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RESEARCH ARTICLE

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Evaluating hypercoagulability in new-onset systemic lupus erythematosus patients using thromboelastography

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Abstract

Background: Thromboelastography (TEG) can reflect the coagulation status in vivo, from clot formation to clot lysis. In the present study, we aimed to evaluate the function of TEG in detecting coagulation in patients with SLE and sought to explore the correlation between clinical and laboratory data.

Methods: A total of 41 patients with new-onset SLE who had not undergone treatment and 56 healthy controls were included. TEG and other laboratory tests were performed, and clinical data were collected.

Results: A significant difference in the TEG reaction time and TEG achievement of clot firmness was observed between the groups. Moreover, these parameters were correlated with the lupus anticoagulant levels, platelet count, 24-hour urinary total protein quantity, and systemic lupus erythematosus disease activity index.

Conclusion: Our study demonstrated the prospective value of TEG in evaluating hypercoagulability in patients with SLE.

KEYWORDS

coagulation, SLE, thromboelastography

1 | INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disease that involves T cell, B cell, and dendritic cell dysfunction, as well as antinuclear autoantibody production.[1] Several clinical and scientific studies of SLE indicate an increased risk of thrombosis in these cases.[2-5] Moreover, patients with SLE may exhibit accelerated atherosclerosis, and higher cardiovascular morbidity and mortality.[6]

The traditional parameters for screening coagulopathy include prothrombin time (PT) and activated partial thromboplastin time (aPTT). However, in Owaidah's study, among patients with positive lupus anticoagulant (LAC) status, 61% exhibited abnormal aPTT results that could not be corrected by mixing with normal plasma; moreover, the PT test yielded normal results in 90% cases.[7] However, there is a limitation of the aPTT and PT test in these cases, as the results may reflect a certain product in the coagulation process, rather than the actual marker they are intended to reflect. Moreover, SLE

Gong, Shi and Zhou are contributed equally to this work.

patients with positive LAC status are at risk of thrombosis, although the aPTT results are often prolonged, in contrast to the coagulation status in vivo. In addition, the aPTT test is not sufficiently sensitive for detecting hypercoagulability. In fact, in patients with SLE, the aPTT test does not reflect coagulation function in vivo, and there is some inconsistency in the results between in vivo and ex vivo.[8]

Thromboelastography (TEG) was first described by Hartert in 1948.[9] This procedure facilitates the functional evaluation of the coagulation cascade, from clot formation to clot lysis,[10,11] and provides data on the entire coagulation system. TEG is based on a biochemistry method, whereas the standard coagulation tests are tested in a chemistry method, so TEG is more capable of reflecting the coagulation status in vivo. Due to the limitations of standard coagulation tests, TEG has become a valuable tool for evaluating the coagulation status.[10] The TEG offers 4 important parameters, including TEG reaction time (TEG:R) that represents clotting factor function, TEG achievement of clot firmness (TEG:K) and TEG kinetics of clot development (TEG:A) that reflect fibrin and platelet (PLT) function, and TEG maximum amplitude (TEG:MA) that indicates the strength and stability of the blood clot. For patients with SLE, especially LAC status, PT and aPTT could not reflect the actual marker they are intended to reflect, and the function of PT and aPTT is limited in patients with SLE. TEG could be a good choice for detecting the coagulation status for lupus patients, since it provides data on the entire coagulation system and is more capable of reflecting the coagulation status in vivo.

In the present study, we used TEG to investigate whether ex vivo clotting is altered in patients with SLE, as TEG can detect hypercoagulability. Moreover, we sought to determine the relationships between clotting parameters and the clinical and laboratory data in SLE.

2 | MATERIALS AND METHODS

2.1 | Patients

We reviewed new-onset SLE patients' clinical and laboratory data from October 2016 to October 2018. All patients were recruited from Ruijin Hospital. A total of 41 patients with new-onset SLE who had not undergone treatment were enrolled, and 56 healthy individuals were included as controls. Patients were diagnosed according to the American College of Rheumatology (ACR) criteria. We excluded healthy individuals with a history of hematological or coagulation disorders, as well as those taking anticoagulant therapy. The study was performed in accordance with the Declaration of Helsinki and the Principles of Good Clinical Practice and approved by the Institutional Review Broad of Ruijin Hospital (ID:2016-62), Shanghai Jiaotong University School of Medicine, Shanghai, China.

2.2 | Sample collection

Blood samples were collected using 18G needles and vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) (for complete blood cell count) and 3.2% trisodium citrate (for TEG and coagulation analyses). Moreover, 24-hour urine collection was performed in a clean dry container. All samples were processed within 45 minutes-3 hour after collection. All the human subjects were collected complied with the World Medical Association Declaration of Helsinki.

2.3 | Laboratory tests

Complete blood counts were obtained using an automated hematology analyzer DXH800 (Beckman Coulter). The reference range for PLT count was 101-320 × 10^9/L.

Routine coagulation screening assays, including PT, aPTT, fibrinogen (Fg), thrombin time (TT), d-dimer (DD), and fibrinogen/fibrin degradation products (FDP), were performed in all the patients using an automated coagulometer CS5100 (Sysmex) according to the manufacturer's instructions. The reference ranges for the parameters were as follows: PT, 10.0-16.0 seconds; aPTT, 22.3-38.7 seconds; Fg, 1.8-3.5 g/L; TT, 14.00-21.00 seconds; FDP, 0-5 mg/L; and DD, <0.55 mg/L.

LAC status was examined using ACL-TOP700 (Instrumentation Laboratory), and a value >1.2 was considered to indicate a positive status. Moreover, 24-hour UTP was performed using an automated chemical analyzer AU5800 (Beckman Coulter); the reference range for the UTP was <150 mg/24 h. C-reactive protein (CRP) levels were determined using an automated immunology analyzer IMMAGE 800 (Beckman Coulter); the reference range was <0.8 mg/L.

2.4 | TEG analysis

Blood clot formation and stability were evaluated via TEG tests using a thromboelastography analyzer (Haemoscope). Citrated whole blood was pre-processed with kaolin, and 20 μL 0.2 mol/L $CaCl_2$ was added into 340 μL of processed blood sample. Thereafter, the tests were conducted automatically after the injection of the blood sample, and graphical results were obtained. Reference ranges for TEG were as follows: TEG reaction time (TEG:R), 5-10 minutes; TEG achievement of clot firmness (TEG:K), 1-3 minutes; TEG kinetics of clot development (TEG:A), 53-72 degrees; and TEG maximum amplitude (TEG:MA), 51-69 mm.

2.5 | Statistical analysis

Statistics analysis was performed using SPSS Statistics version 22. Student's *t* test was performed for continuous normally distributed data, and Wilcoxon's rank test was performed for non-normally distributed data. The correlation between variables was analyzed with Spearman's rho analysis test. A *P* value of .05 was considered as significant.

TABLE 1 Clinical characteristics of the patients

	SLE	Healthy control	*P* value
Sex (F/M)	36/5	10/46	.000
Age (y, mean [range])	43.4 (17-69)	63.2 (48-78)	.000
Disease duration (mo, mean [range])	25.8 (0.25-480)	NA	
SLEDAI (mean [range])	5.55(0-17)	NA	
Oral ulcers (number [%])	6 (14.6)	0	
Arthritis (number [%])	22 (53.7)	0	
Vasculitis (number [%])	4 (9.8)	0	
LAC positive (number [%])	11 (26.8)	0	
Platelet count (mean ± SD, $\times 10^9$/L)	133.71 ± 60.25	193.67 ± 44.94	.000
CRP (mean ± SD, mg/L)	0.95 ± 2.02	0.24 ± 0.18	.040
UTP (mean ± SD, mg/24 h)	714.162 ± 1506.59	NA	
Positive thromboembolic history (number [%])	1 (2.4)	0	
Prolong PT and/or aPTT (number [%])	3 (7.3)	0	
Proteinuria (number [%])	11 (26.8)	0	

Abbreviations: CRP, C-reactive protein; LAC, lupus anticoagulant; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index; UTP, 24-hour urinary total protein quantity.

3 | RESULTS

3.1 | Clinical characteristics

Table 1 shows the clinical characteristics of the 2 groups. Of the 41 patients, 36 were women, 5 were men, and the mean age was 43.4 years. One patient had a positive history of thromboembolism. Among the patients with SLE, the platelet count and CRP levels were 133.71 ± 60.25 × 10^9/L and 0.95 ± 2.02 mg/L, as compared to 193.67 ± 44.94 × 10^9/L and 0.24 ± 0.18 mg/L, respectively, in the healthy control group (*P* = .000 and .040, respectively).

3.2 | Results of TEG and routine coagulation screening assays

Table 2 indicates the results of all the TEG tests and routine coagulation screening assays. In the patients with SLE, the TEG:R values were slightly lower than that in the reference range. The values of several parameters differed between the groups, although most of the values were within the reference range. However, no difference was found between the two groups of SLE patients with positive and negative anticoagulant lupus (data not shown). These findings suggest that there are significant differences in the rate of clot formation between SLE patients and healthy control patients.

All the traditional coagulation screening assays, except for TT, indicated significant differences between the 2 groups. Moreover, the FDP and DD results in patients with SLE were higher than the reference ranges. In addition, the PT and aPTT results in the patients with SLE were slightly greater than those in the healthy controls.

TABLE 2 TEG parameters and results of routine coagulation screening assays in SLE patients and healthy controls

	SLE (n = 41)	Healthy control (n = 56)	*P* Value
R (min)	4.97 ± 0.95	5.70 ± 0.86	.000
K (min)	1.41 ± 0.42	1.93 ± 0.43	.000
A (degrees)	70.03 ± 5.60	68.36 ± 5.44	.154
MA (mm)	64.37 ± 5.88	63.06 ± 4.48	.249
PT (s)	12.07 ± 1.11	10.85 ± 0.63	.000
aPTT (s)	32.62 ± 9.09	28.56 ± 1.88	.009
Fg (g/L)	2.99 ± 0.94	2.58 ± 0.50	.014
TT (s)	18.39 ± 1.60	18.36 ± 1.04	.912
FDP (mg/L)	6.63 ± 9.52	1.34 ± 0.86	.001
DD (mg/L)	1.71 ± 2.49	0.31 ± 0.21	.001

Abbreviations: A, TEG kinetics of clot development; aPTT, activated partial thromboplastin time; DD, d-dimer; FDP, fibrinogen/fibrin degradation products; Fg, fibrinogen; K, TEG achievement of clot firmness; MA, TEG maximum amplitude; PT, prothrombin time; R, TEG reaction time; SLE, systemic lupus erythematosus; TEG, thromboelastography; TT, thrombin time.

3.3 | Relationships between TEG parameters and clinical/laboratory data

Several clinical and laboratory parameters were recorded, and their relationship with the TEG parameters was examined. As shown in Figure 1, TEG:R was correlated with the LAC status (r^2 = .603, *P* = .000), TEG:K was negatively correlated with PLT and UTP levels (r^2=−.443 and −.387, *P* = .005 and .018, respectively), TEG:A was correlated with PLT and UTP levels (r^2 = .435 and .424, *P* = .006 and .009, respectively),

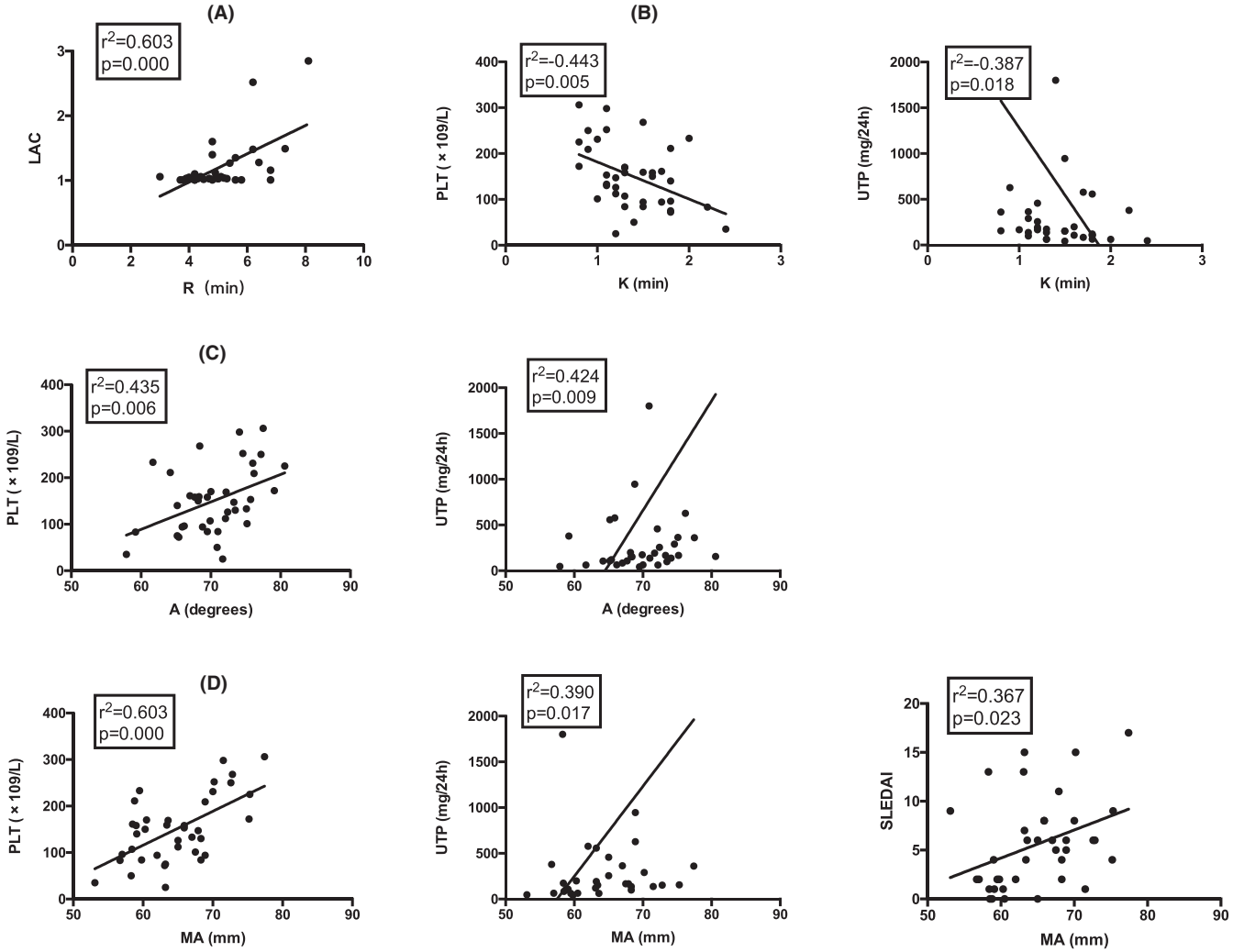

FIGURE 1 Correlation between the clinical and laboratory results in SLE patients and TEG parameters. Only the variables with significant correlations are shown. *, significant difference. LAC stands for lupus anticoagulant; PLT stands for platelet count; UTP stands for 24-hour urinary total protein quantity; SLEDAI stands for systemic lupus erythematosus disease activity index

and TEG:MA was correlated with the PLT, UTP, and SLEDAI values (r^2 = .603, .390, and .367; *P* = .000, .017, and .023, respectively).

We divided the patients with SLE into 2 groups based on the SLEDAI values (0-4 and ≥5), and found that all the 4 TEG parameters were significantly different between the 2 groups (Figure 2; TEG:R: 5.44 ± 1.19 vs 4.69 ± 0.83; TEG:K: 1.52 ± 0.35 vs 1.27 ± 0.39; TEG:A: 68.51 ± 4.68 vs 72.18 ± 5.26; TEG:MA: 62.29 ± 5.29 vs 66.99 ± 5.62; *P* = .028, .046, .030, and .012, respectively).

4 | DISCUSSION

In the present study, we found that the TEG parameters differed between the SLE patients and healthy controls. Moreover, the TEG parameters were correlated with the clinical and laboratory data of patients with SLE.

SLE is a multifactorial, autoimmune rheumatic disease. As is well known, inflammatory manifestation is the most typical feature of SLE, and clinicians primarily focus on controlling the progression of inflammation in these cases. However, SLE complications that can increase mortality and reduce the quality of life should also be carefully considered.[12,13] Among these, thrombosis is a well-defined and important clinical complication in SLE.[2-5] In particular, cerebral and cardiovascular ischemic events are major causes of irreversible damage and death in patients with SLE.[14-16] Previous studies have found that the probability of arterial ischemic events is 5.1%-8.5% within 5 years of diagnosis in patients with SLE.[2] With regard to venous events, the estimated probability is 3.7%-10.3% within 5 years of diagnosis.[2] Compared with the general population, patients with SLE exhibit a 4.5- to 12.7-fold increased risk of deep vein thrombosis and a 3.0- to 19.7-fold increased risk of pulmonary embolism.[17,18] In the present study, only 1 patient had a positive history of thromboembolism. Although this prevalence is lower than that usually reported, in the present study, we included new-onset patients who probably had a relatively shorter disease duration and an insufficiently long follow-up duration. Moreover, the overall number of participants was small.

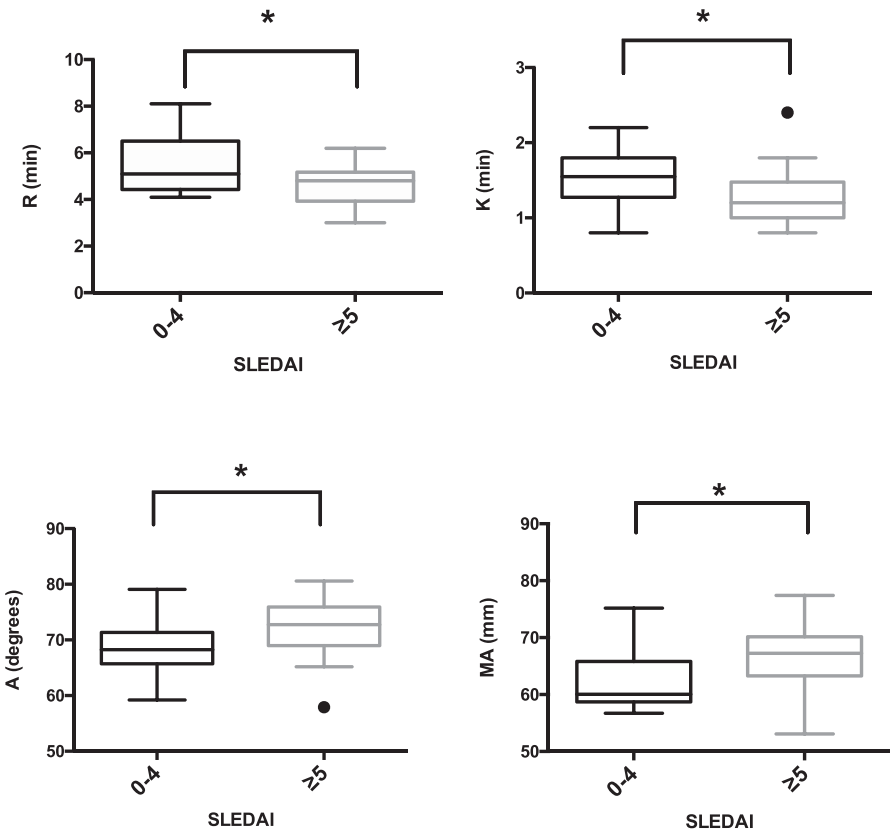

FIGURE 2 Comparison between the 2 groups of SLE patients, divided based on the SLEDAI values. *, significant difference. SLEDAI stands for systemic lupus erythematosus disease activity index. Black bar stands for SLEDAI 0-4 group, gray bar stands for SLEDAI ≥ 5 group

The traditional parameters for evaluating coagulation function, especially PT and aPTT, are not sufficiently sensitive for the detection of the LAC status. More importantly, they are not specific to LAC and LAC is not the only coagulation parameter responsible for the hypercoagulability in SLE.[19] The use of these parameters is limited in patients with SLE. In the present study, there was a significant difference in the PT and aPTT values between the SLE cases and healthy controls, in patients with SLE, the results were prolonged, indicating the interference of LAC. The FDP and DD tests exhibited a difference between the 2 groups, and the average values in patients with SLE were higher than the reference ranges, indicating a trend toward the development of thrombosis.

As these well-known assays are not ideal for evaluating the coagulation status of patients with SLE, perhaps in the future TEG could be the method of choice for assisting clinicians. In a study on Behcet's disease (BD), a significant difference in the TEG parameters was observed between BD patients and the control group.[20] In Cansu's study, the researchers concluded that TEG can be used in clinical practice to predict thrombotic events in patients with rheumatoid arthritis (RA).[21] Therefore, we discussed whether TEG could be helpful to evaluate the risk of thrombotic events in patients with BD and RA, and if so, the manner in which TEG can facilitate the evaluation of patients with SLE. In the present study, TEG:R and TEG:K were significantly different between the 2 groups, which indicated that the function of thrombin in patients with SLE was enhanced; thus, these patients were at risk of thrombosis. This finding was inconsistent with that determined by Collins et al,[22] who found that the TEG parameters were similar between 11 patients with SLE and 13 healthy controls. We believe that this inconsistency may result from the small number of patients involved in the studies, as it might only partly reflect the disease pattern in those cases. Moreover, as the patients in the 2 studies were from different ethnic groups, the probability of thrombosis occurrence may differ.[2]

The TEG parameters were correlated with other clinical and laboratory data, such as LAC status, PLT level, and UTP and SLEDAI values. Patients with positive LAC status,[23] higher PLT levels,[24] UTP >500 mg/24 h,[25] and higher SLEDAI[26] values are known to have a greater risk of thrombosis. Figure 2 shows the change in the parameters with disease severity in the active disease patient group. Thus, together with these tests, TEG could help clinicians identify the group of patients with a high risk of thrombosis.

5 | CONCLUSIONS

In conclusion, our data show that TEG parameters were different between SLE patients and healthy cases. But up to now, no thrombotic events were found in the study, so we do not know the correlation between the clinical events and the laboratory findings, this was a major limitation of this study. Future studies should include additional newly diagnosed SLE patients and longer follow-up, in order to appropriately evaluate the utility of TEG in predicting the risk of thrombosis in patients with SLE.

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