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Serum Angiopoietin-2 and Soluble Thrombomodulin in Patients with Systemic Lupus Erythematosus and their Relation to Disease Activity and Renal Affection

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Abstract

Background: *Several lines of evidence demonstrate excessive endothelial activation in systemic lupus erythematosus (SLE). Angiopoietin-2 (Ang-2) and soluble thrombomodulin (sTM) are two biomarkers of endothelial cell activation that may be clinically useful in SLE.*

Objective: *to examine the clinical utility of two proposed biomarkers of endothelial activation; angiopoietin-2 and soluble thrombomodulin in SLE correlating their serum concentration with disease activity and renal affection.*

Subjects and methods: *Ang-2 and sTM were measured in sera obtained from 60 patients with SLE (6 male patients and 54 female) and 30 healthy controls by ELISA methodology. Disease activity was calculated according to the SLE Disease Activity Index (SLEDAI). Patients were classified according to disease activity and renal involvement.*

Results: *Both Ang-2 and sTM concentrations were increased in patients with SLE in comparison to controls (P<0.001). Both Ang-2 and sTM were significantly higher in the active group and active nephritis subgroup in comparison to inactive group and active patients without nephritis, respectively (P<0.001). Both markers were positively correlated with SLEDAI and protein/ creatinine ratio. Serum*

Ang-2 was superior to serum sTM as predictor of SLE activity and discriminator of lupus nephritis

Conclusion: *Elevated concentrations of circulating sTM and Ang-2 can serve as biomarker of disease activity and renal affection in SLE. Serum Ang-2 was superior to serum sTM as predictor of SLE activity and nephritis. Further studies should be conducted to put serum Ang-2 as a candidate drug target where functional blockade could seem to protect against endothelial damage in such disease.*

Key words: *Systemic lupus erythematosus, angiopoietin-2, soluble thrombomodulin, SLEDAI.*

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the formation of immune complexes (ICs), which contain a complex mixture of autoantigens nucleic acids, nucleic acids-associated proteins and corresponding autoantibodies. In SLE, ICs are deposited in multiple organs. Vasculopathy and vasculitis in SLE are typical complications and are associated with deposition of ICs on endothelium, endothelial activation and inflammatory cell infiltration[1]. Endothelial activation is characterized by phenotypic changes from a quiescent, unresponsive to a responsive state.[2] The activated endothelial cell phenotype is permeable, prothrombotic, and pro-inflammatory.[3] The activational state of the endothelial layer is a major determinate for the initiation; localization; extent and propagation of inflammatory damage.[4] Therefore, factors of endothelial cell activation or damage are of considerable interest.

In the past few years, angiopoietin-2 (Ang-2) has emerged as a key mediator of endothelial cell activation.[4] Ang-2 protein is produced by endothelial cells themselves and stored in endothelial cell Weibel–Palade Bodies (WPBs) and thus, is readily available following endothelial stimulation. The release of Ang-2 results in rapid destabilization of the endothelium, suggesting that Ang-2 functions as an autocrine negative regulator of the quiescent resting endothelium. Moreover, Ang-2 triggers an inflammatory response by activating the endothelium and inducing permeability.[5] The Ang-2 receptor system has been recently identified as a major regulator of vascular responsiveness to inflammatory stimuli. It has been reported that the endothelial activation is emerging as a key event for leukocyte recruitment and accelerated atherosclerosis in SLE. Assuming that endothelial activation in SLE represents an Ang-2 dependent process, the amount of Ang-2 within the circulation should presumably reflect the extent of activated endothelial surface.[6]

Another endothelial marker, thrombomodulin (sTM) is present in large quantities on the surface of the endothelium, particularly in the microcirculation, where it acts as an anticoagulant. The TM-thrombin complex catalyzes the generation of the anticoagulant molecule activated protein C, and prevents thrombin from converting fibrinogen to fibrin and from exerting other procoagulant effects.[7] Soluble TM (sTM) is an

established marker of endothelial cell damage.[8,9] The vascular inflammatory damage in SLE whereby the endothelial cells are affected includes local secretion of pro-inflammatory cytokines like tumour necrosis factor-α (TNF-α) or interleukin-1 (IL-1), ICs deposition, overproduction of nitric oxide (NO) and increasing expression of adhesion molecules[10] leading to adherence of neutrophils and activation of the endothelium. After adherence, neutrophils secrete several enzymes, such as myeloperoxidase and elastase, which cause endothelial injury reflected by increasing the amount of sTM release into the circulation.[11] So increase levels of sTM in serum after endothelial injury has been reported as an evidence for a pro-thrombotic state in SLE.[12]

Peripheral blood biomarkers of endothelial cell activation may therefore be clinically useful in systemic lupus erythematosus. The aim of this study was to examine the clinical utility of two proposed biomarkers of endothelial activation angiopoietin-2 and soluble thrombomodulin in SLE, correlating their serum concentrations with disease activity and renal affection.

SUBJECTS AND METHODS

Patients and controls

Sixty SLE patients fulfilling the updated American College of Rheumatology (ACR) revised criteria for the classification of SLE[13] were recruited from the rheumatology outpatient clinic and internal medicine department, rheumatology division, Ain Shams University Hospital, Cairo. Patients with other inflammatory diseases or malignancy were excluded. There were 6 male patients and 54 female patients, their ages ranged from 20 to 39 and the median was 24 years.

Full history taking and clinical examination were performed for all patients. Assessment of disease activity was done using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI).[14] SLE Patients were divided according to disease activity into two groups: inactive SLE group including 20 patients (SLEDAI < 6) and active SLE group including 40 patients (SLEDAI ≥ 6) which further subdivided according to presence or absence of nephritis into: active SLE with nephritis (n=28), active SLE without nephritis (n=12). Active LN was defined as having histopathological evidence of immune complex-mediated glomerulonephritis, plus the current renal portion of SLEDAI. This consists of the four kidney-related items of the SLEDAI which are hematuria (> 5 RBCs/HPF after exclusion of stone, infection or other causes), pyuria (> 5 WBCs / HPF after exclusion of infection), proteinuria (> 0.5 gm/24 hour, either of new onset or recent increase of more than 0.5 gm/24 hour) and urinary casts (granular or red blood cell).[14] Thirty age and sex-matched apparently healthy controls with no evidence of any rheumatologic disorder or chronic medical illness were also included. The control group included 27 females and 3 males their ages ranged from 23 to 31 years and the median was 26 years.

Laboratory testing

Complete blood count (CBC) using coulter counter (Coulter LH 750 analyzer), erythrocyte sedimentation rate (ESR; in mm/hour) was determined in the first hour by the Westergren method. Quantitative measurement of serum anti-double stranded-DNA (Anti-dsDNA) antibodies was determined by EIA (anti-dsDNA Kit, ORGENTEC Diagnostika GmbH ELISA kit (Carl-Zeiss Strarabe49), Germany). Levels of complement 3 and 4 (C3 and C4) were measured by nephelometric method using Minineph™ human C3 and C4 kit (Binding site Group Ltd, Birmingham, UK). Serum creatinine, blood urea nitrogen (BUN), complete urine analysis and measurement of protein creatinine ratio (pr-cr) were also performed.

Assessment of Angiopoietin-2 levels: Using Quantikine® Human Angiopoietin-2 Immunoassay ELISA kit (R&D Systems, Inc Minneapolis), USA.

Assessment of soluble thrombomodulin levels: Using Quantikine® Human Thrombomodulin/ BDCA-3 Immunoassay ELISA kit (R&D Systems, Inc Minneapolis), USA.

Statistical Analysis:

IBM SPSS statistics (V. 20.0, IBM Corp., USA, 2011) was used for data analysis. Data were expressed using median, 25^{th} and 75^{th} percentiles. Nominal data were expressed as frequency and percentage. Comparisons between patients and controls and between patients' subgroups were done using Mann-Whitney test. The correlations between Ang and sTM concentrations and different parameters were calculated with Spearman's rank correlation test. Receiver operator characteristic (ROC) procedures identified optimal cut-off values for both markers to differentiate between SLE patients with and without activity and patients with and without LN. *P*-value <0.05 was considered significant and *P*-value < 0.001 was highly significant.

RESULTS

Sixty patients with SLE were analyzed. The main demographic and clinical characteristics are shown in **(table 1).**

Both Ang-2 and sTM concentrations increased in patients with SLE in comparison to controls (P<0.001). The median serum concentration of both markers were significantly higher in different SLE patients' subgroups compared to healthy controls (P<0.001) **(Table 2).**

Table 3 and figure 1 show the results of markers measurements in active and inactive groups. Both Ang-2 and sTM serum were significantly higher in the active group (P<0.001). **Table 4 and figure 2** show the results of markers measurements in active patients with and without nephritis subgroups. Both Ang-2 and sTM serum were significantly higher in active patients with nephritis subgroup (P<0.001).

Using correlation studies among active SLE group, serum sTM levels showed highly significant positive correlations to serum BUN,

creatinine, protein/creatinine ratio and SLEDAI score (r= 0.75, 0.78, 0.79 and 0.75, respectively) (P<0.001). Similarly, Ang-2 showed highly significant positive correlations to serum BUN, creatinine, protein/creatinine ratio and SLEDAI score (r= 0.72, 0.70 and 0.96, respectively) (P<0.001), in addition to a significant positive correlation to anti-dsDNA levels (r= 0.72, P=0.05) **(Table 5).**

Diagnostic performance study using Receiver-operating characteristic (ROC) curve analysis and multiple cut-off levels for detection of SLE activity and nephritis revealed that sTM with an optimum cut-off level of >600 pg/mL has diagnostic sensitivity, specificity, positive and negative predictive values and efficacy of 87.5%, 95%, 97.2%,79.2% and 90%, respectively for discriminating active from inactive patients. While diagnostic validity tests for Ang-2 have shown to be 100% for all criteria (sensitivity, specificity, positive and negative predictive values and efficacy), with an optimum cut-off level of >580 pg/mL **(Table 6)**. For discrimination of LN the optimum cut-off level for sTM was >800pg/mL, with diagnostic sensitivity, specificity, positive and negative predictive values and efficacy of 96.4%, 83.3%, 93%, 91% and 92.5%, respectively, while the optimum cut-off level of Ang-2 was >925 pg/mL, with 100% for all diagnostic validity criteria (sensitivity, specificity, positive and negative predictive values and efficacy) **(Table 7).**

Table 1 Demographic and clinical manifestations as defined by SLEDAI scoring system of patients with SLE

Parameter	**SLE patients (n = 60)**
Demographic data	
Age (years), median(25^{th}-75^{th})percenties	24(20- 39)
Sex , female, %	90%
Clinical manifestations	
Vasculitis, %	5%
Arthritis, %	23.3%
Myositis, %	1.6%
Urinary Casts, %	6.6%
Hematurea, %	5%
Proteinurea , %	46.6%
New Rash, %	8.3%

Alopecia, %	8.3%
Mouth Ulcers, %	13.3%
Pleurisy, %	1.6%
Low Complement, %	58.3%
Anti-DNA antibodies, %	43.3%
Thrombocytopenia, %	10%
Leucopenia, %	23.3%

SLEDAI, Systemic Lupus Erythematosus Disease Activity Index

Table 2. Comparison between controls and different SLE patients' subgroups as regards serum Ang-2 and sTM levels:

Studied subgroups	Ang-2 level (pg/mL) median (25th-75th) percentiles	p. value controls/patients' subgroups	sTM level (pg/mL) median (25th-75th) percentiles	p. value controls/patients' subgroups
Controls n:30	270(190-376.2)	—	100 (95-150)	—
All patients' group n:60	880(492.5-1187.5)	<0.001**	850 (600-1075)	<0.001**
Active n:40	1055(870-1600)	<0.001**	1000 (812.5-1500)	<0.001**
Inactive n:20	455(405-497.5)	<0.001**	450 (275-600)	<0.001**
Active Nephritis n:28	1250(1050-1775)	<0.001**	1150 (1000-1537.5)	<0.001**
Active without Nephritis n:12	765(727.5-847.5)	<0.001**	725 (600-837.5)	<0.001**

P > 0.05: Non-significant difference

P < 0.05: Significant difference (*)

p <0.001: Highly significant difference (**)

Table 2. Comparison between active & inactive SLE patients' groups as regards median serum Ang-2 and sTM levels :

Group / Parameter	**Active n:40 median (25^{th}-75^{th}) percentiles**	**Inactive n:20 median (25^{th}-75^{th}) percentiles**	**P. value**
Ang-2 (pg/mL)	1055(870-1600)	455(405-497.5)	<0.001**
TM(pg/ml)	1000(812.5-1500)	450(275-600)	<0.001**

P > 0.05: Non-significant difference

P < 0.05: Significant difference (*)

P <0.001: Highly significant difference (**)

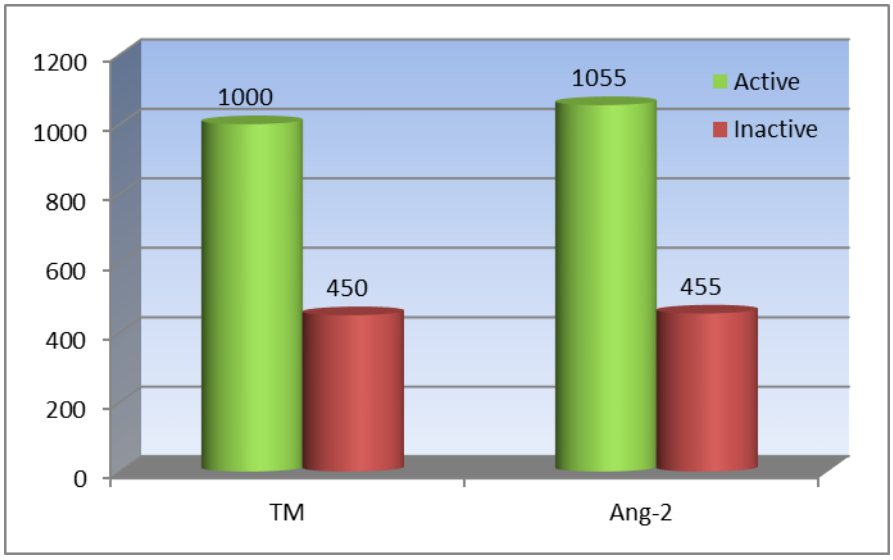

Figure 1. Comparison between active & inactive SLE patients' groups as regards median serum sTM and Ang-2 levels

Table 3. Comparison of Serum Ang-2 and sTM in active with nephritis versus active without nephritis SLE patients' subgroups:

Subgroup / Parameter	Active patients n: 40		P. value
	with Nephritis n:28 median (25^{th}-75^{th}) percentiles	**Without Nephritis n:12 median (25^{th}-75^{th}) percentiles**	
Ang-2 (pg/mL)	1250(1050-1775)	765(727.5-847.5)	<0.001**
TM (pg/mL)	1150(1000-1537.5)	725(600-837.5)	<0.001**

P > 0.05: Non-significant difference

P < 0.05: Significant difference (*)

P<0.001: Highly significant difference (**)

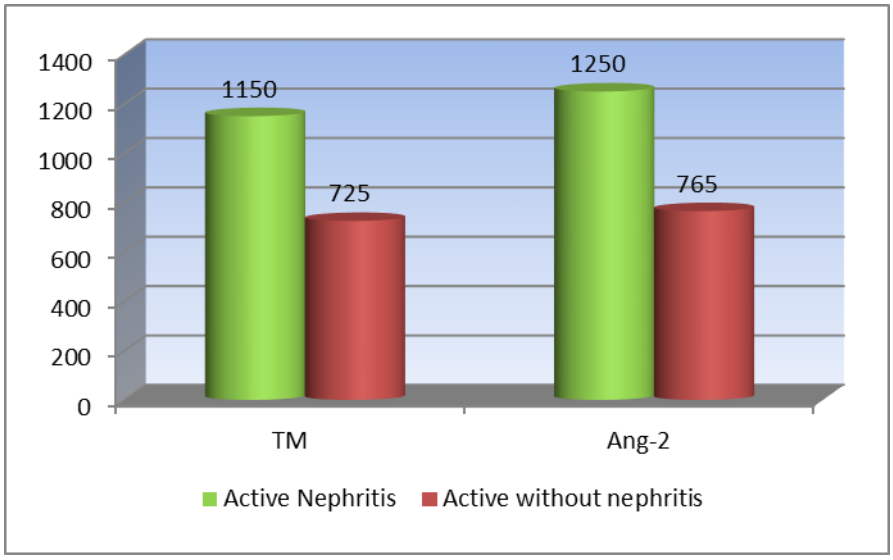

Figure 2. Comparison of median serum levels of Ang-2 and sTM between active with nephritis and active without nephritis SLE patients' subgroups

Table 5. Correlations between both serum Ang-2 and sTM and different studied laboratory parameters among active SLE group

Parameter	Ang-2		sTM	
	r value	p value	r value	p value
Hb (gm/dl)	-0.19	>0.05	-0.11	>0.05
TLC(109/L)	0.11	>0.05	0.13	>0.05
Lympocytes (109/L)	-0.14	>0.05	-0.22	>0.05
Platelets(109/L)	0.16	>0.05	0.19	>0.05
ESR (mm/hr)	0.20	>0.05	0.17	>0.05
Protein/Creatinine	0.79	<0.001**	0.79	<0.001**
BUN (mg/dL)	0.72	<0.001**	0.75	<0.001**
Creat (mg/dL)	0.70	<0.001**	0.78	<0.001**
C3 (mg/dL)	-0.29	>0.05	-0.30	>0.05
C4 (mg/dL)	-0.26	>0.05	-0.29	>0.05
Anti-dsDNA (IU/ml)	0.38	0.05*	0.30	>0.05
SLEDAI Score	0.96	<0.001**	0.75	<0.001**

Table 6. Diagnostic performance of serum sTM and Ang-2, C3, C4 and Anti-dsDNA titre as regards disease activity

Parameter	Cutoff level	Specificity (%)	Sensitivity (%)	p- (%)	p+ (%)	Efficacy (%)
TM (pg/ml)	> 600	95	87.5	79.2	97.2	90
Ang-2 (pg/mL)	>580	100	100	100	100	100
C3 (mg/dL)	< 115	95	92.5	86.4	97.4	93.3
C4 (mg/dL)	< 16	100	85	76.9	100	90
Anti-dsDNA (IU/ml)	.> 24	90	100	100	95.2	96.7

P: predictive values

Table 7. Diagnostic performance of serum sTM, and serum Ang-2, C3, C4 and Anti-nDNA titre as regards kidney affection in SLE

Parameter	Cutoff level	Specificity (%)	Sensitivity (%)	p- (%)	p+ (%)	Efficacy (%)
TM (pg/ml)	>800	83.3	96.4	91	93	92.5
Ang-2 (pg/mL)	> 925	100	96.4	92.3	100	97.5
C3 (mg/dL)	< 75	50	64.3	37.5	75	60
C4 (mg/dL)	< 14	50	60.7	35.3	73.9	57.5
Anti-dsDNA (IU/ml)	> 130	83.3	75	58.8	91.3	77.5

P: predictive values

DISCUSSION

Endothelial dysfunction in SLE is produced by the clustering of traditional risk factors, adverse effects of treatment and SLE itself as an independent risk factor[15] in which immunologically mediated vascular endothelial cell activation is regarded as a potential pathophysiological mechanism of systemic organ damage.[16]

Ang-2 is released by vascular endothelial cells and is an important cytokine that participates in physiological activities and pathophysiological events of endothelial cells.[17] Also TM is a cell surface glycoprotein and presents in vascular endothelial cells specifically. It is considered as a molecular marker of endothelial injury.[18]

In the present study, serum Ang-2 and sTM levels showed a highly significant increase in active SLE patients compared to inactive SLE patients and healthy controls, which agreed with those described previously.[6,10,19-21] Elevated levels of Ang-2 and sTM in SLE may be explained by the fact that SLE is characterized by inflammatory vascular damage whereby the endothelial cells (ECs) are affected.

It was suggested that endothelial activation in active SLE may represent an Ang-2 dependent process; the amount of Ang-2 within the circulation can reflect the extent of activated endothelial in the surface, especially in kidney endothelium.[22] Moreover, sTM which is normally a component of vascular EC membrane is easily

released to plasma in patients with active SLE due to persisting EC detachment in active SLE patients. It was suggested that elevated sTM levels reflect EC damage rather than activation, because EC–leucocyte adhesion and interaction after the activation of inflammatory cytokines might result in the release of TM from the EC surface.[23]

Increased sTM level in plasma and loss of its function can occur by several mechanisms, including cytokine-induced suppression of TM (TNFα and IL), activated neutrophil-elastase-dependent proteolytic release of sTM from the endothelial membrane,[24] immune complexes deposition, overproduction of NO and oxidation of functionally critical amino acids in the extracellular TM domain.[25]

These findings support our finding in the present study, as we have demonstrated a significant positive correlation of sTM level with SLEDAI score. However, no significant correlations could be obtained between sTM and other parameters of disease activity such as anti-dsDNA, C3 and C4.These results agreed with *Horáka et al.* who found no significant correlation between sTM and anti-dsDNA in most subgroups.[26] So they concluded that although serum sTM as well as anti-dsDNA antibody concentration express the disease activity, but their correlation is not strong enough and serum levels of TM reflect the fact, that this free molecule is not a product of the autoimmune stimulation like anti-dsDNA, neither plays role in immune complex clearance as complement components, but it is released by ECs injury.[27]

Regarding serum Ang-2, active SLE patients showed statistically significant positive correlations with SLEDAI score and anti-dsDNA titre. Our results also agreed with those of *Kümpers et al.*[6] Meanwhile, *El-Banawy et al.* showed that serum Ang-2 levels were positively correlated with SLEDAI score; however they could not demonstrate significant correlation to anti-dsDNA positivity.[21] This may be related to difference in method used in assessment of anti-dsDNA result. Accordingly, we suggest that the quantitative assessment of anti-dsDNA (unlike study of ***El-Banawy et al., 2011***) might better allow the detection of the correlation between serum anti-dsDNA and Ang-2 levels.

To elucidate the relation between serum Ang-2, serum sTM and renal affection, active patients with lupus nephritis were compared to active patients without (n=28, 12 respectively). A highly significant elevation of both serum Ang-2 and sTM among the nephritic group was demonstrated. These results were compatible with those of other authors[16,21,23,28-30] who suggested that decreased renal clearance of TM is not enough to explain the increased sTM concentration in patients with LN as it is execreted by kidneys but it is due to increased endothelial synthesis and expression of TM, in particular in the kidneys. Also, *David et al.* reported that the kidney endothelium has been identified as a rich source of Ang-2, so that

chronic kidney disease might directly result in increased Ang-2 release from the kidney and/or from distant systemic endothelium.[31]

In LN patients, serum Ang-2 and serum sTM levels were positively correlated with proteinuria which was previously reported.[21,23] In accordance *Kümpers et al.* showed a close correlation between circulating Ang2 concentrations and vascular barrier function, using proteinuria as a surrogate marker for glomerular endothelial permeability.[6] These findings are consistent with the role of Ang2 in destabilizing endothelial cell integrity, causing proteinuria.[32]

Diagnostic performance study revealed that serum Ang-2 was superior to serum sTM and anti-dsDNA as predictor of SLE activity (ppvs of 100%vs. 97.2 and 95.2%, respectively and efficacies of 100% vs. 90% and 96.7% respectively). Also for discrimination of lupus nephritis, serum Ang-2 was much better than serum sTM and anti-dsDNA (ppvs of 100%vs. 93%and 91.3%, respectively and efficacies of 97.5% vs. 92.5% and 77.5% respectively).

In conclusion, elevated concentrations of circulating sTM and Ang-2 can serve as biomarkers of disease activity and renal affection in SLE. Serum Ang-2 was superior to serum sTM and anti-dsDNA as predictor of SLE activity and nephritis. Further studies should be conducted to to put serum Ang-2 as a candidate drug target where functional blockade could seem to protect against endothelial damage in such disease.

REFERENCES

1. **Sun W, Jiao Y, Cui B, Gao X, Xia Y, Zhao Y**. Immune complexes activate human endothelium involving the cell-signaling HMGB1-RAGE axis in the pathogenesis of lupus vasculitis. Lab Invest. 2013 Jun; 93(6):626-38.
2. **Aird WC**. Spatial and temporal dynamics of the endothelium. J Thromb Haemost. 2005; 3:1392-1406.
3. **Page AV and Liles WC**. Biomarkers of endothelial activation/dysfunction in infectious diseases. Virulence. 2013 Apr 19; 4(6).
4. **Fiedler U, Reiss Y, Scharpfenecker M, Grunow V, Koidl S, Thurston G, Gale NW, Witzenrath M, Rosseau S, Suttorp N, Sobke A,Herrmann M, Preissner KT, Vajkoczy P and Augustin HG.** Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. Nat Med 2006; 12:235–239.
5. **Lemieux C, Maliba R, Favier J, Théorêt JF, Merhi Y, Merhi Y and Sirois MG**. Angiopoietins can directly activate endothelial cells and neutrophils to promote proinflammatory responses. Blood 2005; 105: 1523-1530.
6. **Kümpers P, David S, Haubitz M, Hellpap J, Horn R, Brocker V, Schiffer M, Haller H and Witte T.** The Tie2 receptor antagonist angiopoietin 2

facilitates vascular inflammation in systemic lupus erythematosus. Ann Rheum Dis 2009; 68: 1638-1643.

7. **Levi M and Van Der Poll T**. Thrombomodulin in sepsis. Minerva Anestesiol 2013; 79:294-8.
8. **Ishii H, Yama H, Kazama M**. Soluble thrombomodulin antigen in conditioned medium is increased by damage of endothelial cells. Thromb Haemostas 1991; 65:618–23.
9. **Sawada K, Yamamoto H, Yago H et al**. A simple assay to detect endothelial cell injury; measurement of released thrombomodulin from cells. Exp Mol Pathol 1992; 57:116–23.
10. **Akbarian M, Jamshidi R, Shahram F, Akhlaghi M, Khosravi S, Almasi S and Davatchi F**. Assessment of serum thrombomodulin in patients with systemic lupus erythematosus. Acta Medica Iranica 2009; 47 (2): 97-102.
11. **Gando S, Kameue T, Matsuda N, Hayakawa M and Hoshino H**. Serial changes in neutrophil-endothelial activation markers during the course of sepsis associated with disseminated intravascular coagulation. Thromb Res 2005; 116: 91-100.
12. **Chan SH, Chen JH, Li YH, Lin LJ and Tsai LM**. Increasing post-event plasma thrombomodulin level associates with worse outcome in survival of acute coronary syndrome. Int J Cardiol 2006; 111: 280-285.
13. **Hochberg MC.** Updating the American college of rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997; 40:1725.
14. **Bombardier C, Gladman D, Urowitz M, Caron D, Chang C**. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum 1992; 35, 630–40.
15. **Ahmad Y, Shelmerdine J, Bodill H, Lunt M, Pattrick MG, Teh LS, Bernstein RM, Walker MG and Bruce IN .** Subclinical atherosclerosis in systemic lupus erythematosus (SLE): the relative contribution of classic risk factors and the lupus phenotype. Rheumatology 2007; 46: 983–988.
16. **Kuryliszyn-Moskal A, Klimiuk PA, Ciolkiewicz M and Sierakowski S .** Clinical significance of selected endothelial activation markers in patients with systemic lupus erythematosus. J Rheumatol. 2008; 35:1307-1313.
17. **Weng HB and Li S .** Early changes of plasma angiopoietin-2 in patients
18. with multiple trauma. World J Emerg Med 2011, *Vol 2, No 4.*
19. **Reinhart K, Bayer O, Brunkhorst F, Meisner M.** Markers of endothelial

damage in organ dysfunction and sepsis. Crit Care Med 2002; 30: S302-S312.

20. **Yuelan *C*, Guojun S, and Xinguo L .** Detection of plasma thrombomodulin in patients with systemic lupus erythematosus and its clinical significance. Chinese Journal of Dermatology 2011; 32 (1) 12-13.
21. **Santos MJ, Carmona-Fernandes D, Canhão H, Canas da Silva J and Fonseca JE.** Early Vascular Alterations in SLE and RA Patients—A Step towards Understanding the Associated Cardiovascular Risk. Plos One 2012; 7 (9): e44668.
22. **El-Banawy HS**, **Gaber EW**, **Maharem DA and Matrawy KA .** Angiopoietin-2, endothelial dysfunction and renal involvement in patients with systemic lupus erythematosus. J Nephrol.2011; doi: 10.5301/jn.5000030.
23. **Fiedler U and Augustin HG .** Angiopoietins: a link between angiogenesis and inflammation. Trends Immunol.2006; 27:552-558.
24. **Al-Yasaky AZ, Mahfouz H, Mahdy M and Zakareya N.** Soluble thrombomodulin (sTM) and human adrenomedullin (AM) in systemic lupus erythematosus (SLE) and their relation to disease activity and renal affection. Egypt Rheumatol.2005; 32 (2): 217-233.
25. **Levi M and van der Poll T.** Thrombomodulin in sepsis. Minerva Anestesiologica 2013; 79 (3): 294-298.
26. **Conway EM.** Thrombomodulin and its role in inflammation. Semin Immunopathol.2012; 34: 107-125.
27. **Horák P, Heřmanová Z, Vašíčková J, Pospíšil Z, Faltýnek V and Ščudla V .** Serial follow up of soluble thrombomodulin levels in patients with systemic lupus erythematosus. Acta Univ Palacki Olomuc Fac Med 2000; 144: 33.
28. **Boehme MWJ, Galle PR, Raeth U, Stremmel W and Scherbaum WA.** Serum thrombomodulin a novel marker of disease activity in systemic lupus erythematosus. Arthritis & Rheumatism 2005; 37 (4): 572-577.
29. **Ho CY, Wong CK, Li EK, Tam LS and Lam CW.** Elevated plasma concentrations of nitric oxide, soluble thrombomodulin and soluble vascular cell adhesion molecule-1 in patients with systemic lupus erythematosus. Rheumatol 2003; 42 (1):117–122.
30. **Hassan AA, Al-Akaad AF and Osman AM .** Serum nitric oxide and thrombomodulin levels in patients with systemic lupus erythematosus: a possible addition to disease activity parameters? El-Minia Med Bull 2008; 19(2): 136-149.
31. **Frijns R, Fijnheer R, Schiel A, Donders R, Sixma J and Derksen R .** Persistent

increase in plasma thrombomodulin in patients with a history of lupus nephritis: endothelial cell activation markers. Journal of Rheumatology 2001; 28: 514-519

32. **David S, Kümpers P, Lukasz A, Fliser D, Martens-Lobenhoffer J, Bode-Böger SM, Kliem V, Haller H and Kielstein JT .** Circulating angiopoietin-2 levels increase with progress of chronic kidney disease. Nephrol Dial Transplant 2010; 25: 2571-2576.
33. **Scharpfenecker M, Fiedler U, Reiss Y and Augustin HG .** The Tie-2 ligand Angiopoietin-2 destabilizes quiescent endothelium through an internal autocrine loop mechanism. J Cell Sci 2005; 118: 771–780.