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Platelet Count, Mean Platelet Volume and Aggregation as Markers of Disease Activity in Rheumatoid Arthritis

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic inflammatory disorder that affects small joints of hands and feet. Different forms of hematological disorders are associated with RA as anemia, neutropenia, thrombocytosis, thrombocytopenia, eosinophilia, and hematological malignancies. Thrombocytosis usually occurs during the active clinical stages of RA. Reactive megakaryocytopoiesis increases circulating platelets count and triggers hyperactivity. Hyperactive platelets target synovial membranes with subsequent local rheumatoid inflammation.

Objectives: To correlate the value of mean platelet volume (MPV), platelet count and platelet hyperactivity with Disease Activity Score (DAS 28 score) and their reflection on cardiovascular system in RA.

Patients and Methods: Fifty newly diagnosed RA patients attending the Clinical Rheumatology Unit during the year 2012 with fifteen age- and sex-matched control subjects were randomly selected. For all, CBC including platelet cont, MPV, platelet aggregation using ADP, echocardiography in addition to ECG, ESR and CRP were done.

Results: MPV, Platelet count, CRP and ESR were significantly higher in RA patients than controls, while Platelet aggregation, hemoglobin level, mean diastolic function and ejection fraction (EF) were significantly lower in RA patients than control group. Significant positive correlations were detected between DAS28 score and both MPV and platelet count, while significant negative correlations were found with both hemoglobin and EF. Significant increase of the platelet aggregation in sero-negative compared to sero-positive rheumatoid arthritis patients was detected.

Conclusion: Platelet count and MPV are inexpensive tests, may be useful for a rapid assessment of disease activity in patients with RA.

Key Words: *Platelet aggregation – MPV – Rheumatoid arthritis.*

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that affects many tissues and organs, but principally attacks synovial joints. Although the cause of rheumatoid arthritis is unknown, autoimmunity plays a pivotal role in both chronicity and progression, so RA is considered a systemic autoimmune disease **[1]**. Hematological manifestations in RA can be broadly categorized into anemia, thrombocytosis, neutropenia, thrombocytopenia, particularly autoimmune and drug induced thrombocytopenia; and hematological malignancies **[2]**.

Anemia associated with RA, known as rheumatoid anemia, is a typical example of anemia of chronic disease **[3]**. In the absence of effective treatment, anemia is highly prevalent among RA patients. The mechanisms involved in rheumatoid anemia include shortening of the erythrocyte lifespan, inadequate bone marrow erythropoiesis in response to anemia, and iron metabolism abnormalities **[4]**. Rheumatoid anemia is usually mild to moderate, normocytic normochromic or, less often, microcytic **[5]**.

Thrombocytosis in rheumatoid arthritis is common and correlates with disease activity. The exact pathogenetic mechanisms remain undetermined. Persistent overproduction of certain thrombocytopoietic factors can induce megakaryocytopoiesis and thrombocytopoiesis **[6]**. The megakaryocytopoiesis and inflammatory cascade of RA share hematopoietic cytokines and respond to a number of colony-stimulating factors. Progenitors of osteoclasts, important

during the development of erosion, are of hematopoietic lineage **[7]**. Moreover, the bone marrow could also be diseased in this inflammatory cascade. Therefore, increased platelet mass could be a reflection of the affected bone marrow. Thrombocytosis usually occurs during the active clinical stages of RA **[8]**. Reactive megakaryocytopoiesis increases circulating platelets count and triggers hyperactivity. Hyperactive platelets target synovial membranes with subsequent local rheumatoid inflammation. Hyperactive platelets interact with other cells, and target the vascular wall. Considerable evidence indicates that patients with RA are prone to premature ischemic heart disease (IHD), myocardial infarction (MI) and heart failure. Disease modifying anti-rheumatic drugs (DMARD) decrease platelet activity **[9]**.

Availability of automated blood cell analyzers has made the measurement of platelet count and morphology common practice. Mean platelet volume (MPV) is emerging as an indicator of platelet reactivity, which could estimate cardiovascular risk **[10]**. Increased MPV is related to acute vascular events such as destabilization of atherosclerotic plaque, unstable angina, MI and paroxysmal atrial fibrillation **[11]**. MPV is an independent risk factor and predictor of MI in predisposed subjects **[12]**.

PATIENTS AND METHODS

Fifty newly diagnosed RA patients, 38 males and 12 females were recruited from Rheumatology Unit of Internal Medicine department of Assuit University hospital. Their ages ranged from 18 to 42 with a mean of 29.2±6.2 and a median of 24 years. Thirty six patients (72%) of them were diagnosed as sero-positive, while the remaining 14 patients (28%) were diagnosed as sero-negative rheumatoid arthritis. Twenty five controls, 8 males and 17 females were included; their ages ranged from 19 to 40 with a mean of 28.5±4.5 and a median of 27 years. Patients with history of bleeding tendency, cardiovascular diseases, anemia, liver diseases or receiving NSAIDs were excluded. All patients were subjected to complete medical history and examination, Disease Activity Score (DAS28) was measured for all patients. Written Informed consents were taken from all subjects; this study was approved by the ethical committee of Assiut University. To all patients and controls the following investigations were performed: CBC including MPV, ESR, CRP, RF, blood urea, serum creatinine, random blood glucose, platelet function tests for platelet aggregation using Platelet Aggregation Profiler (PAP-4, USA); Reagent: ADP. Also, Complete M-mode, 2-dimensional, and Doppler echocardiography was performed at rest.

Statistical methods: The data obtained were calculated and statistically analyzed by using SPSS data analysis program. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Patients with RA showed significantly increased ($p<0.001$) MPV (fL) and platelet count ($x10^9$/L) than controls, while platelet aggregation tests using ADP, hemoglobin level (gm/dl), and Ejection fraction (%) were significantly lower ($p<0.001$) in patients than control group (Table 1). The mean levels of ESR (in the 1st and 2nd hours), CRP in mg/L and RF in u/mL of the studied patients were significantly higher ($p<0.001$) when compared with controls (Table 2). Also 21 patients (42%) showed grade I diastolic dysfunction and 14 (28%) showed grade II diastolic dysfunction, while all controls showed normal diastolic function ($p<0.001$).

According to the Disease Activity Score (DAS28) 36 patients (72%) showed moderately active RA >3.2 ≤5.1, and 14 (28%) showed very active RA >5.1, while all control group showed RA ≤3.2. There was a significant positive correlation between DAS28 score and MPV and platelet count ($r=0.58$, $p=0.0001$ and $r=0.408$, $p=0.003$ respectively), while a significant negative correlation was detected between DAS28 score and hemoglobin level ($p=0.000$ and $r=–0.798$) and between DAS28 score and EF ($p=0.011$ and $r=–0.358$) (Fig. 1). No correlation was encountered between DAS28 on one side and ESR 1st and 2nd hour, CRP or platelet aggregation on the other side ($r=0.117$, 0.082, 0.064 and 0.096) respectively.

From the total 50 RA patients, 36 patients (72%) were diagnosed as sero-positive, while the remaining 14 patients (28%) were diagnosed as sero-negative rheumatoid arthritis. A significant increase ($p<0.05$) of the platelet aggregation

in sero-negative rheumatoid arthritis was detected when compared to sero-positive rheumatoid arthritis patients. The DAS28 was significantly higher (p<0.05) in sero-positive when compared to sero-negative rheumatoid arthritis patients, while no significant differences were detected between sero-positive and sero-negative patients as regards MPV, platelet count, ESR, CRP, WBC count, EF or diastolic function (Table 3).

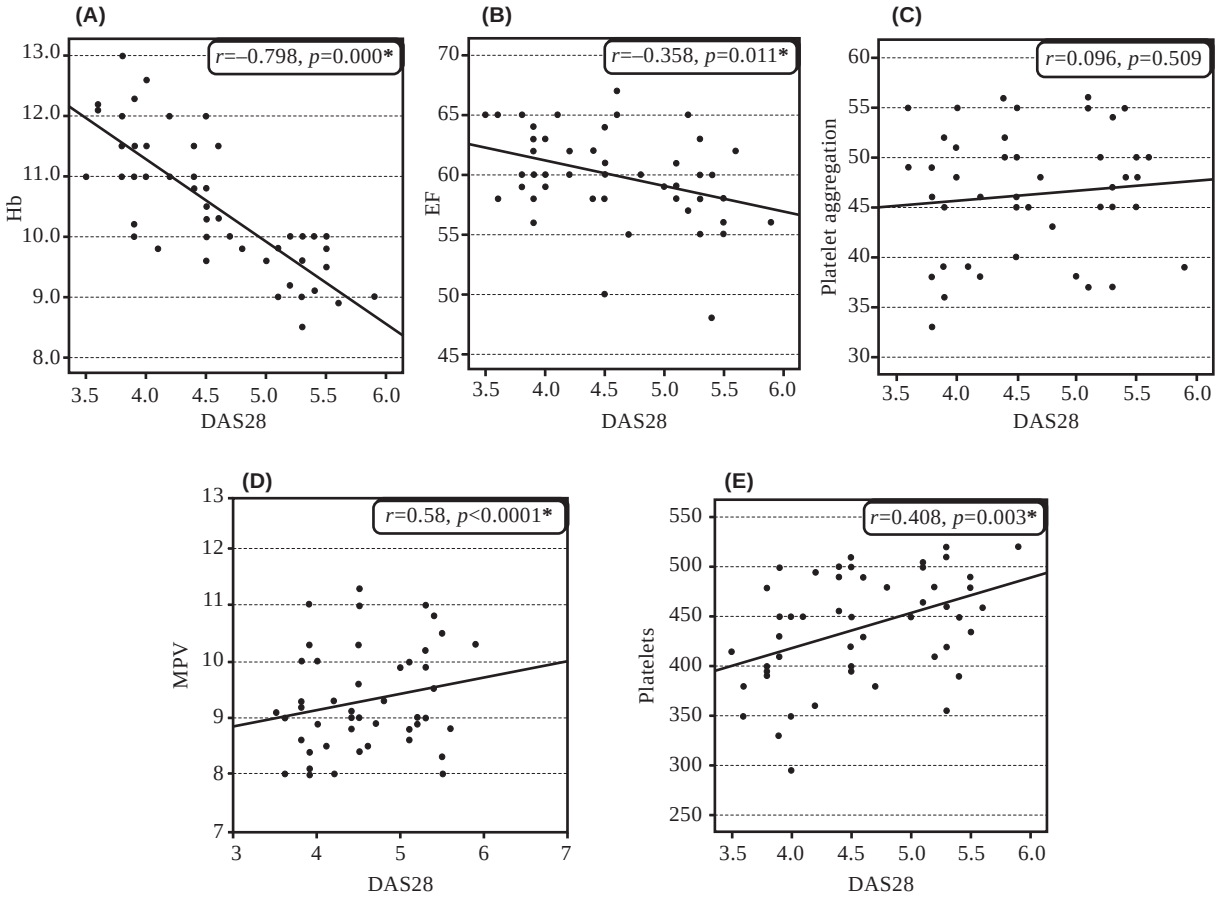

Fig. (1): Correlation between DAS28 score and (A) Hemoglobin (Hb), (B) Ejection Fraction (EF), (C) Platelet aggregation, (D) Mean Platelet Volume (MPV) and (E) Platelet count.

Table (1): Comparison of Hb level, Platelet Count, MPV, platelet aggregation and Ejection Fraction between Rheumatoid Arthritis patients and controls.

Variables	Patients (n=50)	Control (n=25)	p-value
MPV (fl)	8-11 (9.3±0.9)*	8-9 (8.2±0.3)	<0.001
Platelet aggregation using ADP (%)	33-56 (46.2±6.0)	55-70 (61.6±5.1)	<0.001
Platelet x10^9/L	295-520 (438.8±54.8)	160-400 (269.2±59.9)	<0.001
Hb (gm/dl)	8.5-13 (10.5±1.1)	12-14.8 (13.2±0.7)	<0.001
EF %	48-67 (59.9±3.7)	58-66 (63.8±2.6)	<0.001

*Range (mean±SD) ADP: Adenosine Diphosphate.

Hb = Hemoglobin level.
MPV = Mean platelet volume.
fl = Femtolite.
EF = Ejection Fraction.

Table (2): ESR, CRP and RF levels in patients and controls.

	Patients (n=50)	Control (n=25)	*p*-value
ESR 1st hour	17.7±35.6*	7.5±1.4	***$p<0.001$
ESR 2nd hour	60.6±19.4	14.7±2.9	***$p<0.001$
CRP	39.8±28.5	3.1±4.3	***$p<0.001$
RF	26.2±25.5	1.2±2.4	***$p<0.001$

ESR : Erythrocytic Sedimentation Rate.
CRP : C-reactive protein.
RF : Rheumatoid factor.
$p<0.001$: Highly significant.
* : Mean±SD.

Table (3): Comparison between sero-positive and sero-negative Rheumatoid Arthritis patients.

Variables	Rheumatoid factor		*p* value
	Negative (n=14)	Positive (n=36)	
ESR1 (mm/h)	34.64±18.76*	36.03±17.58	NS
ESR2 (mm/h)	60.57±19.73	60.64±19.61	NS
CRP	46.14+39.27	37.28+23.23	NS
WBCs $x10^9$/L	6.92±1.79	6.71±1.19	NS
Platelet $x10^9$/L	440±95.5	438.31±89.56	NS
MPV (Fl)	9.06±1.24	9.39±0.79	NS
Platelet aggregation using ADP (%)	43.29±6.38	47.39±5.49	<0.05
DAS28	4.20±0.55	4.72±0.64	<0.05

* : Mean±SD.
ESR : Erythrocyte Sedimentation Rate.
CRP : C-reactive protein.
WBCs : White Blood Cells
MPV : Mean platelet volume.
fl : Femtoliter.
ADP : Adenosine Diphosphate.
NS : Not significant.

DISCUSSION

Hematological manifestations in RA can be broadly categorized into areas of anemia, thrombocytosis, leukocytosis, thrombocytopenia, particularly autoimmune and drug induced thrombocytopenia even hematological malignancies **[2]**.

In the current study patients with RA showed significantly increased mean platelet count than controls with positive correlation between DAS28 score and platelet count, a finding which is similar to the results of Kisacik et al. **[13]**. The pathogenesis of increased platelet count in those patients is mostly due to enhancement of cytokines release with increased disease activity **[14,15]**.

Rheumatoid patients in the current study are mostly demonstrating a pattern of normocytic normochrmoic anemia, their mean hemoglobin level is lower than that documented by Yazici et al. **[16]**, but similar to Kisacik et al. **[13]** study. Moreover, there was strong positive correlation between DAS28 score and the degree of anemia. Severity of anemia and thrombocytosis is associated with the disease activity in RA **[14]**. These hematopoietic presentations particularly thrombocytosis are presumably mediated by cytokines and growth factors, including Il-1, IL-3, IL-4, IL-6, IL-11 and TNF-α. Amongst these mediators, IL-6 is one of the major cytokines responsible for inflammation in RA, which has also a regulatory function on acute phase response **[15]**. Moreover, some authors suggested that IL-6 is the primarily responsible cytokine in secondary thrombocytosis; therefore, it is reasonable to find an association between disease activity, acute phase markers and platelet characteristics in inflammatory disorders. Some studies suggested participation of platelets in the inflammation of RA **[14]**. There is evidence of platelet activation in RA **[16]** and MPV reflects platelet activation **[17]**. Mean platelet volume is an important platelet histogram index reported by hospital laboratories in daily clinical practice. Several previous reports showed the utility of MPV as a marker of platelet activation based mainly on the fact that in this activation process the platelets change their shape and the volume. Otherwise, MPV as a marker of platelets activation has been demonstrated to have prognostic importance in patients with cardiovascular disease and a large volume may be regarded as a marker of platelets activation **[18,19]**. Mean platelet volume (MPV) in the current study was significantly higher in RA patients; this finding is similar to the study of Gasparyan et al. **[9]**.

Yazici et al. **[16]** demonstrated a significant decrease in platelet count and MPV after treatment by tocilizumab for 6 months. Another study done by Kisacik et al. **[13]** revealed that MPV is significantly decreased in active rheumatoid

arthritis patients than control (osteoarthritis patients) with mild degree of increase after treatment. They suggested that small MPV may reflect accelerated maturation and short life span of platelets in active RA. The difference between the 2 previous studies may be attributed to technical reasons, age of patients, sample size and/or inclusion of patients with co-morbidity.

In the current study, MPV was correlated with DAS28 score in RA patients; furthermore, other disease activity markers demonstrated significant associations. Otherwise, certain studies previously revealed discordance between ESR and CRP levels and disease activity in RA **[20]**. Therefore, MPV is an inexpensive test that may be useful for a rapid, at a glance assessment of disease activity in patients with RA.

In the current study we reported a positive correlation between platelet aggregation test using ADP and DAS28 score. This is coinciding with Mac Mullan et al. **[21]** study. However it is worth mentioning that the cause of weak platelet aggregation may be attributed to that most of our patients might have received empirical treatment including non steroidal anti inflammatory drugs (NSAIDs) before attending to the outpatient clinic.

By using Doppler Echocardiography, 68% of patients were suffering from diastolic dysfunction. Early diastole depends on active relaxation of the ventricle, as well as passive properties of the ventricle that include wall thickness, chamber geometry, and myocardial stiffness **[22]**. Relaxation is an energy dependent process and influenced by load **[23]**. The cause of diastolic dysfunction in our patients is not well recognized. It may be explained by ischemia due to premature atherosclerosis of the coronary arteries which may be attributed to increase in MPV and platelet hyperactivity. Early detection of ischemia for these patients may need stress ECG which is practically difficult as many of RA patients have knees arthritis. Stress echocardiography can be an alternative with sensitivity reaching 80% in many patients but should be done under careful monitoring to avoid fatal arrhythmia. Coronary angiography is the gold standard for detection of coronary arterial atherosclerosis, but it is invasive and expensive. Multi Slice Computed Tomography (MSCT) coronary angiography can be a substitution and it has got good negative results to exclude ischemia, but the major disadvantage for using it is that it may give false positive results especially in patients having calcification in their coronaries.

Moreover, diastolic dysfunction in these patients may be explained by myocardial fibrosis causing restrictive filling of the heart and reducing diastolic volume of either or both ventricles. Constrictive pericarditis could be an explanation in some patients and should not be missed. Constriction is due to chronic inflammation and fibrosis of the pericardium often superimposed by calcification resulting in decreased ventricular compliance. There is increased end-diastolic pressure for any given end diastolic volume. The increased pressure affecting both ventricles equally and effectively decreases diastolic filling and thus end-diastolic volume of both ventricles. The increased pressure is transmitted backward and results in elevated pulmonary and systemic pressures. Equalization of end diastolic pressures in all four cardiac chambers is the hallmark of constrictive pericarditis. Other causes of diastolic dysfunction were excluded before collecting data from patients as morbid obesity, elderly persons, and uncontrolled diabetes mellitus to avoid misinterpretation.

In the current study, only 4% of patients had ejection fraction (EF) <55% (below normal range) with systolic wall motion abnormalities. As in patients with high output state due to anemia, the heart's systolic function index and EF are expected to be higher than in normal subjects. So, it has been recommended that a normal LVEF should be above 60% in anemic patients. Results of the current study are similar to that of Rudominer et al. **[24]** in which the vast majority of their patients had preserved EF. Also, diastolic dysfunction, lower LV mass, higher pulmonary arterial pressure and higher left atrial volume index.

In the current study, there was no difference between sero-positive and sero-negative patients as regards ESR, CRP, WBC count, platelet count and MPV. However, the mean DAS 28 of sero-positive patients was significantly higher than that of sero-negative patients. The same applies for platelet aggregation. ESR and CRP correlates closely with clinical disease activity in patients

with RA. However, certain studies revealed discordance between ESR or CRP levels and the disease activity in RA **[20]**.

Conclusion: Platelet count and MPV are inexpensive tests, may be useful for a rapid assessment of disease activity in patients with RA.

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