Increased circulating soluble CD40 levels in patients with slow coronary flow phenomenon: an observational study

Koroner yavaş akım hastalarında kanda artmış CD40 konsantrasyonu: Bir gözlemsel çalışma

Murtaza Emre Durakoğlugil, Sinan Altan Kocaman[1], Mustafa Çetin[1], Aynur Kırbaş, Aytun Çanga[1], Turan Erdoğan, Yüksel Çiçek*

From Departments of Cardiology and *Biochemistry, Faculty of Medicine, Recep Tayyip Erdoğan University, Rize-*Turkey*
[1]Clinic of Cardiology, Rize Education and Research Hospital, Rize-*Turkey*

Abstract

Objective: Slow coronary flow (SCF) is an angiographic finding characterized with delayed opacification of epicardial coronary arteries without obstructive coronary disease. CD40/CD40 ligand (CD40L) signaling seems closely related to atherosclerosis due to increased inflammation and prothrombotic state. We investigated whether soluble CD40 (sCD40), an indirect marker of CD40/CD40L dyad, is related to SCF.

Methods: The present study was cross-sectional and observational, consisting of seventy individuals who underwent coronary angiography with suspicion of CAD and had angiographically normal coronary arteries of varying coronary flow rates. The relationship between sCD40, C-reactive protein (CRP) and SCF phenomenon was investigated. Fifty patients with isolated SCF (mean age: 56±10 years) and 20 age- and gender-matched control participants with normal coronary flow (NCF) and normal coronary arteries (NCA), (mean age: 55±10 years) were included in the study. We used logistic regression analysis to determine the predictors of SCF.

Results: The clinical characteristics were not statistically significant different between SCF and NCA group. Serum CRP levels were also similar between two groups. Serum sCD40 level was significantly higher in the SCF group compared to control group (74±31 vs. 59±16 pg/mL, p=0.014). In multiple regression analyses, mean coronary diameter strongly (OR: 7.358, 95% CI: 1.990-27.20, p=0.003) and sCD40 (OR: 1.044, 95% CI: 1.006-1.084, p=0.023) weakly predicted SCF.

Conclusion: This study revealed, significantly increased serum sCD40 levels in patients with SCF. Although we cannot conclude the underlying pathological process of SCF, we believe that these findings may be pivotal for further studies searching the specific roles of CD40/CD40L signaling on SCF phenomenon in coronary vasculature. *(Anadolu Kardiyol Derg 2013; 13: 39-44)*

Key words: Slow coronary flow, soluble CD40, C-reactive protein, coronary angiography, regression analysis

Özet

Amaç: Koroner yavaş akım (KYA) epikardiyal koroner arterlerin obstrüktif hastalık olmaksızın yavaş dolması ile karakterize bir durumdur. CD40/CD40 ligandı (CD40L) sistemi artmış enflamasyon ve protrombotik yatkınlık sebebiyle aterosklerozla yakın ilişkili gibi görünmektedir. Biz, CD40/CD40L ikilisinin indirekt bir göstergesi olan serum çözünmüş CD40 (çCD40) düzeylerinin KYA ile ilişkisini araştırmayı hedefledik.

Yöntemler: Çalışmamız gözlemsel ve kesitsel nitelikte olup, koroner arter hastalığı şüphesi ile koroner anjiyografi yapılan ve değişken akım hızlarında normal koroner arterler saptanan 70 hasta içeriyordu. KYA, çCD40 ve C-reaktif protein (CRP) arası ilişki incelendi. Çalışmaya KYA'sı olan 50 hasta (ort. yaş 56±10) ve koroner akımı ve koroner arterleri normal (NKA) 20 yaş-cinsiyeti uyumlu kontrol hastası (Ort. yaş 55±10) alındı. KYA'ın öngörücülerini belirlemede lojistik regresyon analizi kullanıldı.

Bulgular: KYA ve NKA grubu arasında klinik karakteristikler ve CRP düzeyleri açısından farklılık saptanmadı. Serum çCD40 düzeyi KYA grubunda NKA grubuna göre anlamlı düzeyde yüksek (74±31 - 59±16 pg/mL, p=0.014) saptandı. Çok değişkenli analizde, ortalama koroner çapı kuvvetli (OR: 7.358, %95 CI: 1.990-27.20, p=0.003) ve çCD40 (OR: 1.044, %95 CI: 1.006-1.084, p=0.023) düzeyi zayıf bir KYA belirteci olarak saptandı.

Sonuç: Çalışmamızda, KYA akım hastalarında artmış çCD40 konsantrasyonun var olduğunu ilk defa ortaya koyduk. Biz, KYA'nın altta yatan patofizyolojik sürecini netleştiremesek de, bulgularımızın KYA fenomeni gelişiminde çCD40/CD40L sisteminin özgül rollerinin daha iyi anlaşılmasında öncül rol oynayabileceğine inanıyoruz. *(Anadolu Kardiyol Derg 2013; 13: 39-44)*

Anahtar kelimeler: Koroner yavaş akım, çözünmüş CD40, C-reaktif protein, koroner anjiyografi, regresyon analizi

Address for Correspondence/Yazışma Adresi: Dr. Murtaza Emre Durakoğlugil, Recep Tayyip Erdoğan Üniversitesi Tıp Fakültesi, Kardiyoloji Anabilim Dalı, İslampaşa Mah., 53100, Rize-*Türkiye* Phone: +90 464 212 30 09 Fax: +90 464 212 30 15 E-mail: emredur@hotmail.com

Accepted Date/Kabul Tarihi: 28.08.2012 **Available Online Date/Çevrimiçi Yayın Tarihi:** 12.10.2012

doi:10.5152/akd.2013.005

Introduction

Slow coronary flow (SCF) is an angiographic finding characterized with delayed opacification of epicardial coronary arteries without obstructive coronary disease (1). SCF is a relatively common angiographic finding with a reported incidence of 1% in patients undergoing coronary angiography for the suspicion of coronary artery disease (CAD) (2). Since the first description in 1972 by Tambe et al. (1), only a limited number of studies have focused on SCF; therefore, the precise pathophysiological mechanisms and the clinical importance of SCF are not fully understood at the moment. Several mechanisms have been proposed for SCF phenomenon, including small vessel disease, microvascular vasomotor dysfunction, diffuse atherosclerosis, and endothelial dysfunction (3-6). Occlusive disease of the small coronary arteries, which may be a form of early-phase atherosclerosis, has also been suggested as a cause (7). Moreover, SCF may cause transient myocardial hypoperfusion in patients with angina and normal coronary arteries, and these patients have higher probability of significant coronary artery disease and an apparently worse prognosis (8).

CD40 ligand (CD40L/CD154), a transmembrane glycoprotein retaining structural homology with tumor necrosis factor-α (TNF-α), was initially revealed on activated T lymphocytes (9). Subsequently, CD40L was demonstrated within platelets with rapid expression after platelet activation (10). The receptor of CD40L, CD40 is also found in immune cells as well as in endothelial cells, smooth muscle cells, macrophages and fibroblasts (11). Recently, CD40 has gained considerable interest due to evidence linking CD40/CD40L dyad with atherosclerosis, inflammation and thrombosis (12).

The physiological role and pathophysiological importance of sCD40 in SCF are unclear. Since SCF phenomenon seems to be an early-form of atherosclerosis and low-grade inflammation plays a major role in the atherosclerotic vascular processes; sCD40 mediated inflammation may also be involved in SCF as well.

The aim of this study was to investigate whether sCD40 level and C-reactive protein (CRP) levels are increased in patients with SCF compared to patients with angiographically normal coronary arteries.

Methods

Study design

The present study was cross-sectional and observational.

Patient population

A total of seventy individuals who underwent coronary angiography with suspicion of CAD, between May 2011 and December 2011 at our institute's outpatient clinic, and had angiographically normal coronary arteries (NCA) of varying coronary flow rates without any atherosclerotic lesion were enrolled. All patients had chest pain or angina equivalent symptoms with either positive treadmill test or myocardial perfusion study. Therefore, our patients fulfill cardiac syndrome-X criteria including (a) angina episodes ensuing exclusively or predominantly on effort and typical enough to suggest CAD; (b) findings compatible with myocardial ischemia or coronary blood flow abnormalities during spontaneous or provoked angina; (c) normal coronary arteries at angiography; (d) absence of other specific forms of cardiac disease.

Clinical characteristics, which consisted of multiple descriptors from each patient's history and physical examination, were collected by physicians from cardiology clinic of each patient at the time of cardiac catheterization and were stored in the database of coronary angiography laboratory at our institution.

Patients with known coronary or peripheral vascular disease, ectatic coronary arteries, non-ischemic dilated cardiomyopathy, renal and hepatic dysfunction, evidence of ongoing infection or inflammation, hematological disorders and known malignancy were excluded from the study. None of the participants in the study was on any vasoactive drugs.

The study was performed in accordance with the principles stated in the Declaration of Helsinki and approved by the local Ethics Committee of Recep Tayyip Erdoğan University, Faculty of Medicine. Informed consent was obtained from all patients prior to the study.

Biochemical measurements

Blood samples were drawn by venipuncture to measure routine blood chemistry parameters after fasting for at least 8 hours. Fasting blood glucose, serum creatinine, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglyceride levels were recorded. Glucose, creatinine, and lipid profile were determined by standard methods. Serum CRP was analyzed using a nephelometric technique (Beckman Coulter Immage 800; Fullerton, CA, USA; normal range 0-0.8 mg/dL).

Blood samples for sCD40 were drawn after coronary angiography and serum, isolated by centrifugation within 1 h at 2500g for 10 minutes, was stored at -80°C. Serum levels of sCD40 were quantified by enzyme-linked immunosorbent assay (ELISA) using commercially available matched antibodies (eBioscience, San Diego, CA, USA). The intra-assay and inter-assay coefficients of variation (CV) are 5.5% and 7.0%, respectively. The sensitivity was calculated to be 1.3 pg/mL.

Coronary angiography and determination of SCF

Coronary angiography was performed by femoral approach using the standard Judkins technique. Coronary angiograms were recorded in right and left oblique planes using cranial and caudal angulations, with a rate of 30 frames/s (Allura Xper FD10, Philips Healthcare, Best, The Netherlands). During coronary angiography, iopromide (Ultravist 370, Schering AG, Berlin, Germany) was used as the contrast agent in all patients and control participants.

The patients were assessed for the presence of SCF during coronary angiography and coronary flow rates were quantified by the thrombolysis in myocardial infarction (TIMI) frame count

method (TFC). For objective quantification of the coronary flow, two independent observers blinded to the clinical data of the study participants, assessed the coronary flow in coronary arteries using TIMI frame count method (13). In this method, the number of cine frames, recorded at 30 frames/s, required for the contrast to reach standard distal coronary landmarks in left anterior descending (LAD), left circumflex (LCX) and right coronary arteries (RCA) are noted. Predefined distal landmarks are the distal bifurcation for the LAD, commonly referred to as the 'pitchfork' or 'whale's tail', the distal bifurcation of the segment with the longest total distance for the LCX, and the first branch of the posterolateral artery for the RCA. The standard mean values for normal visualization of coronary arteries are described as 36.2±2.6 frames for LAD, 22.2±4.1 frames for LCX and 20.4±3 frames for RCA. As the LAD coronary artery is usually longer than the other major coronary arteries, the TIMI frame count for this vessel is often higher. Therefore, the TIMI frame count for LAD is divided by 1.7 to obtain the corrected TIMI frame count. The standard corrected mean value (cTFC) for LAD coronary artery is 21.1±1.5 frames. All participants with a corrected TIMI frame count greater than the two standard deviation of the previously published range for the particular vessel were considered to have SCF (13). The mean TIMI frame count for each patient and control participant was calculated by dividing the sum of the TIMI frame count of the corrected LAD, LCX and RCA by three. Vessel lengths and ostial diameters of the three coronary arteries were measured using quantitative coronary angiography (QCA).

Statistical analysis

The SPSS statistical software (SPSS 20.0 for windows, Inc., Chicago, IL, USA) was used for all statistical calculations. Continuous variables are given as mean±SD; categorical variables were defined as percentage. Data were tested for normal distribution using the Kolmogorov-Smirnov test. The Student's t-test was used for the univariate analysis of normally distributed continuous numerical variables and Mann-Whitney U test was used for non-normally distributed numerical variables, and the Chi-square test for the categorical variables. Logarithmic (Ln) transformation of serum sCD40 concentration was required because data was not normally distributed. Independent variables with a significance of $p<0.10$ in univariate analyses were re-analyzed by multiple logistic regression analyses with enter method. All tests of significance were two-tailed. Statistical significance was defined as $p<0.05$.

Results

Clinical characteristics of the study population

The clinical characteristics of the patients are presented in Table 1. The clinical characteristics including age, gender, smoking, presence of hypertension and diabetes mellitus were not statistically significant different between SCF and NCA group ($p>0.05$). Similarly, fasting glucose and lipid parameters did not differ significantly between groups ($p>0.05$).

Table 1. Demographic and clinical characteristics of study participants

Variables	SCF (n=50)	NCF (n=20)	*p
Age, years	57±12	56±11	NS
Male gender, n (%)	30 (60%)	13 (65%)	NS
BMI, kg/m^2	31±5	29±4	NS
Hypertension, n (%)	26 (52%)	9 (45%)	NS
Diabetes mellitus, n (%)	10 (20%)	2 (10%)	NS
Hyperlipidemia, n (%)	24 (48%)	9 (45%)	NS
Cigarette smoking, n (%)	18 (36%)	9 (45%)	NS
Family history of CAD, n (%)	14 (28%)	9 (45%)	NS
Fasting glucose, mg/dL	112±46	100±23	NS
Total cholesterol, mg/dL	189±36	190±26	NS
Triglycerides, mg/dL	148±94	125±62	NS
HDL cholesterol, mg/dL	42±11	44±10	NS
LDL cholesterol, mg/dL	117±31	120±25	NS
CRP, mg/dL	0.62±1.0	0.55±0.4	NS
Soluble CD40, pg/mL	74±31	59±16	0.014
TIMI frame count measurements			
LAD	60±28	29±7	<0.001
LAD (corrected)	35±17	17±4	<0.001
LCx	29±11	20±3	<0.001
RCA	41±23	19±5	<0.001
Mean	35±12	19±3	<0.001
The length of epicardial coronary arteries			
LAD, mm	170±20	170±19	NS
LCx, mm	127±30	124±29	NS
RCA, mm	183±39	166±32	NS
Diameters of coronary arteries			
LAD, mm	3.96±0.66	3.60±0.53	0.026
LCx, mm	3.66±0.65	3.29±0.74	0.044
RCA, mm	3.71±0.81	3.10±0.68	0.003
Coronary flow velocities			
LAD, mm/s	102.8±47.0	186.6±57.0	<0.001
LCx, mm/s	148.7±59.6	203.7±61.5	0.001
RCA, mm/s	165.9±73.9	280.1±76.8	<0.001

Data are presented as mean ± SD, median (interquartile range) and as number (percentage).
*Student's t-test, Mann-Whitney U test and Chi-square test
BMI - body mass index, CRP-C - reactive protein, HDL - high density lipoprotein, LAD - left anterior descending artery, LCx - left circumflex artery, LDL - low-density lipoprotein, NCF - normal coronary flow, NS - not significant, RCA - right coronary artery, SCF - slow coronary flow

TIMI frame count of the study population

Patients with SCF had higher TIMI frame counts in each of the major coronary arteries compared to controls as expected ($p<0.05$). Vessel lengths did not differ between groups; however, patients with SCF had larger coronary arteries and slower coronary flow rates ($p<0.05$).

The relationship of CRP, sCD40 and SCF

Serum CRP levels were also similar between two groups. Mean serum sCD40 level was significantly higher in the SCF group compared to NCA group (74±31vs. 59±16 pg/mL, p=0.014, statistical power for analysis was 82%). In multiple logistic regression analyses, mean coronary diameter strongly (OR: 7.358, 95% CI: 1.990-27.20, p=0.003) and sCD40 (OR: 1.044, 95% CI: 1.006-1.084, p=0.023) weakly predicted SCF (Table 2).

Discussion

In the present study, we revealed significantly higher sCD40 levels in patients with slow coronary flow compared to patients with angiographically normal coronary arteries. To our knowledge, this is the first report demonstrating the relationship of SCF with sCD40.

Although the exact mechanism of SCF is not consistently determined, there are several suggested mechanisms involved in pathogenesis of SCF. The first hypothesis that small vessel dysfunction contributes to the pathogenesis of SCF was proposed by Tambe et al. (1) and was confirmed by Mangieri et al. (3), who demonstrated microvascular abnormalities in patients with SCF (3). Their histopathological examinations showed evidence of small vessel abnormalities such as endothelial thickening due to cell edema, capillary damage, and reduced luminal diameter of the small vessels. In addition, Kurtoğlu et al. (14) reported an improvement in microvascular tone and coronary flow with microvascular vasodilators, suggesting a functional increase in microvascular resistance. On the contrary, in addition to microvessel disease, intravascular ultrasound studies identified epicardial coronary artery disease as a pathophysiological factor for SCF (4,15,16). Abnormal slow flow pattern in coronary arteries has been concluded to be a manifestation of diffuse atherosclerotic disease due to endothelial injury without creating an angiographically visible coronary lesion (16); therefore, SCF may be an early manifestation of diffuse atherosclerosis involving both microvascular system and epicardial coronary arteries (4). Additionally, inflammation (17,18), platelet function disorder (19,20), and imbalance of vasoactive substances (15, 21) have also been implicated in the pathogenesis of the SCF phenomenon. Serum paraoxonase (PON), a high-density lipoprotein bound antioxidant enzyme, acts against atherosclerosis and endothelial dysfunction. Yıldız et al. (22) reported an independent association between serum PON activity with the mean TIMI frame count (TFC); suggesting that reduced serum PON activity may be a biochemical marker of SCF. Enli et al. (23), demonstrated significantly increased serum malondialdehyde, erythrocyte superoxide dismutase, and decreased erythrocyte-reduced glutathione levels in patients with SCF compared to patients with normal coronary flow. These findings indicate that free radical damage as well as endothelial dysfunction may also take place in the pathogenesis of SCF. In contrast to these data, recently Kopetz et al. (24) could not demonstrate any differences in endothelial function, inflammatory proteins (myeloperoxidase and high sensitive CRP), oxidative stress biomarkers (malondialdehyde and homocysteine), and asymmetric dimethylarginine levels in patients with SCF compared to healthy controls. Therefore, the mechanisms and pathogenesis of SCF remains controversial.

Table 2. The independent relationship of soluble CD40 with slow coronary flow phenomenon

Variables	Slow coronary flow (Dependent variable)	
	OR (95%CI)	*p
sCD40, pg/mL	1.044 (1.006-1.084)	0.023
Ln (sCD40)	18.11 (1.694-193.5)	0.017
Mean coronary diameter	7.358 (1.990-27.20)	0.003
Constant	0.000	0.002
Nagelkerke R Square	0.321	

*Logistic regression analyses with enter method for independent variables which were included if they were significantly different in the univariate analyses
sCD40 - soluble CD40

Evidence suggests that both CD40 and CD40L are expressed in endothelial cells, smooth muscle cells and macrophages of atherosclerotic lesions, displaying an increased prevalence within advanced, rupture-prone lesions (25). The physiologic interaction of CD40L with CD40 causes a diversity of reactions including increased expression of adhesion molecules, secretion of interleukine-1 (IL-1), IL-6 and TNF-α; all of which in turn recruit more inflammatory cells aggravating local micro-inflammation (11, 26). Furthermore, CD40/CD40L induces enhanced expression of tissue factor and matrix metalloproteinases (MMPs) (27, 28); therefore maintains a prothrombotic milieu and decreases plaque stability. CD40 signaling also results in increased production of reactive oxygen species that antagonizes endothelial nitric oxide (NO) activity and thus produces endothelial dysfunction (29). All of the previously mentioned actions support the important role of CD40/CD40L dyad in endothelial dysfunction, inflammation and eventually atherosclerosis.

Although CD40 is expressed on the cell surface, we measured sCD40 which is produced from pre-CD40 mRNA by alternative splicing to type II CD40, the major alternative isoform that lacks membrane associated endodomain (30). Contin et al. (31) showed that sCD40 may also be produced following stimulation of membrane-bound CD40 by MMP-mediated proteolytic cleavage, implicating a potential negative feedback control of CD40 activity. Moreover, they demonstrated that the engagement of membrane-bound CD40 on the surface of B cells by anti-CD40 antibody led to increased sCD40 release, whereas non-activated cells produced minimal sCD40. Additionally, it is known that cell lines that have high CD40 expression release larger amount of sCD40 in culture supernatants (32). Therefore, elevated concentrations of plasma sCD40 may reflect activated CD40/CD40L system. Since SCF appears to be related to platelet disorders, inflammation, and endothelial dysfunction; CD40/CD40L dyad, which is also closely related to pre-mentioned factors seems to take place in SCF pathogenesis as well.

There is not any specific treatment targeting CD40/CD40L system so far. However, statin treatment, known for diverse anti-inflammatory and immunomodulatory effects, has also been demonstrated to inhibit both CD40 expression and CD40-associated activation of vascular cells (33). On the contrary, oxidized low-density lipoprotein has been shown to induce CD40/CD40L expression in human atheroma, which could be diminished by statins (34). Therefore, statins may decrease expression of the CD40/CD40L dyad either directly or by lowering lipoprotein levels.

In our study, an interesting finding is that CRP was not related to SCF. This issue is still a matter of controversy (17). Even though SCF has been related to inflammatory process, a recent study, comparing SCF patients with normal coronary angiograms also found similar CRP levels (35), which is in line several studies (24, 36). On the contrary, there are studies that report increased CRP levels in patients with SCF (37, 38). These conflicting results might stem from heterogeneity of recruited patients and/or clinical state of the SCF phenomenon.

Study limitations

Our study has several limitations. First, the study population was relatively small. A larger study population would provide a higher statistical power. The main limitation of our study is the observational nature, which does not explain the exact mechanism of the relationship between increased sCD40 and SCF. In the current study, the patients did not undergo IVUS (intravascular ultrasonography) to detect atherosclerotic changes in the coronary arteries. Hence, the coexistence of non-obstructive CAD in patients with "isolated" SCF cannot be established absolutely. Nevertheless, in clinical practice, isolated SCF patients do not undergo IVUS routinely and SCF is usually diagnosed with visual assessment of coronary angiography. Other inflammatory cytokines, except CRP, might be measured to clarify possible causative mediators.

Conclusion

In conclusion, our study demonstrated significantly increased serum sCD40 levels in patients with SCF. Although we cannot conclude the underlying pathologic process of SCF, we believe that these findings may be pivotal for further studies searching the specific roles of CD40/CD40L signaling on SCF phenomenon in coronary vasculature.

Conflict of Interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contribution: Concept - M.E.D., M.Ç., A.Ç.; Design - Y.Ç., A.K.; Supervision - Y.Ç., A.K.; Resource - M.Ç., A.Ç., M.E.D.; Data Collection&/or Processing - M.E.D., S.K., T.E.; Analysis&/or Interpretation - M.E.D., S.K.; Literature Search - M.E.D., S.K.; Writing - M.E.D.; Critical Reviews - S.K., T.E., A.Ç.

References

1. Tambe AA, Demany MA, Zimmerman HA, Mascarenhas E. Angina pectoris and slow flow velocity of dye in coronary arteries--a new angiographic finding. Am Heart J 1972; 84: 66-71. [CrossRef]
2. Goel PK, Gupta SK, Agarwal A, Kapoor A. Slow coronary flow: a distinct angiographic subgroup in syndrome X. Angiology 2001; 52: 507-14. [CrossRef]
3. Mangieri E, Macchiarelli G, Ciavolella M, Barilla F, Avella A, Martinotti A, et al. Slow coronary flow: clinical and histopathological features in patients with otherwise normal epicardial coronary arteries. Cathet Cardiovasc Diagn 1996; 37: 375-81. [CrossRef]
4. Cin VG, Pekdemir H, Camsar A, Çiçek D, Akkuş MN, Parmaksız T, et al. Diffuse intimal thickening of coronary arteries in slow coronary flow. Jpn Heart J 2003; 44: 907-19. [CrossRef]
5. Sezgin AT, Sığırcı A, Barutçu I, Topal E, Sezgin N, Özdemir R, et al. Vascular endothelial function in patients with slow coronary flow. Coron Artery Dis 2003; 14: 155-61. [CrossRef]
6. Rıza Erbay A, Turhan H, Yaşar AS, Ayaz S, Şahin O, Senen K, et al. Elevated level of plasma homocysteine in patients with slow coronary flow. Int J Cardiol 2005; 102: 419-23. [CrossRef]
7. Mosseri M, Yarom R, Gotsman MS, Hasin Y. Histologic evidence for small-vessel coronary artery disease in patients with angina pectoris and patent large coronary arteries. Circulation 1986; 74: 964-72. [CrossRef]
8. Fragasso G, Chierchia SL, Arioli F, Carandente O, Gerosa S, Carlino M, et al. Coronary slow-flow causing transient myocardial hypoperfusion in patients with cardiac syndrome X: long-term clinical and functional prognosis. Int J Cardiol 2009; 137: 137-44. [CrossRef]
9. Armitage RJ, Fanslow WC, Strockbine L, Sato TA, Clifford KN, Macduff BM, et al. Molecular and biological characterization of a murine ligand for CD40. Nature 1992; 357: 80-2. [CrossRef]
10. Henn V, Slupsky JR, Grafe M, Anagnostopoulos I, Förster R, Müller-Berghaus G, et al. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. Nature 1998; 391: 591-4. [CrossRef]
11. Schonbeck U, Libby P. The CD40/CD154 receptor/ligand dyad. Cell Mol Life Sci 2001; 58: 4-43. [CrossRef]
12. Santilli F, Basili S, Ferroni P, Davi G. CD40/CD40L system and vascular disease. Intern Emerg Med 2007; 2: 256-68. [CrossRef]
13. Gibson CM, Cannon CP, Daley WL, Dodge JT Jr, Alexander B Jr, Marble SJ, et al. TIMI frame count: a quantitative method of assessing coronary artery flow. Circulation 1996; 93: 879-88. [CrossRef]
14. Kurtoğlu N, Akcay A, Dindar I. Usefulness of oral dipyridamole therapy for angiographic slow coronary artery flow. Am J Cardiol 2001; 87: 777-9, A8.
15. Pekdemir H, Polat G, Cin VG, Camsari A, Çicek D, Akkuş MN, et al. Elevated plasma endothelin-1 levels in coronary sinus during rapid right atrial pacing in patients with slow coronary flow. Int J Cardiol 2004; 97: 35-41. [CrossRef]
16. De Bruyne B, Hersbach F, Pijls NH, Bartunek J, Bech JW, Heyndrickx GR, et al. Abnormal epicardial coronary resistance in patients with diffuse atherosclerosis but "Normal" coronary angiography. Circulation 2001; 104: 2401-6. [CrossRef]
17. Li JJ, Xu B, Li ZC, Qian J, Wei BQ. Is slow coronary flow associated with inflammation? Med Hypotheses 2006; 66: 504-8. [CrossRef]
18. Turhan H, Saydam GS, Erbay AR, Ayaz S, Yaşar AS, Aksoy Y, et al. Increased plasma soluble adhesion molecules; ICAM-1, VCAM-1, and E-selectin levels in patients with slow coronary flow. Int J Cardiol 2006; 108: 224-30. [CrossRef]

19. Lanza GA, Andreotti F, Sestito A, Sciahbasi A, Crea F, Maseri A. Platelet aggregability in cardiac syndrome X. Eur Heart J 2001; 22: 1924-30. [CrossRef]
20. Gökçe M, Kaplan S, Tekelioğlu Y, Erdoğan T, Küçükosmanoğlu M. Platelet function disorder in patients with coronary slow flow. Clin Cardiol 2005; 28: 145-8. [CrossRef]
21. Çamsarı A, Pekdemir H, Çicek D, Polat G, Akkuş MN, Döven O, et al. Endothelin-1 and nitric oxide concentrations and their response to exercise in patients with slow coronary flow. Circ J 2003; 67: 1022-8. [CrossRef]
22. Yıldız A, Gür M, Yılmaz R, Demirbağ R, Polat M, Selek S, et al. Association of paraoxonase activity and coronary blood flow. Atherosclerosis 2008; 197: 257-63. [CrossRef]
23. Enli Y, Türk M, Akbay R, Evrengül H, Tanrıverdi H, Kuru O, et al. Oxidative stress parameters in patients with slow coronary flow. Adv Ther 2008; 25: 37-44. [CrossRef]
24. Kopetz V, Kennedy J, Heresztyn T, Stafford I, Willoughby SR, Beltrame JF. Endothelial function, oxidative stress and inflammatory studies in chronic coronary slow flow phenomenon patients. Cardiology 2012; 121: 197-203. [CrossRef]
25. Lutgens E, Daemen MJ. CD40-CD40L interactions in atherosclerosis. Trends Cardiovasc Med2002; 12: 27-32. [CrossRef]
26. Kiener PA, Moran-Davis P, Rankin BM, Wahl AF, Aruffo A, Hollenbaugh D. Stimulation of CD40 with purified soluble gp39 induces proinflammatory responses in human monocytes. J Immunol 1995; 155: 4917-25.
27. Malik N, Greenfield BW, Wahl AF, Kiener PA. Activation of human monocytes through CD40 induces matrix metalloproteinases. J Immunol 1996; 156: 3952-60.
28. Zhou L, Stordeur P, de Lavareille A, Thielemans K, Capel P, Goldman M, et al. CD40 engagement on endothelial cells promotes tissue factor-dependent procoagulant activity. Thromb Haemost 1998; 79: 1025-8.
29. Urbich C, Dernbach E, Aicher A, Zeiher AM, Dimmeler S. CD40 ligand inhibits endothelial cell migration by increasing production of endothelial reactive oxygen species. Circulation 2002; 106: 981-6. [CrossRef]
30. Tone M, Tone Y, Fairchild PJ, Wykes M, Waldmann H. Regulation of CD40 function by its isoforms generated through alternative splicing. Proc Natl Acad Sci U S A 2001; 98: 1751-6. [CrossRef]
31. Contin C, Pitard V, Itai T, Nagata S, Moreau JF, Dechanet-Merville J. Membrane-anchored CD40 is processed by the tumor necrosis factor-alpha-converting enzyme. Implications for CD40 signaling. J Biol Chem 2003; 278: 32801-9. [CrossRef]
32. De Paoli P, Cozzi M, Tedeschi R, Gloghini A, Cilia AM, van Kooten C, et al. High CD40 membrane expression in AIDS-related lymphoma B cell lines is associated with the CD45RA+, CD45RO+, CD95+ phenotype and high levels of its soluble form in culture supernatants. Cytometry 1997; 30: 33-8. [CrossRef]
33. Mulhaupt F, Matter CM, Kwak BR, Pelli G, Veillard NR, Burger F, et al. Statins (HMG-CoA reductase inhibitors) reduce CD40 expression in human vascular cells. Cardiovasc Res 2003; 59: 755-66. [CrossRef]
34. Schonbeck U, Gerdes N, Varo N, Reynolds RS, Horton DB, Bavendiek U, et al. Oxidized low-density lipoprotein augments and 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors limit CD40 and CD40L expression in human vascular cells. Circulation, 2002; 106: 2888-93. [CrossRef]
35. Arı H, Arı S, Erdoğan E, Tiryakioğlu O, Huysal K, Koca V, et al. The effects of endothelial dysfunction and inflammation on slow coronary flow. Turk Kardiyol Dern Ars 2010; 38: 327-33.
36. Yazıcı M, Aksakal E, Demircan S, Şahin M, Sağkan O. Is slow coronary flow related with inflammation and procoagulant state? Anadolu Kardiyol Derg 2005; 5: 3-7.
37. Xia S, Deng SB, Wang Y, Xiao J, Du JL, Zhang Y, et al. Clinical analysis of the risk factors of slow coronary flow. Heart Vessels 2011; 26: 480-6. [CrossRef]
38. Barutçu I, Sezgin AT, Sezgin N, Güllü H, Esen AM, Topal E, et al. Increased high sensitive CRP level and its significance in pathogenesis of slow coronary flow. Angiology 2007; 58: 401-7. [CrossRef]