100.0%	

Table 5 the Relation of Haematological Parameters to the Aberrant Expression

Variable	CD	N	Mean	S.D	t-test	P value
RBC×10^{12}	Negative	14	3.32	0.99	0.201	0.843
	Positive	11	3.40	0.82		
Hb g/dl	Negative	14	8.06	2.61	0.408	0.687
	Positive	11	7.68	1.87		
WBC×10^{9}	Negative	14	31.9	11.2	6.68	**0.002***
	Positive	11	7.8	4.6		
Malignant cells%	Negative	14	67.4	8.4	10.7	**0.001***
	Positive	11	34.2	6.7		
Platelet×10^{9}	Negative	14	67.98	33.48	0.135	0.894
	Positive	11	66.37	23.27		

Table 6 The Relation of The Three Groups of Aberrancy to Haematological Parameters (at presentation)**.**

Variable	CD marker	No.	Mean	S.D	F test (ANOVA)	P value
Hb g/dl	CD7	7	8.13	2.14	0.508	0.620
	CD 19	1	7.00	-----		
	CD 7, 19	3	6.85	1.34		
**WBC count×10^{9}	CD7 total	10	8.48	3.1	14.86	0.01*
	CD7 alone	7	6.8	3.9		
	CD19 total	4	9.6	1.3		
	CD19 alone	1	1.3	-----		
	BothCD7, 19+	3	12.4	1.4		
Malignant cells%	CD7 total	10	34.50	7	5.5	0.989
	CD7 alone	7	34.57	8		
	CD19 total	4	33.50	6		
	CD19alone	1	31	------		
	Both CD 7, 19	3	34.33	7		
Platelet count×10^{9}/l	CD7	7	61.87	11.66	1.106	0.377
	CD 19	1	50.00	------		
	CD 7, 19	3	82.33	41.40		

** There was a significant relation between the 3 parameters (CD7 alone, CD19 alone and both CD7, CD 19) with mean of WBC count.

Table 7 The Incidence of aberrant expression of markers according to FAB classification.

FAB Category	N. of AML Patients	CD7		CD19		Both CD7 andCD19	
		No.	Incidence %	N	Incidence %	N	Incidence %
M_0	3	-	0	1	33.3	-	0
M_1	7	3	42.8	1	14.2	1	14.2
M_2	5	4	80	1	20	1	20
M_3	3	1	33.3	-	0	-	0
M_4	2	-	0	-	0	-	0
M_5	3	2	66.6	1	33.3	1	33.3
M_6	1	-	0	-	0	-	0
M_7	1	-	0	-	0	-	0
Total	25	10	40	4	16	3	12

Table 8 Incidence of CD7 in various studies

Study	Incidence of CD7 Positivity
El-Sissy et al (Saudi study)	11.8%
Bahia et al (Brazilian study)	25.7%
Kita et al (Japanese study	19%
Julius et al (USA study)	32.6%
Chang et al (Canadian study)	37%
Khurram et al (Pakistani study)	37.03%
Auewarakul et al (Thailand study)	27%

Table 9 Incidence of CD19 in various studies.

Study	Incidence of CD19 Positivity
El-Sissy et al (Saudi study)	11.8%
Bahia et al (Brazilian study)	8.6%
Chang et al , 2004 (Canadian study)	5%
Khurram et al (Pakistani study)	11.1%
Auewarakul et al (Thailand study)	4%

Discussion

4.1. Age and Gender:

The mean age of all patients included in this study was 37 ± 18.07, with a median of 33 year old and range of 15-70 years, which was in agreement with Iraqi studies [13-16], Iranian study [17] and Saudi study [18].

In this study 56% of the adult AML patients were male with a male to female ratio1.3 :1 which was in accordance with that reported by the Iraqi ministry of health [19] and other Iraqi studies [16, 18,20] as well as other studies worldwide [17,21,22] .

4.2. Clinical Presentation:

Easy fatigability and fever were the most common presenting symptoms in adult AML patients; 28.3% and 26.6% respectively. The least symptoms were nausea and vomiting, 1.6% for both. Whereas, the most common physical findings were hepatosplenomegaly, pallor and bleeding tendency with frequency of 13.3% for all. The least finding was lymphadenopathy, 3.4%. These results were in agreement with different Iraqi studies [56, 58, 62, and 63] and were similar to the data reported abroad [23].

4.3. Aberrant CD7 and CD19 markers expression:

In this study aberrant expression of lymphoid associated antigens CD7 and CD19 were studied for their biological and clinical significance with comparison to previous studies.

Some studies have shown significant correlation between clinical and laboratory characteristics and aberrant lymphoid antigen expression such as CD7, CD19 and CD56. Most of these studies concerned with the prognostic significance of aberrant CD7 expression.

4.3.1Association with Age, Gender and Hepatosplenomegaly:

Regarding the age, patients with $CD7^+$ and $CD19^+$ AML were younger (mean age 33.45±17.16 SD) than those without aberrant expression (mean age 40.78±18.72 SD). This observation was similar to the studies of Chang et al, [24], Kita et al [25] and Khurram et al [26] but inconsistent with the studies of Saxana et al [27] and Del Poeta et al [28] in which no restricted age distribution was found.

Regarding gender, CD7 was detected mostly in male; however this relation did not reach the level of significance; which was in agreement with Saxana et al [27] and Chang et al [24] and Khurram et al [26] study, but inconsistent with the study of Kita et al [25].

Regarding hepatosplenomegaly, 7 out of 14 patients without aberrant antigens expression showed hepatosplenomegaly. While all the patients with aberrant antigens expression showed hepatosplenomegaly which was in agreement with Saxsana et al al [27] and Chang et al [24] study, but inconsistent with the study of Kita et al[25].

4.3.2Association with Hematological Parameters:

In the current study, the percent of blast cells and WBC count in patients harboring aberrant markers were significantly lower than those without aberrant expression, and the lowest WBC count was detected in those having CD7 and higher count in those having both CD7 and CD19. Most of the studies concerning CD7 and CD19 expression in AML showed significant high WBCs and blast count [13-16]. This disagreement in those results may be due to small sample size and environmental factors.

No significant difference in RBC, HB and platelet count in AML cases with or without CD7 and CD19 aberrant markers expression (**table 5**).

According to the FAB classification 7/25 (28%) of AML patients were of M1 subtype followed by M2 subtype 5/25 (20%) and the least frequent were detected in M6 and M7 (one patient for each) (**figure 8**).

Moreover all M2 subtype 5/5 (100%) and 4/7(57%) M1subtype had CD7 expression; this was explained byEl-Sissy et al [29], Saxana et al [27] ,Kita et al [25] and Juluis et al [30] who proposed that CD7 expression associated with early FAB AML subtypes because CD7 is expressed on early hematopoietic progenitors as the earliest surface antigen of T-cells and on malignant precursor T-cells [4, 31, 32].

In this study, 40% of AML cases expressed CD7; this frequency is comparable to other studies (**Table 8**)

Relation to Induction Therapy

The majority of previous studies showed a significant association of the aberrant CD7 antigen with low remission rate and biological aggressiveness in a significant proportion of AML cases. In this study we follow the AML patients after 2 weeks of completion of the standard induction chemotherapy. Detection of complete remission was assessed by morphological examination of the BM. 3/10(30%) patients harboring CD7+ show no response to induction therapy and two of them died during the induction therapy. This observation was similar to Sexana et al [27], Kita et al [25], Del Poeta et al [38] and Julius et al [30] studies which showed that complete remission rate was significantly lower in CD7+ cases portend poor prognosis. Del Poeta also showed that the overall survival and disease free survival rate of CD7+ AML were lower than those who were CD7-negative cases [28]. Similarly Chang et al [33] emphasized that the Patients expressing CD7 had significant shorter disease free survival (DFS) and post-remission survival (PRS) than patients without CD7.

The poor prognosis referred to CD7 was further emphasized by its presence of poor prognostic marker FLT3-ITD [34]. Rausei-Mills et al [34], stated that there was close association between aberrant CD7 expression and FLT3/ITD mutation in the myoblasts of FLT3/ITD+ AML, thus he suggested that FLT3/ITD- mediated leukemic transformation occurs in the more early stage of myeloid progenitor cells.Ogata et al. [35], stated that the proportion of CD7+ cases increased stepwise from the cases with favorable cytogenetic to the cases with intermediate and unfavorable cytogenetic, and CD7-positivity adversely affected the survival only in cases with unfavorable cytogenetic, therefore they recommended that CD7 expression in AML should be interpreted in association with the cytogenetic.

CD19

CD19 is a transmembrane glycoprotein of the Ig superfamily expressed by B cells from the time of heavy chain rearrangement until plasma cell differentiation [32, 36].

In this study, 16% of AML cases expressed CD19, a frequency higher than that reported in previous studies (**Table 9**).

Table 4.2 revealed that the incidence of CD19+ in AML was comparable to that of the Saudi study, El.Sissy et al [29] and Pakistani study Khurram et al [26] which may be due to racial and environmental factors.

Two of four cases of CD19 were detected in M1 and M2, however no study had revealed CD19+ to early FAB classification, large sample may clarify this argument.

Bain had concluded that CD19 expression is associated with AML that either associated with t (8:21) (good prognosis) [16, 40] or with AML associated with t (9:22) (poor prognosis) [37].

Thus the impact of CD19 expression on prognosis in AML should be further investigated and explored and we may propose that AML patients with CD19+ should be send for cytogenetic study to assess their prognosis.

CD7 and CD19 co-expression

In this study the co-expression of both CD7 and CD19 was detected in 3 out of 25 patients with AML. Khurram et al [30] had found that the paired aberrancy of CD7 and CD19 was found in 1 out of 27 cases of AML which was near to the result of this study.

Conclusion

1. The incidences of aberrant expression of CD7, CD19 and both CD7 andCD19 in 25 AML patients were 40%, 16%, and12% respectively.
2. CD7 expression was detected mainly in males whereas the expression of CD19 was distributed among males and females.
3. Total WBC count and blast cell percent were lower in patients harbouring aberrant expression in comparison to those without aberrant expression.
4. CD7 was detected mainly in early FAB classification (M1 and M2).
5. Six out of twenty five AML patients had no response to standard therapeutic regimen; three of them were harbouring CD7 and no one had CD19 alone, thus we may propose that CD7 is associated with poor response to induction therapy.

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