Results

Results

Demographic, clinical and laboratory data of AML patients and the results of the present study are presented in Tables (12-27) and Figures (26-35).

A-Demographic Features of Patients:

40 patients were enrolled in this study divided into 2 groups; the 1st one included 30 AML patients and the 2nd one included 10 patients with other blood diseases (e.g. Hypersplenism, Idiopathic thrombocytopenic purpura), their ages ranged from 17 to 66 years, with a mean age of 34.3±15.2 years. They were 15 males and 15 females with a male to female ratio of 1:1 (Table 12), (Figure 26).

Table (12): Demographic data of the studied groups:

	Group I (De Novo) (N=30)		Group II (Control) (N=10)		Test of Significance	P
Age (Means) (17-66)	34.3±15.2		37±10.5		St. "t" 2.2	>0.05
Gender	No	%	No	%	-	-
Male	15	50.0	5	50.0		
Female	15	50.0	5	50.0		

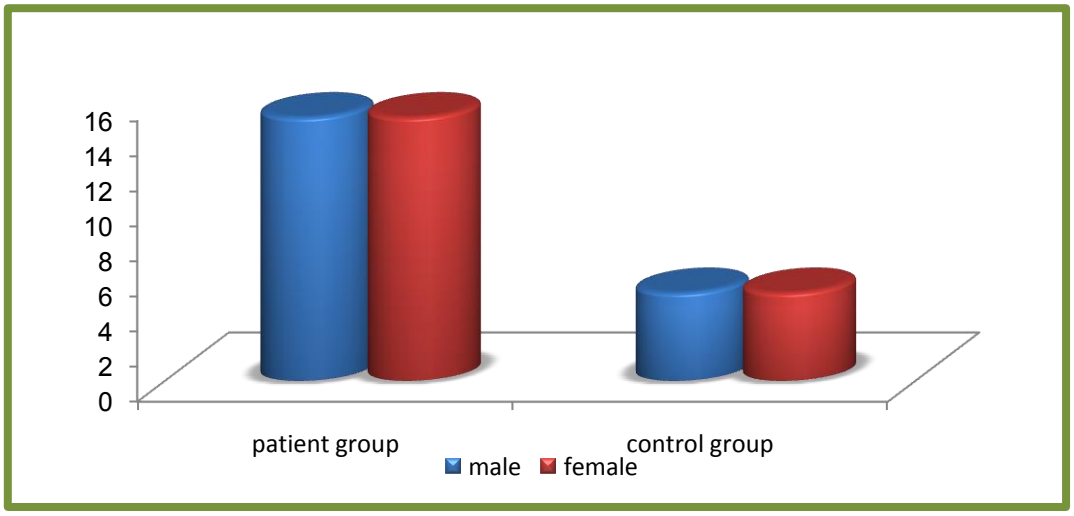

Figure (26): The distribution between genders

B- Clinical and Laboratory Features of Patients:

(1) Clinical features:

(Table 13) and (Figure 27) show that out of the 30 patients, 13 (43.3%) patients presented with pallor, 19 (63.3%) with fever, 8 (26.7%) had bleeding tendency and 13 (43.3%) complained of severe bone aches. On examination, hepatosplenomegaly was observed in 23 (76.7%) of patients, while 8 (26.7%) had marked splenomegaly, lymphadenopathy was detected in 5 (16.7%) patients, CNS manifestations found in 2 (6.7%) and mediastinal mass in 2 (6.7%).

Table (13): Clinical data of the patients groups:

Clinical data	De novo AML group			
	+ ve		- ve	
	No	%	No	%
Pallor	13	43.3	17	56.7
Fever	19	63.3	11	36.7
Bleeding tendency	8	26.7	22	73.3
Lymph nodes	5	16.7	25	83.3
Hepatosplenomegaly	23	76.7	7	23.3
CNS manifestation	2	6.7	28	93.3
Mediastinal mass	2	6.7	28	93.3
Bone pain	13	43.3	17	56.7

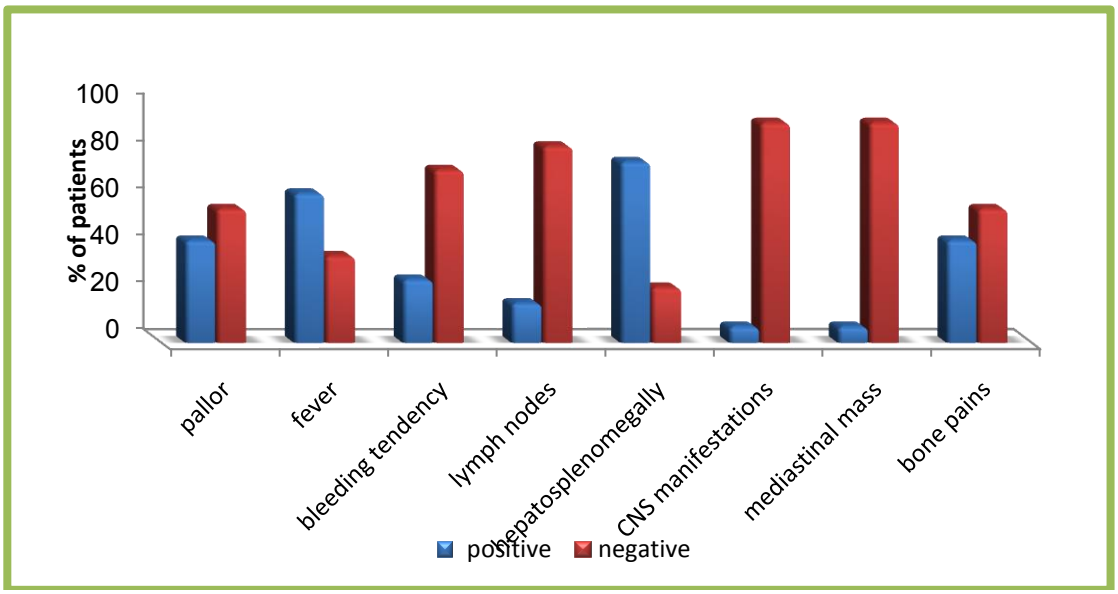

Figure (27): The percentage of the clinical data

(2) Hematological data:

The Hb level in (Table 14) and (Figure 28) ranged from 4.6 to 11.2 g/dl (mean 8.3±1.6, median 8.7), WBC count from 1.1 to 272 x10^9/L (mean 71.9±80.6, median 36.6) (Figure 29), platelets count from 18 to 711 x10^9/L (mean 101.9±103.7, median 72) and LDH from 342 to 4922mg/dl (mean1414.4±1064.9, median 1000). The range of blasts in PB was from 2 to 92% (mean 45.1±27.8, median 42), while that of blasts in BM was from 22 to 95% (mean 61.1±20.8).

Table (14): Laboratory data findings of the studied groups:

Laboratory data	Range	Group I (De Novo) (N=30)	Group II (Control) (N=10)	Test of significance	p
TLC (×10^9/L)	1.1-272 x10^9/L	71.9±80.6	7.1±1.9	MWU=51	<0.01*
Hb (g/dl)	4.6-11.2 g/dl	8.3±1.6	12.5±1.1	St. "t" =7.6	<0.001**
Platelets (×10^9/L)	18-711 x10^9/L	101.9±103.7	199.2±53.1	MWU=35	<0.001**
LDH (mg/dl)	342-4922mg/dl	1414.4±1064.9	-	-	-
% of Blasts in PB	2-92%	45.1±27.8	-	-	-
% of Blasts in BM	22-95%	61.1±20.8	-	-	-

*: significant, **: highly significant, ***: highly significant

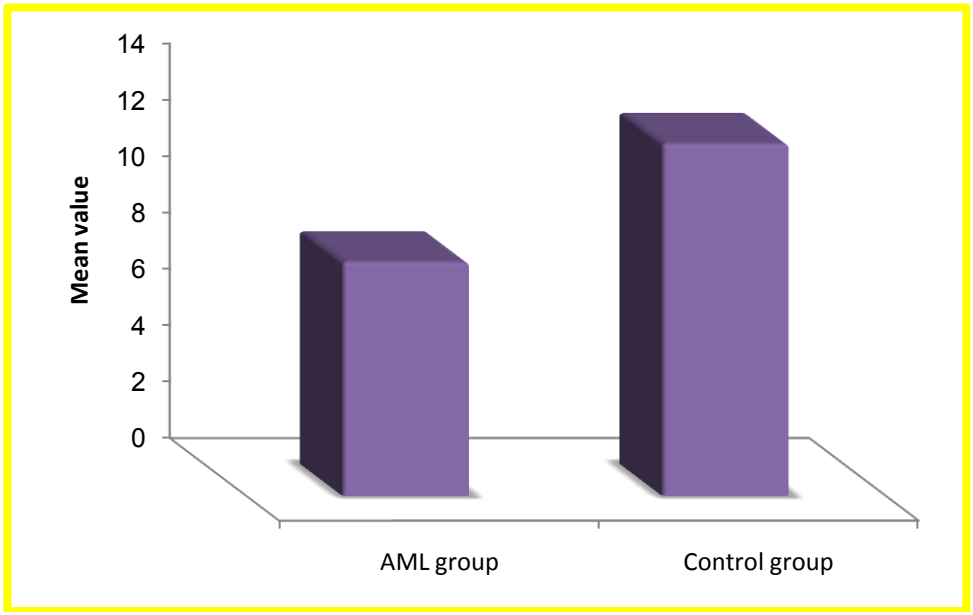

Figure (28): Hb% among AML studied patients and control group

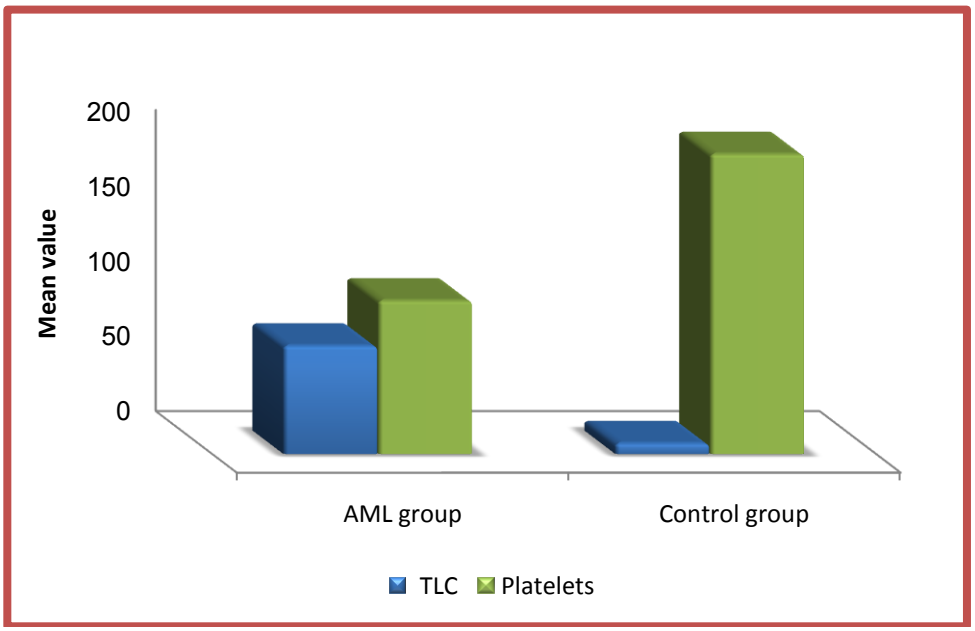

Figure (29): TLC and PLT among AML studied patients

(3) Cytochemical and immunophenotypic features:

Cytochemical reaction for myeloperoxidase (MPO) activity was positive in 29 (97%) cases.

Immunophenotypically, blasts of 25 (83.3%) were positive for CD33 expression, 20 (66.7%) for CD34 expression, 29 (97%) for intracellular MPO staining. Besides, 27 (73.3%) patients were positive for CD13 expression, while 21 (70%) patients were positive for HLA-DR expression. 16 (53.3%) patients were positive for CD64 expression and 4 (13.3%) patients were positive for CD14 expression.

Aberrant expression of each of the lymphoid markers CD20 and CD5 was detected in only one case, while that of CD7 was found in 14 (46.7%) patients, CD22 in 9 (30%), CD10, CD19 and CD79a in 3 (10%). TDT is positive in 5 (16.7%) cases. None of the patients were positive for CD3. (Table 15) shows the range, mean and median of expression of each of the previous markers.

Table (15): Frequency of monoclonal antibodies in AML group:

		No	%	means(%)	t	P
CD34	**+ve**	20	66.7	37.2±17.5	6.2	<0.001**
	-ve	10	33.3	2.6±2.3		
CD13	**+ve**	22	73.3	43.0±15.1	5.8	<0.001**
	-ve	8	26.7	10.6±6.8		
CD33	**+ve**	25	83.3	45.2±15.2	4.4	<0.001**
	-ve	5	16.7	20.6±5.1		
HLA-DR	**+ve**	21	70.0	45.7±16.3	6.3	<0.001**
	-ve	9	30.0	10.1±7.1		
CD22	**+ve**	21	30.0	36.8±20.8	6.45	<0.001**
	-ve	9	70.0	6.9±4.0		
CD10	**+ve**	3	10.0	56.9±22.5	12.8	<0.001**
	-ve	27	90.0	3.3±3.4		
CD7	**+ve**	14	46.7	38.2±16.2	7.0	<0.001**
	-ve	16	53.3	8.5±4.7		
CD5	**+ve**	1	3.0	27.9±0.0	7.9	<0.001**
	-ve	29	97.0	3.2±3.1		
CD3	**+ve**	0	0.0	--	--	--
	-ve	30	100.0	2.6±2.7		
CD79a	**+ve**	3	10.0	55.0±30.5	8.5	<0.001**
	-ve	27	90.0	7.1±4.5		
CD20	**+ve**	1	3.0	50.0±0.0	13.4	<0.001**
	-ve	29	97.0	5.1±3.3		
MPO	**+ve**	29	97.0	34.2±17.1	1.8	>0.05
	-ve	1	3.0	3.5±0.0		
TDT	**+ve**	5	16.7	42.62±6.8	7.7	<0.001**
	-ve	25	83.3	2.8±2.9		
CD19	**+ve**	3	10.0	51.7±21.3	11.9	<0.001**
	-ve	27	90.0	3.7±3.5		
CD64	**+ve**	16	53.3	44.4±15.4	7.8	<0.001**
	-ve	14	46.7	10.5±5.7		
CD14	**+ve**	4	13.3	27.9±5.9	11.7	<0.001**
	-ve	26	86.7	3.1±2.6		
CD133	**+ve**	17	56.7	28.4±14.4	6.3	<0.001**
	-ve	13	43.3	2.8±2.3		

**: High significant

(4) FAB classification percentage:

According to morphology, cytochemistry and immunophenotyping, patients were classified into 3 (10%) cases FAB-M_1, 8 (26.7%) cases FAB-M_2, 3 (10%) cases FAB-M_3, 11 (33.3%) cases FAB-M_4, 3 (10%) cases FAB-M_5 and 2 (6.7%) cases BAL (Table 16 and Figure 30).

Table (16): Frequency of FAB and immunophenotyping subtypes in patients group:

	AML group	
	n = 30	**(%)**
M_1	3	10.0
M_2	8	26.7
M_3	3	10.0
M_4	11	36.7
M_5	3	10.0
BAL	2	6.7

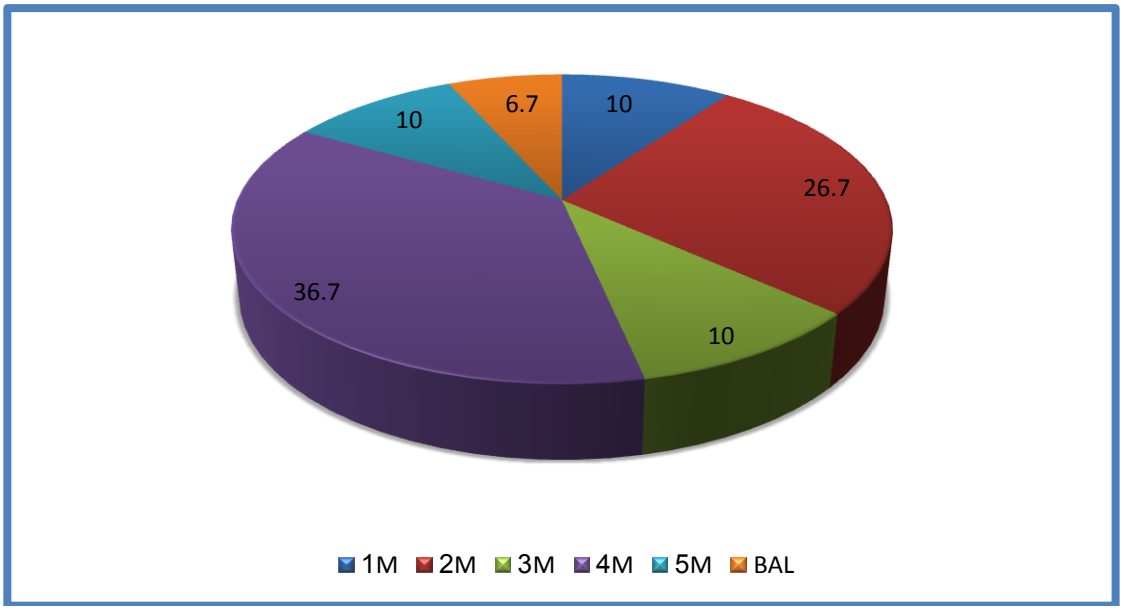

Figure (30): The pie diagram showing FAB distribution among the studied group

(4) Clinical outcome:

Nineteen (63.3%) patients showed good response to chemotherapy and achieved complete remission till the end of follow up period (12 months). Six (20%) patients died. Five (16.7%) patients developed resistance to chemotherapy (Tables 17 and Figure 31).

Table (17): Frequency of outcome status among AML group:

Outcome	No	%
Complete remission	19	63.3
Resistant	5	16.7
Died	6	20.0
Total	30	100

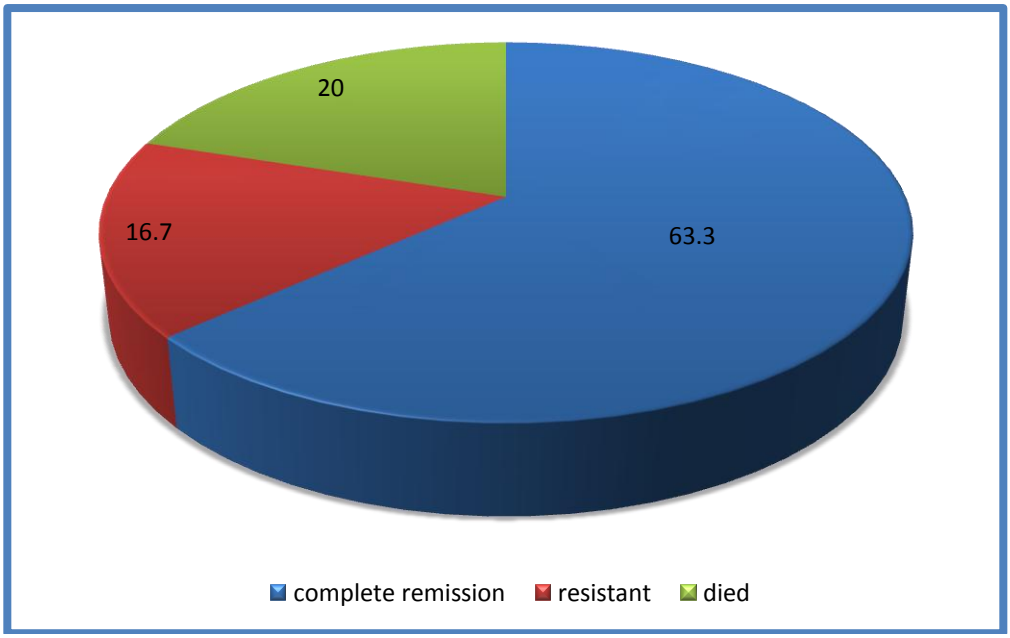

Figure (31): Pie diagram showing the percentage of outcome among patients group

C- Association between CD133 Expression and Various Demographic, Clinical and Laboratory Features of Patients:

1. CD133 expression:

(Table 18) shows that the frequency of expression of CD133 in was 17 (56.7%) cases positive (mean 28.4±14.4) and 13 (43.3%) cases negative (mean 2.8±2.3) in group I (the patient group) and the CD133 expression in group Π (the control group) was negative in all cases. There were significant differences between the expressions of CD133 in the control group when compared with the AML group (Figure 32).

Table (18): Percentages of CD133 expression in the studied groups:

Clinical data	Group I (N=30)			Group Π (N=10)		Test of significance	P
	Range	No	%	No	%		
Positive cases	28.4±14.4	17	56.7	0	0.0	Corrected (X^2)	<0.01*
Negative cases	2.8±2.3	13	43.3	10	100.0		

*: Significant

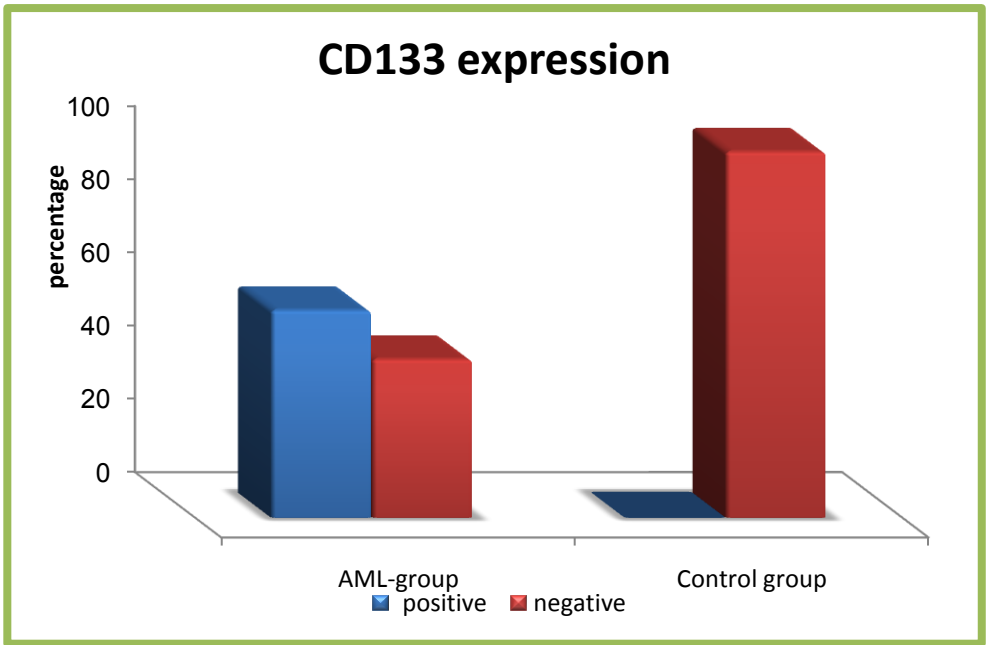

Figure (32): Percentages of CD133 expression among the studied groups

2. CD133 expression and some demographic data of patients:

No significant association was elicited in (Table 19) between CD133 positive expression and any of the studied demographic or clinical parameters of patients ($p>0.05$).

Table (19): Distribution of percentage of CD133 expression and some demographic data in the AML patients:

		N=30	CD133				X^2	p
			+ve		-ve			
			No	%	No	%		
Age	adult	30	17	56.6	13	43.4	Corrected (X^2)=0.001	>0.05
Gender	Male	15	8	47.1	7	53.8	X^2=0.14	>0.05
	Female	15	9	52.9	6	46.2		

3. CD133 and some clinical data:

Concerning the clinical data (Table 20), there was a statistically significant positive association between CD133 positive expression and bone pain (p=0.05). In this study the hepatosplenomegally increased in patients with $CD133^+$ (17 cases from 23 +ve cases).

Table (20): Relation between CD133 percentage and some clinical data in the studied group:

Parameter		(n)	CD133				χ^2	p
		N=30	+ve		-ve			
			No	%	No	%		
Pallor	**+ve** **-ve**	13 17	7 10	41.2 58.8	6 7	46.2 53.8	0.07	>0.05
Fever	**+ve** **-ve**	19 11	9 8	52.9 47.1	10 3	76.9 23.1	corr.X^2 0.94	>0.05
Bleeding tendency	**+ve** **-ve**	8 22	5 12	29.4 70.6	3 10	23.1 76.9	corr.X^2 0.0	>0.05
Lymph nodes	**+ve** **-ve**	5 25	3 14	17.6 82.4	2 11	15.4 84.6	corr.X^2 0.0	>0.05
Hepatosplenomegaly	**+ve** **-ve**	23 7	15 2	88.2 11.8	8 5	61.5 38.5	corr.X^2 1.6	>0.05
CNS manifestation	**+ve** **-ve**	2 28	2 15	11.8 88.2	0 13	0.0 100.0	corr.X^2 0.3	>0.05
Mediastinal mass	**+ve** **-ve**	2 28	1 16	5.9 94.1	1 12	7.7 92.3	corr.X^2 0.0	>0.05
Bone Pain	**+ve** **-ve**	13 17	10 7	58.8 41.2	3 10	23.1 76.9	corr.X^2 3.8	=0.05*

*: Significant

4. CD133 and the laboratory data:

As shown in (Table 21) no significances detected between CD133 and TLC, PLT and Hb. Also no significance concerning the FAB classification M_1, M_2, M_3, M_4, M_5 and BAL but there is statistical significance in FAB M_3 and FAB M_4 ($p<0.05$).

Table (21): Relation between CD133 percentage and the laboratory data in the studied group:

	No=30	CD133 (%)				p
		-ve (n=13)		+ve (n=17)		
		No	%	No	%	
TLC^{X2}: >50×10^9/L	13	7	53.8	6	35.3	>0.05
<50×10^9/L	17	6	46.2	11	64.7	
Hb ^{X2}: >10g/dl	4	3	23.1	1	5.9	>0.05
<10g/dl	26	10	76.9	16	94.1	
PLT ^{X2}: ≤30×10^9/L	6	1	7.7	5	29.4	>0.05
>30×10^9/L	24	12	92.3	12	70.6	
FAB:						
M_1^{FET} Count	3	0		3		>0.05
% within CD133		0.0%		17.6%		
M_2^{FET} Count	8	5		3		>0.05
% within CD133		38.5%		17.6%		
M_3^{FET} Count	3	3		0		<0.05*
% within CD133		23.1%		0.0%		
M_4^{FET} Count	11	3		8		<0.05*
% within CD133		23.1%		47.1%		
M_5^{FET} Count	3	1		2		>0.05
% within CD133		7.7%		11.8%		
BAL^{FET} Count	2	1		1		>0.05
% within CD133		7.7%		5.9%		

*: Significant, X^2: Chi-square test, FET: Fisher exact test.

5. Correlation between CD133 expression and some prognostic factors in AML:

No statistically significant associations were elicited between CD133 expression and some of the studied standard prognostic factors concerning the age and other laboratory data ($p>0.05$) (Table 22). $CD133^+$ cells in this study are inversely proportional to PLT, Hb and TLC (Figures 33, 34 and 35).

Table (22): Correlation between the CD133 and some prognostic parameters in AML patients:

	CD133	
	r (spearman's)	**p**
Age (years)	-0.22	>0.05
TLC ($\times 10^9$/L)	-0.18	>0.05
Hb (g/dl)	-0.11	>0.05
LDH (mg/dl)	0.29	>0.05
Platelets ($\times 10^9$/L)	-0.31	>0.05
BM blasts (%)	0.3	>0.05

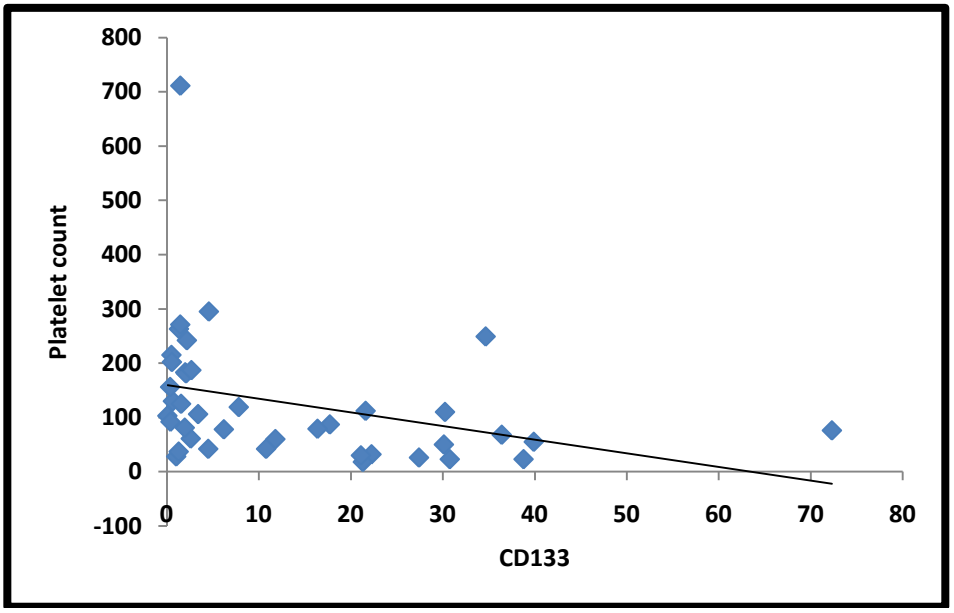

Figure (33): Correlation between CD133 expression and PLT count among AML patients

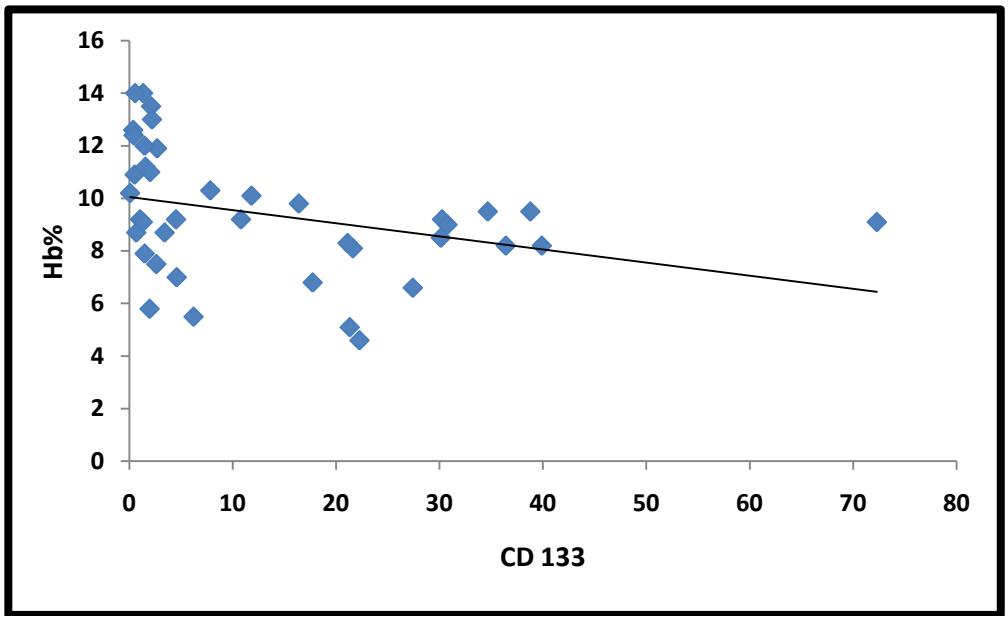

Figure (34): Correlation between CD133 and Hb% among AML patients

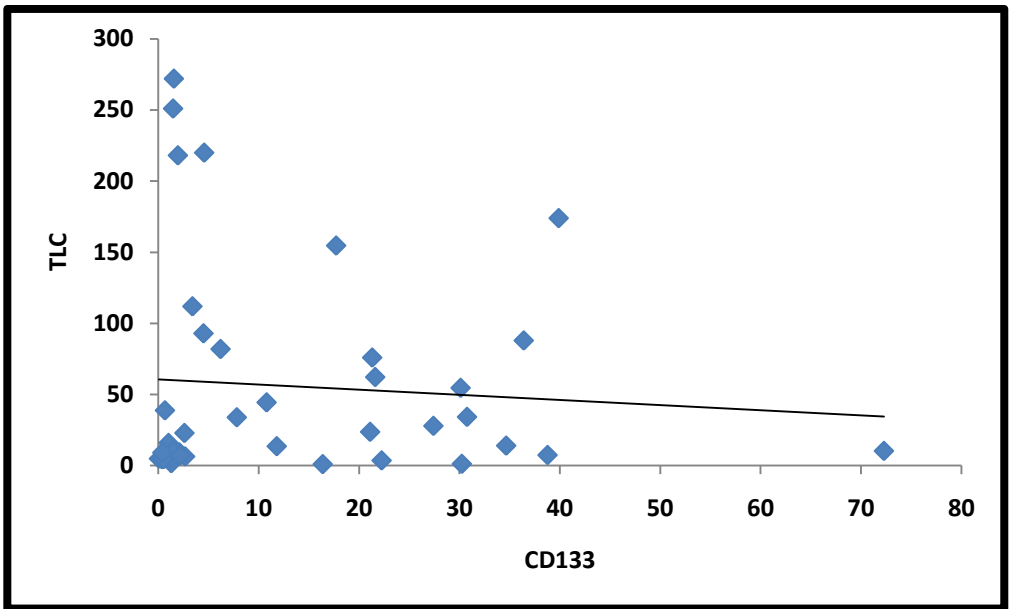

Figure (35): Correlation between CD133 and TLC among AML patients

6. Correlation between CD133 expression and monoclonal antibodies:

There are significant associations between CD133 and HLA-DR, CD3, CD7 and TDT ($p<0.05$), high significance for CD13 ($p<0.01$) (Figure 36) and very high significance for CD34 ($p<0.001$) (Figure 37 and Table 23).

Table (23): Correlation between the CD133 and monoclonal antibodies:

	CD133%		Significance
	r	p	
CD34 (%)	0.601	<0.001***	Highly Significant
CD13 (%)	0.47	<0.01**	Highly Significant
CD33 (%)	0.05	>0.05	NS
HLA-DR (%)	0.37	<0.05*	S
CD22 (%)	0.32	>0.05	NS
CD10 (%)	0.17	>0.05	NS
CD7 (%)	0.45	<0.05*	S
CD5 (%)	0.22	>0.05	NS
CD3 (%)	0.37	<0.05*	S
CD79a (%)	-0.19	>0.05	NS
CD20 (%)	0.03	>0.05	NS
MPO (%)	-0.02	>0.05	NS
TDT (%)	0.36	<0.05*	S
CD19 (%)	0.15	>0.05	NS
CD64 (%)	-0.01	>0.05	NS
CD14 (%)	0.08	>0.05	NS

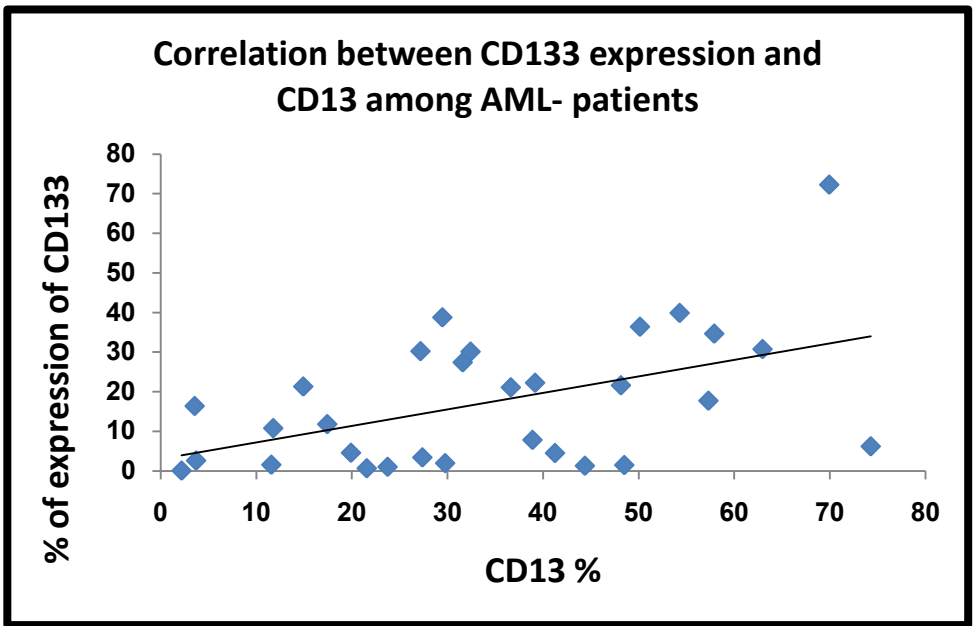

Figure (36): Correlation between CD133 expression and CD13 among AML patients

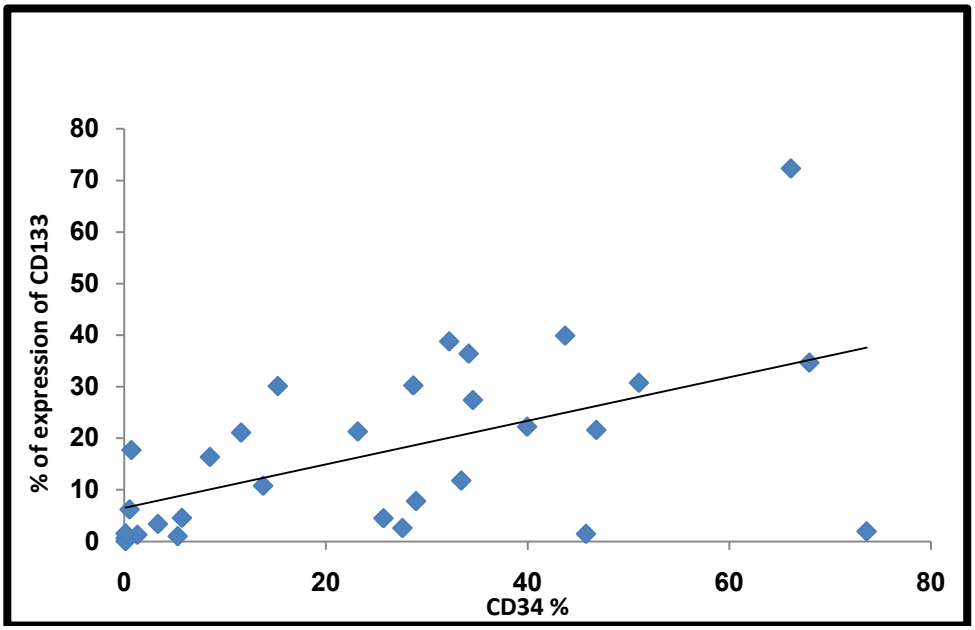

Figure (37): Correlation between CD133 expression and CD34 among AML patients

7. Association between CD133 expression and the clinical outcome:

(Figure 38 and 39) illustrates that no significant association was found between positive CD133 expression and treatment outcome; p>0.05. In despite of absence of significance there is increasing CD133 mean among the cases with chemotherapeutic resistance (Table 24 and 26).

Table (24): Differences of the percentage of CD133 expression between the AML subgroups according to their clinical outcome:

	CR		Resistance		Dead		Test of significant	p
	No	%	No	%	No	%		
CD133							Z_1= 0.51	>0.05
+ve (n=17)	11	64.7	4	23.5	2	11.8	Z_2= 1.15	>0.05
-ve (n=13)	8	61.5	1	7.7	4	30.8	Z_3= 1.3	>0.05
CD133 (means)	16.5±12.6		26.2±26.5		11.1±16.8		ANOVA= 1.3	>0.05

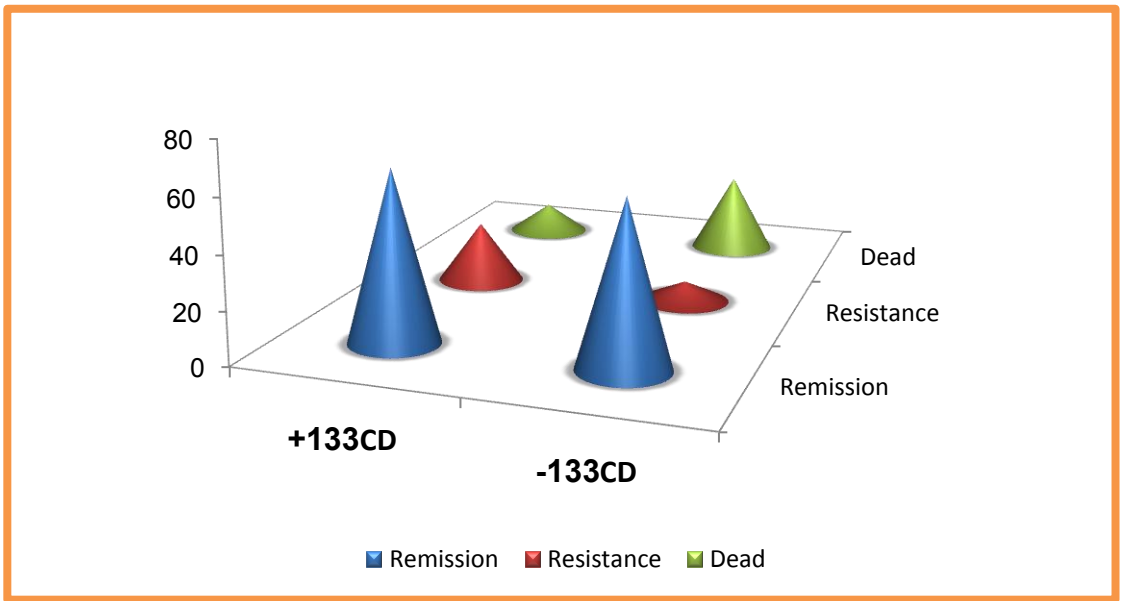

Figure (38): Percentage of CD133 expression according to clinical outcome

8. Association between CD133 expression and overall survival:

The Kaplan-Meier plot in (figure 39) and the data in (table 25) illustrating the high significance correlation between CD133 expression and the overall survival of the patients indicating that increasing CD133 leads to decrease the survival by the time.

Table (25): Cox regression analysis of overall survival:

Variable	Medians	Hazard Ratio	*p*	95% CI
$CD133^{+}$ (17)	7 ±0.129	0.4454	0.0183**	0.1920 to 1.0335
$CD133^{-}$ (13)	10 ±0.128			

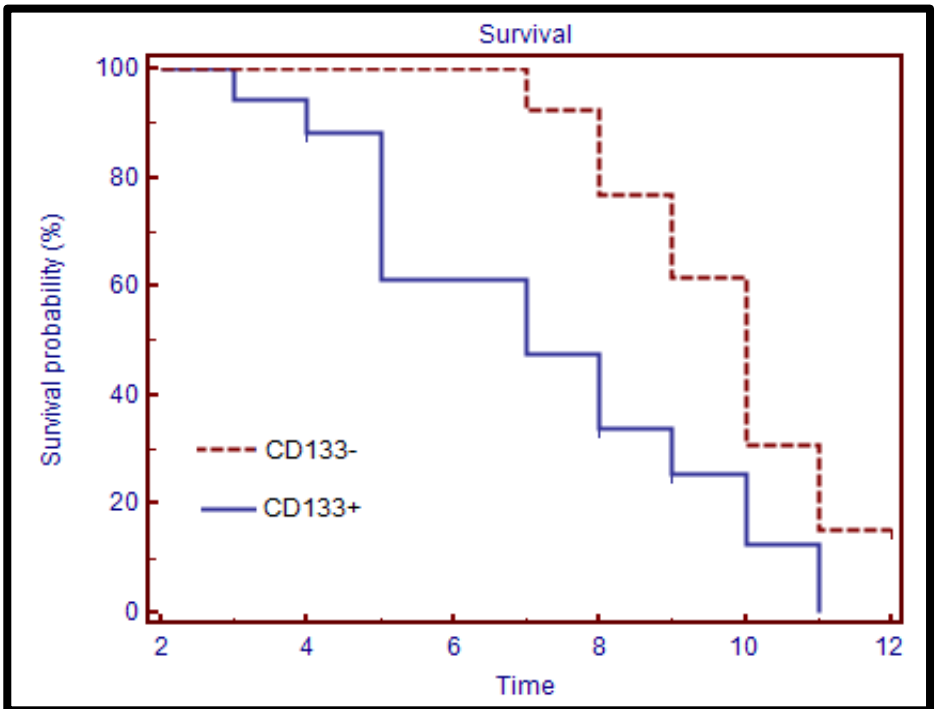

Figure (39): Kaplan-Meier plot comparing survival based on $CD133^{+}$ or $CD133^{-}$ of AML patients. Median survival of $CD133^{+}$ (17 patients): 7 months; median survival of $CD133^{-}$ (13 patients): 10 months; P <0 .01 (log-rank test).

9. Frequency of monoclonal antibodies with relation to CD133:

The significant correlation is present in both CD34 and CD7 ($p<0.05$) but none of the other is significant ($p>0.05$) (Table 26).

Table (26): Frequency of monoclonal antibodies of immunophenotyping in AML:

		CD133				χ^2	p
		-ve (n=13)		+ve (n=17)			
		No	%	No	%		
CD34(%)	**+ve**	5	38.5	15	88.2	Corr.X^2 6.13	<0.05*
	-ve	8	61.5	2	11.8		
CD13 (%)	**+ve**	9	69.2	13	76.5	Corr.X^2 0.001	>0.05
	-ve	4	30.8	4	23.5		
CD33 (%)	**+ve**	11	84.6	14	82.4	Corr.X^2 0.0	>0.05
	-ve	2	15.4	3	17.6		
HLA-DR (%)	**+ve**	7	53.8	14	82.4	Corr.X^2 1.7	>0.05
	-ve	6	46.2	3	17.6		
CD22 (%)	**+ve**	2	15.4	7	41.2	Corr.X^2 1.3	>0.05
	-ve	11	84.6	10	58.8		
CD10 (%)	**+ve**	1	7.7	2	11.8	Corr.X^2 0.0	>0.05
	-ve	12	92.3	15	88.2		
CD7 (%)	**+ve**	3	23.1	11	64.7	Corr.X^2 5.1	<0.05*
	-ve	10	76.9	6	35.3		
CD5 (%)	**+ve**	0	0.0	1	5.9	Corr.X^2 0.0	>0.05
	-ve	13	100.0	16	94.1		
CD3 (%)	**+ve**	0	0.0	0	0.0	--	--
	-ve	13	100.0	17	100.0		
CD79a (%)	**+ve**	1	7.7	2	11.8	Corr.X^2 0.0	>0.05
	-ve	12	92.3	15	88.2		
CD20 (%)	**+ve**	0	0.0	1	5.9	Corr.X^2 0.01	>0.05
	-ve	13	100.0	16	94.1		
MPO (%)	**+ve**	13	100.0	16	94.1	Corr.X^2 0.0	>0.05
	-ve	0	0.0	1	5.9		
TDT (%)	**+ve**	1	7.7	4	23.5	Corr.X^2 0.43	>0.05
	-ve	12	92.3	13	76.5		
CD19 (%)	**+ve**	1	7.7	2	11.8	Corr.X^2 0.0	>0.05
	-ve	12	92.3	15	88.2		
CD64 (%)	**+ve**	7	53.8	9	52.9	Corr.X^2 0.002	>0.05
	-ve	6	46.2	8	47.1		
CD14 (%)	**+ve**	2	15.4	2	11.8	Corr.X^2 0.0	>0.05
	-ve	11	84.6	15	88.2		