Endocan, Novel Potential Biomarker for Systemic Sclerosis: Results of a Pilot Study

Paul Bălănescu,^{1,2,3*} Anca Lădaru,⁴ Eugenia Bălănescu,¹ Theodor Voiosu,^{2,3}

Cristian Băicuș,^{2,3,5} and Gheorghe Andrei Dan^{2,3,5}

¹CDPC Clinical Immunology Department, Colentina Clinical Hospital, Bucharest, Romania

²Internal Medicine Chair, University of Medicine and Pharmacy “Carol Davila,” Bucharest, Romania

³Clinical Research Unit, RECIF (Réseau d’Epidémiologie Clinique Internationale Francophone), Bucharest, Romania

⁴Pediatrics Department, Institute for Mother and Child Protection “Alfred Rusescu,” Bucharest, Romania

⁵Department of Internal Medicine, Colentina Clinical Hospital, Bucharest, Romania

Background: Systemic sclerosis (Ssc) is an autoimmune disease characterized by vascular alterations of small arteries and microvessels with subsequent tissue fibrosis. Endocan is expressed by endothelial cells and associated with endothelial dysfunction; therefore it could be a potential biomarker for Ssc patients. **Methods:** Twenty-one Ssc patients and 20 sex- and age-matched healthy controls were recruited for the study. Serum endocan levels were determined using ELISA method in all patients and controls. **Results:** Serum endocan levels were superior in Ssc patients (median 2.53 (1.10–7 ng/ml)) compared with controls (0.79 (0–2 ng/ml), $P < 0.05$). Higher serum endocan expression was seen in diffuse Ssc subset and associated with the presence of digital ulcers and daily Raynaud’s phe-

nomenon ($P < 0.05$). Higher serum endocan levels were associated with a modified Rodnan skin score > 14 and longer disease duration ($P < 0.05$). Values of areas under the receiver operating curves showed that serum endocan had good discriminative power for Ssc diagnosis, differentiating diffuse from limited subset type and differentiating patients with modified Rodnan skin score above and under 14 (area under curve: 0.94, 0.81, 0.75, respectively). **Conclusion:** The results of this pilot study suggest endocan as a potential biomarker for microvascular manifestations and complications in Ssc patients. These encouraging results could promote future prospective studies in order to determine the exact role played by endocan as a biomarker for Ssc. *J. Clin. Lab. Anal.* **30**:368–373, 2016. © 2015 Wiley Periodicals, Inc.

Key words: systemic sclerosis; endocan; biomarker; vascular alteration; Raynaud’s phenomenon; ischemic digital ulcer; skin fibrosis

INTRODUCTION

Systemic sclerosis (Ssc) is an autoimmune disease characterized by cutaneous thickening associated with various degree of tissue fibrosis, chronic inflammatory infiltrate in various organs, fibroproliferative vasculopathy, and profound alterations of the immune system characterized by autoantibodies synthesis (1). The physiopathology of Ssc is still incompletely understood, but vascular involvement clearly plays one important role. Vascular alterations of small arteries and microvessels determine that tissue hypoxia lead to tissue fibrosis with various internal organ damages (2). Intensive research is focused upon biomarker discovery and validation in Ssc

patients, as biomarkers that assess Ssc severity are lacking (3).

Grant sponsor: Sectoral Operational Programme Human Resources Development (SOP HRD) financed from the European Social Fund and by the Romanian Government; Grant number POSDRU/159/1.5/S/137390.

*Correspondence to: Paul Bălănescu, CDPC Clinical Immunology Department, Colentina Clinical Hospital, 19–21 Stefan cel Mare street, Bucharest 020125, Romania. E-mail: plbalanescu@gmail.com

Received 23 July 2014; Accepted 27 June 2015

DOI 10.1002/jcla.21864

Published online in Wiley Online Library (wileyonlinelibrary.com).

Endocan, originally known as endothelial cell specific molecule-1 (ESM-1) (4), is a proteoglycan expressed by endothelial cells. Its main function is inhibition of leukocyte recruitment. First, endocan was studied in order to establish its role in tumorigenesis. Many types of cancers such as clear cell renal carcinoma (5), non-small-cell lung cancer (6), hepatic carcinoma (7), gastric cancer (8), nasopharyngeal carcinoma (9), and colorectal cancer (10) express high levels of endocan and this was associated with a poorer prognosis. Apart from its role in tumorigenesis, recent evidence suggests endocan as a valuable serum biomarker in a wide variety of conditions. Serum endocan is an important biomarker in infectious diseases as some recent reports suggest. In sepsis, serum endocan levels increase dramatically and is associated with poor prognosis (11). In addition, endocan could be an important stratifying biomarker for patients with acquired community pneumonia, as suggested by a recent paper (12). Endocan also seems to be involved in pathogenesis of psoriasis as it correlates with disease severity and cardiovascular impairment (13).

Due to the fact that endocan is implicated in endothelial damage, it is expected that it could be a potential biomarker for Ssc patients as Ssc pathophysiology is centered upon vascular involvement and endothelial dysfunction (14). To our knowledge, there is no study in the literature that explores endocan expression in Ssc patients. Therefore, an observational study was conducted to determine whether endocan serum levels are increased in Ssc patients and if endocan could be a possible biomarker for some of the Ssc clinical characteristics.

MATERIAL AND METHODS

Patients and Controls

Twenty-one consecutive patients (19 females, 2 males, 53 ± 14.7 years) diagnosed with Ssc according to the classification criteria proposed by the American College of Rheumatology (ACR) (15), who attended Colentina Clinical Hospital between January 2013 and May 2014, were enrolled. All patients underwent full clinical examination and they also had evaluation of systemic involvement as follows. Interstitial lung disease was evaluated with chest radiography and high-resolution computer tomography (CT). All patients underwent respiratory function tests (flow spirometry and diffusing capacity for carbon monoxide (DLCO)). Pulmonary hypertension was assessed indirectly by means of increased mean pulmonary arterial pressure (above 35 mmHg at rest) (16) at transthoracic echocardiography. Joint involvement was clinically evaluated and graded as arthritis or polyarthralgias accordingly. Patients were evaluated for cardiac involvement with cardiac ultrasonography

and electrocardiogram (EKG). Cutaneous involvement was determined by defining the presence/absence and frequency of Raynaud's phenomenon, modified Rodnan skin score (17), presence/absence of teleangiectasias, presence/absence of ischemic digital ulcers (defined by loss in epithelialization and tissue that involved subcutaneous tissue), and presence/absence of calcinosis (defined by calcium deposits in soft tissues that were visible and confirmed by the X-ray) (18). The frequency of Raynaud's phenomenon was defined as daily or less frequent. Disease activity score was assessed using European League Against Rheumatism (EULAR) Scleroderma Trial and Research group criteria (EUSTAR criteria) (19).

Data about inflammatory profile of the Ssc patients were also recorded (erythrocyte sedimentation rate, C-reactive protein levels). Patient treatment in the moment of blood sampling was also recorded. Some patients did not have any therapy, others were on steroid therapy or calcium blockers or angiotensin-converting enzyme inhibitors (see Table 1).

Twenty sex- and age-matched healthy controls (18 females, 2 males, 52 ± 14.5 years) were enrolled from patients referred to Colentina Clinical Hospital. They underwent physical examination and laboratory tests that excluded any medical condition that could induce increased serum endocan levels (infection, malignancy, other inflammatory conditions). They had no previous record of Raynaud's phenomenon. All patients and controls signed an informed consent according to the Declaration of Helsinki and the study was approved by the local ethics committee.

Serum Autoantibodies and Endocan Analysis

Blood samples from patients and controls were stored at -80°C for further analysis. Antinuclear antibodies (ANA) were detected by indirect immunofluorescence. Commercial enzyme-linked immunosorbent assay (ELISA) kits were used according to the manufacturer's instructions to determine anti-Scl70 and anticentromere antibodies (Euro Diagnostica, Malmo, Sweden).

Serum endocan was determined using a sandwich commercial ELISA kit from Lunginnov (Lille, France) according to the manufacturer's instructions. Briefly, samples from healthy controls were diluted twofold and patient samples were diluted fivefold into dilution buffer provided by the kit. Minimum level of detection of the assay was 0.132 ng/ml. All samples were assayed in duplicate. The absorbance was measured at 450 nm on a spectrophotometer (BioRad, Hercules, CA) and a four-parameter logistic regression curve was used for standard curve fitting analysis. Results were expressed as nanograms per milliliter (ng/ml).

TABLE 1. Characteristics of Ssc Patients

Patient characteristics (<i>n</i>)	All Ssc patients (21)	Diffuse subtype Ssc (11)	Limited subtype Ssc (10)	<i>P</i> -value
Age (mean $\pm$ SD, years)	53 $\pm$ 14.7	53 $\pm$ 11	51.8 $\pm$ 18.55	NS
Female/male (number of patients)	19/2	10/1	9/1	NS
Duration of disease (median, min–max; months)	60 (5–204)	60 (5–204)	42 (6–168)	NS
Interstitial lung disease (number of patients)	9 (43%)	7 (63%)	2 (20%)	NS
Percent of predicted DLCO (median, min–max)	88 (38–91)	76 (31–90)	90 (45–91)	0.04
Pulmonary hypertension (number of patients)	3 (14%)	2 (18%)	1 (10%)	NS
Percentage of predicted forced vital capacity (mean $\pm$ SD)	89.75 $\pm$ 12.72	88.2 $\pm$ 17.04	95.5 $\pm$ 4.19	NS
Raynaud's phenomenon (number of patients)	21 (100%)	11 (100%)	10 (100%)	NS
Digital ulcers (number of patients)	6 (28%)	6 (55%)	0	0.01
Calcinosis (number of patients)	4 (19%)	4 (36%)	0	NS
Teleangiectasias (number of patients)	15 (72%)	7 (63%)	8 (80%)	NS
Sclerodactyly (number of patients)	20 (96%)	11 (100%)	9 (90%)	NS
Finger flexion (number of patients)	10 (48%)	9 (82%)	1 (10%)	0.02
Modified Rodnan score (median, min–max)	14 (3–34)	21 (6–34)	6 (3–14)	<0.001
ANA (number of patients)	21 (100%)	11 (100%)	10 (100%)	NS
Anticentromere antibodies (number of positive patients)	9 (43%)	0	9 (90%)	<0.001
Anti-Scl70 antibodies (number of positive patients)	10 (48%)	10 (91%)	0	<0.001
CRP (median, min–max; mg/l)	2 (0.37–32)	2 (0.37–31.50)	2.1 (0.5–32)	NS
ESR (median, min–max; mm/1h)	15 (6–55)	16 (9–55)	13.5 (6–38)	NS
EUSTAR score (median, min–max)	2.5 (0.5–8)	4.5 (0.5–8)	1 (0.5–4)	0.005
Steroid therapy (number of patients)	10 (48%)	7 (64%)	3 (30%)	NS
Calcium blockers (number of patients)	5 (24%)	3 (27%)	2 (20%)	NS
ACE inhibitors (number of patients)	4 (19%)	1 (9%)	3 (30%)	NS

NS, not significant; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ACE, angiotensin-converting enzyme.

Statistical Analysis and Sample Size Considerations

Continuous variables were presented as mean $\pm$ SD if they were normally distributed, or as median and minimum and maximum values if they were nonnormally distributed. To test normality of the continuous variables, Kolmogorov–Smirnov tests were applied. Fischer's exact tests were used to determine whether two categorical variables are associated. Difference in serum concentration markers among groups was determined using Mann–Whitney *U* test. Spearman's rank correlation coefficient was used in order to assess linear relationship among the study variables. Receiver operating curves (ROCs) were determined for endocan levels as diagnostic tests for specific clinical outcomes. A *P*-value ≤ 0.05 was considered significant. Statistical analysis was performed using the Statistical Software for Social Sciences (SPSS), version 16, for Windows (Chicago, IL).

Because Ssc is a relatively rare disease and recruitment rates are low in medical centers, we performed a power analysis in order to determine the sample size necessary to perform this preliminary study (using G*Power 3.1.3 software). In order to highlight an effect size of 1.68 with allocation rate of patients and controls of about 2/1, for an α error probability at 0.01 and for a study power of 0.90, we would need a minimum of ten controls and 20 Ssc patients (total estimated sample size of 30 participants).

RESULTS

Twenty-one Ssc patients and 20 sex- and age-matched healthy controls were recruited for the study. Table 1 summarizes the general characteristics of Ssc patients. Eleven patients had diffuse subtype Ssc and ten patients had limited subtype Ssc. The most frequent clinical manifestations of Ssc patients were Raynaud's phenomenon (present in all 21 patients) and sclerodactyly (present in 20 of 21 patients). All Ssc patients tested positive for ANA. In the limited subtype of disease, nine of ten patients had anticentromere antibodies present; and in the diffuse subtype, ten of 11 patients had anti-Scl70 antibodies. These two autoantibodies were mutually exclusive—that is, they were not both present in the same patient.

Serum endocan levels were higher in Ssc patients than in healthy controls (median 2.53 (1.10–7 ng/ml) vs. 0.79 (0–2 ng/ml), *P* < 0.001, Mann–Whitney *U* test, Fig. 1. In order to test the capacity of serum endocan to discriminate between Ssc patients and healthy controls, an ROC analysis was performed. Using a cut-off value of 1.88 ng/ml for serum levels of endocan according to the ROC curve, the biomarker had 100% specificity and 80% sensitivity to discriminate between Ssc patients and healthy controls. Area under the curve (AUROC) was 0.94 (95% CI, between 0.86 and 1).

Within the Ssc patient group, clinical characteristics of patients were correlated with serum endocan levels in

Fig. 1. Serum endocan levels in healthy controls and Ssc patients. Serum endocan levels are significantly higher in Ssc patients ($P < 0.001$, Mann-Whitney U test).

Fig. 2. Serum endocan levels in Ssc disease subtypes. Serum endocan levels are significantly higher in diffuse Ssc subtype ($P = 0.02$, Mann-Whitney U test).

order to determine if some disease characteristics were associated with serum endocan levels. Serum endocan levels were higher in patients with diffuse subtype Ssc compared with patients with limited Ssc (median 2.65 (2.05–6.05 ng/ml) vs. 1.65 (1.10–7 ng/ml), $P = 0.02$, Mann-Whitney U test, Fig. 2). In order to test the capacity of serum endocan to discriminate between Ssc disease subtypes, an ROC analysis was also performed. Using a cut-off value of 2 ng/ml for serum levels of endocan according to the ROC curve, the biomarker had 80% specificity and 100% sensitivity to discriminate between diffuse and limited Ssc subtypes. AUROC was 0.81 (95% CI, between 0.58 and 1).

Ssc patients with digital ulcers had higher endocan levels compared with those without ulcers (median 3.32

(2.05–5.20 ng/ml) vs. 1.95 (1.10–7 ng/ml), $P = 0.04$, Mann-Whitney U test). Interestingly, patients with daily Raynaud's phenomenon had higher serum endocan levels compared with patients with less-frequent manifestations (median 2.62 (1.35–7 ng/ml) vs. 1.5 (1.10–4.95 ng/ml), $P = 0.038$, Mann-Whitney U test). However, serum endocan levels did not correlate with the number of digital ulcers. Patients with telangiectasias had lower serum endocan level compared with Ssc patients without telangiectasias (median 1.95 (1.10–5.20 ng/ml) vs. 3.8 (2.05–7 ng/ml), $P = 0.035$, Mann-Whitney U test).

Patients who had a modified Rodnan skin score >14 (used by EUSTAR score) had also higher serum endocan levels (median 2.65 (2.05–5.02 ng/ml) vs. 1.80 (1.10–7 ng/ml), $P = 0.04$, Mann-Whitney U test). An ROC analysis was performed to determine the capacity of serum endocan levels to discriminate between modified Rodnan skin score below and above the cut-off of 14. Therefore, for Ssc patients, using a cut-off value of 2.32 ng/ml, endocan had 75% specificity and 88.9% sensitivity to discriminate between modified Rodnan skin scores above and below 14. AUROC was 0.75 (95% CI, between 0.55 and 0.98).

Serum endocan levels were higher in patients who had Ssc duration more than 3 years (3.32 (1.10–7 ng/ml) vs. 1.8 (1.10–2.65 ng/ml), $P = 0.048$, Mann-Whitney U test). Serum endocan levels did not correlate with inflammatory status of the Ssc patients, pulmonary manifestations, or disease activity score. Also, no association was found between serum endocan and current Ssc treatment.

DISCUSSIONS

Endocan seems to be a promising biomarker for Ssc patients, as it is expressed mainly by endothelial cells. Serum endocan levels were higher in Ssc patients compared with healthy controls, suggesting endocan as a potential biomarker for Ssc. Endocan is already suggested as potential biomarker for other autoimmune diseases such as psoriasis (13), Behcet disease (20), and inflammatory bowel disease (21). In such autoimmune diseases, serum endocan levels correlated with the inflammatory response and autoimmune disease activity scores. However, this was not the case for Ssc patients, where surprisingly endocan did not correlate with the inflammatory response. Much of the limited existing literature on endocan is in the setting of acute (and vigorous) inflammation such as sepsis and, more recently, in some medications such as antihypertensive medications. A recent paper (22) reported amlodipine and valsartan to decrease endocan levels in newly diagnosed hypertensive patients. In Ssc patients, there were no differences in serum endocan levels between treated patients with calcium blockers and not-treated patients, as there were no differences in serum endocan levels between

patients treated with ACE inhibitors and not-treated patients.

Serum endocan levels could discriminate between diffuse and limited Ssc subsets, as its serum levels were higher in diffuse Ssc patients. Furthermore, higher levels of endocan were associated with various clinical manifestations of Ssc patients, mainly microvascular ones. Higher endocan levels were found in patients with digital ulcers, although it did not correlate with the number of digital ulcers in patients with this clinical manifestation. Serum endocan levels correlated with the presence of daily Raynaud's phenomenon and its levels were lower in telangiectasia patients. Other research groups demonstrated that endocan correlates with endothelial dysfunction expressed by vascular abnormalities (flow-mediated vasodilation and carotid intima media thickness) being increased during endothelial damage and is suggested as a biomarker of endothelial dysfunction (23, 24). Therefore, endocan could be a valuable surrogate biomarker for tailoring therapies that target ischemic manifestations of Ssc, mainly ischemic digital ulcers and Raynaud's phenomenon. According to these results, endocan could influence Ssc pathogenesis and clinical manifestations through endothelial function. It is possible that microvascular changes that are characteristic for Ssc would trigger endocan shedding and expression, apart from atherosclerosis, which was previously suggested (25). As Ssc patients associate high vascular endothelial growth factor (VEGF) levels (26), endocan could be upregulated via VEGF in Ssc patients, as it was previously demonstrated that VEGF determines transcription of endocan gene (27).

Higher serum endocan levels were observed in patients with a modified Rodnan skin score >14. This threshold is used in EUSTAR criteria in determining the severity of Ssc. Endocan is a circulating proteoglycan and a previous report showed that proteoglycans (heparan sulfates) enhance the activity of dermal fibroblasts to form scleroderma skin (28). Therefore, endocan could be a biomarker for assessing skin fibrosis in Ssc patients. Biomarkers for skin fibrosis are in great demand, as for the moment there is no validated biomarker that correctly assesses Ssc cutaneous fibrosis.

Although higher levels of endocan were observed in Ssc patients with Rodnan scores >14 and in patients with digital ulcers, it did not correlate with EUSTAR scores. This could be explained by the fact that serum endocan levels did not correlate with DLCO, pulmonary manifestations, and inflammatory status of patients.

Serum endocan levels also associated with longer disease duration. This aspect is important, as probably endocan would not be a suitable biomarker for the assessment of very early Ssc. As Ssc progresses, vascular changes and endothelial dysfunction also progress and could determine higher endocan expression.

The study has some obvious limitations. First of all, it is yet a small sample size to detect all the significant differences and correlations of serum endocan within the Ssc patient group. In addition, other angiogenesis biomarkers (such as VEGF) were not evaluated within this group in order to determine correlations with endocan. Finally, this pilot study measured only in one point serum endocan levels, without further followup of patients. Future studies should focus upon the relationship between endocan and other angiogenesis biomarkers in Ssc, as endocan seems to be a promising biomarker, at least for the microvascular manifestations and cutaneous fibrosis. In addition, prospective studies evaluating endocan's capability for prediction of microvascular manifestations are warranted.

CONCLUSION

This is the first study in the literature that describes upregulation of serum endocan levels in Ssc patients compared with healthy controls, suggesting that endocan could play a role in Ssc patient evaluation. These encouraging results could promote future prospective studies in order to determine the exact role played by endocan as a biomarker in Ssc and its implications in microvascular complications of the disease.

REFERENCES

- Bernatsky S, Joseph L, Pineau CA, Belisle P, Hudson M, Clarke AE. Scleroderma prevalence: Demographic variations in a population-based sample. *Arthritis Rheum* 2009;61(3):400–404
- Flavahan NA, Flavahan S, Mitra S, Chotani MA. The vasculopathy of Raynaud's phenomenon and scleroderma. *Rheum Dis Clin North Am* 2003;29:275–291
- Bălănescu P, Lădaru A, Bălănescu E, Băicuș C, Dan GA. Systemic sclerosis biomarkers discovered using mass-spectrometry-based proteomics: A systematic review. *Biomarkers* 2014;19(5):345–355.
- Lassalle P, Molet S, Janin A, et al. ESM-1 is a novel human endothelial cell-specific molecule expressed in lung and regulated by cytokines. *J Biol Chem* 1996;271(34):20458–20464
- Leroy X, Aubert S, Zini L, et al. Vascular endocan (ESM-1) is markedly overexpressed in clear cell renal cell carcinoma. *Histopathology* 2010;56:180–187
- Grigoriu BD, Depontieu F, Scherpereel A, et al. Endocan expression and relationship with survival in human nonsmall cell lung cancer. *Clin Cancer Res* 2006;12(15):4575–4582.
- Huang GW, Tao YM, Ding X. Endocan expression correlated with poor survival in human hepatocellular carcinoma. *Dig Dis Sci* 2009;54(2):389–394.
- Liu N, Zhang LH, Du H, et al. Overexpression of endothelial cell specific molecule-1 (ESM-1) in gastric cancer. *Ann Surg Oncol* 2010;17(10):2628–2639.
- Yu PH, Chou SF, Chen CL, et al. Upregulation of endocan by Epstein-Barr virus latent membrane protein 1 and its clinical significance in nasopharyngeal carcinoma. *PLoS One* 2013;8(12):e82254

10. Zuo L, Zhang SM, Hu RL, et al. Correlation between expression and differentiation of endocan in colorectal cancer. *World J Gastroenterol* 2008;14(28):4562–4568.
11. Mihajlovic DM, Lendak DF, Brkic SV, et al. Endocan is useful biomarker of survival and severity in sepsis. *Microvasc Res* 2014;93:92–97.
12. Kao SJ, Chuang CY, Tang CH, et al. Plasma endothelial cell-specific molecule-1 (ESM-1) in management of community-acquired pneumonia. *Clin Chem Lab Med* 2013;9:1–7.
13. Balta I, Balta S, Demirkol S, et al. Elevated serum levels of endocan in patients with psoriasis vulgaris: Correlations with cardiovascular risk and activity of disease. *Br J Dermatol* 2013;169(5):1066–1070.
14. Tani C, Bellando Randone S, Guiducci S, Della Rossa A. Systemic sclerosis: A critical digest of the recent literature. *Clin Exp Rheumatol* 2013;31(2 Suppl 76):172–179.
15. Subcommittee for scleroderma criteria of the American Rheumatism Association diagnostic and therapeutic criteria committee: Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum* 1980;23:581–590.
16. Galie N, Hoeper MM, Humbert M, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension: The task force for the diagnosis and treatment of pulmonary hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). *Eur Heart J* 2009;30:2493–2537.
17. Clements P, Lachenbruch P, Seibold J, et al. Inter- and intraobserver variability of the total thickness score (modified Rodnan TSS) in systemic sclerosis. *J Rheumatol* 1995;22:1281–1285.
18. Amanzi L, Braschi F, Fiori G, et al. Digital ulcers in scleroderma: Staging, characteristics and sub-setting through observation of 1614 digital lesions. *Rheumatology (Oxford)* 2010;49:1374–1382.
19. Valentini G, D'Angelo S, Della Rossa A, Bencivelli W, Bombardieri S. European Scleroderma Study Group to define disease activity criteria for systemic sclerosis. IV. Assessment of skin thickening by modified Rodnan skin score. *Ann Rheum Dis* 2003; 62(9): 904–905.
20. Balta I, Balta S, Koryurek OM, et al. Serum endocan levels as a marker of disease activity in patients with Behçet disease. *J Am Acad Dermatol* 2014;70(2):291–296.
21. Voiosu T, Bălănescu P, Benguş A, et al. Serum endocan levels are increased in patients with inflammatory bowel disease. *Clin Lab* 2014;60(3):505–510.
22. Celik T, Balta S, Karaman M, et al. Endocan, a novel marker of endothelial dysfunction in patients with essential hypertension: Comparative effects of amlodipine and valsartan. *Blood Press* 2015;24(1):55–60.
23. Yilmaz MI, Siriopol D, Saglam M, et al. Plasma endocan levels associate with inflammation, vascular abnormalities, cardiovascular events, and survival in chronic kidney disease. *Kidney Int* 2014. doi: 10.1038/ki.2014.227.
24. Balta S, Mikhailidis DP, Demirkol S, et al. Endocan – A novel inflammatory indicator in newly diagnosed patients with hypertension: A pilot study. *Angiology*. 2014;65(9):773–777.
25. Balta S, Mikhailidis DP, Demirkol S, Celik T, Ozturk C, Iyisoy A. Endocan and atherosclerosis. *Angiology*. 2015;66(5):490.
26. Liakouli V, Cipriani P, Marrelli A, Alvaro S, Ruscitti P, Giacomelli R. Angiogenic cytokines and growth factors in systemic sclerosis. *Autoimmun Rev* 2011;10(10):590–594.
27. Shin JW, Huggenberger R, Detmar M. Transcriptional profiling of VEGF-A and VEGF-C target genes in lymphatic endothelium reveals endothelial-specific molecule-1 as a novel mediator of lymphangiogenesis. *Blood* 2008;112(6):2318–2326.
28. Chen Y, Leask A, Abraham DJ, et al. Heparan sulfate-dependent ERK activation contributes to the overexpression of fibrotic proteins and enhanced contraction by scleroderma fibroblasts. *Arthritis Rheum* 2008;58(2):577–585.