

## platelets levels and platelet to lymphocyte ratio in patients with Acute myeloid leukemia before and after chemotherapy

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### Abstract

Acute Myeloid Leukemia is blood disorder that characterizing by the overproduction of abnormal cells (blasts) in bone marrow and affects the myeloid linages that responsible of production on normal blood cells the rapid growth of abnormal cells that impede the normal production of platelet and courses thrombocytopenia and bleeding tendency. The present study (50) samples were collected from bone marrow and from peripheral blood. According to results that platelets count and platelet crit in patients before treatment was significantly ( $p < 0.05$ ) lower than in patients after treatment and below the lower limit of normal range. However, platelet-large cell ratio in patients group before treatment is significantly higher than in patients after treatment and above the upper limit of normal range. While platelet/lymphocyte ratio (PLR) reveals that this ratio in patients before treatment is  $48.2 \pm 68.6$  which is significantly lower than  $159.5 \pm 117.2$  in patients after treatment .The current study administrate that the chemotherapy impact on the levels of platelets and the influence the effect of patient's characters and subtypes on the platelets outcomes also the platelet to lymphocyte ratio could being prognostic factor that reflect the health status of patients and their response to treatment.

### Introduction:

Cancer is a major worldwide public health concern because of its highly aggressive nature, dismal prognosis, and low rate of survival[1]Acute myeloid leukemia (AML) is a heterogeneous hematological cancer that is primarily distinguished by the blocking of myeloid cells differentiation and development of immature myeloid progenitors in the bone marrow of patient[2] the most prevalent form of acute leukemia in adults is AML and interpretations for about a third of all diagnosed leukemia, with its incidence that increasing with age[3] .The number of patients with AML is a significant increase in the number the last twenty years in Iraqi populations the increasing in the frequency of AML could be connected to many factors like environmental modifications mostly ionizing radiation which is most important etiological elements that responsible of acute leukemia particularly AML, which

reflects one of the considerable concerns of wars in Iraq [4]. Platelets are small cells that padding holes in the blood vessels [5]. Platelets are essential for thrombosis, a variety of bleeding disorders and hemostasis [6]. Consequently, thrombocytopenia, or alterations in platelet count, is linked to a high rate of morbidity, there are two primary mechanisms that can lead to thrombocytopenia: inadequate production in the bone marrow and excessive platelet breakdown in peripheral circulation. [7] Leukemic cells devoid of megakaryocytic differentiation destroy normal hematopoiesis, which lowers platelet counts [8]

## **Materials & Methods**

### **Sample collection**

Samples were carried out from August 2022 to October 2023 from Baghdad Teaching Hospital /Medical City/ Baghdad. The overall (50) AML patients were enrolled in this study samples collected from bone marrow and from peripheral blood, 28 of AML patients were male while the (22) of them were female. The age of AML patients on the onset of the disease ranged from (14) to (70) year, and (16) of patients were newly diagnosis of AML before treatment and (36) patients of AML after treatment

Bone marrow aspiration was used to diagnose the patients and peripheral blood, the morphology by using FAB classification in this study the subtypes were included (M3, M2, M4 and M5) as following:

- Patients are presented with M3 subtype (Acute promyelocytic leukemia) were 18 of the total patients numbers
- 17 M5 subtype (Acute monocytic leukemia),
- 13 M2 subtype (Acute myelocytic leukemia),
- and only 2 of them with M4 subtype (Acute myelomonocytic leukemia).

### **Blood Samples**

From each of (50) patients 5 ml of hole vein blood samples were taken by via a disposable syringe, the blood samples were attained by using ethylene diamine tetra-acetic acid (EDTA), is a complete blood count (CBC) anticoagulant tube.

### **Bone marrow aspiration**

The Bone marrow aspiration (50) samples was collected from the posterior iliac crest after sterilization with sterile technique and uses some of local anesthesia to reduction the pain.

### **Preparation of marrow smears**

1. Drop of marrow aspirated on the slid the marrow fragments are pulled behind by using anther clean slid and leave trail of cells behind them.
2. After drying, the film was fixed and stained by Leishman stain, as for blood film.
3. In low power ( $\times 10$ ) the cellularity was resolute, megakaryocytes identified.
4. With high power ( $\times 40$ ,  $\times 100$ ) oil immersion, myeloid and erythroid cell maturation stages were seen and identified. The reading done by a specialist hematologist.

### **Statistical Analysis**

Statistical analysis was performed with Microsoft excel sheet 2016 and loaded into Statistical Package for the Social Sciences (SPSS) version 28 statistical program values were

expressed as mean  $\pm$  standard deviation (M  $\pm$  SD), percentages and Pearson's correlation coefficient, P < 0.05 was considered statistically significant.

## Results and Discussion

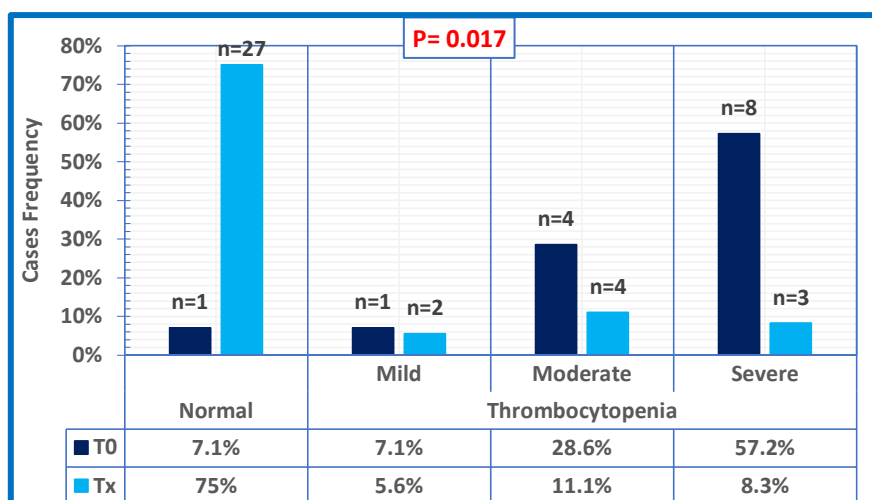
According to results of CBC, Table 1 shows that platelets count in patients of newly diagnosis (T0) is  $52 \pm 37.2 \times 10^9/L$ , which is significantly lower than  $233 \pm 139 \times 10^9/L$  in patients of after treatment (Tx) and also below the lower limit of normal range ( $150 \times 10^9/L$ ). Similarly, platelet crit (PCT) in patients of T0 ( $0.04 \pm 0.03\%$ ) is significantly lower than Tx ( $0.22 \pm 0.12\%$ ) and below the lower limit of normal range ( $0.11\%$ ). However, the platelet-large cell ratio (P-LCR) in patients of T0 group ( $39.5 \pm 14\%$ ) is significantly higher than  $30.6 \pm 12.5\%$  in Tx group and also above the upper limit of normal range ( $35\%$ ).

**Table 1:** Platelets parameters in both groups of AML patients

Platelet Parameters	Normal Reference	T0 (n=14)	Tx (n=36)	P value
Count ( $\times 10^9/L$ )	150 - 450	$52 \pm 37.2$	$233 \pm 139$	< 0.0001
MPV (fl)	6.5 - 12	$8.3 \pm 2.7$	$8.5 \pm 1.2$	0.709
PCT (%)	0.11 - 0.28	$0.04 \pm 0.03$	$0.22 \pm 0.12$	< 0.0001
P-LCR (%)	15 - 35	$39.5 \pm 14$	$30.6 \pm 12.5$	0.041

MPV= mean platelet volume, PCT= platelet crit, P-LCR= platelet-large cell ratio

Moreover, Figure 1 reveals that 57.2% of patients in T0 group are presented with severe thrombocytopenia (platelets count  $< 50 \times 10^9/L$ ), 28.6% with moderate thrombocytopenia (platelets count  $50 - 100 \times 10^9/L$ ), 7.1% with mild thrombocytopenia (platelets count  $101 - 140 \times 10^9/L$ ), and only one patient (7.1%) of them was presented with normal count. The frequency of these cases are significantly different from those in Tx group (8.3%, 11.1%, 5.6%, and 75% respectively).



**Fig1:** Frequency of cases with different types of thrombocytopenia in both groups of AML patients

On the other hand, calculation of platelet/lymphocyte ratio (PLR) as an indicator of inflammatory status reveals that this ratio in patients of T0 group is  $48.2 \pm 68.6$  which is significantly lower than  $159.5 \pm 117.2$  in patients of Tx group Table 2. Also, this table shows that 64.3% of patients in T0 group have PLR below the lower limit of normal range, which is significantly higher than 11.1% in patients of Tx group.

Table 2: Platelet/lymphocyte ratio (PLR) in ALM patients of both groups

Platelet/lymphocyte ratio (PLR)		T0 (n=14)	Tx (n=36)	P value
M $\pm$ SD		48.2 $\pm$ 68.6	159.5 $\pm$ 117.2	0.001
Category (n, %)	Normal	4 (28.6%)	20 (55.6%)	0.0005
	Low	9 (64.3%)	4 (11.1%)	
	High	1 (7.1%)	12 (33.3%)	
Normal range of PLR= 37 - 173				

To determine the relationship of thrombocytopenia and high PLR with other patient's feature, Table 3 shows that patients with subtype M2 have significant lower cases of thrombocytopenia ( $r = -0.3417$ ,  $P = 0.015$ ) and significant more cases with high PLR ( $r = 0.3238$ ,  $P = 0.021$ ). However, all the rest features reveal non-significant correlation with thrombocytopenia and high PLR.

**Table3:** Correlation of thrombocytopenia and high PLR cases versus gender, age, and subtypes of all AML patients

Correlation feature versus	of	Thrombocytopenia		High PLR	
		r	P	r	P
Gender		-0.1071	0.459	0.2036	0.156
Age		0.0553	0.702	0.0516	0.721
AML subtype	M2	-0.3417	0.015	0.3238	0.021
	M3	0.1746	0.225	-0.0637	0.660
	M4	-0.1809	0.208	-0.0783	0.588
	M5	0.2143	0.135	-0.2029	0.157

Abnormal hematopoiesis is categorized by the increasing in immature cells in bone marrow and peripheral blood [9], and leukaemia is malignancy disorder of the hematopoietic stem cells that can affect adults and children and all lines off blood component production [10] The importance of PLT numbers at diagnosis for prognosis in AML residual contentious the level of platelets in recent study observed that had high significant differences between patients and apparently normal reference the platelet count was significant lower in patients in initial stage of dieses before Chemotherapy because there is no sufficient of megakaryocytes that can normally differential to platelets due to it replacement by blast cells and causes the stat of thrombocytopenia this study was consistent with the study of [11,12] in which the Thrombocytopenia was a significant indicator of AML ,

This is caused by the leukemic cells building up in the bone marrow and inhibiting the growth of platelets and other normal hematopoietic cells. Also study of Al-Dulaimi et al., reported that thrombocytopenia is a well-established AML finding and had friendship with bleeding because of falling platelet count [13], furthermore Comparable patterns of alterations in hematological parameters were demonstrated by [14,15].

The Platelet parameters specifically MPV, PCT, and P-LCR could clarify the behaviors of platelet and may give a diagnostic and prognostic evidence of patient's state in many clinical situations Depending on platelet production and activation several parameters of platelets change. Consequently, platelet indices can be utilized to indicate the reason of the thrombocytopenia. MPV is parameter that measures the average platelet size, PCT, which is the volume used by the platelets in the blood and is reported as a percentage, is crucial for identifying platelet-measurable disorders and P-LCR can be used to track platelet activity since it is a sign of larger platelets in circulation[16] In present study were a significant decreased in PCT and P-LCR, had significant increase between patients and normal Reference these finding in agreement with [17], while MPV had no significantly changes and this the opposite of study of Tığlıoğlu et al., who reported that those with greater MPV at diagnosis were determined to have a negative influence in overall survival[18] , These findings may indicate that these indicators are used in conjunction with additional clinical and laboratory data, perhaps avoiding bone marrow biopsy and aspiration in these patients.

The PLT counts at diagnosis is vary widely in patients with AML and the results showed that there was verity of thrombocytopenia among the patients the results ranging from mild to moderate to severe thrombocytopenia this diversity it is reasonable due to the prognosis of AML patients' is influenced by both the disease and the patient-specific factors. The recognizing of the level of PLT might be useful as prognostic in AML according to study of Zhang et al., Patients with medium platelet count (50–120 \_ 109/L) obligated longer overall survival and disease-free survival than those whom had lower or higher platelet count (<50 \_ 109/L or >120 \_ 109/L), respectively [19]. Additionally, Chen et al., reported that treatment response is more adaptable the non-response rates to treatment were high in patients with lower platelet counts after chemotherapy[20].

The PLR could be another indicator also may be use for diagnosis and assessment to the status of patients because PLR are important for prognosis in many medical illnesses because they show how the patient's immune system interacts with the tumor microenvironment [21] The decrease in platelet count and lymphocyte this might have direct effect on the immunity response and return the medical conditions of the patients the current study showed that there were significantly lower in PLR value in non-treated patients and the this value became higher in group pf patient whom had treatment these results in agreement with study of [22] and study of [23].

Moreover Woelfinger et al. reported patients with high pre-HSCT PLR, showed a better overall survival, fewer relapses lower non-relapse mortality [24].

According, to result the thrombocytopenia and had high PLR could affect any age and gender and also the sub type of AML could affect by thrombocytopenia and had high PLR except M2 sub type the effect of thrombocytopenia was less than other sub type and high PLR is due to that the patients with M2 sub type had less thrombocytopenia that mean might had mild or normal level of platelets and lymphocytes.

## **Conclusion**

This study demonstrates that the relationship between platelet count at diagnosis and after treatment can be helpful in evaluation the condition of the patients. total count and the platelet to lymphocyte ratio could be simple, easily, available and cost effective prognostic tests, that may be clinically useful to help risk-stratify patients with AML, in order to better adjust the treatment intensity

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## مستويات الصفائح الدموية ونسبة الصفائح الدموية إلى الخلايا الليمفاوية في المرضى الذين يعانون من سرطان الدم النخاعي الحاد قبل وبعد العلاج الكيميائي

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البحث مستل من اطروحة دكتوراه الباحث الاول

### الخلاصة:

سرطان الدم النخاعي الحاد هو اضطراب في الدم يتميز بالإفراط في إنتاج الخلايا غير الطبيعية (الأرومات) في نخاع العظام ويؤثر على الروابط النخاعية المسؤولة عن إنتاج خلايا الدم الطبيعية والنمو السريع للخلايا غير الطبيعية التي تعيق الإنتاج الطبيعي للصفائح الدموية وتؤدي إلى نقص الصفائح وميل النزيف. تم جمع (50) عينة من الدراسة الحالية من نخاع العظم ومن الدم المحيطي. أظهرت النتائج أن عدد الصفائح الدموية وكريات الصفائح الدموية لدى المرضى قبل العلاج كان أقل بشكل ملحوظ ( $p < 0.05$ ) منه في المرضى بعد العلاج وأقل من الحد الأدنى للمستوى الطبيعي. ومع ذلك، فإن نسبة الصفائح الدموية إلى الخلايا الكبيرة في مجموعة المرضى قبل العلاج أعلى بكثير منها في المرضى بعد العلاج وأعلى من الحد الأعلى للمستوى الطبيعي. بينما تكشف نسبة الصفائح الدموية إلى الخلايا الليمفاوية (PLR) أن هذه النسبة في المرضى قبل العلاج هي  $68.6 \pm 48.2$  وهي أقل بكثير من  $117.2 \pm 159.5$  في المرضى بعد العلاج. وتشير الدراسة الحالية إلى تأثير العلاج الكيميائي على مستويات الصفائح الدموية وتأثيرها على تأثير خصائص المريض وأنواعه الفرعية على نتائج الصفائح الدموية، كما يمكن أن تكون نسبة الصفائح الدموية إلى الخلايا الليمفاوية عاملاً إنذارياً يعكس الحالة الصحية للمرضى واستجابتهم للعلاج.

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