Levels of tissue factor pathway inhibitor in lupus patients correlate with lupus activity and endothelial damage markers

Tissue factor (TF) is a low molecular weight glycoprotein considered a major regulator of coagulation.[1] Tissue factor pathway inhibitor (TFPI) appears to play a primary role in regulating TF-induced coagulation, as it is a potent inhibitor of activated factor VII/TF complexes. TFPI is expressed by the endothelium under normal physiologic conditions. The plasma concentration of TFPI is low, and only small amounts circulate in plasma as full-length molecule (free-TFPI).[2]

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Levels of TF and total-TFPI have been described in patients with antiphospholipid syndrome (primary and secondary to systemic lupus erythematosus [SLE]) have been described to be higher than those in healthy controls.[4] The aim of our study was to analyze the TF/TFPI system in SLE patients. We also studied some markers of endothelial damage to assess the relationship between the TF/TFPI system and endothelial injury. Finally we studied the relationship between the TF/TFPI system and lupus activity.

We included 62 SLE patients (57 women, 32.2±15.9 years) who fulfilled the American College of Rheumatology 1982 revised criteria for SLE. Exclusion criteria for this study were: a present or recent thrombotic event (<12 months), oral anticoagulation, pregnancy, infectious processes or malignancy. The control group consisted of 23 healthy subjects with similar age and sex. Disease activity was determined in all patients with the overall *Systemic Lupus Activity Measure* (SLAM) score (32 items graded between 0-1 or 0-3).[5]

Plasma levels of soluble TF, total- and free-TFPI were measured using an enzyme immunoassay (ELISA) technique (Asserachrom Diagnostica STAGO, France). Antiphospholipid antibodies (APA) were determined by ELISA (Diagnostica STAGO, France). Lupus anticoagulant (LA) was tested by the platelet neutralization procedure (Staclot PNP, Diagnostica STAGO, France). Plasma levels of von Willebrand factor (VWF) were measured by an immunologic test (LIA test, Boehringer Manheim, Germany), and antigenic tissue-type plasminogen activator (t-PA) and its inhibitor (PAI-1) levels by ELISA (Asserachrom kit, Diagnostica STAGO, France).

Continuous variables were tested for normal distribution. Results are expressed as a median (25^{th}-75^{th} percentiles) or mean±standard deviation. Statistical analyses were performed by the Mann-Whitney-U test and Student's t test for independent samples. Spearman's and Pearson's test were used to identify correlations. The differences and correlation coefficient with a 2-tailed p value <0.05 were considered significant.

Thirty-two patients were APA and/or LA positive. Thirteen patients fulfilled criteria for the diagnosis of antiphospholipid syndrome (7 miscarriages, 6 thrombocytopenia, 3 thrombosis). Research indices are shown in Table 1.

We found a positive correlation between free-TFPI levels and VWF (r:0.305; p=0.016). We also found a positive correlation between disease activity and total-TFPI (r:0.379, p=0.003), free-TFPI (r:0.452, p<0.001), t-PA (r:0.400, p=0.001) and PAI-1 levels (0.470, p<0.001). We have found that SLE patients had elevated plasma levels of total- and free-TFPI and endothelial cell markers, with a positive correlation between free-TFPI and VWF. An important part of TFPI originates from the enhanced synthesis and/or release in stimulated endothelial cells.[6] The free form of TFPI may reflect the level of endothelial cell-associated TFPI, and it is thought to play the most important antithrombotic role.[7] It has been found that free-TFPI levels correlate with endothelial cell markers such as TM, VWF and t-PA,[8] and it has been demonstrated that endothelial cell marker levels are raised in SLE patients and correlate with lupus activity.[9] Precisely, in our patients t-PA, PAI-1 and free-TFPI levels (the only active pool of TFPI) correlated positively with disease activity. In spite of the antithrombotic role of TFPI, caused by its inhibition of TF/FVIIa, increased levels of TFPI might represent endothelial dysfunction and could reflect disease activity.

Table 1. Clinical and biological parameters of patients and controls.

	Patients	*Controls*	*p*
No.	62	23	
Age	32.2±15.9	33.9±10.4	
Age diagnosis	29.2±13.7	–	
Evolution (months)	73.3±161.0	–	
SLAM	5.9±4.4	–	
t-PA (ng/mL)	2.1 (1.7-3.1)	1.6 (1.4-2.2)	0.004
PAI-1 (ng/mL)	25.7 (14.3-44.5)	20.6 (14.3-31.2)	0.13
VWF (ng/mL)	129.5±58.3	86.9±34.8	0.002
TF (pg/mL)	52.2 (46.6-67.8)	56.2 (50.1-67.8)	0.40
Free-TFPI (ng/mL)	21.9±10.6	14.3±5.8	<0.001
Total-TFPI (ng/mL)	80.2 (67.9-95.1)	60.2 (46.6-74.8)	<0.001

	APA/LA (+) (n=33)	*APA/LA (-) (n=29)*		
TF (pg/mL)	53.5 (46.6-65.9)	51.8 (46.6-74.1)	–	0.794
Free-TFPI (ng/mL)	21.4±10.7	22.4±10.6	–	0.573
Total-TFPI (ng/mL)	79.5 (66.9-93.6)	81.9 (71.8-95.8)	–	0.494

t-PA: tissue plasminogen activator; PAI-1: plasminogen activator inhibitor; TM: thrombomodulin; VWF: von Willebrand factor; TF: tissue factor; TFPI: tissue factor pathway inhibitor; APA/LA (+): Antiphospholipid and/or lupus anticoagulant positive patients. APA/LA (-): Antiphospholipid and/or lupus anticoagulant negative patients.

A previous study showed elevated levels of TF and total-TFPI in antiphospholipid syndrome patients, suggesting that TFPI may play a protective role of hypercoagulability in response to the elevated levels of TF,[4] but the authors did not determine free-TFPI levels. Moreover, a poor correlation between total- and free-TFPI has been described.[8] In our study group, we did not find any correlation between the two molecules (r:0.127; p=0.326). Finally, it has been reported that antibodies to β-2-glycoprotein I in antiphospholipid patients suppress TFPI-dependent inhibition of TF-induced coagulation, resulting in increased generation of activated factor X.[10] Interestingly, our patients had elevated levels of TFPI irrespectively of the presence of APA or LA.

In conclusion, SLE patients have high levels of TFPI, which could indicate endothelial injury. The elevated levels of free-TFPI may be a reflection of the whole activation of the system as it correlates both with endothelial damage markers (VWF) and lupus activity (SLAM score). Hence, the TFPI system seems to be a good marker of endothelial damage and reflect disease activity.

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