

RAPID COMMUNICATION

Endothelial function in patients with slow coronary flow and normal coronary angiography

Luis Ulisses Signori,^{I,II} Alexandre Schaan de Quadros,^{II} Graciele Sbruzzi,^{II} Thiago Dipp,^{II} Renato D. Lopes,^{III} Beatriz D'Agord Schaan^{II,IV}

^IUniversidade Federal do Rio Grande, Instituto de Ciências Biológicas, Rio Grande/RS, Brazil. ^{II}Instituto de Cardiologia do Rio Grande do Sul/Fundação Universitária de Cardiologia, Porto Alegre/RS, Brazil. ^{III}Duke University Medical Center, Duke Clinical Research Institute, Durham, North Carolina, USA. ^{IV}Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Department of Internal Medicine and Hospital das Clínicas de Porto Alegre, Endocrine Division, Porto Alegre/RS, Brazil.

Email: l.signori@hotmail.com
Tel.: 55 53 3201-7362

INTRODUCTION

Atherosclerotic heart disease usually manifests as angina and is diagnosed by stress imaging tests and coronary angiography (1), but some patients with typical angina and documented myocardial ischemia have normal coronary arteries (2), a clinical picture called cardiac syndrome X (3). Endothelial (4) and microvascular (5) dysfunction have been suggested to play a pathogenic role in this situation. Patients with slow coronary flow (SCF) (6) and endothelial dysfunction (7) are both at increased risk for cardiovascular events. Several methods to measure endothelial injury can provide clinical opportunities to identify these patients (8), but the evaluation of endothelial function in arterial and venous vascular beds has not yet been performed. The aim of this study was to evaluate the arterial and venous endothelial functions in patients with stable angina and normal coronary anatomy but SCF on a cardiac angiogram.

MATERIALS AND METHODS

This case-control study was previously approved by the institutional ethics committee and involved 21 patients referred for coronary angiography to evaluate coronary artery disease. The inclusion criteria were angina, a myocardial perfusion defect (stress imaging test) and normal coronaries (coronary angiography). The exclusion criteria were myocardial infarction in the last 60 days, heart transplantation, a revascularization procedure, left ventricular dysfunction, systemic inflammatory diseases, autoimmune diseases, obesity, smoking, hematological disorders, hemodialysis and the current use of anticoagulant, corticosteroid or immunosuppressive therapy.

The patients underwent elective coronary angiography using the standard Judkins technique (9). The coronary arteries were visualized in the left and right oblique planes using cranial and caudal angles. Left ventriculography was performed in the right anterior oblique view. The injection

of contrast medium was recorded at a speed of 30 frames/s. Coronary flow was quantified objectively by two independent observers, who were blinded to the clinical characteristics of the participants. The TIMI frame count was assessed as described in the literature, and the longer left anterior descending artery frame counts were corrected by dividing by 1.7 to derive the corrected TIMI frame count (CTFC). The patients with a CTFC greater than two standard deviations from the normal published range for the particular vessel were accepted as having SCF (10). The average TIMI frame count for each subject was calculated by adding the TIMI frame counts for the left anterior descending artery, left circumflex artery, and right coronary artery and then dividing the value obtained by three.

The participants had their blood collected in the fasting state to measure glycemia, total cholesterol, high-density lipoprotein cholesterol (HDL-c), triglycerides (automated enzymatic commercial kits; Roche, Mannheim, Germany), insulin (enzyme immunoassay commercial kits; Abbot-Murex, Park, IL, USA), and C-reactive protein (nephelometry, nephelometer BN100, Dade Behring Inc., Marburg, Germany). Low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald formula. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as previously described (11). The patients were instructed not to use medications for 72 h before the endothelial evaluations.

Venous endothelial function was evaluated by the dorsal hand vein technique (12,13). A 23-gauge butterfly needle was inserted into a vein on the back of the hand. A continuous infusion (Harvard infusion pump, South Natick, MA) of saline solution (rate, 0.3 mL/min) was started. A tripod holding a linear variable differential transformer (model 025 MHR; Shaevitz Engineering, Pennsauken, NJ) was mounted on the hand to measure the diameter of the vein. The readings were taken at a congestive pressure of 40 mmHg by the inflation of a blood pressure cuff placed on the upper portion of the arm under study. The diameter of the vein during the saline infusion with the cuff inflated was defined as 100% relaxation. The vein was precontracted by infusing increasing doses (7 min each) of phenylephrine (37-25000 ng/mL) until the dose that produced approximately 70% constriction (ED_{70%}) of the vein was identified; this dose was used as a reference for the subsequent experiments. The vasodilation produced by six doses (12-12000 ng/mL) of

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No potential conflict of interest was reported.

acetylcholine (endothelium-dependent) and three doses (156-3125 ng/mL) of sodium nitroprusside (endothelium-independent) was analyzed (3 min each). The individual effects were analyzed as the percentage of maximum venodilation (13).

Flow-mediated vasodilation (FMD) was measured to evaluate the arterial endothelium-dependent vasodilation using a high-resolution vascular ultrasound (EnVisor CHD; Philips, Bothell, WA, USA) and a 3-12 MHz linear-array transducer (L12-3, Philips, Bothell, WA, USA). Briefly, the change in the brachial artery diameter after 60 s of reactive hyperemia was compared with a baseline measurement after the deflation of a cuff that had been placed around the forearm and inflated to 50 mmHg above the systolic blood pressure for 5 min (14). The diameter increase after a sublingual nitroglycerin spray (0.4 mg) was used as a measurement of endothelium-independent vasodilation. The vessel diameter responses to reactive hyperemia and to nitroglycerin were expressed as the percentage changes relative to the diameter immediately before cuff inflation and to the diameter immediately before drug administration.

The data are presented as the means ± SD. The distributions of the variables were determined using Shapiro–Wilk tests of normality. GraphPad Software Prism 4.0 was used for comparisons with ANOVA (using the Bonferroni *post hoc* test), Fisher’s exact test, the Mann-Whitney test and the unpaired t test, as appropriate, and for Pearson correlations. A 5% level of significance was set for all tests performed.

RESULTS

Of the 21 patients enrolled in our study, nine (cases) were considered to be patients with SCF, and 12 (controls) had normal coronary flow; their characteristics are presented in Table 1. The SCF group had a higher body mass index, triglycerides, glycemia and HOMA-IR and lower HDL-c compared with the control group. Metabolic syndrome was more common in the SCF group. The usage patterns of aspirin, statins, angiotensin converting enzyme inhibitors, calcium channel blockers and beta-blockers were similar between the groups.

The CTFC was higher in the patients with SCF than in the controls for each major epicardial coronary artery: left anterior descending artery (36 ± 3 vs. 47.3 ± 24), left circumflex artery

(22 ± 4 vs. 31.4 ± 6), and right coronary artery (20 ± 3 vs. 34.9 ± 11). In addition, the average TIMI frame count was higher in the patients with SCF than in the controls (26 ± 4 vs. 37.9 ± 12).

Endothelium-dependent venodilation, measured as the maximum venodilation by acetylcholine, was 40% lower in the SCF group (Table 2). Venoconstriction induced by phenylephrine and venodilation induced by sodium nitroprusside (endothelium-independent venodilation) were similar between the groups. The dose of phenylephrine needed to reach ED_{70%} and the doses of acetylcholine and sodium nitroprusside needed to reach maximum venodilation were similar between the groups. Endothelial arterial function assessed by FMD was 45% lower in the SCF group (p = 0.022). Nitroglycerin-induced vasodilation was similar between the groups. Brachial artery flow-mediated vasodilation was inversely correlated with the C-reactive protein level (r = -0.668; p = 0.049).

DISCUSSION

Here, we show that patients with SCF, normal coronary arteries and documented myocardial ischemia display arterial and venous endothelial dysfunction, which was not observed in similar patients with normal coronary flow. In addition, we show that venous endothelial dysfunction is observed in patients with SCF and normal coronary arteries.

Multiple abnormalities have been reported to explain cardiac syndrome X, including endothelial dysfunction (15), increased oxidative stress (16) and vascular inflammation (17). The observation that SCF has also been associated with metabolic abnormalities (18) and endothelial dysfunction (19) may link these abnormalities. Previous studies showed arterial endothelial dysfunction in patients with SCF (15,17,20). One of these studies reported that CTFC correlates with endothelial function, even in individuals with normal coronary flow (15). However, both the arterial and venous endothelium can be injured in patients with cardiovascular risk factors (21,22), and their treatment can reverse the endothelial dysfunction in both vascular beds (22), which could occur in patients with SCF. Under physiological conditions, the venous endothelium is subjected to a lower *shear stress* and O₂ concentration compared with the arterial endothelium (23), but arterial vasodilation is reduced when the venous endothelium is injured (24),

Table 1 - Baseline clinical and laboratory characteristics of the study subjects.

Parameters	Controls (n = 12)	Slow Coronary Flow (n = 9)	p-value
Gender (F/M)	4/8	3/6	0.999
Age (years)	52.2 ± 10	58.0 ± 10	0.230
Body mass index (kg/m ²)	25.4 ± 3	29.9 ± 5	0.044
Systolic blood pressure (mmHg)	122.8 ± 10	133.3 ± 18	0.116
Diastolic blood pressure (mmHg)	78.1 ± 9	82.2 ± 9	0.343
Hemoglobin (g/dL)	13.8 ± 1	14.7 ± 1	0.608
C-reactive protein (mg/dL)	0.29 ± 0.2	0.48 ± 0.3	0.145
Total cholesterol (mg/dL)	198.7 ± 36	183.2 ± 55	0.475
Triglycerides (mg/dL)	88.9 ± 26	131.1 ± 52	0.047
LDL-c (mg/dL)	121.4 ± 30	113.5 ± 54	0.703
HDL-c (mg/dL)	59.5 ± 14	43.4 ± 8	0.006
Fasting plasma glucose (mg/dL)	86.6 ± 8	99.2 ± 10	0.019
Insulin (µU/mL)	5.4 ± 3	10.3 ± 6	0.051
HOMA-IR	2.1 ± 1	4.7 ± 3	0.045

Data are shown as the means ± SD. HOMA-IR = homeostasis model assessment of insulin resistance.

Table 2 - Endothelial function evaluated in the venous and arterial beds in the study subjects.

Parameter	Controls (n = 12)	Slow Coronary Flow (n = 9)	p-value
Venoconstriction (% phenylephrine)	72.4 ± 5	73.6 ± 10	0.744
E _{max} (% acetylcholine)	87.2 ± 34	52.7 ± 27	0.019
E _{max} (% sodium nitroprusside)	142.7 ± 39	139.9 ± 35	0.440
Drug concentration			
ED _{70%} (phenylephrine, ng/mL)	175 ± 167	452 ± 788	0.560
E _{max} (ng/mL, acetylcholine)	4500 ± 4031	2948 ± 3834	0.196
E _{max} (ng/mL, sodium nitroprusside)	1496 ± 619	1822 ± 781	0.237
Flow-mediated vasodilation (%)	13.3 ± 5	7.5 ± 5	0.022
Nitroglycerin-induced vasodilation (%)	18.8 ± 6	13.4 ± 7	0.104

Data are shown as the means ± SD. ED_{70%} = percentage of venoconstriction; E_{max} = maximum effect.

suggesting that substances produced by the venous endothelium can influence arteriolar tonus (24,25). Thus, venous endothelium dysfunction can contribute to the microvascular spasm observed in patients with SCF.

Endothelial dysfunction and increased reactive oxygen species may trigger the production of cytokines and cell adhesion molecules (26). Patients with SCF present decreased plasma concentrations of adiponectin (27) and increased serum levels of soluble adhesion molecules (ICAM-1, VCAM-1 and E-selectin) (19) and C-reactive protein (28). Our data revealed that C-reactive protein was associated with arterial endothelial dysfunction, suggesting additional repercussions of systemic inflammation in the arterial bed.

Lipid profile changes, insulin resistance and the frequency of metabolic syndrome were more frequent in patients with SCF and endothelial dysfunction, as demonstrated in the literature (19,29), suggesting a common ground for these alterations (18).

Our study has several limitations: the sample size was small, it was a single-center study and only patients with stable conditions were selected. The observational nature of the study does not allow cause and effect relationships to be determined. Nonetheless, the completeness of the evaluations is a strength that can provide new insights into this puzzling issue that should be tested and validated in larger studies.

In conclusion, patients with SCF and normal coronary arteries present venous and arterial endothelial dysfunction, suggesting that this condition might be a systemic vascular phenomenon.

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AUTHOR CONTRIBUTIONS

Signori LU, Quadros AS, Shruzzu G, Dipp T, Lopes RD and Schaan BD conceived and designed the study and were responsible for the manuscript draft, data analysis and interpretation, critical revision for the important intellectual content of the manuscript and approval of the final version of the manuscript.

REFERENCES

- [Guidelines of chronic coronary disease: stable angina]. Sociedade Brasileira de Cardiologia. *Arq Bras Cardiol*, 2004;83(Suppl 2):6-43.
- Kemp HG, Kronmal RA, Vlietstra RE, Frye RL. Seven year survival of patients with normal or near normal coronary arteriograms: a CASS registry study. *Journal of the American College of Cardiology*. 1986;7(3):479-83, [http://dx.doi.org/10.1016/S0735-1097\(86\)80456-9](http://dx.doi.org/10.1016/S0735-1097(86)80456-9).
- Crea F, Lanza GA. Angina pectoris and normal coronary arteries: Cardiac syndrome X. *Heart*. 2004;90(4):457-63.
- Bugiardini R, Manfrini O, Pizzi C, Fontana F, Morgagni G. Endothelial function predicts future development of coronary artery disease - A study of women with chest pain and normal coronary angiograms. *Circulation*. 2004;109(21):2518-23, <http://dx.doi.org/10.1161/01.CIR.0000128208.22378.E3>.
- Antonios TF, Kaski JC, Hasan KM, Brown SJ, Singer DR. Rarefaction of skin capillaries in patients with anginal chest pain and normal coronary arteriograms. *Eur Heart J*. 2001;22(13):1144-8, <http://dx.doi.org/10.1053/euhj.2000.2442>.
- Agostini F, Iannone MA, Mazzucco R, Cionini F, Baccaglioni N, Lettieri C, et al. Coronary flow velocity pattern assessed by transthoracic Doppler echocardiography predicts adverse clinical events and myocardial recovery after successful primary angioplasty. *J Cardiovasc Med (Hagerstown)*. 2006;7(10):753-60, <http://dx.doi.org/10.2459/01.JCM.0000247323.57536.4d>.
- Gokce N, Keaney JF Jr, Hunter LM, Watkins MT, Nedeljkovic ZS, Menzoian JO, et al. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J Am Coll Cardiol*. 2003;41(10):1769-75, [http://dx.doi.org/10.1016/S0735-1097\(03\)00333-4](http://dx.doi.org/10.1016/S0735-1097(03)00333-4).
- Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. *Circulation*. 2007;115(10):1285-95.
- Tanriverdi H, Evrengul H, Tanriverdi S, Kuru O, Selici D, Enli Y, et al. Carotid intima-media thickness in coronary slow flow: relationship with plasma homocysteine levels. *Coronary artery disease*. 2006;17(4):331-7, <http://dx.doi.org/10.1097/00019501-200606000-00002>.
- 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(5):879-88.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28(7):412-9, <http://dx.doi.org/10.1007/BF00280883>.
- Aellig WH. A new technique for recording compliance of human hand veins. *Br J Clin Pharmacol*. 1981;11(3):237-43.
- Signori LU, da Silva AM, Plentz RD, Moreno H Jr, Irigoyen MC, Schaan BD. Reversal of postprandial endothelial dysfunction by cyclooxygenase inhibition in healthy volunteers. *J Cardiovasc Pharmacol*. 2009;54(1):90-3, <http://dx.doi.org/10.1097/FJC.0b013e3181abc26f>.
- Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*. 2002;39(2):257-65, [http://dx.doi.org/10.1016/S0735-1097\(01\)01746-6](http://dx.doi.org/10.1016/S0735-1097(01)01746-6).
- Sezgin AT, Sigirci A, Barutcu I, Topal E, Sezgin N, Ozdemir R, et al. Vascular endothelial function in patients with slow coronary flow. *Coron Artery Dis*. 2003;14(2):155-61, <http://dx.doi.org/10.1097/00019501-200304000-00008>.
- Leu HB, Lin CP, Lin WT, Wu TC, Lin SJ, Chen JW. Circulating mononuclear superoxide production and inflammatory markers for long-term prognosis in patients with cardiac syndrome X. *Free radic biol & medicine*. 2006;40(6):983-91.
- Lin CP, Lin WT, Leu HB, Wu TC, Chen JW. Differential mononuclear cell activity and endothelial inflammation in coronary artery disease and cardiac syndrome X. *Int J Cardiol*. 2003;89(1):53-62.
- Turhan H, Erbay AR, Yasar AS, Bicer A, Sasmaz H, Yetkin E. Impaired coronary blood flow in patients with metabolic syndrome: documented by Thrombolysis in Myocardial Infarction (TIMI) frame count method. *Am Heart J*. 2004;148(5):789-94, <http://dx.doi.org/10.1016/j.ahj.2004.05.016>.
- Turhan H, Saydam GS, Erbay AR, Ayaz S, Yasar 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(2):224-30, <http://dx.doi.org/10.1016/j.ijcard.2005.05.008>.

20. Sezgin N, Barutcu I, Sezgin AT, Gullu H, Turkmen M, Esen AM, et al. Plasma nitric oxide level and its role in slow coronary flow phenomenon. *Int Heart J*. 2005;46(3):373-82, <http://dx.doi.org/10.1536/ihj.46.373>.
21. Silva AM, Schaan BD, Signori LU, Plentz RD, Moreno H Jr, Bertoluci MC, et al. Microalbuminuria is associated with impaired arterial and venous endothelium-dependent vasodilation in patients with Type 2 diabetes. *J Endocrinol Invest*. 2010;33(10):696-700.
22. de Sousa MG, Yugar-Toledo JC, Rubira M, Ferreira-Melo SE, Plentz R, Barbieri D, et al. Ascorbic acid improves impaired venous and arterial endothelium-dependent dilation in smokers. *Acta Pharmacol Sin*. 2005;26(4):447-52.
23. Migliacci R, Becattini C, Pesavento R, Davi G, Vedovati MC, Guglielmini G, et al. Endothelial dysfunction in patients with spontaneous venous thromboembolism. *Haematologica*. 2007;92(6):812-8, <http://dx.doi.org/10.3324/haematol.10872>.
24. Hester RL, Hammer LW. Venular-arteriolar communication in the regulation of blood flow. *Am J Physiol Regul, Integr and Comp Physiol*. 2002;282(5):R1280-5.
25. Saito Y, Eraslan A, Lockard V, Hester RL. Role of venular endothelium in control of arteriolar diameter during functional hyperemia. *The American journal of physiology*. 1994;267(3 Pt 2):H1227-31.
26. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation*. 2001;104(22):2673-8, <http://dx.doi.org/10.1161/hc4601.099485>.
27. Selcuk H, Selcuk MT, Temizhan A, Maden O, Saydam GS, Ulupinar H, et al. Decreased plasma concentrations of adiponectin in patients with slow coronary flow. *Heart and vessels*. 2009;24(1):1-7, <http://dx.doi.org/10.1007/s00380-008-1074-5>.
28. Lanza GA, Sestito A, Cammarota G, Grillo RL, Vecile E, Cianci R, et al. Assessment of systemic inflammation and infective pathogen burden in patients with cardiac syndrome X. *The American journal of cardiology*. 2004;94(1):40-4.
29. Binak E, Gunduz H, Sahin M, Kurtoglu N, Dindar I. The relation between impaired glucose tolerance and slow coronary flow. *Int J Cardiol*. 2006;111(1):142-6, <http://dx.doi.org/10.1016/j.ijcard.2005.09.007>.