The Relationship Between Coronary Collateral Circulation and Serum Endocan Levels in Patients With Coronary Chronic Total Occlusions

Koroner Kronik Total Oklüzyonu Olan Hastalarda Serum Endokan Düzeyi ile Koroner Kollateral Dolaşım Arasındaki İlişki

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ABSTRACT

Introduction: Endocan, a dermatan sulfate proteoglycan, is synthesized and secreted by vascular endothelium and it is involved in pathogenesis of vascular diseases, organ-specific inflammations, and angiogenesis. We aimed to investigate the relationship between serum endocan level and development of coronary collateral circulation (CCC) in patients with stable angina pectoris.

Materials and Method: Patients with stable angina with at least one total coronary occlusion (n= 44) and patients who had normal coronary artery (n = 29) were included in the study. Collateral degree was evaluated according to Rentrop and Cohen's classification. Patients who had grade 0 or 1 collateral filling were included in the poorly-developed collateral group (n =19), and those with grade 2 or 3 collateral filling were included in the well-developed collateral group (n = 23).

Results: Endocan levels were significantly elevated in patients with well CCC group compared to those with poor CCC and control groups ($p < 0.001$). Endocan level is higher in patients with poorly-developed CCC than in the control group. Endocan was only found as an independent predictors of well CCC in regression analysis (OR 1.002, 95% confidence interval (CI) 1.001–1.005; $p = 0.001$). ROC analysis detected an optimal cutoff point for endocan level of 1773,9 ng/L that predicted the presence of well development CCC with a sensitivity of 84% and specificity of 63% (AUC:0.749, 95%CI: 0.654-0.849, $p<0.001$).

Conclusion: Elevated endocan level is associated with better CCC in patients with CTO. Endocan may play an important role in the development of CCC.

Keywords: Endocan, Coronary collateral circulation, Angiogenesis, Coronary artery disease

ÖZET

Giriş: Bir dermatan sülfat proteoglikan olan endokan, vasküler endotelyum tarafından sentezlenir ve salgılanır ayrıca vasküler hastalıkların, organ-spesifik inflamasyon ve anjiyogenezin patogenezinde rol oynar. Çalışmamızda, stabil angina pektorisli hastalarda serum endokan düzeyi ile koroner kollateral dolaşım (KKD) gelişimi arasındaki ilişkiyi araştırmayı amaçladık.

Hastalar ve Metod: Çalışmamıza en az bir kronik total tıkanıklığı olan stabil angina hastaları (n = 44) ve normal koroner arteri olan kontrol grubu (n = 29) dahil edildi. Kollateral derecesi, Rentrop ve Cohen sınıflandırmasına göre değerlendirildi. Evre 0-1 kollateral doluşa sahip olan hastalar zayıf gelişmiş KKD grubuna (n=19) , evre 2-3 kollateral doluşa sahip olan hastalar iyi gelişmiş KKD grubuna (n=29) dahil edildi.

Bulgular: İyi gelişmiş KKD grubunda, zayıf gelişmiş KKD grubuna ve kontrol grubuna göre endokan düzeyleri anlamlı olarak yüksek bulundu (p<0.001). Zayıf gelişmiş KKD olan hastalarda endokan düzeyi kontrol grubuna göre daha yüksektir. Endokan düzeyi, regresyon analizinde iyi gelişmiş KKD için tek bağımsız öngördürücü idi (OR 1.002, 95% confidence interval (CI) 1.001–1.005; p = 0.001). İyi gelişmiş KKD öngörmede endokan düzeyi optimal kestirim değeri ≥1773, 9 ng/L olarak bulundu. Endokan düzeyi ≥1773,9 ng/L olması halinde iyi gelişmiş kollateral varlığını %84 duyarlılık ve %63 özgüllükle öngörmekteydi. (AUC:0.749, 95%CI: 0.654-0.849, p<0.001).

Sonuç: Koroner kronik total oklüzyonu olan hastalarda KKD artmış endokan düzeyi ile ilşkilidir. KKD gelişiminde endokanın önemli rolü olabilir.

Anahtar Kelimeler: Endokan, Koroner kollateral dolaşım, Anjiogenez, Koroner arter hastalığı

Geliş Tarihi: 01.06.2018 - **Kabul Tarihi:** 26.11.2018

Introduction

Development of CCC is an adaptation mechanism of myocardium to protect itself from tissue damage due to chronic ischemia and infarction **(**1**).** Well-developed collateral circulation was shown to decrease cardiovascular mortality and morbidity by reducing recurrent myocardial infarction, fatal arrhythmia, ventricular failure and ventricular aneurysm formation (2) Although diabetes mellitus, hypertension, smoking, exercise, chronic inflammation, oxidative stress, duration and severity of myocardial ischemia are well known triggers of collateral development, angiogenesis significantly varies even among individuals with the same clinical status (3, **4**). Vascular growth factors such as vascular endothelial growth factor (VEGF), and transforming growth factor β (TGF-β) and immune system components like monocytes, macrophages, neutrophiles, lymphocytes have an important role in development of CCC. However, various mechanisms involved in development of CCC are still not clear (5).

Endocan (Endothelial-specific molecule 1) is a dermatan sulfate proteoglycan, synthesized and secreted by activated vascular endothelium, and plays role in pathogenesis of vascular diseases, organ-specific inflammations, tumor adhesion, migration and angiogenesis (**6, 7, 8)**. A previous study demonstrated that endocan levels increased in patients with acute coronary syndromes. Authors asserted that increased levels of endocan are not associated with the severity of coronary artery disease assessed with Gensini and SYNTAX scores **(9).** Contradictory results exist about relationship between serum endocan levels and development of collateral circulation in literature **(10, 11) .**

In the present study, we aimed to clarify the controversial consequences of the relationship between the endocan level and CCC. Additionally, we also compared patients with well and poor CCC to the control group.

Material and Methods

Study population

A total of seventy three (n =73) patients who underwent elective coronary angiography with signs of ischemia (positive treadmill exercise test, ischemia in myocardial perfusion scintigraphy, patients with recently detected left ventricular wall motion abnormalities, or patients with stable angina pectoris, etc.) were enrolled to the study. Forty four patients had CTO in at least 1 major epicardial coronary artery and twenty nine subjects had normal coronary anatomy. Patients with acute/chronic infective or inflammatory disease, connective tissue disease, chronic kidney disease (serum creatinine >1.5 mg/dL), history of coronary artery bypass grafting, acute coronary syndrome within last one year, severe valvular heart disease, hepatic and hemolytic disorders, active malignancy and patients

receiving immunosuppressive treatments were excluded. Transthoracic echocardiography was performed in all patients and left ventricular ejection fraction was calculated using modified Simpson's method. Demographic, clinical, laboratory parameters and angiography results of patients were recorded. Local ethical committee approved the study protocol, and written consent was obtained from each patient.

Coronary angiography and evaluation of coronary collateral circulation

Coronary angiography was performed with the standard Judkins technique. Coronary angiograms and the degree of coronary collateral development were evaluated by two experienced interventional cardiologists. The grades of coronary collateral filling was classified according to the Cohen-Rentrop method as follows; *Grade 0*, no filling of any collateral; *Grade 1*, filling of side branches by collateral vessels without viewing of the epicardial segment; *Grade 2,* local filling of the epicardial artery by collateral vessels; *Grade 3*, complete filling of the major epicardial coronary artery by a collateral vessel **(12).** If the patient had more than one vessel with collateral circulation, classification of collateral grade was performed according the best collateral filling. CTOs are described as complete interruption of antegrade coronary blood flow on angiography with a duration of more than 3 months (**13)**. SYNTAX score was calculated as previously described in literature (**14)**. More than 50% stenosis in at least one coronary artery was accepted as stenotic coronary artery disease and the numbers of diseased vessels were recorded as one, two or three vessels disease. Patients with grades 0 and 1 were grouped as poor CCC (n= 19) whereas patients with grades 2 and 3 as good CCC (n= 25). Twenty nine healthy subjects with normal coronary anatomy included in the study as control group.

Routine laboratory tests and measurements of serum endocan levels

Blood for routine hematologic and biochemical tests were collected before the procedure after 12 h of fasting period. The serum levels of creatinine, urea and the hematological values were determined using the standard methods. Patient's serums were separated after centrifugation at 1500 g for 10 minutes and stored at -80 C until analysis. Serum endocan levels were calculated using an enzyme-linked immunosorbent assay (ELISA) kit with high sensitivity and specificity for the detection of human Endocan *(Human endothelial cell-specific molecule 1 Elisa Kit, Shanghai Yehua Biological Technology, Shanghai, P.R. China).* All the blood samples were routinely tested by ELISA in duplicate, and the results were averaged.

Statistical analysis

The data collected during the research were analyzed using the SPSS 17.0 statistical package program (IBM Corp. Armonk, NY). Descriptive statistics were depicted as mean ±standard deviation or

median (inter-quartile range [IQR]) for continuous variables, and as the number of cases (n) and percentages (%) for categorical variables. Normality distribution was evaluated using the Kolmogorov–Smirnov test. The relationships among the parameters were assessed using Pearson's or Spearman's correlation analysis according to the normality of the data. One-Way ANOVA test was used to show the differences between the groups in continuous numeric parameters with normal distribution and compare endocan levels in accordance with the Rentrop–Cohen classification. Student t test was used to compare parametric continuous variables and Mann-Whitney U test was used to compare nonparametric continuous variables. Logistic regression analysis was performed in order to identify independent predictors of well CCC. Variables with a p-value of < 0.1 in univariate logistic regression analysis and variables that considered have clinical importance were included in a multivariate logistic regression model. The receiver–operating characteristic (ROC) curve was used to show the sensitivity and specificity of endocan levels and optimal cutoff value for predicting well CCC. A *p*-value less than 0.05 was considered to be statistically significant.

Results

A total of 73 subjects (25 well CCC, 19 poor CCC and 29 healthy control) were included to the study. Clinical, laboratory, angiographic and demographic characteristics of the groups are demonstrated in Table 1. Baseline characteristics, laboratory parameters and conventional risk factors for coronary artery disease were not different between all groups (p > 0.05). SYNTAX score, number of vessel disease or localization of CTO were similar in patients with poor and well CCC groups (p > 0.5). Endocan levels were significantly elevated in patients with well CCC group compared to those with poor CCC and control groups (Table 1 and Figure 1, p < 0.001). On the other hand, serum endocan levels were significantly lower in control group compared to patients with well CCC and poor CCC (p<0.001, p=0.04 respectively) (Figure 1). Furthermore, as per the correlation analysis, the relationship between endocan and SYNTAX score (Spearman's rho = -0.137, p = 0.380) was found to be non-significant. Moreover, endocan (OR 1.002, 95% confidence interval (CI) 1.001–1.005; p = 0.001) was only found as an independent predictors of well CCC in multivariate logistic regression analysis including age, syntax score and presence of diabetes (Table 2). In ROC analysis, a cut point of 1773,9 ng/L identified well-developed collateral with a 84% sensitivity and 63% specificity (AUC:0.749, 95%CI: 0.654-0.849, p<0.001) (Figure 2).

Discussion

In present study, we found a significant relationship between serum endocan levels and well CCC in patients with stable coronary artery disease. Although some studies compared endocan levels in patients with well and poor collaterals, to the best of our knowledge, this is the first study which included a normal coronary artery group (10,11) Serum endocan levels were significantly increased in patients with CTO independent of the collateral's grade compared with patients with normal

coronaries. Increase in serum endocan level was an independent predictor of development good CCC.

Endocan is an endothelium-derived soluble dermatan sulfate proteoglycan, secreted by inflamed endothelium, that can bind various bioactive molecules related with intercellular signaling and adhesion, organ-specific inflammations, tumor progression, angiogenesis. Thus endocan regulates differentiation, proliferation, adhesion and migration of different cell types (**15**, **16).** Endothelial dysfunction and vascular inflammation are considered as the main pathological processes in the initiation and progression of atherosclerosis (**17)**. In this context, Kose M et al. demonstrated that serum endocan levels are significantly increased in patients with acute coronary syndromes **(9).** Moreover, Ye M. et al proposed that increase in serum endocan level was an independent predictor of coronary slow flow **(18).** Recent study demonstrated increased endocan levels in patients with coronary ectasia which is associated with endothelial dysfunction **(19).** Another study showed that patients with cardiac syndrome-X had higher serum endocan levels, therefore endocan levels might be a good marker of microvascular disease **(20).** Aforementioned emphasized association between microvascular and macrovascular coronary artery disease and serum endocan levels.

Two studies found relationship between coronary collateral development and serum endocan level. Semet E et al. showed association of serum endocan levels with coronary collateral development (**10)**. The study concluded levels of VCAM-1 (vascular cell adhesion molecule- 1), a potential angiogenetic molecule, were increased in patients with good collatarel and positively correlated with serum endocan level. Moreover, serum endocan levels negatively correlated with hs-CRP (high sensitivity C-reactive protein). Gok M. et al. suggested that low endocan levels were independently associated with good coronary collateral circulation **(11).** Furthermore, they have found that endocan levels significantly and positively correlated with hsCRP unlike the previous study. Our results support the hypothesis that serum levels of endocan are higher in patients with good CCC compatible with study of Emet S. et al. Unlike these two studies, presence of the control group in our study makes it more powerful, however low number of participants is the most important limitation.

Previous studies demonstrated that elevated serum CRP levels were associated with impaired CCC (21). If the endocan is a critical molecule that plays a role in the formation of new vessel, it is thought that serum CRP levels should be negatively correlated with serum endocan levels. (7). We think, lack of CRP or any other inflammatory marker in our study does not constitute a significant limitation, since these markers do not provide additional information to comment on this topic. It seems more reasonable that serum endocan levels are higher in patients with good collateral development and negatively correlated with CRP levels, as Emet S et al. and our study have determined (10).

This study showed that a higher serum endocan level was associated with well CCC in patients with stable coronary artery disease who had at least one CTO. Serum endocan levels were an independent predictor for good collateral development. Moreover, serum endocan levels were lower in

patients with normal coronary artery compared to patients with poor and well CCC. Nevertheless our study points out the possible effect of a novel marker in neovascularization which should be investigated with further large studies.

Study Limitations

This study has several limitations. First, small sample size is a major limitation of our study. Secondly, degree of coronary collateral development was assessed visually during routine angiography in a semiquantitative way using the Rentrop score. Intravascular hemodynamic assessment (coronary flow index) might change our results. Peripheral blood sample was used to detect serum endocan levels. Coronary artery or coronary sinus sampling may be more accurate. Finally, lack of inflammation markers (hs-CRP, interleukins, fibrinogen, etc.) may also be considered as a limitation.

References

1 - **Hansen JF** (1989) Coronary collateral circulation: clinical significance and influence on survival in patients with coronary artery occlusion. Am Heart J 1989; 117:290–295

2 - **Nathoe HM**, Koerselman J, Buskens E, et al. Determinants and prognostic significance of collaterals in patients undergoing coronary revascularization. *Am J Cardiol* 2006; 98: 31–35.

3 - **Pohl T**, Seiler C, Billinger M, et al. Frequency distribution of collateral flow and factors influencing collateral channel development. Functional collateral channel measurement in 450 patients with coronary artery disease. *J Am Coll Cardiol* 2001; 38: 1872–1878.

4 - Seiler C: The human coronary collateral circulation. Eur J Clin Invest 2010; 40: 465–476.

5 - **Hakimzadeh N**, Verberne HJ, Siebes M, Piek JJ. The future of collateral artery research. Curr Cardiol Rev. 2014;10(1):73–86

6 - Sarrazin S, Adam E, Lyon M, et al. Endocan or endothelial cell specific molecule-1 (ESM-1): a potential novel endothelial cell marker and a new target for cancer therapy. Biochim Biophys Acta Rev Cancer. 2006;1765(1):25-37.

7 -Bechard D, Scherpereel A, Hammad H, Gentina T, Tsicopoulos A, Aumercier M, Pestel J, Dessaint JP, Tonnel AB, Lassalle P (2001) Human endothelial-cell specific molecule-1 binds directly to the integrin CD11a/CD18 (LFA-1) and blocks binding to intercellular adhesion molecule-11. J Immunol 167:3099–3106

8 - Menon P, Kocher ON, Aird WC. Endothelial cell specific molecule-1 (ESM-1), a novel secreted proteoglycan stimulates vascular smooth muscle cell proliferation and migration. Circulation. 2011;124(21):A15455.

9 - **Kose M,** Emet S, Akpinar TS, Kocaaga M, Cakmak R, Akarsu M, Yuruyen G, Arman Y, Tukek T.Serum Endocan Level and the Severity of Coronary Artery Disease: A Pilot Study. Angiology. 2015 Sep;66(8):727-31.

10 -Emet S, Elitok A, Onur I, Kocaaga M, Bilge AK, Oflaz H, Umman B, Oncul A, Bugra Z, Adalet K, Mercanoglu F. Endocan: a novel biomarker associated with well-developed coronary collateral circulation in patients with stable angina and chronic total occlusion. J Thromb Thrombolysis. 2017 Jan;43(1):60-67. doi: 10.1007/s11239-016-1424-6.

11- Gok M, Kundi H, Kiziltunc E, Topcuoglu C, Ornek E. Endocan Levels and Coronary Collateral Circulation in Stable Angina Pectoris: A Pilot Study. Angiology. 2018 Jan;69(1):43-48.

12- Rentrop KP, Thornton JC, Feit F, et al: Determinants and protective potential of coronary arterial collaterals as assessed by an angioplasty model. Am J Cardiol 1988; 61: 677–684.

13- Stone GW, Kandzari DE, Mehran R, et al. Percutaneous recanalization of chronically occluded coronary arteries: a consensus document: part I. *Circulation* 2005; 112: 2364–2372.

14 - Sianos G, Morel MA, Kappetein AP, et al. The SYNTAX Score: an angiographic tool grading the complexity of coronary artery disease. EuroIntervention 2005;1(2):219–27.

15 - Lassalle P, Molet S, Janin A, Heyden JV, Tavernier J, Fiers W, Devos R, Tonnel AB. ESM-1 is a novel human endothelial cellspecific molecule expressed in lung and regulated by cytokines. J Biol Chem 1996;271:20458–64.

16 - Aparci M, Isilak Z, Uz O, Yalcin M, Kucuk U. Endocan and endothelial dysfunction. Angiology 2015;66:488–9.

17 - Zakynthinos E, Pappa N. Inflammatory biomarkers in coronary artery disease. J Cardiol. 2009;53(3):317-333.

18 - **Ye MF,** Zhao ZW, Luo YK, Dong XF, Yan YM.Elevated endocan concentration is associated with coronary slow flow.Scand J Clin Lab Invest. 2016 Sep;76(5):345-8.

19 - **Turan T**, Akyuz AR, Aykan AC, Kul S, Cirakoglu OF, Aslan AO, Gul I, Uçar U, Demir S, Celik S.Plasma Endocan Levels in Patients With Isolated Coronary Artery Ectasia.Angiology. 2016 Nov;67(10):932-936.

20- **Efe SC**, Demirci K, Ozturk S, Gurbuz AS, Poci N, Kilicgedik A, Guler A, Yilmaz MF, İzgi IA, Kirma C. Serum endocan levels in patients with cardiac syndrome X. Herz. 2017 Jun 6. doi: 10.1007/s00059-017-4580-3.

21 - Gulec S, Ozdemir A, Maradit Kremers H, Dincer I, Atmaca Y, Erol C. Elevated levels of C-reactive protein are associated with impaired coronary collateral development. Eur J Clin Invest. 2006;36(6):369-375.

Table 1. Demographic characteristics, laboratory parameters and endocan levels of patients and control subjects

Variables	Control Group (n=29)	CTO with well CCC(n= 25)	CTO with poor CCC(n= 19)	P value
Age, years (mean±std)	58.1±10.5	63.0±8.6	60.2±9.5	0.183
Sex,Male(n/%)	19 (65.5)	18 (72)	15 (78.9)	0.6
Hypertension(n/%)	17 (58.6)	11 (44)	12 (63.2)	0.39
DiabetesMellitus(n/%)	17 (58.6)	16 (64)	13 (68.4)	0.783
Dyslipidemia(n/%)	13 (44.8)	7 (28)	9 (47.4)	0.331
Smoking (n/%)	13 (44.8)	10 (40)	9 (47.4)	0.879
Urea (mg/dl)	34.7±9.7	33.8±7.3	37.5±9.7	0.365
Creatinine (mg/dl)	0.79±0.21	0.79±0.17	0.89±0.21	0.154
Hemoglobin (g/dl)	13.7±1.7	13.7±1.8	14.2±1.4	0.504
Platelet (1000/mm³)	215 (196-256)	226 (191-255)	230 (207-281)	0.569
Leukocyte (1000/mm³)	8.6 (7.7-11.1)	8.2 (7.1-9.2)	8.2 (6.7-9.7)	0.309
Medications (n,%)				
Beta-Blocker	7 (24.1)	22 (88)	17 (89.5)	**<0.001**
ACEi-ARB	16 (55.2)	16 (64)	13 (68.4)	0.625
Statin	13 (44.8)	13 (52)	9 (47.4)	0.869
Number of DiseaseVessels (n,%)				
One vessel disease		10 (40)	8 (42.1)	0.888
Two vessel disease		10 (40)	6 (31.6)	0.565
Three vessel disease		5 (20)	5 (26.3)	0.62
Position of chronic total occlusion (n,%)				
LAD		6 (24)	6 (31.6)	0.576
Cx		6 (24)	3 (15.8)	0.504
RCA		13 (52)	10 (52.6)	0.967
Syntax Score (mean±std)		12.1±6.3	12.5±5.5	0.802
EndocanLevels (ng/L)	1408.1 (710.4-1553.6)	2194.1 (1849-3979.3)	1657.3 (928.8-2128.8)	**<0.001**

*__ACE-I:__angiotensin converting enzyme inhibitor; __ARB:__angiotensin receptor blockers; __Cx:__circumflex coronary artery; __CTO:__ chronic total occlusion. __LAD:__left anterior descending coronary artery. __RCA:__ right coronary artery

 DOI: 10.5578/khj.67698

Table 2. Multivariate Logistic Regression

	p value	***Exp(B)***	***95% C.I.for EXP(B)***	
			Lower	***Upper***
Age	0.123	1.070	0.982	1.166
Syntax Score	0.958	1.003	0.891	1.130
Diabetes Mellitus	0.234	2.620	0.536	6.789
Endocan	0.001	1.002	1.001	1.005

 DOI: 10.5578/khj.67698

Figure 1: Serum endocan concentration in control subjects and in patients with well and poor coronary collateral circulation (CCC)

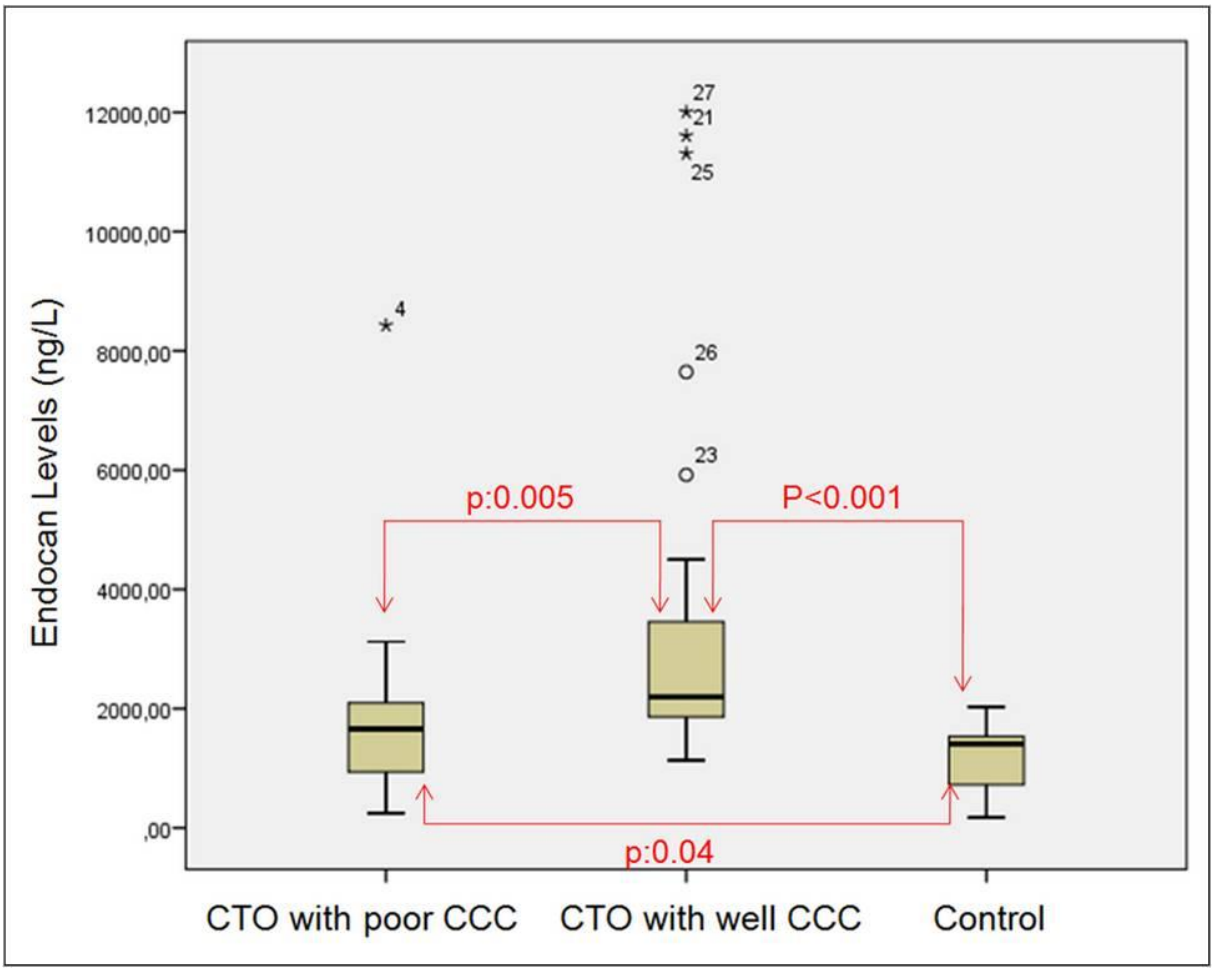

Figure 2: Receiver operating characteristic (ROC) curve analysis for endocan in coronary collateral circulation