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Reduced systemic microvascular density and reactivity in individuals with early onset coronary artery disease

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ABSTRACT

Objective: This study sought to test whether patients with early-onset coronary artery disease (EOCAD, $n = 30$) showed systemic microvascular rarefaction and endothelial dysfunction in comparison to age- and sex-matched healthy controls (CTL, $n = 30$), as evaluated by skin video-capillaroscopy.
Methods: Functional capillary density (FCD) was defined as the number of spontaneously perfused capillaries per square millimeter of skin area and assessed by high-resolution intra-vital color microscopy in the dorsum of the middle phalanx. Capillary recruitment (capillary reserve) was evaluated using post-occlusive reactive hyperemia (PORH) after arm ischemia for 3 min.
Results: The mean capillary density at rest was significantly reduced in patients with EOCAD compared to controls (CTL 95 ± 20 and EOCAD 80 ± 18 capillaries/mm^2, $P = 0.0040$). During PORH, capillary density was also markedly reduced in EOCAD patients (CTL 96 ± 18 and EOCAD 71 ± 20 capillaries/mm^2, $P < 0.0001$). Moreover, the capillary density in EOCAD patients was significantly reduced during PORH (EOCAD at rest 80 ± 19 and EOCAD during PORH 71 ± 20 capillaries/mm^2, $P = 0.0073$).
Conclusions: Patients with EOCAD presented systemic capillary rarefaction and impaired microvascular endothelial function. Thus, the early detection of these microvascular alterations in young adults at an increased risk of coronary artery disease could be useful as a surrogate marker of subclinical atherosclerosis.

Introduction

The evaluation of systemic microvascular reactivity and density is essential for investigation into the pathophysiology of cardiovascular diseases and the stratification of cardiovascular risk (Rizzoni et al., 2011; Struijker-Boudier et al., 2007). Additionally, the cutaneous microcirculation is now considered an accessible and representative vascular bed for the assessment of systemic microcirculatory reactivity (Holowatz et al., 2008; Roustit and Cracowski, 2013). In fact, cutaneous microvascular reactivity has been correlated with microvascular function in different vascular beds, regarding both the magnitude and underlying mechanisms (De Boer et al., 2012; Holowatz et al., 2008; Roustit and Cracowski, 2013).

Microvascular rarefaction has been closely correlated with cardiovascular and metabolic diseases, including arterial hypertension, diabetes, obesity and metabolic syndrome (De Boer et al., 2012; Debbabi et al., 2006; Kaiser et al., 2013). Moreover, individuals at an increased coronary artery disease risk present impaired systemic microvascular function, characterized primarily by capillary rarefaction in the skin (IJzerman et al., 2003). Thus, the early detection of subclinical disease, characterized by microvascular rarefaction, could provide an opportunity for early intervention, thereby preventing future cardiovascular events.

Interestingly, it was recently shown that patients with myocardial infarction demonstrated reduced arteriolar and capillary density in the non-ischemic myocardium remote from the site of infarction, as compared to patients without previous infarction (Campbell et al., 2013). These patients also presented with decreased myocardial angiogenesis, as demonstrated by the reduced expression of pro-angiogenic factors (Campbell et al., 2013). In this context, it was also suggested that the acute coronary syndrome depended not only on the presence of vulnerable plaques but also on the presence of myocardial microcirculatory dysfunction and consequent reduction in coronary blood flow (Lerman et al., 2007).

Although most prevalent in elderly individuals, coronary artery disease also affects younger adults (Rubin and Borden, 2012). Early-onset (premature) coronary artery disease (EOCAD), defined by the occurrence of an acute coronary syndrome in patients younger than 45 years of age at diagnosis for men and 55 years of age for women, has been associated with the same classical risk factors and etiologies of coronary artery disease in older people (Rubin and Borden, 2012). Accordingly, the majority of coronary events in young adults are related to

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atherosclerosis, and these patients frequently present with positive family histories for coronary artery disease (Rubin and Borden, 2012).

We recently showed that patients with EOCAD presented altered endothelium-dependent and -independent skin microvascular vasodilator responses compared to healthy subjects. Moreover, these microvascular alterations were concurrent with an increase in carotid intima-media thickness (Souza et al., 2014), which is an established imaging marker of atherosclerosis used to assess the risk of future cardiovascular events (Nambi et al., 2010).

In the current study, our primary aim was to test whether patients with EOCAD showed cutaneous (systemic) microvascular rarefaction in comparison to age- and sex-matched healthy controls (CTL), as evaluated by skin video-capillaroscopy. The early detection of these microvascular alterations in young adults at an increased risk of coronary artery disease could be useful as a surrogate marker of subclinical atherosclerosis.

Materials and methods

Subjects

The main results of this study, including the complete clinical characteristics of the healthy controls and patients with EOCAD, were previously published (Souza et al., 2014). This cross-sectional study included 30 patients with EOCAD aged 42.1 ± 0.6 years, who were younger than 45 years of age at diagnosis for men and younger than 55 years of age at diagnosis for women (Iribarren et al., 2006), and 30 healthy age- and sex-matched individuals. In the group of EOCAD patients, 33% presented with diabetes, 90% presented with arterial hypertension, 87% presented with dyslipidemia, and 87% had previous acute myocardial infarction. In the group of control subjects, there was one hypertensive individual, and the other abovementioned pathologies were absent. EOCAD was defined by the occurrence of any acute coronary syndrome, including ST elevation or non-ST elevation myocardial infarction or unstable angina (all defined by characteristic history, electrocardiographic and cardiac enzymes abnormalities), or by the diagnosis of obstructive coronary artery disease on coronary angiography (defined as ≥50% stenosis of any epicardial coronary artery) in patients with stable angina. According to the study protocol, the patients and controls underwent clinical examinations and testing of cutaneous microvascular density. For biochemical testing, venous blood samples were obtained in the morning after 12 h of fasting. The patients took their usual medications on the morning of the tests, excepting direct vasodilators, which were administered immediately after the microcirculatory tests.

The present study was undertaken in accordance with the Helsinki Declaration of 1975, as revised in 2000, and was approved by the Institutional Review Board (IRB) of the National Institute of Cardiology of Rio de Janeiro, Brazil, under protocol number 0332/1105211. Once considered eligible, all of the subjects read and signed the informed consent form approved by the IRB.

Capillaroscopy by intra-vital microscopy

The microcirculatory tests were performed in an undisturbed quiet room with a defined stable temperature (23 ± 1 °C) after a 20-minute rest in the supine position. The period of acclimatization lasts until skin temperature has stabilized (Shore, 2000); and we had previously shown that after 15–20 min of acclimatization skin temperature stabilizes around 29 °C (Tibirica et al., 2007b).

The dorsum of the non-dominant middle phalanx was used for image acquisition, while keeping the patient sitting comfortably. Room temperature was monitored and adjusted if necessary using air conditioning, considering that outside temperature was usually >25 °C. The arm was positioned at the level of the heart and immobilized using a vacuum cushion (a specially constructed pillow filled with polyurethane foam that can be molded to any desired shape by creating a vacuum, from AB Germa, Kristianstad, Sweden).

Capillary density, i.e., the number of perfused capillaries per square millimeter of skin area, was assessed by high-resolution intra-vital color microscopy (Moritex, Cambridge, UK), as previously described and validated (Antonios et al., 1999c; Debbabi et al., 2006; Francischetti et al., 2011; Kaiser et al., 2013; Tibirica et al., 2007b). We used a video-microscopy system with an epi-illuminated fiberoptic microscope containing a 100-W mercury vapor lamp light source and an M200 objective with a final magnification of 200×. Images were acquired and saved for posterior off-line analysis using a semi-automatic integrated system (Microvision Instruments, Evry, France) (Fig. 1). The mean capillary density for each patient was calculated as the arithmetic mean of visible (i.e., spontaneously perfused) capillaries in three contiguous microscopic fields of 1 mm^2 each. For post-occlusive reactive hyperemia (PORH), a blood pressure cuff was then applied around the patient's arm and inflated to suprasystolic pressure (50 mm Hg greater than systolic arterial pressure) to completely interrupt the blood flow for 3 min. This time of occlusion has already been shown to effectively recruit capillaries in an endothelium-dependent manner (Antonios et al., 1999a, 1999c; Tibirica et al., 2007b). After cuff release, images were again acquired and recorded over the subsequent 60–90 s, during which time the maximal hyperemic response was expected to occur.

The mean number of skin spontaneously perfused capillaries at rest is considered to represent the functional capillary density, as previously described (Antonios et al., 1999a; Shore, 2000). On the other hand, the number of perfused capillaries during post-occlusive reactive hyperemia represents functional capillary recruitment, resulting from the release of endothelial mediators and consequent arteriolar vasodilation (Antonios et al., 1999a).

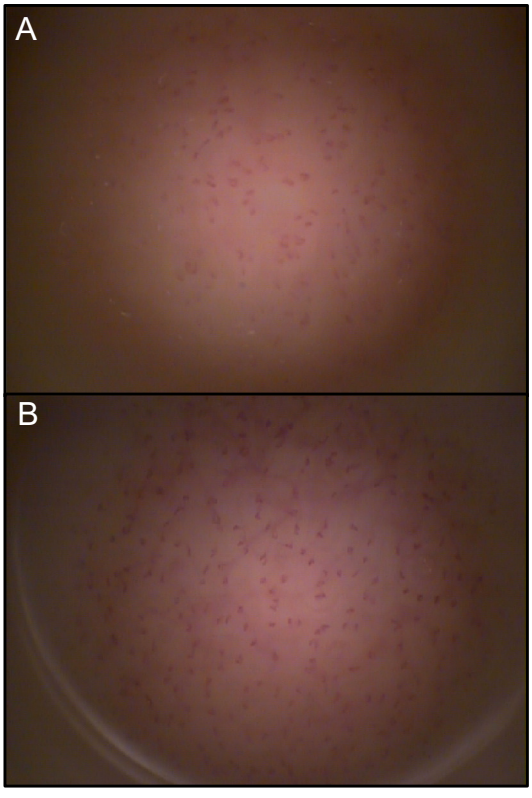

Fig. 1. Representative images of perfused capillaries of the skin of the finger, obtained with high-resolution intra-vital color video microscopy and shown at a final magnification of 200×. For each patient, the mean capillary density was calculated as the arithmetic mean of the number of spontaneously perfused capillaries in three contiguous microscopic fields of 1 mm^2 each. (A) Image from a patient presenting with early-onset coronary artery disease and (B) image from a healthy control subject. Images were obtained during post-occlusive reactive hyperemia.

Statistical analysis

Values are expressed as the means ± SDs. Sample distribution was analyzed using the Shapiro–Wilk test. Because all of the values of capillary density were normally distributed, the comparison between the means of basal values and post-occlusive reactive hyperemia (PORH) values of the control subjects with those of the EOCAD group of patients was performed using an unpaired two-tailed Student's *t*-test. The comparison between basal values and those measured after PORH was performed using a paired two-tailed Student's *t*-test. The null hypothesis was rejected for $P < 0.05$. The statistical package employed was Prism, version 5.0 (GraphPad Software Inc. La Jolla, CA, USA).

Results

Capillary density

The mean capillary density at rest was significantly reduced in patients with EOCAD compared to controls (CTL 95 ± 20 and EOCAD 80 ± 18 capillaries/mm^2, $P = 0.0040$, Fig. 2). During PORH, the capillary density was also markedly reduced in EOCAD patients (CTL 96 ± 18 and EOCAD 71 ± 20 capillaries/mm^2, $P < 0.0001$), with levels lower than those observed at baseline (EOCAD at rest 80 ± 19 and EOCAD during PORH 71 ± 20 capillaries/mm^2, $P = 0.0073$). In contrast, the capillary density did not change significantly in control subjects during PORH (96 ± 18 capillaries/mm^2) compared to the baseline values (95 ± 20 capillaries/mm^2).

Discussion

The main findings of this study were as follows: (i) cutaneous functional capillary density at rest and during PORH was reduced in patients with EOCAD compared to healthy controls; and (ii) capillary recruitment after PORH was also impaired in EOCAD patients.

Vascular endothelial function has been increasingly used as an effective surrogate marker of atherosclerosis risk (Yeboah et al., 2009). For instance, an independent association of cardiovascular events with hyperemic brachial artery velocity, which is the microvascular stimulus for flow-mediated dilation (FMD) but is not itself FMD, was demonstrated in a study of primary prevention in healthy men (the Firefighters and Their Endothelium or "FATE" study) (Anderson et al., 2011).

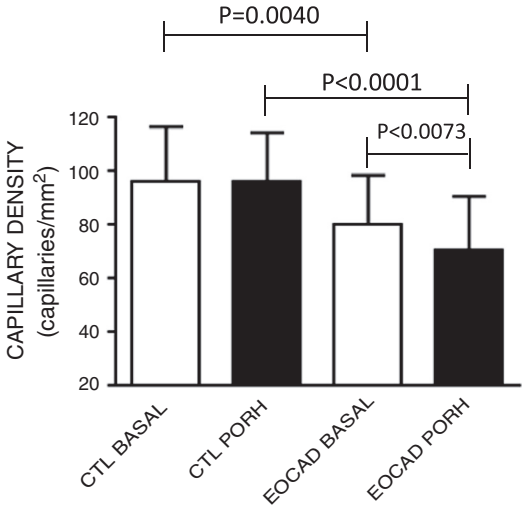

Fig. 2. Functional capillary density at baseline (BASAL) and during post-occlusive reactive hyperemia (PORH) in healthy control subjects (CTL, $n = 30$) and patients presenting with early-onset coronary artery disease (EOCAD, $n = 30$). Values represent means ± SDs. Paired or unpaired Student's two-tailed *t*-tests were used when appropriate.

Capillary rarefaction appears to be related to end-organ damage, as suggested by the association between hypertensive myocardial disease and reduced myocardial capillary density (Strauer, 1990), as well as by that between left ventricular hypertrophy and skin microcirculatory dysfunction independent of blood pressure levels (Strain et al., 2010). In fact, capillary rarefaction at the level of nutritive capillary beds provokes impaired tissue perfusion and seems to be involved in end-organ damage and its complications, which involve several tissues and vascular beds, including the eye (retinopathy), the brain (lacunar stroke), the kidneys (microalbuminuria) and the heart (cardiac failure)(De Boer et al., 2012; Levy et al., 2008). Moreover, a poorer prognostic role has been attributed to the finding of microcirculatory dysfunction, even in patients who are initially free of cardiovascular disease (Anderson et al., 2011).

In the present study, functional capillary density, represented by spontaneously perfused capillaries at baseline, was significantly reduced in patients with EOCAD by approximately 16% as compared to control subjects. Thus, in accordance with a previous study in an older population (IJzerman et al., 2003), our results suggest that coronary artery disease in young adults is also accompanied by capillary rarefaction. Our results are also in agreement with those of clinical studies in hypertensive patients showing capillary deficits ranging from 10 to 30% (Antonios et al., 1999b, 1999c; Debbabi et al., 2010; Serne et al., 2001), which varied according to the territory studied, the methodology employed for capillary loop visualization and the severity of hypertension. The recruitment of capillaries by PORH, which is related to microvascular endothelial function, was markedly reduced by approximately 26% in EOCAD patients, indicating the presence of endothelial dysfunction in these patients. In fact, capillary density was significantly reduced during PORH in EOCAD patients, most likely because of the imbalance between the release of vasodilator and vasoconstrictor endothelial mediators that characterize microvascular endothelial dysfunction (Serne et al., 2007). In fact, microvascular dysfunction can be considered a predictor of prognosis and of an increased incidence of cardiovascular events (Levy et al., 2008). The Framingham risk score has also been shown to be inversely correlated with skin capillary recruitment (IJzerman et al., 2003), maximal skin capillary density (Bonadonna et al., 1998) and coronary flow reserve (Wang et al., 2006). Moreover, impaired flow-mediated microvascular (capillary) recruitment was shown to be involved in the pathophysiology of different conditions, including hypertension, diabetes, insulin resistance and obesity (Serne et al., 2006).

In contrast, in the population of control subjects in the present study, it appeared that most skin capillaries were continuously and maximally perfused, as the increase in capillary density during PORH was only approximately 1%. In previous studies using video-capillaroscopy, using the same methodology that was used in the present investigation, we showed that maximal capillary recruitment in younger healthy subjects ranged between 6 and 7% (Francischetti et al., 2011; Kaiser et al., 2013; Tibirica et al., 2007a, 2007b), although we do not yet have any plausible explanation for this discrepancy. It is also noteworthy that basal values of capillary density (spontaneously perfused capillaries) in healthy volunteers can vary considerably between different studies. The main reason for these discrepancies is considered to be the high spatial variability of capillary density in different regions of the skin (Roustit et al., 2010). Another methodological issue could be the reproducibility of the values obtained with the mean counting of the total number of capillaries in three microscopic fields. We had previously shown that, using our methodology, the intra-observer repeatability of data analysis obtained by reading the same images blindly on two separate occasions is rather high (coefficient of variability 4.3%). The same is true concerning the inter-observer reproducibility, when a second observer independently assesses the capillary density of the same images (coefficient of variability 3.3%)(Francischetti et al., 2011).

The limitations of this study must also be considered. Approximately one-third of the patients with EOCAD presented with type 2 diabetes.

Because it is well known that patients with diabetes have impaired systemic microvascular dysfunction (Serne et al., 2006; Tibirica et al., 2007b), this association could have influenced the results observed in EOCAD patients. However, it was not possible to perform sub-group analyses because of the reduced number of diabetic patients and the consequent lack of statistical power. Finally, for medical and ethical reasons, the patients took their usual medication in the morning of the microcirculatory tests, excepting direct vasodilators, which could have influenced the results. Nevertheless, it is important to note that medications used in the treatment of coronary artery disease, arterial hypertension, dyslipidemia and diabetes, as is the case of the present study, are well-known to improve microvascular endothelial function. Thus, the capillary rarefaction observed in the present study in EOCAD patients could have been underestimated.

In conclusion, the evaluation of cutaneous capillary density may represent a simple, useful and noninvasive method for investigating the presence of subclinical atherosclerosis, which could be employed to assess individuals at risk for coronary artery disease. However, larger studies are needed to confirm our findings and expand them to other populations with increased cardiovascular risks.

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Conflicts of interest

The authors have no conflicts of interest to disclose.

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