Regulatory T Cells (CD4, CD25, FOXP3) and Acute Myeloid Leukemia (AML)

Alhussein Al Araby Ahmed Almeshaal *1, Nahla Abd Elmoneim Hamed 2, Nabil Ahmed El-Halawani 2, Rania Shafik Swelem 3, Dalia Ahmed Mohammed Nafie 2

1 MBBCh, Tripoli, Libya

2 Internal Medicine Department, Faculty of Medicine, Alexandria University, Egypt

3 Clinical and Chemical Pathology Department, Faculty of Medicine, Alexandria University, Egypt

Corresponding Author: Alhussein Al Araby Ahmed Almeshaal

Email: a.almishal@zu.edu.ly

Abstract

Background: Acute myelogenous leukemia represents a group of clonal hematopoietic stem cell disorders that result from genetic alterations in normal hematopoietic stem cells.It has been demonstrated that CD4 , CD25 and FoxP3 positivity characterize Treg cells in the peripheral blood (PB) using multicolor flow cytometry. Recent study,suggest that T reg may play a role in the pathogenesis of acute myeloid leukemia and measurements of T reg frequency may have clinical value in the clinical outcome.**Aim:**to measure Treg cells(CD4,CD25,Foxp3+ve)in newly diagnosed acute myeloid leukemia patients(AML) and to correlate it with cytogenetic studies for the evaluation of clinical outcome.**Patients and Methods:** This study was conducted on patients with newly diagnosed de novo AML they included25 AML pt and another 25 adults healthy group. All patients included in our study were subjected to full history taking, complete clinical examination ,routine laboratory investigations including complete blood count and bone marrow aspiration .Diagnostic peripheral blood and bone marrow samples were analyzed. All samples were studied for cytochemical staining ,immunophenotyping and finally detection of Treg cells by flow cytometry. Treg frequency was higher in AML patients at diagnosis compared to healthy controls. . However. Patients who achieved CR after induction chemotherapy had lower Treg levels compared with patients that had persistent leukemia. In Cytogenetic analysis We have not detected any significant difference in the percentage of circulating Treg between the patients who have an normal karyotype and those with abnormal karyotypes at presentation.**Conclusion:** There was an increase frequency of CD4CD25 and foxp3 Treg cells in PB in patients with AML.Patients who achieved CR after induction chemotherapy had lower Treg levels compared with patients that had persistent leukemia.

Keywords: Regulatory T Cells, Acute Myeloid Leukemia

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Introduction

Acute myelogenous leukemia represents a group of clonal hematopoietic stem cell disorders that result from genetic alterations in normal hematopoietic stem cells. These alterations induce differentiation arrest and excessive proliferation of abnormal leukemic cells or blasts. **(1,2)**

AML accounts for approximately 25% of all leukemias in adults in the Western world, and therefore is the most frequent form of leukemia. It is by far the most common type of acute leukemia. Worldwide, the incidence of AML is highest in the U.S., Australia, and Western Europe. **(3)**

According to National Cancer Institute, Cairo University; 349 new AML cases were diagnosed in the period 2002-2003 representing 1.9% of all cancers. **(4)**

The immune system has evolved numerous mechanisms of peripheral T cell immunoregulation, including a network of regulatory T cells (Treg), to modulate and down-regulate immune responses at various times and locations and in various inflammatory circumstances. The generation of CD4+CD25+ Treg cells in the immune system is genetically controlled, influenced by antigen recognition, and various signals, in particular, cytokines such as interleukin-2 and transforming growth factor-Beta 1, control their activation, expansion, and suppressive effector activity. **(5)**

The role of Treg cells in hematologic malignancies such as nonHodgkin lymphoma being less established. Contrary to these observations, the follicular lymphoma patients with higher Treg cell numbers in their tumors had a better response to therapy and improved overall survival. **(6)** More recently, patients with chronic lymphocytic leukemia demonstrated significantly increased frequencies of CD4+CD25highFoxP3+ Treg cells, with highest frequencies in untreated or progressing patients presenting with extended disease**. (7)**

It has been demonstrated that CD4, CD25 and FoxP3 positivity characterize Treg cells in the peripheral blood (PB) using multicolor flow cytometry. Recent study,suggest that T reg may play a role in the pathogenesis of acute myeloid leukemia and measurements of T reg frequency may have clinical value in the clinical outcome. **(8)**

The aim of the present work is to measure Treg cells(CD4,CD25,Foxp3+ve)in newly diagnosed acute myeloid leukemia patients(AML) and to correlate it with cytogenetic studies for the evaluation of clinical outcome.

Subjects and Methods

This study included 50 subjects divided into two groups: -

Group 1: 25 newly diagnosed cases of AML

Group 2: 25 healthy subjects

Inclusion criteria:

1- Sex: Both Males and Females
2- Age: Above 20 year and below 60 years.
3- FAB classification:All French-American-British (FAB) subtypes with the Exception *Acute promyelocytic leukemia (M3)*

Exclusion criteria:

1. Advanced liver disease.
2. Advanced renal disease.
3. Pregnancy.
4. *Acute promyelocytic leukemia(*M3).
5. Elderly patients.

All patients in this study were subjected to the following:

1. Full history taking including:

- Age and sex.
- The presenting symptoms including fever, bone pains, manifestation of anemia, infection and bleeding.
- Occupational history.
- History of prolonged exposure to irradiation.
- Drug history especially the cytotoxic drug therapy for other malignant conditions.

2. Complete clinical examination:

Assessing the presence or absence of splenomegaly, hepatomegaly, lymphoadenopathy, manifestation of anemia, infection, bleeding, gum hypertrophy and extramedullary infiltration

3. Sample collection:

Blood samples were obtained by venipuncture using sterile disposable plastic syringe.About 8

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milliters of venous blood were withdrawn aseptically after disinfection of the skin using 70% alcohol and betadine ,and divided into the following:

- 2ml were delivered into a vacutainer ethylenediamine tetra-acetic acid (EDTA) tube for CBC.
- 4ml were delivred into avacutainer ethylenediamine tetra-acetic acid (EDAT) tube for immunophenotyping for diagnosis of AML and Human Regulatory T- cell detection by flowcytometry.
- 2ml were delivered into a vacutainer sodium heparin tube for cytogentic karyotyping

4. Complete blood count (CBC):(9,10)

Complete blood pictures were performed on an automated cell counter (ADVIA2120). PB films were stained with leishman stain and were used for the morphological identification of various cell types.

5. Bone marrow aspiration (11)

Bone marrow aspiration was performed. The diagnosis of acute leukemia was primarily based on the morphologic and cytochemical characteristics of blast cells. The sample was used to prepare bone marrow films. The remaining was sent for immunophenotyping. Myeloperoxidase (MPO) stain was also performed on BM films when needed.

6. Immunophenotyping by flow cytometry (12, 13,14)

The detailed characterization of hemopoietic cell was obtained by analyzing the expression of a given set of antigens in a cell population.

Immunophenotyping was performed on MACSQuant Flowcytometer analyzer equipped with MACS Quantify software version 2.3. using a panel of M0Abs specific to both myeloid and lymphoid and for cytoplasmic clusters of differentiation and antigens.

7- Detection of Human Regulatory T Cell by Multi-Color Flow Cytometry Kit (15-16)

This kit contains three conjugated antibodies. that can be used for single step staining of human regulatory T cells.

- CD25-PE (Clone 24212; mouse IgG2A)
- CD4-PerCP (Clone 11830; mouse IgG2A)
- FoxP3-APC (goat IgG)

The kit also contains Flow Cytometry FoxP3 Staining Buffer (120 mL), which contains 1% formaldehyde and 0.2% sodium azide, and Flow Cytometry Staining Buffer (50 mL), which contains BSA and 0.1% sodium azide.

8- Cytogentic study: (17-18)

Chromosomes were prepared from dividing cells (mitotic cells). Cells were cultured in RPMI 1640, supplemented with fetal bovine Serum, L-glutamine, penicillin and streptomycin.

Statistical analysis of the data

Data were fed to the computer using the Predictive Analytics Software (PASW Statistics 18).

Qualitative data were described using number and percent. Association between categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Monte Carlo correction.

Quantitative data were described using median, minimum and maximum as well as mean and standard deviation.

The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test. D'Agstino test was used if there was a conflict between the two previous tests. If it reveals normal data distribution, parametric tests were applied. If the data were abnormally distributed, non-parametric tests were used.

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For normally distributed data, comparison between two independent population were done using independent t-test.

For abnormally distributed data, Mann-Whitney Test (for data distribution that was significantly deviated from normal) were used to analyze two independent population. If more than two population were analyzed Kruskal Wallis test to be used. Correlations between two quantitative variables were assessed using Spearman coefficient.

Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

Results:

The present study included 25 newly diagnosed patients with AML and 25 healthy persons as control. AML patients included 16 (64%) males and 9 (36) females and control group included 15 (60%) males and 10 (40) females. AML patient age ranged from 20-52 years with a mean of 30.72 ± 9. 46 years. and control group age ranged from 21 -46years with a mean of 31.92 ± 7.82years. (Table 1)

Blast percentage in the peripheral blood: The percentage of blast in the peripheral blood ranged from 14% to 91% with a mean 38.88 ± 21.69%. Blast percentage in the bone marrow: The percentage of blast in the bone marrow (BM) ranged from 30.0 % to 90.0 % with a mean of 53.48 ± 18.11 % (Table 2)

Of all AML patients ,8 cases were M1 (32%),7 cases were M2 (28%),6 cases were M4 (24%), and 4 was M5 (16%) (Figure 1).

Percentages of CD4, CD4+CD25+ and CD4+CD25, FOXP3 T cells in the peripheral blood of NC and AML patients. The percentage of Treg was increased in AML patients significant to that of NC $P < 0.001$. Increased frequency ofCD4+CD25high T cells in the peripheral blood of patients with AML before treatment, gating strategy used to identify the CD4+CD25high T reg. CD4+T cells with mean fluorescence intensity of CD25 expression. Phenotypic characteristic of CD4+CD25high Treg obtained from PBMC of AML patients and NC, gating strategy used to identify the CD4+T- cells and then coexpression of foxp3/CD25high Treg cells in CD4+high cell (Table 3, Figure 2).

Of the 25 samples with karyotypes analyzed,16 had normal karyotypes (64%), and 9 samples with abnormal karyotypes (36%).

According to our study,of 25 cases with karyotypes study ,8 cases were classified as M1,among which 4 cases (25%) had normal karyotyping and 4 cases(44.4%) had abnormal karyotyping.7cases were classified as M2,among which 5 cases (31.3%) had normal karyotyping and 2 cases(22.2%) had abnormal karyotyping. ,6 cases were classified as M4, among which 4 cases (25%) had normal karyotyping and 2 cases (22.2%) had abnormal karyotyping. ,4 cases were classified as M5, among which 3 cases (18.8%) had normal karyotyping and 1 case (11.1%) had abnormal karyotyping (Figure 3).

Among FAB classification We have not detected any significant difference in the percentage of circulating Treg between different subtypes of FAB classification. (Table 4).

We have not detected any significant difference in the percentage of circulating Treg cells with the Blast cells percent in Bone marrow (Table 5).

Frequency of base line Treg in AML patients after treatment. Patients who achieved CR after induction chemotherapy had lower Treg levels compared with patients that had persistent leukemia., significant differences is presented in Treg of Complete remission patients and persistent leukemia at($P = 0.016$) (Table 6).

In Cytogenetic analysis we have not detected any significant difference in the percentage of circulating Treg between the patients who have a normal karyotype and those with abnormal karyotypes at presentation.

But there was a statistically significant difference between the two groups regarding CD4+% ($p=0.036$) (Table 7).

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Table (1): Comparison between the two studied groups according to demographic data

	Cases		Control		Test of sig.
	No	%	No	%	
Sex					
Male	16	64.0	15	60.0	p = 0.771
Female	9	36.0	10	40.0	
Age					
Min. – Max.	20.0 – 52.0		21.0 – 46.0		
Mean ± SD	30.72 ± 9.46		31.92 ± 7.82		tp = 0.627
Median	26.0		32.0		

p: p value for comparing between the two studied groups
2: Chi-square test
t: Student t-test

Table (2): Descriptive analysis of the studied cases according to Blast cells percentage in the Bone marrow and peripheral blood:

	Min. – Max.	Mean ± SD	Median
Blast cells in Bone marrow %	30.0 –90.0	53.48 ± 18.11	51.0
Blast cells peripheral blood%	14.0 – 91.0	38.88 ± 21.69	35.0

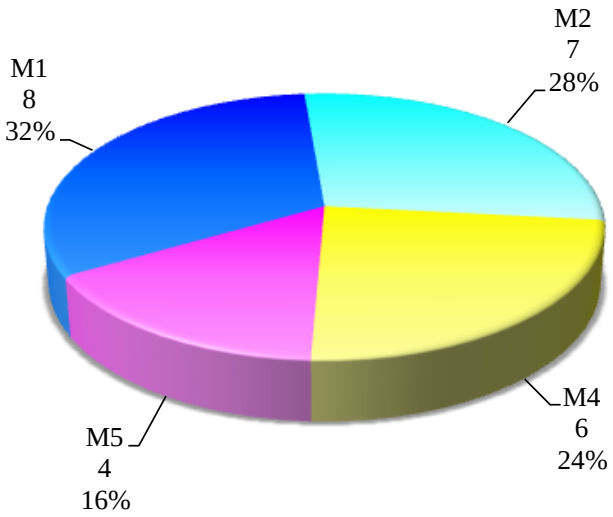

Figure (1): Distribution of the studied cases according to FAB Classification

Table (3): Comparison between the two studied groups according to T cell regulation Distribution

	Cases %	Control%	MWp
CD4 of total lymphocytes cell Min. – Max. Mean ± SD Median	 0.54 – 55.38 9.39 ± 11.17 4.66	 6.90 – 28.32 15.64 ± 8.10 9.93	 0.002*
CD4/ CD25 of CD4 Min. – Max. Mean ± SD Median	 0.14 – 22.28 6.80 ± 6.84 3.28	 1.30 – 6.28 3.50 ± 1.90 2.70	 0.290
CD4/ CD25 and FOXP3 Min. – Max. Mean ± SD Median	 0.01 – 1.49 0.32 ± 0.43 0.11	 0.0 – 0.14 0.02 ± 0.04 0.01	 <0.001*

p value for comparing between the two studied groups
MW: Mann Whitney test
*: Statistically significant at p ≤ 0.05

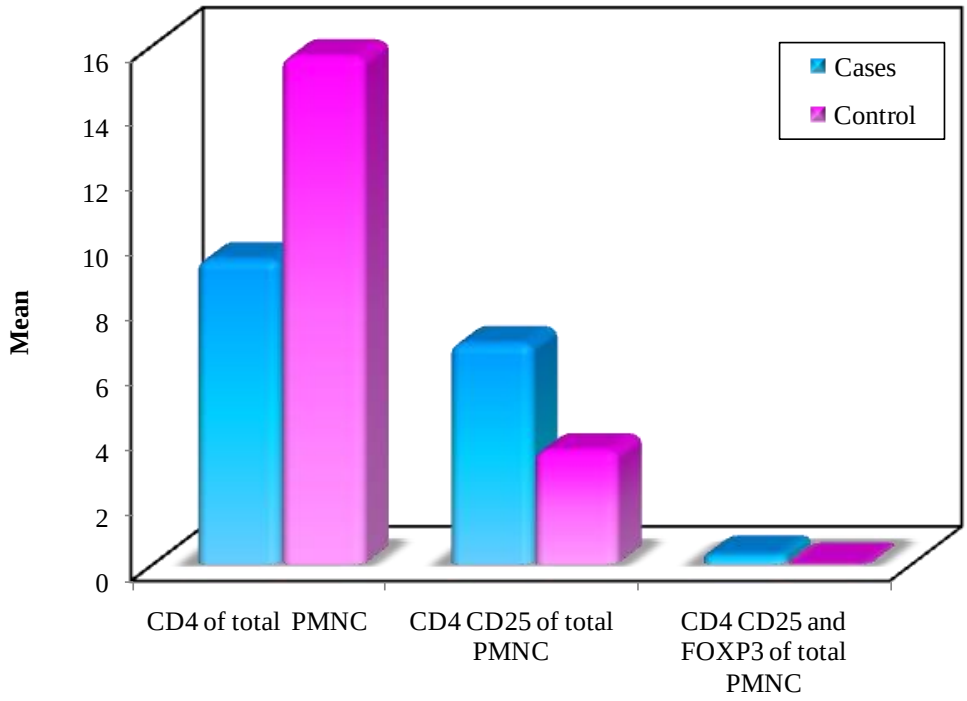

Figure (2): Comparison between the two studied groups according to Treg cell Distribution

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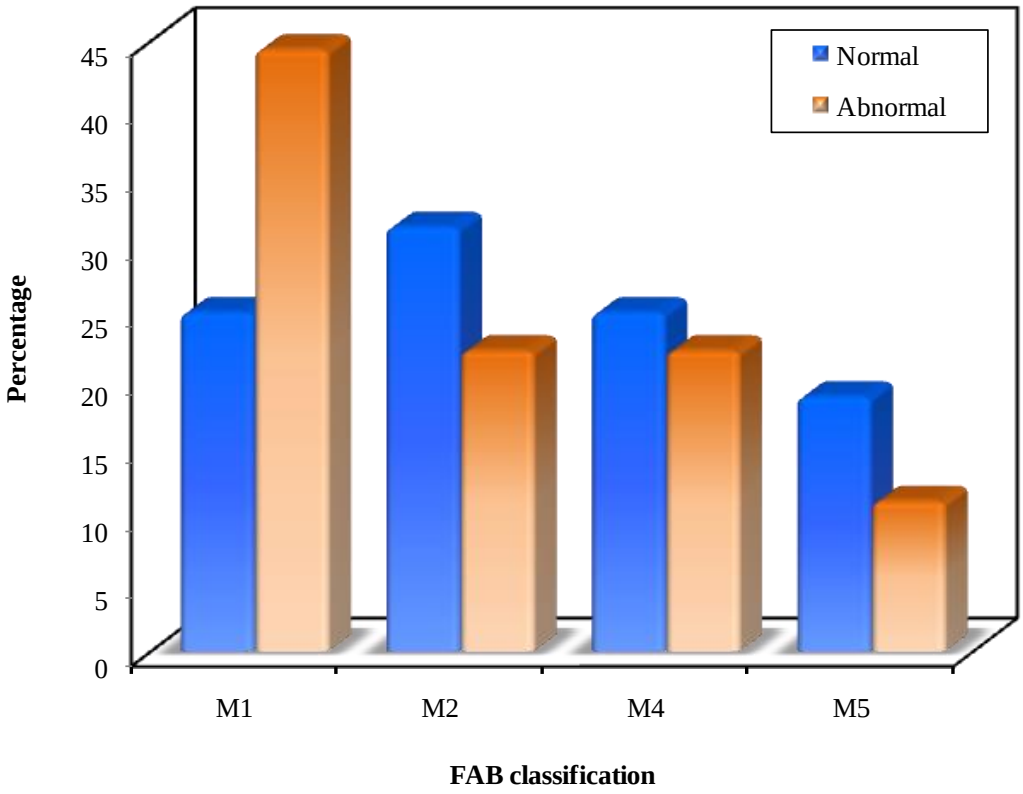

Figure (3): Relation between Cytogentic Karyotyping and FAB Classification

Table (4): Relation between Treg cell and FAB classification

	FAB classification				KWp
	M1	M2	M4	M5	
CD4 of total lymphocytes					
Min. – Max.	0.54 – 55.38	1.70 – 19.50	1.74 – 20.40	1.17 – 10.40	
Mean ± SD	11.66 ± 18.16	9.13 ± 6.46	9.58 ± 7.36	5.03 ± 3.88	0.769
Median	4.10	10.45	7.78	4.27	
CD4 CD25 of CD4					
Min. – Max.	0.24 – 22.28	0.14 – 12.50	1.17 – 21.30	2.09 – 16.50	
Mean ± SD	6.79 ± 8.89	5.36 ± 4.81	8.38 ± 7.37	6.99 ± 6.56	0.785
Median	1.98	2.73	7.52	4.69	
CD4 CD25 and FOXP3					
Min. – Max.	0.01 – 1.30	0.03 – 1.49	0.01 – 1.10	0.02 – 0.29	
Mean ± SD	0.29 ± 0.43	0.43 ± 0.58	0.36 ± 0.40	0.12 ± 0.12	0.765
Median	0.15	0.09	0.25	0.09	

p: p value for comparing between Treg cell and FAB classification
KW: Kruskal Wallis test
*: Statistically significant at p ≤ 0.05

Table (5): Correlation between T reg cell and Blast cells in Bone marrow

	Blast cell percent in Bone marrow	
	rs	p
CD4 of total lymphocytes	0.181	0.386
CD4/ CD25 of CD4	-0.503*	0.010
CD4/ CD25 and FOXP3	-0.233	0.263

rs: Spearman coefficient
*: Statistically significant at p ≤ 0.05

Table (6): The relation between T reg cells baseline and therapy response

	Complete remission-pt (n = 9)	persistent leukemia (n = 16)	MWp
CD4 of total PMNC			
Min. – Max.	0.54 – 11.95	1.70 – 55.38	
Mean ± SD	5.73 ± 4.54	11.46 ± 13.26	0.258
Median	3.90	7.78	
CD4 CD25 of total PMNC			
Min. – Max.	0.24 – 16.50	0.14 – 22.28	
Mean ± SD	5.02 ± 5.51	7.81 ± 7.46	0.396
Median	2.26	5.65	
CD4 CD25 and FOXP3 of total PMNC			
Min. – Max.	0.01 – 0.37	0.04 – 1.49	
Mean ± SD	0.10 ± 0.12	0.44 ± 0.49	0.016*
Median	0.04	0.24	

p: p value for comparing between the two studied groups

Table (17): Correlation between Treg cell and Cytogenetic Karyotyping result

	Classification		MWp
	Normal (n = 16)	Abnormal (n = 9)	
CD4 of total lymphocytes			
Min. – Max.	0.54 – 55.38	1.17 – 11.95	
Mean ± SD	12.25 ± 13.02	4.31 ± 3.32	0.036*
Median	10.43	3.49	
CD4/ CD25 of CD4			
Min. – Max.	0.24 – 22.28	0.14 – 21.30	
Mean ± SD	5.67 ± 5.68	8.82 ± 8.52	0.821
Median	3.01	6.26	
CD4 /CD25 and FOXP3			
Min. – Max.	0.01 – 1.10	0.02 – 1.49	
Mean ± SD	0.24 ± 0.34	0.46 ± 0.55	0.202
Median	0.09	0.24	

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Discussion

Acute myloid leukemia (AML) is a malignant neoplasm of hematopoietic cell characterized by autonomous proliferation and impaired differentiation of hematopoietic progenitors leading to the accumulation of immature myeloid precursors in the bone marrow. It is clinically, cytogentically and molecularly heterogenous. **(19)**

Our understanding of the pathophysiology of AML has rapidly increased over the past two decades.Karyotype diagnosis provides the most important prognostic information is cytogentic data that assign patients to distinct prognostic groups. The detection and characterization of chromosomal alterations in AML has also provided means to establish a new pathogenesis-oriented classification of the disease (the WHO classification). **(20,21)**

However, by conventional chromosomal banding analysis approximately 50% of the AML patients lack clonal chromosome aberrations,and discrimination between prognostically different subsets of patients by using molecular genetic approaches is of major importance**.(22)**

The AML microenviroment is immunosuppressive and antiapoptotic, favoring the survival of malignant hematopoietic cells**.(23)** In vitro studies have shown that AML cells secrete factors, which inhibit T cell activation and proliferation and limit proinflammatory T helper-1 (Th-1) cytokine production**.(24)** This suppressive effect is reversed, however, when Tregs and other T lymphocytes are removed from the microenvironment in vitro, leading to augmented immune responses to AML**.(24)**

Collectively, these data indicate that the AML microenvironment contains a variety of immune suppressive elements, including secreted factors and immune cells, namely Tregs, which appear to be mediators of immune suppression. **(25)**

The human immune system maintains the balance between protecting the host from pathologic microorganisms and avoiding excess immune reactions that could be harmful to the host. **(26)** Aberrant immune reactions can cause serious damage to host, such as various autoimmune and inflammatory diseases. Naturally occurring thymus-derived CD4+CD25+ regulatory T-cells (Tregs) play a critical role in the control of immune responses in various clinical settings, including autoimmune diseases, allergic disorders, infections, transplantations, and cancers. **(27-28)**

Recent studies suggested that number and locality of Treg might have prognostic value in various cancers**. (29)** Treg have been classically defined based on high FoxP3 expression**. (30)** In humans, relatively homogeneous populations can be isolated from PB by sorting the 1%–2% brightest CD25 cells within the CD4 T-cell population**. (31,32)** However, the flow cytometry-based method accurately gate for CD4. **(33,34)**

From a technical point of view, BM processing is more difficult due to fat tissues and lower lymphocyte proportions; thus, sampling from PB would be better than sampling from BM for Treg measurement in routine clinical settings. **(35)**

In comparison with solid malignancies, relatively little information is available about functional characteristics of Treg or their clinical significance in patients with acute leukemia. **(36)**

Several studies have described the presence of abnormalities in the immune system of patients with AML leukemia. **(37,38)**

The aim of the present study, was to investigate the frequency of circulating Treg in 25 newly diagnosed untreated AML patients and 25 healthy control. to correlate it with cytogenetic studies for the evaluation of clinical outcome

Regarding the demographic data such as age and sex the present study showed no significant difference between AML cases and healthy controls

Peripheral blood sample from AML patients (9 females and 15 males) and 25 HV (10 females and 15 males) with mean age of (30.72 years) for AML patients and for healthy controls (31.92 years) were analyzed.

In this study, we evaluated the frequency, of Treg obtained from the peripheral circulation of

patients with AML at diagnosis. The subset of Treg was defined as CD4+CD25/foxp3. by human regulatory cell multi-color flow cytometry kit

As evident from the results Treg frequency in the peripheral blood of AML patients. The percentages of circulating CD4+ Tcells Treg was higher in the healthy controls (15.64 ± 8.10) than is AML patients (9.39 ± 11.17). However, no significant difference in CD4+CD25+ Tcells was observed in AML patients and healthy controls (mean ± SD, 6.80 ± 6.84versus 3.50 ± 1.90), respectively. Similarly, the percentage of circulating CD4+CD25+ Foxp3+ Treg was higher. in AML patients (0.32 ± 0.43; 0.01 – 1.49%) compared with NC (0.02 ± 0.04; range, 0.0 – 0.14%).

Human CD4+CD25+ Treg cells has been isolated from human peripheral blood, thymus, lymph nodes and cord blood. **(39)** The increase frequency of Treg in the peripheral blood in the present study was similar to that reported by Wang et al. who shown an increased frequency of Treg in the peripheral blood of AML patients and a concomitant increase in their frequency in the bone marrow. **(40)**

Another study conducted by **Zhang et al. (8**) between November 2007 and February 2010, on 182 newly diagnosed patients with AML (age range: 15 to 81 years, mean 46.4.,) from the First Affiliated Hospital of Wenzhou Medical College. The age-matched control group comprised of 20 healthy controls (age range: 19 to 66 years, mean 43.4)

The frequency of circulating Treg cells was slightly higher in patients with AML than those of HVs, which is similar to the previous studies**. (34,40)**

Miroslaw et al. (36) conducted a study on AML patients and healthy volunteers. Samples of venous blood (20-50 mL) were obtained from 31 newly diagnosed AML patients (18 females and 13 males) before any treatment and 25 age-matched healthy volunteers. showed an elevated frequency of Treg in the peripheral blood in patients with AML than normal controls. **(34)**

Combining the data from all studies reported to date, including the current study, it is obvious that An elevated frequency of Treg in the peripheral blood was detected in AML cases.

In **Miroslaw et al study, (36)** Cytogenetic analysis was done in all newly diagnosed AML patients, and cytogenetic abnormalities were grouped according to the published criteria. **(41)** We have not detected any significant difference in the percentage of circulating Treg between the patients who have a normal karyotype and those with abnormal karyotypes at presentation (p= 0.202) .

To gain further insights into the role Treg played in acute leukemia, we also evaluated the frequency of Treg after induction chemotherapy. We found that patients who achieved CR after induction chemotherapy had a significantly lower Treg frequency at diagnosis compared with patients who did not respond and had persistent leukemia or who died.

AML cases in our study was classified according to FAB classification which divides AML into 8 subtypes (M0-M7). **(42,43,44)**

In our study, the patients fell into four of eight subtypes of the FAB classification 8 M1 (32%),7 M2(28%), 6 M4(24%) and 4 M5(16%).

Regarding T reg cells frequency in different FAB subtypes , We have not detected any significant difference in the percentage of circulating Treg between different subtypes of FAB classification. Leukaemic cells, may infiltrate spleen, gum and lymph nodes and circulate in the bloodstream. **(45)** As regards splenomegaly, the results of the studied cases showed that 20% has splenomegaly. Swollen gums occurred in our studied with average of 28% ,and lymph node enlargement occurred in, 28% of cases.

The normal percentage of blasts in the bone marrow is less than 5%. in the M1,M2 and M4 subtypes of classification blasts are more than or equal to 20% .In the M5 subtype ,monoblasts are more than or equal to 80% **.(46)** The malignant cell in AML is the myeloblast . In normal haematopoiesis ,the myeloblast is an immature precursor of myeloid white blood cells :a normal myeloblast will gradually mature into a mature white blood cell .However ,in AML,a single myeloblast accumulates genetic changes which freeze the cell in its immature state .The

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uncontrolled growth of an immature clone of cells ,leads to the clinical entity of AML**.(47)** The mean percentage of blasts in peripheral blood in our studied group was38.88 ± 21.69 ,and the mean of blasts in the bone marrow was 53.48 ± 18.11.

Regardding T reg cells We did not detect any corelation in the percentage of circulating Treg cells with the Blast cells in Bone marrow.

Control group haemoglobin values were 12.0 – 15.40 g/dl.The level of haemoglobin in our study group was significantly lower , with a mean value of 7.56 ± 2.19 g/dl. .The clinical signs and symptoms of AML result from the fact that ,as the leukemic clone of cells grows ,it tends to displace or interfere with the development of normal blood cells in the bone marrow .This leads to neutropenia ,anaemia and thrombocytopenia**.(48)**

The platelet count was237.55 ± 48.29 x109/l in healthy control,with a mean value of 70.64 ± 46.98 x109/l in studied group.As regards the mean value of white cell count of healthy control, WBCS was 13.02 ± 0.92 x109/l, and leukaemic patients had a mean value of 11.27 ± 32.12 x109/l .

In the present study, the percentage of Treg was increased in AML patients significant to that of healthy control. There was no correlation with Treg levels and cytogenetic subgroups. Higher pretreatment Treg PB frequencies predicted poor response to induction therapy. The frequencies Tregs at diagnosis were lower in patients who had achieved CR compared to those with persistent leukemia or who died.

All studies consistently showed that the frequency of Tregs is increased in patients with AML compared with healthy control patients and, some of these studies indicated that increased Tregs at diagnosis of AML is poor prognostic.

Conclusion

There was an increase frequency of CD4CD25 and foxp3 Treg cells in PB in patients with AML. Patients who achieved CR after induction chemotherapy had lower Treg levels compared with patients that had persistent leukemia. In addition, the circulating Treg frequencies have no significant correlation with cytogentic karyotyping.

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