## Changes of Platelet Indices in Patients with Systemic Lupus Eythematosus and their Correlation with Disease Activity

WAFAA M. SHERIF, M.Sc.  $^1$ ; RASHA A. ABDEL NOOR, M.D.  $^2$ ; MOHAMED ATTIA, M.D.  $^3$  and NASHWA NOR EL-DEEN, M.D.  $^4$ 

The Departments of Internal Medicine<sup>1</sup>, Internal Medicine & Rheumatology<sup>2</sup>, Clinical Pathology<sup>3</sup> and Internal Medicine & Hematology<sup>4</sup>, Faculty of Medicine, Tanta University, Tanta, Egypt

#### **Abstract**

Background: Systemic lupus erythematosus (SLE) is autoimmune disease needs continuous assessment of disease activity. There is no single biomarker used for that purpose. Platelet indices have recently been found to be a simple inflammatory marker used in the assessment of systemic inflammation in many diseases like, rheumatoid arthritis, ankylosing spondylitis and inflammatory bowel diseases.

Aim of Study: To evaluate platelet indices [mean platelet volume (MPV), platelet distribution width (PDW) and platelet-crit (PCT)] as markers of disease activity in patients with SLE.

Patients and Methods: In this cross sectional study we tested 100 subjects; 20 healthy control and 80 SLE patients recruited from rheumatology unit in Internal Medicine Department, Tanta University, Tanta, Egypt. Then the lupus patients were divided according to SLEDAI into 20 patients without flare (SELEDAI <4), 17 patients mild flare (SELEDAI 4–<8), 25 patients moderate flare (SELEDAI 8–<12) and 18 patients severe flare (SELEDAI > 12). Platelet indices (MPV, PDW, PTC) were assessed in all of them and their correlation to SLEDAI score were analyzed.

Results: MPV and PDW had no clinical significance in assessing lupus activity, PCT significantly lower with SLE patients with severe flare and it had a significant negative correlation with SELEDAI score with cutoff value equals <0.173, sensitivity 61.11%, specificity 79.03%, PPV 45.8% and NPV 87.5%. Finally there was no significant difference between lupus nephritis and lupus without nephritis patients in Platelet indices.

*Conclusion:* Plateletcrit (PCT) can be used as a new marker for SLE activity.

**Key Words:** SLE – Lupus activity –SLEDAI – Platelet indices – Mean platelet volume – Platelet-crit – Platelet distribution width.

Correspondence to: Dr. Rasha A. Abdel Noor, E-Mail: rashaabdelnoor@yahoo.com rashaabdelnoor@med.tanta.edu.eg

#### Introduction

**SYSTEMIC** lupus ervthematosus (SLE) is a multisystem autoimmune disease. Considering its remitting and relapsing nature, it is important to have a biomarker to monitor its disease activity [1,2]. There is no single biomarker for that purpose till now but there are many validated measures, including Systemic Lupus Erythematosus Disease Activity Index (SLEDAI-SLENA), European Consensus Lupus Activity Measurement (ECLAM), British Isles Lupus Activity Group(BILAG) ... etc [3-6]. These tools have been found to be beneficial in day to day practice. Most of these activity indices use multiple parameters like clinical and laboratory parameters some of them are simple, easy to use in routine clinical practice like urine analysis, platelet and leucocyte counts, others may not be simply assessed during routine follow-up of patients like Anti-dsDNA titer, C3, C4. Platelet indices [Mean platelet volume (MPV) and platelet distribution width (PDW) and platelet-crit (PCT)] are a group of platelet parameters determined in automatic CBC profiles; they are related to platelets' morphology and proliferation kinetics and represent platelet activation. Mean platelet volume (MPV) is the mode of the measured platelet volume. Typically, the average mean cell volume is 7.2-11.7 fL in healthy subjects is an indicator of platelet function. It reflects activation of the platelets and it has been found to be related with inflammatory conditions. Under normal circumstances when platelet production is decreased, young platelets become bigger and more active so there is an inverse relationship between platelet size and number [7,8]. Platelet indices has been studied as an inflammatory marker in several diseases. Some studies have reported that MPV increases in myocardial infarction and cerebrovascular disease;

while in contrast, it decreases in active rheumatologic diseases including rheumatoid arthritis, ankylosing spondylitis and ulcerative colitis [9,12]. Platelet distribution width (PDW) is an indicator of volume variability in platelets size and is increased in the presence of platelet anisocytosis [13]. Platelet-crit (PCT) is the volume occupied by platelets in the blood as a percentage and calculated according to the formula PCT= Platelet count x MPV/10,000 The normal range for PCT is 0.22-0.24%. In healthy subjects, platelet mass is closely regulated to keep it constant [14].

The present study aimed to investigate changes of mean platelet volume, platelet distribution width and platelet-crit in patient with SLE and their correlation with disease activity.

#### **Patients and Methods**

This cross sectional study was conducted on 100 subjects; 80 SLE patients were recruited from rheumatology unit of Internal Medicine Department (inpatient wards and outpatient clinics) of Tanta University Hospitals and 20 age and sex matched healthy persons as a control group in the period between May 2017 to April 2018.

### Inclusion criteria:

Patients with 4 or more criteria of the 2012 SLICC criteria (the Systemic Lupus International Collaborating Clinic) [15] and disease activity was assessed by the SLE Disease Activity Index (SLEDAI) [16].

## Exclusion criteria:

Other autoimmune diseases, malignancies and Infections:

This study is in agreement with the ethical guidelines of the Declaration of Helsinki and it follows the ethical standards of Tanta Faculty of Medicine. Informed consents from all patients were obtained in accordance with the local ethical committee. Privacy of all patients' data was granted as there was a code number for every patient file that includes all investigations.

All controles (group 1) and the patients (group 2) were subjected to:

Thorough history taking, Complete clinical examination, searching for signs of lupus activity.

#### Laboratory investigations including:

Complete blood count including (Mean platletvolume, platelet distribution width and platelet-

crit) were determined by using BCC-3000 Auto Hematology Analyzer within two hours of collection. Blood Urea and serum creatinine, Liver function tests, Erythrocyte Sedimentation Rate (ESR), C-reactive protein (CRP), Urine analysis, 24 hour urine proteins. Anti-Nuclear Antibody (ANA), Anti-ds DNA titer was done by ELISA.

*Reference value:* Negative: <40 U/ml. Equivocal: 40-60 U/ml. Positive: >60 U/ml.

Serum complement levels (C3 & C4) and Renal biopsy with histo-pathological examination, grading and determination of activity and chronicity indices of Lupus Nephritis.

Disease activity was assessed using the SLE disease activity index (SLEDAI) score and the SLE patients were divided into 4 subgroups:

- a- Without flare (were SELEDAI <4).
- b- Mild flare (4 < 8).
- c- Moderate flare (8 < 12).
- d- Severe flare (>or=12).

## Statistical analysis:

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level.

## Results

Our SLE patients are divided according to SLEDAI score into 4 groups and the demographic data, diseases duration are shown in Tables (1,2).

Regarding the CBC parameters and plateletindices, there was significant difference in Hb%, Hematocrit value, platelet count and platelet-crit between control and patient groups with marked decrease in platelet-crit in SLE patients with severe lupus flare and this is shown in Tables (3,4).

Table (1): Distribution of the studied cases according to SELEDAI score.

SELEDAI score	No.	%
Without flare (<4)	20	25.0
Mild flare (4– <8)	17	21.3
Moderate flare (8–12)	25	31.3
Severe flare (>12)	18	22.5
Min. – Max.	0.0-2	28.0
Median	8.	0

Table (2): Comparison between the different studied groups according to demographic data.

	SLE patients (n=80)											
		ontrol =20)	flare	thout e (<4) =20)	(4-	l flare - <8) =17)	flare	oderate e (8–12) n=25)	flare	evere (>12) =18)	Test of Sig.	p
	No.	%	No.	%	No.	%	No.	%	No.	%		
Sex: Male Female	6 14	30.0 70.0	3 17	15.0 85.0	1 16	5.9 94.1	1 24	4.0 96.0	0 18	0.0 100.0	$\chi^2 = 9.471 *$	$MC_{p}=0.021*$
Age (years): Min. – Max. Mean ± SD.		0–45.0 ±31.75		0-51.0 ±30.20		0-50.0 ±30.41		0–54.0 ±27.32.		0-55.0 ±32.22.	F= 0.943	0.443
		SLE patients (n=80)										
Duration (month)		thout flare (4) (n=20)		Mild flare (4– <8) (n=17)	2		ate flare (n=25)		Severe fla		Н	p
Min. – Max. Median		2.0–288.0 ).0		0.25–288. 24.0	0	0.25- 24.0	-180.0		0.25–132 30.0	2.0	8.333*	0.040*
Sig. bet. grps.		$p_1$ =0.023	*, p <sub>2</sub> =(	0.01 1 *, <b>p</b> 3:	=0.028	*,p <sub>4</sub> =0.9	72, <i>p</i> <sub>5</sub> =	0.915, p	o <sub>6</sub> =0.879			

<sup>:</sup> Chi square test.

Table (3): Comparison between the different studied groups according to CBC.

			SLE pat	ients (n=80)			
CBC	Control (n=20)	Without flare (<4) (n=20)	Mild flare (4– <8) (n=17)	Moderate flare (8–12) (n=25)	Severe flare (>12) (n=18)	Test of Sig.	p
Hgb (g/dl): Min.– Max. Mean $\pm$ SD.	10.20 - 14.50 11.97±1.34	5.30-14.0 10.68±2.52	8.30-14.40 11.17±1.50	6.20-15.0 10.02±2.08	7.10–14.0 10.56±2.06	F=2.984*	0.023*
pl Sig. bet. grps		0.239 $p_2$ =0.942, $p_3$	0.732 <sub>3</sub> =0.796, p <sub>4</sub> =1.00	0.011 * 0, <i>p</i> <sub>5</sub> =0.345, <i>p</i> <sub>6</sub> =0.	0.186 890, <i>p</i> <sub>7</sub> =0.900		
MCV (fl) Min. – Max. Mean ± SD.	76.0–87.80 82.52±3.46	62.0-94.0 81.47±7.45	70.20–97.0 82.31±6.59	71.0–95.0 80.17±6.11	79.0–97.0 85.14±4.52	2.00	0.101
HCT (%) Min. – Max. Mean ± SD. pl Sig. bet. grps	31.0–42.0 36.11±3.76	17.90–42.0 32.71±7.13 0.370 p <sub>2</sub> =0.980, p <sub>3</sub>	24.0–42.0 33.81±4.47 0.762 3=0.796, p <sub>4</sub> =0.75	16.50–46.70 30.61±6.65 0.021 * 8, p <sub>5</sub> =1.000, p <sub>6</sub> =0.	21.90–45.80 32.33±6.37 0.290 426, $p_7$ =0.879	F=2.556*	0.044*
PLT (x10 <sup>3</sup> /ul): Min. – Max. Median pl Sig. bet. grps	197.0–313.0 269.5	97.0–431.0 258.5 0.401 p <sub>2</sub> =0.425,p <sub>3</sub>	89.0-780.0 277.0 0.994 =0.924, p <sub>4</sub> =0.030	46.0–663.0 229.0 0.327 0, p <sub>5</sub> =0.354, p <sub>6</sub> =0.0	50.0–484.0 169.0 0.003* 004, p <sub>7</sub> =0.029*	H = 11.411*	0.022*
WBCs $(x10^3/ul)$ : Min. – Max. Mean $\pm$ SD.	4.40–10.0 6.81±1.78	3.0–26.0 7.84±5.0	3.30–15.0 7.08±3.25	2.50–15.0 7.69±2.95	3.80–13.90 7.57±3.27	H = 0.995	0.911

MC: Monte Carlo.

F: ANOVA test.

p: p-value for comparing between the different groups.

 $<sup>\</sup>chi^2$ : Chi square test. MC: Monte Carlo. F. ABOVA (88).

\* : Statistically significant at  $p \le 0.05$ .

H: Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test).

p: p-value for comparing between the different groups.
p2: p-value for comparing between without flare and mild flare.
p4: p-value for comparing between without flare and severe flare.

p5: p-value for comparing between mild flare and moderate flare.
p6: p-value for comparing between mild flare and severe flare.

p7: p-value for comparing between mild flare and moderate flare.
p7: p-value for comparing between mild flare and severe flare.

F: ANOVA test, Pairwise comparison bet. each 2 groups was done using Post HocTest (Tukey).

H: Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test).

p: p-value for comparing between the different groups.
p2: p-value for comparing between without flare and mild flare.
p4: p-value for comparing between without flare and severe flare.
p6: p-value for comparing between mild flare and severe flare.
p6: p-value for comparing between mild flare and moderate flare.
p7: p-value for comparing between moderate flare and severe flare.
p7: p-value for comparing between moderate flare and severe flare.

Table (4): Comparison between the different studied groups according to platelet-indices.

		SLE patients (n=80)					
	Control (n=20)	Without flare (<4) (n=20)	Mild flare (4– <8) (n=17)	Moderate flare (8–12) (n=25)	Severe flare (>12) (n=18)	Test of Sig.	p
MPV (fl): Min. – Max. Mean ± SD.	9.10-10.50 9.94±0.30	8.80–10.90 9.84±0.64	8.0–10.80 9.67±0.72	8.90-13.60 10.07±1.02	7.80–13.90 10.41±1.76	F=1.369	0.251
PDW (%): Min. – Max. Mean ± SD.	10.70–18.80 14.27±2.17	5.80-18.0 12.94±3.0	9.40–18.80 13.35±2.27	9.10–19.70 13.81±2.98	8.90-19.40 12.68±3.06	F= 1.088	0.367
PCT (%): Min. – Max. Median p1	0.027–0.307 0.257	0.102–0.3 83 0.255 0.394	0.095–0.632 0.271 0.898	0.062–0.616 0.224 0.264	0.050-0.440 0.159 0.001*	H= 14.073	0.007*
Sig. bet. grps		<i>p</i> <sub>2</sub> =0.490, <i>p</i> <sub>3</sub>	=0.828, p <sub>4</sub> =0.011,	$p_5 = 0.351, p_6 = 0.00$	2, <i>p</i> <sub>7</sub> =0.014*		

F: ANOVA test. H:Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test). p: p-value for comparing between the different groups.  $p_{\parallel}$  p-value for comparing between control group and each other groups.

Our results showed also no significant differences as regard platelet indices between lupus nephritis and lupus without nephritis patients (Table 5).

There was also significant negative correlation between platelet crit (PCT) % and SLEDAI score

(Table 6, Fig. 1) and the receiver operating characteristic curve (ROC) curve showed that plateletcrit has a cutoff value equal ≤0.173, 61.11% sensitivity 79.03% specificity, 45.8% PPV and 87.5% NPV in differentiating lupus patients with severe flare from other lupus patients (Table 7, Fig. 2).

Table (5): Comparison between the control group, and lupus nephritis patients and lupus patients without nephritis according to platelet indices.

	Control (n=20)	Non nephritis (n=54)	Nephritis (n=26)	Test of Sig.	p
MPV (fl):					0.947
Min Max.	9.10-10.50	8.0-13.60	7.80-13.90	F=0.054	
Mean $\pm$ SD.	$9.94 \pm 0.30$	$9.99 \pm 1.01$	$10.03 \pm 1.34$		
PDW (%):					0.323
Min. – Max.	10.70-18.80	5.80-19.50	8.90-19.70		
Mean $\pm$ SD.	14.27±2.17	13.20±2.72	$13.31 \pm 3.14$	F=1.145	0.130
PCT (%):		0.05.0.5	0.07.0.52		0.130
Min. – Max.	0.03-0.31	0.06-0.63	0.05-0.62	H = 4.077	
Median	0.26	0.24	0.20		

F: ANOVA test. H: Kruskal Wallis test.

p:p-value for comparing between the different groups.

Table (6): Correlation between SELEDAI score and platlet indices in cases group (n=80).

	rs	р
MPV (fl):	0.083	0.465
PDW (%):	-0.045	0.694
PCT (%):	-0.267*	0.017*

r : Spearman coefficient. \*: Statistically significant at *p*≤0.05.

Table (7): Agreement (sensitivity, specificity) for PCT (%) to predict severe flare patient.

	Cutoff	Sensitivity	Specificity	PPV	NPV
PCT (%)	≤0.173	61.11	79.03	45.8	87.5

p2: p-value for comparing between without flare and mild flare.

p3:p-value for comparing between without flare and moderate flare. p4: p-value for comparing between without flare and severe flare.  $p_5$ : p-value for comparing between mild flare and moderate flare.

p6: p-value for comparing between mild flare and severe flare. \* : Statistically significant at  $p \le 0.05$ .

p7: p-value for comparing between moderate flare and severe flare.

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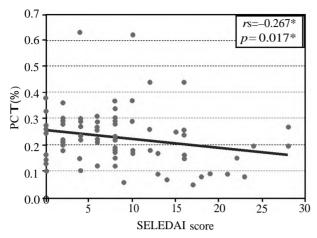


Fig. (1): Correlation between SELEDAI score and PCT (%) in cases group (n=80).

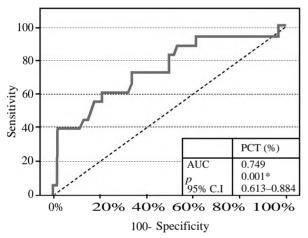


Fig. (2): ROC curve for PCT (%) to predict severe flare patient

## **Discussion**

Systemic lupus erythematosus is an autoimmune disease with frequent flares and remissions needs continuous monitoring of its activity. Some studies reported the association of platelet indices with some inflammatory and rheumatological diseases as rheumatoid arthritis, ankylosing spondylitis, behcets disease, systemic sclerosis and familial meditrenan fever (FMF) [9,12,17-22] but few studies tested this relation with SLE patients [23-25]. So we tried in this work to study the relation between SLE activity and platelet indices. We assessed MPV, PDW, PCT in 100 subjects; 20 healthy control and 80 SLE patients recruited from rheumatology unit in internal medicine department, Tanta University. The lupus patients divided into 20 patients without flare (SELEDAI <4), 17 patients mild flare (SELEDAI 4– <8), 25 patients moderate flare (SELEDAI 8–<12) and 18 patients severe flare (SELEDAI  $\geq 12$ ). Although we found that patients with moderate flare had lower mean values of hemoglobin and Heamatocrite than control group and the platelet count showed lower median values in severe flare patients than control group, MPV showed no statistical significance among studied groups and this in agreement with, El-Garf et al., 201 6 who found that there was no significant difference in the MPV between juvenile SLE patients with low disease activity and those with active disease [26]. On the conterary some studies as Yavuz [27], Çankaya et al., [28], Sarkar [29] who reported higher MPV level in the active phase of SLE patients compared to their inactive disease period. Also Rupa [30], Safak [24], Khan et al., [31] reported that MPV was lower in active SLE patients than in inactive SLE patients.

This heterogenicity may be due to the difference in clinical characteristics, treatment strategies, sexual ratio and race, we could not explore associations between these factors and MPV level. The potential mechanism of decreasing MPV level in active SLE patients may attribute to the release of bioactive molecules of pro-inflammatory platelets and plasma platelet-derived micro-particle in the presence of inflammation. At a matter of fact, overproduction of pro-inflammatory cytokines can suppress bone marrow following the production of small-size platelet [32]. In regard to what cause the elevated MPV, active SLE patients tend to have vascular events and thrombocytopenia with a higher frequency resulting increasing MPV [33,34].

In our study also PDW showed no statistical significance among studied groups and this not in agreement with Chen et al., [35] who noticed higher PDW values in SLE patients than healthy controls and the positive relationship between PDW and disease activity but they reported that there are no other reports or studies on PDW in patients with SLE [35].

PCT showed lower values in lupus patients with severe flare than other groups with statistical significance (p=0.007) between studied groups. There were no reported results regarding PCT in lupus but Metin et al., showed that the platelet-crit values seemed to be higher during active rheumatoid arthritis [36].

When we tried to find any relation or difference between lupus patients with and without nephritis in platelet indices our results showed no clinical significance between lupus patients with and without nephritis and this not in agreement with Yavuz et al., [27] who reported that MPV was superior to erythrocyte sedimentation rate, CRP and C3 to predict disease flares in juvenile SLE also Li L et

al., [37] reported that MPV might be disease activity indicators for SLE nephritis. Lastly Çankayaetal determined that MPV increases during the active period of SLE patients with renal involvement [28].

We noticed also no correlation between MPV, PDW and SELEDAI but strong negative correlation between PCT and SELEDAI with cutoff value equals <0.173, sensitivity 61.11%, specificity 79.03%, PPV 45.8% and NPV 87.5% in differentiating severe flare patients from other lupus patients and this in agreement with El-Garf etal who showed no correlation between MPV and SELEDAI [26], and not in agreement with Yavuz et al., who noticed that MPV was positively correlated with SLEDAI [27] and Delgado study where there was negative correlation between MPV and SELEDAI [23]. Chen et al., showed positive correlation between PDW and SELEDAI [35].

This study has some limitations as it is a single center study. In addition, the relative small sample size might affect its external validation. Another limitation that we did not report the patient medications and their effect on platelet indices. Also the selection of SLE patients who have different clinical complications, such as thrombocytopenia, or anti-phospholipid syndrome and vascular events may have different effect on these parameters.

So, we can conclude that mean platelet volume (MPV) and platelet distribution width (PDW) have no clinical significance in assessing lupus activity but Platelet-crit (PCT) can be used as a possible marker for SLE activity with cutoff value equals ≤0. 173, sensitivity 61.11%, specificity 79.03%, PPV 45.8% and NPV 87.5%.

Conflicts of interest: No conflict of interest.

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# تغيرات مؤشرات الصفائح الدموية في مرضى الذئبة الحمراء وأرتباطها بنشاط المرض

يعد مرض الذئبة الحمراء هو أحد الأمراض المناعية التى تهاجم أجهزة الجسم المختلفة ومن المهم أن يكون هناك دائماً مؤشر حيوى الرصد نشاط المرض. مؤشرات الصفائح الدموية هى تحاليل بسيطة روتينية تظهر صورة الدم فقد تستخدم كعلامة إلتهابية بسيطة فى عدة أمراض مثل الأمراض الروماتيزمية كمرض الروماتويد المفصلى وتقرحات القولون.

الهدف من الدراسة: التعرف على مؤشرات الصفائح الدموية في مرضى الذئبة الحمراء ومدى أرتباطها بنشاط المرض.

المرضى وطرق البحث: تم القيام بهدا البحث كدراسة مقارنة في مستشفيات جامعة طنطا. وتم إدراج مائة شحص في الدراسة. وتم أختيار المرضى من قسم الأمراض الباطنة (عيادات وعنابر وحدة الأمراض الروماتيزمية) خلال الفترة من مايو ٢٠١٧ إلى أبريل ٢٠١٨. وتم تقسيم المرضى إلى مجموعتين: المجموعة الأولى: مجموعة أصحاء وعددهم عشرون المجموعة الثانية: مرضى الذئبة الحمراء وعددهم ثمانون وتم تقسيم المرضى وفقاً لمؤشر سليداى سلينا إلى مجموعات فرعية: غير نشطة – قليلة النشاط – معتدل النشاط – شديدة النشاط، وتم خضوع جميع المرضى التى شملتها الدراسة إلى: دراسة التاريخ المرضى – إجراء الفحص الأكلينيكي – إجراء فحوصات المعملية الروتينية – متوسط حجم الصفائح وعرض توزيع الصفائح – سرعة ترسيب وسى أر بي – تحليل بول – تحاليل مناعية للذئبة – تجميع بول ٢٤ ساعة – وقياس كمية البروتينات – معامل سي٣ وسي٤ – تقييم مشاط المرض بإستخدام معيار سليداى سلينا.

أظهرت النتائج: إنخفاض نو قيمة إحصائية بين النسبة المئوية لحجم عينة الدم المشغولة بالصفائح في مريض الذئبة الحمراء شديد النشاط مقارنة بمرضى الذئبة الحمراء والأصحاء وأيضاً هناك علاقة عكسية بين النسبة الئوية لحجم عينة الدم المشغولة بالصفائح ونشاط مرضى الذئبة الحمراء المقاس بمعيار السليداي سلينا.

الأستتتاج والخلاصة: من تقييم نتائج هذه الرسالة ومقارنتها بنتائج الأبحاث الأخرى في نفس المجال يمكن التوصل إلى إستخدام النسبة المئوية لحجم عينة الدم المشغولة بالصفائح كدلالة لقياس نشاط مرض الذئبة الحمراء.