

The association between serum angiogenin and osteopontin levels and coronary collateral circulation in patients with chronic total occlusion

^{ID} Kadri Murat Gürses, ^{ID} Muhammed Ulvi Yalçın¹, ^{ID} Duygu Koçyiğit², ^{ID} Muhammed Said Beşler*,
^{ID} Hande Canpınar**, ^{ID} Banu Evranos*, ^{ID} Hikmet Yorgun*, ^{ID} Mehmet Levent Şahiner*,
^{ID} Ergün Barış Kaya*, ^{ID} Necla Özer*, ^{ID} Dicle Güç**, ^{ID} Kudret Aytemir*, ^{ID} Lale Tokgözoğlu*

Department of Basic Medical Sciences, Faculty of Medicine, Adnan Menderes University; Aydın-Turkey

¹Department of Cardiology, Faculty of Medicine, Selçuk University; Konya-Turkey

²Cardiology Clinics, Afyonkarahisar Dinar State Hospital; Afyonkarahisar-Turkey

Departments of *Cardiology, and **Basic Oncology, Cancer Institute, Faculty of Medicine, Hacettepe University; Ankara-Turkey

ABSTRACT

Objective: A well-developed coronary collateral circulation lowers both in-hospital and long-term morbidity and mortality limiting the infarct. Angiogenin (AGN) and osteopontin (OPN) are known to be potent inducers of angiogenesis. The aim of the present study was to investigate the relationship between serum ANG and OPN levels and collateral filling grade in subjects with stable coronary artery disease (SCAD).

Methods: A total of 122 age- and gender-matched consecutive patients who were found to have total occlusion (n=70) and no significant stenosis in epicardial coronary arteries (n=52) who underwent coronary angiography due to SCAD between January 2015 and July 2017 were included in the study. AGN and OPN levels were measured using enzyme-linked immunosorbent assay. Coronary collateral circulation was graded using Rentrop's classification of collateral filling.

Results: A total of 52 patients (61.60±11.78 years, 61.5% male) without significant epicardial coronary artery stenosis and 70 patients (62.87±8.24 years, 65.7% male) with totally occluded coronary arteries were included in the study. Subjects with total occlusion had significantly higher levels of AGN [122.00 (79.00–623.00) pg/mL vs. 98.00 (18.00–160.00) pg/mL, p<0.001] and OPN [1863.50 (125.00–6500.00) pg/mL vs. 451.00 (112.00–1850.00) pg/mL, p<0.001] than those without significant stenosis. In addition, AGN [127.00 (87.00–623.00) pg/mL vs. 110.00 (79.00–188.00) pg/mL, p=0.011] and OPN [2681.00 (126.00–6500.00) pg/mL vs. 649.00 (125.00–4255.00) pg/mL, p=0.001] levels were significantly higher in patients with better developed collaterals. Serum AGN and OPN levels were found to be significantly associated with coronary collateral development.

Conclusion: AGN and OPN are associated with better developed coronary collateral circulation and may have therapeutic implications for the promotion of coronary collateral development. (*Anatol J Cardiol* 2019; 22: 77-84)

Keywords: coronary collateral circulation, osteopontin, angiogenin

Introduction

Ischemic heart disease (IHD) is one of the leading causes of death worldwide. A well-functioning coronary collateral circulation has been shown to have a favorable impact on mortality, myocardial infarction recurrence, and adverse cardiovascular events in patients with chronic IHD (1-3). Angiogenesis and arteriogenesis are the main mechanisms for coronary collateral development. Previous studies have shown a relationship between

biomarkers related to angiogenesis and arteriogenesis and coronary collateral development (3-7).

Angiogenin (AGN) is a ribonuclease and has been shown to induce blood vessel formation (8). Transplantation of autologous mesenchymal stem cells overexpressing the AGN gene to the heart in a chronic ischemia model was associated with the improvement of heart perfusion and function (9). Previous studies have also demonstrated that AGN is a marker of three-vessel coronary artery disease (CAD) (10). In addition, AGN level increases

Address for correspondence: Dr. Muhammed Ulvi Yalçın, Selçuk Üniversitesi Tıp Fakültesi, Kardiyoloji Anabilim Dalı, Konya-Türkiye

Phone: +90 332 221 00 00 Fax: +90 332 323 67 23 E-mail: ulviyalcin@gmail.com

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in acute coronary syndrome (ACS) and has a prognostic value in patients with ACS (11).

Osteopontin (OPN) is a 34 kDa, phosphorylated sialic acid-rich non-collagenous matricellular protein that functions in a variety of biological processes, including inflammation, immunity, wound repair, tumorigenesis, cell adhesion, cell migration, bone mineralization, and remodeling (12). It is a unique component of the extracellular matrix that may play an important role in the control of vascular growth. The role of OPN in ischemic limb revascularization has been demonstrated in a previous study (13). In addition, decreased OPN expression has been related with impaired neovascularization, whereas overexpression of OPN has been found to be increased during angiogenesis (14).

The relationship between serum AGN and OPN levels with coronary collateral circulation in patients with stable CAD (SCAD) has not been evaluated yet in the literature. The aim of the present study was to compare AGN and OPN levels in patients who were found to have either chronic total occlusion (CTO) or no significant epicardial coronary artery stenosis during coronary angiography due to SCAD. In addition, the present study aimed to investigate the relationship between AGN and OPN levels and collateral filling grade in subjects with CTO.

Methods

This was an observational study. A total of 122 patients who were admitted to our outpatient clinics with stable angina pectoris and scheduled for elective coronary angiography between January 2015 and July 2017 were enrolled in the study. Of the 122 patients, 70 had total occlusion in at least one major coronary artery, and 52 age- and gender-matched subjects with no significant epicardial coronary artery stenosis were classified as the control group. Stable angina pectoris was diagnosed according to the American College of Cardiology/American Heart Association criteria (15). The decision of coronary angiography was made based on a positive non-invasive stress test or presence of high clinical suspicion for severe coronary artery stenosis. The study was approved by the Institutional Ethics Committee in compliance with the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all participants.

Baseline demographic and clinical characteristics including age, gender, body mass index (BMI), history of smoking, hypertension, diabetes mellitus, and family history of CAD were recorded for all patients. Diabetes mellitus was defined as plasma glucose level ≥ 126 mg/dL at fasting or ≥ 200 mg/dL at any measurement or the current use of a glucose-lowering agent. Hypertension was defined as either the current use of anti-hypertensive medication or the documentation of a systolic blood pressure ≥ 140 mm Hg and/or a diastolic blood pressure ≥ 90 mm Hg. Family history of CAD was defined as the presence of CAD or sudden cardiac death in a first-degree relative before the age of 55 years for men and 65 years for women.

Patients with recent ACS (within the last 6 months), previous revascularization, heart failure with reduced ejection fraction (left ventricular ejection fraction $< 40\%$), sign and/or symptoms of decompensated heart failure, symptomatic peripheral vascular disease (transient ischemic attack, stroke, intermittent claudication, peripheral revascularization, or amputation), evidence of ongoing infection or inflammation, chronic kidney disease (serum creatinine > 1.4 mg/dL), chronic obstructive pulmonary disease, previous diagnosis of malignancy, BMI < 20 kg/m² or > 30 kg/m², and patients with diabetes receiving insulin treatment were excluded from the study.

Coronary angiography

Standard Judkins technique was used for coronary artery visualization. At least two orthogonal plane images were obtained for each coronary artery. The coronary angiograms of the study population were examined again for collateral vessels by two experienced interventional cardiologists from our institute who were totally blinded to the study. CTO was defined as a complete interruption of coronary artery flow.

Coronary collateral grading was determined according to Rentrop's method (16), with grade 0, no filling of any collateral vessels; grade 1, filling of side branches of the artery to be perfused by collateral vessels without visualization of epicardial segment; grade 2, partial filling of the epicardial artery via collateral vessels; and grade 3, complete filling of the epicardial artery via collateral vessels (17). The CTO group was divided into two groups according to the degree of collateral development. Patients who were graded as 0 or 1 were classified as the poor collateral group, whereas patients graded as 2 or 3 were classified as the better developed collateral group.

Laboratory analysis

Venous peripheral blood samples for complete blood count and biochemistry panel were withdrawn following 12 h of fasting before coronary angiography. Samples were centrifuged at 1600 rpm for 15 min and stored at -80 °C. After the completion of patient recruitment, frozen serum samples were rapidly thawed, brought to room temperature of 24 °C, and assayed for the presence of OPN (Human OPN PicoKine™ ELISA Kit; Boster, CA, USA) and AGN (Human ANG PicoKine™ ELISA Kit; Boster) by using ELISA kits according to the manufacturer's instructions. Serial dilutions of known concentrations of human OPN and AGN were used to construct a standard curve of the analytes. The intensity of the color in each well was measured on a microplate reader (Molecular Devices, SpectraMax Plus, UK). Serum OPN and AGN levels were then estimated by extrapolation from a log:log linear regression curve determined from the serially diluted OPN ranging from 5000 pg/mL to 156 pg/mL and AGN ranging from 5000 pg/mL to 78 pg/mL.

Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences software (IBM SPSS Statistics for

Windows, version 20.0; IBM Corp., Armonk, NY, USA). Normally distributed parameters were presented as mean ± standard deviation, and skewed parameters were expressed as median (interquartile range: minimum–maximum). Categorical variables were presented as percentage (%). Kolmogorov–Smirnov test was used to test the normality of distribution. Mann–Whitney U test or Student’s t-test was used to compare continuous variables, where appropriate. Chi-square test was used to compare categorical variables. Binary logistic regression analysis was used to determine the independent associates of better developed coronary collaterals. The validity of the multiple regression model was tested by calculating the Variance Inflation Factors for the variables included in the models. A p value <0.05 was considered statistically significant.

Results

In the patient group, there were 70 patients with coronary total occlusion in at least one major coronary artery. The age of the patient group was 62.87±8.24 years. The patient group was composed of 65.7% male. In the control group, there were 52

patients with no significant epicardial coronary artery stenosis. The age of the control group was 61.60±11.78 years. The control group was composed of 61.5% male. Baseline demographic, clinical, laboratory, and echocardiographic parameters of the study population are shown in Table 1. Baseline characteristics did not differ between the two groups except high-density lipoprotein cholesterol, AGN, and OPN levels. Subjects with CTO had significantly higher levels of AGN [122.00 (79.00–623.00) vs. 98.00 (18.00–160.00) pg/mL, p<0.001] and OPN [1863.50 (125.00–6500.00) vs. 451.00 (112.00–1850.00) pg/mL, p<0.001] than the control group. Smoking and AGN and OPN levels were found to be independently associated with the presence of CTO in the multiple binary logistic regression analysis (Table 2).

When patients with CTO were grouped according to their Rentrop grade, 23 patients were classified in the poor collateral group (grades 0 and 1), and 47 patients were classified in the better developed collateral group (grades 2 and 3). Baseline characteristics were similar between these two groups except for the duration of ischemic symptoms and the number of affected coronary arteries (Table 3). Among laboratory parameters, only AGN and OPN levels differed significantly between the two groups (Table 3). AGN [127.00 (87.00–623.00) vs. 110.00 (79.00–188.00)

Table 1. Baseline demographic, clinical, laboratory, and echocardiographic parameters of the study population

	Control group (n=52)	Chronic total occlusion group (n=70)	P
Clinical parameters			
Gender, male, n (%)	32 (61.5)	46 (65.7)	0.635
Age, years	61.60±11.78	62.87±8.24	0.483
BMI, kg/m ²	26.07±3.16	26.65±3.53	0.357
Smoking, n (%)	19 (36.5)	38 (54.3)	0.052
Family history of CAD, n (%)	19 (36.5)	28 (40.0)	0.698
Diabetes mellitus, n (%)	13 (25.0)	25 (35.7)	0.206
Hypertension, n (%)	32 (61.5)	47 (67.1)	0.522
Laboratory parameters			
WBC count, ×10 ⁹ /L	8.30±1.96	7.92±2.33	0.964
Fasting blood glucose, mg/dL	109.56±51.30	121±43.19	0.184
Total cholesterol, mg/dL	201.96±45.42	190.05±46.05	0.158
LDL cholesterol, mg/dL	125.88±33.58	119.06±38.53	0.309
HDL cholesterol, mg/dL	44.50±12.16	39.16±7.22	0.003
Triglyceride, mg/dL	157.86±70.95	159.17±80.00	0.926
Angiogenin, pg/mL	98.00 (18.00-160.00)	122.00 (79.00-623.00)	<0.001*
Osteopontin, pg/mL	451.00 (112.00-1850.00)	1863.50 (125.00-6500.00)	<0.001*
Echocardiographic parameters			
LV end-diastolic diameter, mm	4.84±0.63	4.94±0.50	0.337
LVEF, %	60.57±4.52	59.25±8.04	0.290

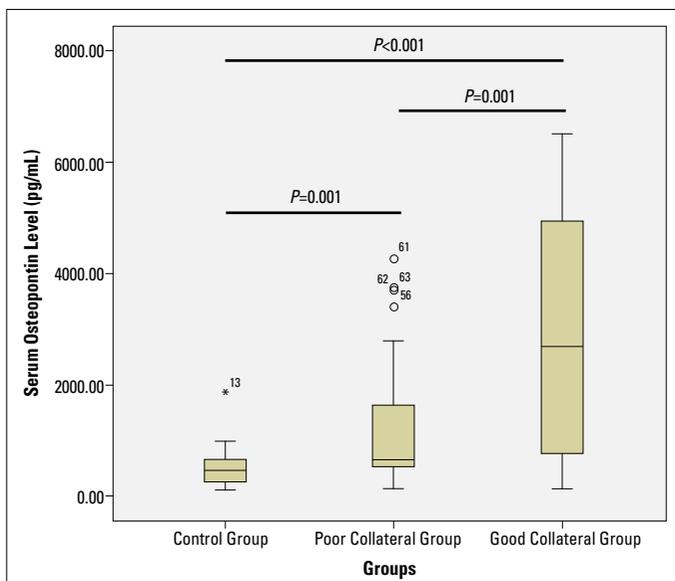
*P<0.0, statistically significant

BMI - body mass index; CAD - coronary artery disease; HDL - high-density lipoprotein; LDL - low-density lipoprotein; LV - left ventricular; LVEF - left ventricular ejection fraction; WBC - white blood cell

Table 2. Binary logistic regression analyses to determine the independent associates of chronic total occlusion

Variables	Univariate		Multiple	
	OR (95% CI)	P	OR (95% CI)	P
Smoking	2.062 (0.989-4.300)	0.053	3.994 (1.012-15.765)	0.048*
Fasting blood glucose, mg/dL	1.006 (0.997-1.014)	0.189	-	-
Total cholesterol, mg/dL	0.994 (0.986-1.002)	0.158	-	-
HDL cholesterol, mg/dL	0.944 (0.906-0.983)	0.005*	-	-
Angiogenin, pg/mL	1.026 (1.012-1.040)	<0.001*	1.046 (1.016-1.077)	0.002*
Osteopontin, pg/mL	1.002 (1.001-1.003)	<0.001*	1.002 (1.001-1.003)	0.007*

*P<0.05, statistically significant
CI - confidence interval; HDL - high-density lipoprotein; OR - odds ratio

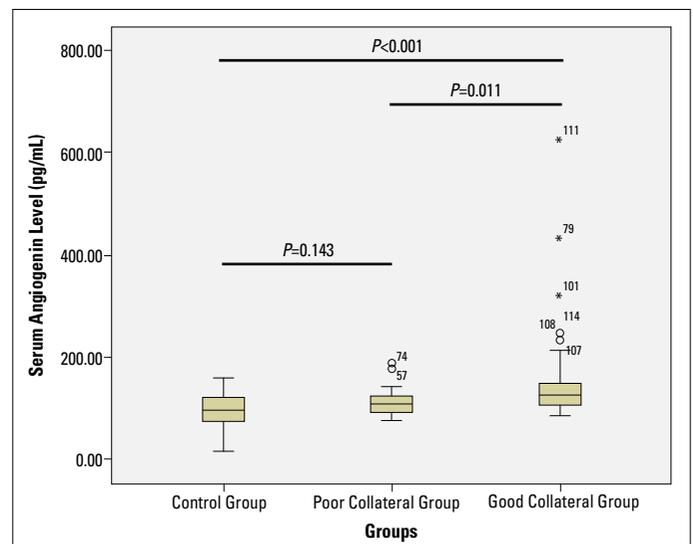
**Figure 1.** Box plot graph depicting osteopontin levels in the control, poor, and good coronary collateral groups

pg/mL, $p=0.011$) and OPN [2681.00 (126.00–6500.00) vs. 649.00 (125.00–4255.00) pg/mL, $p=0.001$] levels were significantly higher in patients with better developed coronary collaterals than in those with poor collateral circulation. Box plot graphs depicting OPN and AGN levels in the control, poor, and good coronary collateral groups, including p values, are shown in Figures 1 and 2.

The multiple binary logistic regression analysis revealed that AGN [odds ratio (OR) 1.032; 95% confidence interval (CI) 1.008–1.057, $p=0.010$] and OPN (OR 1.001; 95% CI 1.000–1.001, $p<0.001$) levels were independently associated with better developed coronary collateral circulation in patients with CTO (Table 4).

Discussion

The present study demonstrated an association between serum AGN and OPN levels and the presence of coronary CTO.

**Figure 2.** Box plot graph depicting angiogenin levels in the control, poor, and good coronary collateral groups

In addition, serum AGN and OPN levels were found to be significantly associated with better coronary collateral circulation. To the best of our knowledge, this is the first study demonstrating the relationship between AGN and OPN levels and the presence of coronary CTO and degree of coronary collateral development.

AGN is a potent angiogenic growth factor related to endothelial cell proliferation. It was first isolated as a tumor angiogenic factor based on its angiogenic activity; therefore, subsequent studies have focused mainly on its angiogenic capacity (18). It has been shown to interact with all steps of angiogenesis, including migration, proliferation, and tube formation (19). Previous studies have suggested that AGN also plays a role in regulating rRNA transcription in various conditions, such as cancer, chronic heart failure, ACS, wound healing, and asthma (11, 20-23).

Tello-Montoliu et al. (11) have demonstrated that plasma AGN levels are significantly increased in ACS, and that high AGN levels are predictive of adverse events during follow-up. AGN-modified mesenchymal stem cells (MSCs) have been shown to

Table 3. Baseline demographic and clinical parameters of the study population regarding Rentrop's classification of coronary collateral filling

	Poor collateral (n=23)	Better developed collateral (n=47)	P
Clinical parameters			
Gender, male, n (%)	15 (65.2)	31 (66.0)	0.951
Age, years	63.30±7.85	62.66±8.5	0.761
BMI, kg/m ²	26.66±2.91	26.65±3.82	0.996
Smoking, n (%)	14 (60.9)	24 (51.1)	0.439
Family history of CAD, n (%)	10 (43.5)	18 (38.3)	0.678
Diabetes mellitus, n (%)	6 (26.1)	19 (40.4)	0.240
Hypertension, n (%)	16 (69.6)	31 (66.0)	0.763
Gensini score	32 (2-32)	32 (4-32)	0.193
Duration of angina pectoris, months	3 (3-72)	25 (3-350)	0.001*
No. of coronary arteries with severe stenosis, n (%)			
1	13 (56.5)	40 (85.1)	0.022*
2	9 (39.1)	7 (14.9)	
3	1 (4.3)	0 (0.0)	
Medications			
Renin-angiotensin system blockers, n (%)	14 (60.9)	28 (59.6)	0.917
Beta blockers, n (%)	16 (69.6)	28 (59.6)	0.416
Calcium channel blockers, n (%)	2 (8.7)	7 (14.9)	0.467
Statins, n (%)	16 (69.6)	26 (55.3)	0.253
ASA, n (%)	19 (82.6)	34 (72.3)	0.347
Glucose-lowering drugs, n (%)	3 (13)	13 (27.7)	0.171
Laboratory parameters			
WBC count, ×10 ⁹ /L	8.21±2.69	7.78±2.15	0.473
Fasting blood glucose, mg/dL	113.13±43.30	124.85±43.08	0.290
Total cholesterol, mg/dL	185.35±48.28	192.35±45.27	0.554
LDL cholesterol, mg/dL	114.65±39.24	121.21±38.41	0.507
HDL cholesterol, mg/dL	38.48±8.97	39.49±6.28	0.586
Triglyceride, mg/dL	161.09±105.89	158.23±65.08	0.890
Angiogenin, pg/mL	110.00 (79.00-188.00)	127.00 (87.00-623.00)	0.011*
Osteopontin, pg/mL	649.00 (125.00-4255.00)	2681 (126.00-6500.00)	0.001*
Echocardiographic parameters			
LV end-diastolic diameter, mm	5.0±0.64	4.9±0.42	0.458
LVEF, %	59.87±4.83	58.95±9.25	0.656
*P<0.05, statistically significant ASA - acetylsalicylic acid; BMI - body mass index; CAD - coronary artery disease; HDL - high-density lipoprotein; LDL - low-density lipoprotein; LV - left ventricular; LVEF - left ventricular ejection fraction; WBC - white blood cell			

enhance the tolerance of engrafted MSCs to hypoxia injury in vitro and improve their viability in infarcted hearts, thus helping to preserve the left ventricular (LV) contractile function and attenuate LV remodeling through vasculogenesis (19). Krecki et al. (10) have reported that AGN is a novel marker of three-vessel CAD, showing a relationship with the angiographic severity of the

disease. In our study, AGN levels were higher in patients with coronary CTO and were independently associated with better developed coronary collateral circulation.

OPN is a transformation-related phosphorylated acidic glycoprotein (12). It is a key player in essential biological phenomena, such as inflammation, autoimmune disease progression,

Table 4. Binary logistic regression analyses to determine the independent associates of better developed coronary collateral circulation

Variables	Univariate		Multiple	
	OR (95% CI)	P	OR (95% CI)	P
Gensini score	1.054 (0.969-1.147)	0.222	-	-
Duration of angina pectoris, months	1.018 (1.00-1.036)	0.051	1.023 (1.00-1.047)	0.052
No. of coronary arteries with severe stenosis, n	0.232 (0.077-0.700)	0.009*	-	-
Glucose-lowering drugs, n	0.392 (0.100-1.546)	0.181	-	-
Angiogenin, pg/mL	1.018 (1.00-1.037)	0.046*	1.032 (1.008-1.057)	0.010*
Osteopontin, pg/mL	1.001 (1.00-1.001)	0.002*	1.001 (1.000-1.001)	<0.001*

*P<0.05, statistically significant
CI - confidence interval; OR - odds ratio

bone remodeling, angiogenesis, aortic stenosis, and tumor cell metastasis (24-28). Although first isolated from mineralized bone matrix, OPN can also be synthesized by several other cells, such as cardiomyocytes, vascular endothelial cells, and fibroblasts. OPN has been detected in human atherosclerotic plaques in the aorta, carotid, and coronary arteries, and it has been shown to be implicated in the development and progression of atherosclerosis (29). Increased levels of OPN have been found to be related to the presence and extent of CAD and to restenosis following percutaneous coronary revascularization (29, 30). Leaw et al. (31) have shown that rat carotid neointimal lesions induced by balloon catheter denudation can be reduced by in vivo neutralization of OPN.

Some studies have implicated the role of OPN in postnatal neovascularization, such as increased mRNA expression at the sites of ischemia-induced retinal neovascularization in mice and impaired neovascularization in blunted OPN expression in a murine model of hind limb ischemia (14, 32). In addition, OPN was shown to be associated with tumor-related angiogenesis, metastasis, and healing after bone fractures (33). In our study, OPN levels were higher in the coronary CTO group and were independently associated with better developed coronary collaterals.

A significant number of patients with coronary CTO are either ineligible or demonstrate suboptimal responses to surgical and percutaneous revascularization approaches. A non-invasive approach aimed at promoting the growth of coronary collateral blood vessels has been proposed as a new treatment strategy in such patients (34). Therefore, identifying new molecules that promote the growth of coronary collateral blood vessels is very important. OPN and AGN were widely studied for their angiogenic potential in different disease conditions (23, 26, 32, 33). In the current study, AGN and OPN levels were revealed to be independently associated with the coronary collateral development in patients with coronary CTO. In addition, OPN itself and molecules affecting OPN expression were shown to improve the angiogenic properties of stem cells in recent studies (35, 36). All these findings suggest that these molecules can constitute fu-

ture therapeutic targets for medical revascularization, and their exact role in coronary collateral development should be clarified in further studies.

Our study has several limitations. First, this is a single-center study, and the small number of the study population has limited some statistical analyses to be performed. Second, this is a hypothesis-generating clinical study, and a mechanistic link between levels of AGN and OPN and the presence of coronary total occlusion and degree of collateral circulation development cannot be proposed with these data.

Conclusion

In conclusion, the present study highlights the association between AGN and OPN levels and coronary collateral circulation in patients with coronary total occlusion. However, the underlying mechanisms remain largely unknown. The results of our study merit further studies to reveal if AGN and OPN are biomarkers or moderators in coronary collateral development.

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B.E., H.Y., M.L.Ş., E.B.K., N.Ö., D.G., K.A., L.T.; Literature search – K.M.G., M.U.Y., D.K., N.Ö., D.G., K.A., L.T.; Writing – K.M.G., M.U.Y., D.K., M.S.B., H.C., B.E., H.Y., M.L.Ş., E.B.K., N.Ö., D.G., K.A., L.T.; Critical review – B.E., H.Y., M.L.Ş., E.B.K., N.Ö., D.G., K.A., L.T.

References

1. Seiler C, Engler R, Berner L, Stoller M, Meier P, Steck H, et al. Prognostic relevance of coronary collateral function: confounded or causal relationship? *Heart* 2013; 99: 1408-14. [CrossRef]
2. Meier P, Hemingway H, Lansky AJ, Knapp G, Pitt B, Seiler C. The impact of the coronary collateral circulation on mortality: a meta-analysis. *Eur Heart J* 2012; 33: 614-21. [CrossRef]
3. Seiler C, Stoller M, Pitt B, Meier P. The human coronary collateral circulation: development and clinical importance. *Eur Heart J* 2013; 34: 2674-82. [CrossRef]
4. Helisch A, Schaper W. Angiogenesis and arteriogenesis--not yet for prescription. *Z Kardiol* 2000; 89: 239-44. [CrossRef]
5. Meier P, Gloekler S, de Marchi SF, Indermuehle A, Rutz T, Traupe T, et al. Myocardial salvage through coronary collateral growth by granulocyte colony-stimulating factor in chronic coronary artery disease: a controlled randomized trial. *Circulation* 2009; 120: 1355-63. [CrossRef]
6. Zorkun C, Akkaya E, Zorlu A, Tandogan I. Determinants of coronary collateral circulation in patients with coronary artery disease. *Anadolu Kardiyol Derg* 2013; 13: 146-51. [CrossRef]
7. Oğuz D, Atmaca Y, Ozdöl C, Ozdemir AO, Kaya CT, Erol C. The relationship between coronary collateral artery development and inflammatory markers. *Anadolu Kardiyol Derg* 2014; 14: 336-41.
8. Shestenko OP, Nikonov SD, Mertvetsov NP. Angiogenin and its role in angiogenesis. *Mol Biol (Mosk)* 2001; 35: 349-71. [CrossRef]
9. Huang SD, Lu FL, Xu XY, Liu XH, Zhao XX, Zhao BZ, et al. Transplantation of angiogenin-overexpressing mesenchymal stem cells synergistically augments cardiac function in a porcine model of chronic ischemia. *J Thorac Cardiovasc Surg* 2006; 132: 1329-38.
10. Kręcki R, Krzemińska-Pakuła M, Drożdż J, Szcześniak P, Peruga JZ, Lipiec P, et al. Relationship of serum angiogenin, adiponectin and resistin levels with biochemical risk factors and the angiographic severity of three-vessel coronary disease. *Cardiol J* 2010; 17: 599-606.
11. Tello-Montoliu A, Marin F, Patel J, Roldan V, Mainar L, Vicente V, et al. Plasma angiogenin levels in acute coronary syndromes: implications for prognosis. *Eur Heart J* 2007; 28: 3006-11. [CrossRef]
12. Icer MA, Gezmen-Karadag M. The multiple functions and mechanisms of osteopontin. *Clin Biochem* 2018; 59: 17-24. [CrossRef]
13. Duvall CL, Weiss D, Robinson ST, Alameddine FM, Guldberg RE, Taylor WR. The role of osteopontin in recovery from hind limb ischemia. *Arterioscler Thromb Vasc Biol* 2008; 28: 290-5. [CrossRef]
14. Lyle AN, Joseph G, Fan AE, Weiss D, Landazuri N, Taylor WR. Reactive oxygen species regulate osteopontin expression in a murine model of postischemic neovascularization. *Arterioscler Thromb Vasc Biol* 2012; 32: 1383-91. [CrossRef]
15. Fihn SD, Blankenship JC, Alexander KP, Bittl JA, Byrne JG, Fletcher BJ, et al.; American College of Cardiology/American Heart Association Task Force on Practice Guidelines; American Association for Thoracic Surgery; Preventive Cardiovascular Nurses Association; Society for Cardiovascular Angiography and Interventions; Society of Thoracic Surgeons. 2014 ACC/AHA/AATS/PCNA/SCAI/STS focused update of the guideline for the diagnosis and management of patients with stable ischemic heart disease: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines, and the American Association for Thoracic Surgery, Preventive Cardiovascular Nurses Association, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons. *J Thorac Cardiovasc Surg* 2015; 149: e5-23.
16. Rentrop KP, Cohen M, Blanke H, Phillips RA. Changes in collateral channel filling immediately after controlled coronary artery occlusion by an angioplasty balloon in human subjects. *J Am Coll Cardiol* 1985; 5: 587-92. [CrossRef]
17. Cohen M, Rentrop KP. Limitation of myocardial ischemia by collateral circulation during sudden controlled coronary artery occlusion in human subjects: a prospective study. *Circulation* 1986; 74: 469-76. [CrossRef]
18. Fett JW, Strydom DJ, Lobb RR, Alderman EM, Bethune JL, Riordan JF, et al. Isolation and characterization of angiogenin, an angiogenic protein from human carcinoma cells. *Biochemistry* 1985; 24: 5480-6. [CrossRef]
19. Liu XH, Bai CG, Xu ZY, Huang SD, Yuan Y, Gong DJ, et al. Therapeutic potential of angiogenin modified mesenchymal stem cells: angiogenin improves mesenchymal stem cells survival under hypoxia and enhances vasculogenesis in myocardial infarction. *Microvasc Res* 2008; 76: 23-30. [CrossRef]
20. Tsuji T, Sun Y, Kishimoto K, Olson KA, Liu S, Hirukawa S, et al. Angiogenin is translocated to the nucleus of HeLa cells and is involved in ribosomal RNA transcription and cell proliferation. *Cancer Res* 2005; 65: 1352-60. [CrossRef]
21. Patel JV, Sosin M, Gunarathne A, Hussain I, Davis RC, Hughes EA, et al. Elevated angiogenin levels in chronic heart failure. *Ann Med* 2008; 40: 474-9. [CrossRef]
22. Steed DL, Trumpower C, Duffy D, Smith C, Marshall V, Rupp R, et al. Amnion-derived cellular cytokine solution: a physiological combination of cytokines for wound healing. *Eplasty* 2008; 8: e18.
23. Hoshino M, Takahashi M, Aoike N. Expression of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin immunoreactivity in asthmatic airways and its relationship to angiogenesis. *J Allergy Clin Immunol* 2001; 107: 295-301. [CrossRef]
24. Senger DR, Wirth DF, Hynes RO. Transformed mammalian cells secrete specific proteins and phosphoproteins. *Cell* 1979; 16: 885-93.
25. Gimba ER, Tilli TM. Human osteopontin splicing isoforms: known roles, potential clinical applications and activated signaling pathways. *Cancer Lett* 2013; 331: 11-7. [CrossRef]
26. Blasberg JD, Goparaju CM, Pass HI, Donington JS. Lung cancer osteopontin isoforms exhibit angiogenic functional heterogeneity. *J Thorac Cardiovasc Surg* 2010; 139: 1587-93. [CrossRef]
27. Grau JB, Poggio P, Sainger R, Vernick WJ, Seefried WF, Branchetti E, et al. Analysis of osteopontin levels for the identification of asymptomatic patients with calcific aortic valve disease. *Ann Thorac Surg* 2012; 93: 79-86. [CrossRef]
28. Scatena M, Liaw L, Giachelli CM. Osteopontin: a multifunctional molecule regulating chronic inflammation and vascular disease. *Arterioscler Thromb Vasc Biol* 2007; 27: 2302-9. [CrossRef]
29. O'Brien ER, Garvin MR, Stewart DK, Hinohara T, Simpson JB, Schwartz SM, et al. Osteopontin is synthesized by macrophage, smooth muscle, and endothelial cells in primary and restenotic human coronary atherosclerotic plaques. *Arterioscler Thromb* 1994; 14: 1648-56. [CrossRef]
30. Ohmori R, Momiyama Y, Taniguchi H, Takahashi R, Kusuhara M, Nakamura H, et al. Plasma osteopontin levels are associated with the presence and extent of coronary artery disease. *Atherosclerosis* 2003; 170: 333-7. [CrossRef]

31. Liaw L, Lombardi DM, Almeida MM, Schwartz SM, deBlois D, Giachelli CM. Neutralizing antibodies directed against osteopontin inhibit rat carotid neointimal thickening after endothelial denudation. *Arterioscler Thromb Vasc Biol* 1997; 17: 188-93. [\[CrossRef\]](#)
32. Takagi H, Suzuma K, Otani A, Oh H, Koyama S, Ohashi H, et al. Role of vitronectin receptor-type integrins and osteopontin in ischemia-induced retinal neovascularization. *Jpn J Ophthalmol* 2002; 46: 270-8.
33. Duvall CL, Taylor WR, Weiss D, Wojtowicz AM, Guldberg RE. Impaired angiogenesis, early callus formation, and late stage remodeling in fracture healing of osteopontin-deficient mice. *J Bone Miner Res* 2007; 22: 286-97. [\[CrossRef\]](#)
34. Lavine KJ, Ornitz DM. Rebuilding the coronary vasculature: hedgehog as a new candidate for pharmacologic revascularization. *Trends Cardiovasc Med* 2007; 17: 77-83. [\[CrossRef\]](#)
35. Carvalho MS, Cabral JM, da Silva CL, Vashishth D. Synergistic effect of extracellularly supplemented osteopontin and osteocalcin on stem cell proliferation, osteogenic differentiation, and angiogenic properties. *J Cell Biochem* 2019; 120: 6555-69. [\[CrossRef\]](#)
36. Tang Z, Xie H, Jiang S, Cao S, Pu Y, Zhou B, et al. Safflower yellow promotes angiogenesis through p-VHL/ HIF-1 α /VEGF signaling pathway in the process of osteogenic differentiation. *Biomed Pharmacother* 2018; 107: 1736-43. [\[CrossRef\]](#)