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# Evaluation of microvascular endothelial function and capillary density in patients with infective endocarditis using laser speckle contrast imaging and video-capillaroscopy

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ABSTRACT

*Objective:* To evaluate the systemic microcirculation of patients with infective endocarditis (IE).
*Methods:* This is a comparative study of patients with definite IE by the modified Duke criteria admitted to our center for treatment. A reference group of sex- and age-matched healthy volunteers was included. Microvascular flow was evaluated in the forearm using a laser speckle contrast imaging system, for noninvasive measurement of cutaneous microvascular perfusion, in combination with skin iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP) to test microvascular reactivity. Microvascular density was evaluated using skin video-capillaroscopy.
*Results:* We studied 22 patients with IE; 15 were male and seven female. The mean age and standard deviation (SD) were 45.5 ± 17.3 years. Basal skin microvascular conductance was significantly increased in patients with IE, compared with healthy individuals (0.36 ± 0.13 versus 0.21 ± 0.08 APU/mmHg; $P < 0.0001$). The increase in microvascular conductance induced by ACh in patients was 0.21 ± 0.17 and in the reference group, it was 0.37 ± 0.14 APU/mmHg ($P = 0.0012$). The increase in microvascular conductance induced by SNP in patients was 0.18 ± 0.14 and it was 0.29 ± 0.15 APU/mmHg ($P = 0.0140$) in the reference group. The basal mean skin capillary density of patients (135 ± 24 capillaries/mm$^2$) was significantly higher, compared with controls (97 ± 21 capillaries/mm$^2$; $P < 0.0001$).
*Conclusions:* The main findings in the microcirculation of patients with IE were greater basal vasodilation and a reduction of the endothelium-dependent and -independent microvascular reactivity, as well as greater functional skin capillary density compared to healthy individuals.

## 1. Introduction

Infective endocarditis (IE) is a disease which may result in severe complications and with a high mortality rate. Its clinical presentation is dynamic and variable; depending on patient age, the presence of comorbidities (especially underlying valvular heart disease), the causative microorganism and the presence of complications (Baddour et al. 2015). In recent years, there has been a change in its epidemiological profile, especially in developed countries. Although younger patients with rheumatic valve disease were the predominant population affected by IE, in the last two decades, older individuals and healthcare-associated infections (including those related to intracardiac devices, valve prosthesis and hemodialysis) and a staphylococcal etiology has become more frequent (Baddour et al. 2015; Habib et al. 2015; Murdoch et al. 2009). However, in developing countries, patients with rheumatic valve disease account for a third of all IE cases, and *viridians* group streptococci are the causative agents in a third of all IE cases (Brandao et al. 2017).

Infective endocarditis is a systemic disease involving the vascular system and is often accompanied by bacteremia or fungaemia. Therefore, it results in a septic state. In addition, acute heart failure or acute-on-chronic heart failure commonly complicates left-sided IE due to valve destruction, which leads to acute valvular regurgitation. Indeed, the most common indication for valve replacement surgery is severe heart dysfunction not amenable to pharmacologic intervention (Baddour et al. 2015; Habib et al. 2015; Murdoch et al. 2009).

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Considering the severity and increasing incidence of IE, it is important to understand further the pathophysiology of the endocarditis syndrome. The use of noninvasive techniques in the early diagnosis of IE and its complications may prove useful. In fact, the evaluation of systemic microvascular reactivity has proven to be very helpful in the investigation of the pathophysiology of cardiovascular and metabolic disorders (Rizzoni et al. 2011; Struijker-Boudier et al. 2007) as well as sepsis (Vincent and Taccone, 2016).

Abnormalities in the microcirculation have been demonstrated through microvascular rarefaction to be present in diseases such as arterial hypertension, diabetes, obesity and metabolic syndrome (De Boer et al. 2012; Francischetti et al. 2011; Kaiser et al. 2013; Karaca et al. 2014; Struijker-Boudier et al. 2007; Tibirica et al. 2007). Moreover, impaired systemic microvascular function, characterized primarily by capillary rarefaction in the skin, has been demonstrated in individuals with increased coronary artery disease risk (IJzerman et al. 2003; Souza et al. 2014).

Laser speckle contrast imaging, which is used to assess skin microvascular reactivity, allows for innovative and reproducible noninvasive evaluation of tissue flow with high real-time spatial resolution in patients with cardiometabolic diseases (Millet et al. 2011; Roustit and Cracowski, 2013)and critically ill patients (De Backer et al. 2013; De Backer et al. 2010). Moreover, cutaneous microvascular reactivity has been correlated to microvascular function in different vascular beds, both in intensity and regarding the underlying mechanisms (Holowatz et al. 2008).

Although IE is the prototype of a septic condition that results in acute heart dysfunction, only one study has addressed microcirculation in a series of patients with infective endocarditis (Piette et al. 1989), and thus far, none have utilized laser speckle contrast imaging or functional skin video-capillaroscopy. Studies have shown microvascular changes in sepsis, in which abnormalities are found in early phases even before the deterioration of hemodynamic parameters (Kiss et al. 2015). These studies show the relationship of microcirculatory changes with organ failure and mortality, which are independent of the systemic hemodynamic variables (Kiss et al. 2015). It is probable that in acute staphylococcal endocarditis, findings similar to those in the studied sepsis models may be encountered (De Backer et al. 2013; Edul et al. 2012).

The goal of this study is to assess microvascular reactivity and density in patients with acute and subacute endocarditis using laser speckle contrast imaging and intravital video-microscopy, respectively.

## 2. Methods

### 2.1. Study design and place

This is a comparative study that included patients with a confirmed diagnosis of infective endocarditis who were admitted to the National Institute of Cardiology (NIC) at the Ministry of Health in Rio de Janeiro, Brazil. The NIC is a national reference center for the treatment and research of cardiovascular diseases. Its staff is composed of cardiologists, cardiothoracic surgeons, infectious diseases' specialists, specialized nursing staff, physiotherapists and pharmacists as well as technical staff. The investigative resources include echocardiography, computed tomography, magnetic resonance imaging and scintigraphy. The NIC has outpatient units, four intensive care units and operating theatres where approximately 1300 cardiac surgeries are performed yearly.

### 2.2. Study participants and recruitment

The present study was conducted in accordance with the Declaration of Helsinki 1975, which was revised in 2000, and was approved by the Institutional Review Board (IRB) of the National Institute of Cardiology in Rio de Janeiro, Brazil under protocol # CAAE 52871216.0.0000 and registered at ClinicalTrials.gov (NCT02940340).Study participants were informed on the nature of the protocol and gave written consent.

The eligibility criteria were as follows: i) confirmed IE according to the modified Duke criteria (Li et al. 2000); ii) inpatient treatment at the NIC; iii) clinical stability at the time of the intervention as evaluated by the investigator; iv) age ≥ 18 years and v) cardiac surgery performed > 15 days prior to the protocol date (Boralessa et al. 1986; Wan et al. 1997). The exclusion criterion was a confirmed previous diagnosis of *diabetes mellitus*.

### 2.3. Study variables

The variables included were as follows: demographic data (sex and age), medical conditions prior to the diagnosis of IE (systemic arterial hypertension, renal failure on conservative or dialytic treatment, smoking, chronic valvular disease, cardiac surgery or percutaneous procedures), predisposing conditions to IE (previous episode of IE, rheumatic valve disease, congenital heart disease, intravenous drug use, valve prosthesis and intracardiac devices), medications in use (angiotensin-converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARB), statins, betablockers, diuretics), data referring to the episode of IE, such as timing of presentation (acute IE was defined as the presentation of signs and symptoms within one month of diagnosis, and subacute IE as that presenting for more than one month at the time of diagnosis), mode of acquisition (community-acquired and health care-related; the second defined as IE occurring > 72 h following hospital admission or acquired within two months of an invasive procedure), etiologic agents. These latter were divided into four groups for analysis: i) viridans group streptococci, including those with blood culture negative, since we have previously shown by PCR of excised valves that viridians streps are the most frequent agents in our scenario IE (Lamas et al. 2016); ii) aggressive staphylococci (including *Staphylococcus aureus* both methicillin susceptible and resistant and *S. lugdunensis*; iii) coagulase negative staphylococci and iv) enterococci. We also evaluated affected structures and left ventricular function, evaluated as normal or moderately to severely compromised at the time of diagnosis of IE; left ventricular ejection fraction was not used as a parameter for heart dysfunction as it is often overestimated in moderate to severe valvular regurgitation, the predominant lesion in IE. Other variables studied were pulmonary artery systolic pressure (PASP), as an indirect measure of myocardial dysfunction, embolic and non-embolic complications (paravalvular abscess, prosthetic dehiscence, atrioventricular block, new cardiac failure, new renal failure); antibiotic and surgical treatment and laboratory data (C-reactive protein levels, CRP, hemoglobin, hematocrit, leukocyte count, and serum creatinine levels). For complete data describing clinical characteristics of patients see Tibirica et al. (2018).

### 2.4. Intervention

The evaluation of microvascular endothelial function in patients with infective endocarditis was performed using laser speckle contrast imaging. These results were compared to those previously obtained from age and sex-matched healthy volunteers (Souza et al. 2014). The systemic microvascular data obtained from this group of healthy volunteers were used as reference microcirculatory values of individuals free of systemic diseases. The healthy volunteers did not present with arterial hypertension, diabetes, dyslipidemia or any other systemic pathology.

### 2.5. Evaluation of microcirculatory reactivity

The microcirculatory tests were performed in the morning between 8 A.M. and 12 P.M. in an undisturbed, quiet room with a defined stable temperature (23 ± 1 °C), following a 20-min rest period in the supine

position. The room temperature was monitored and adjusted if necessary using air conditioning. The acclimatization period lasted until the patient's skin temperature stabilized (Shore 2000). We have previously demonstrated that following 15–20 min of acclimatization, the skin temperature stabilizes at approximately 29 °C (Tibirica et al. 2007).

### 2.6. Evaluation of skin microvascular flow and reactivity

Microvascular reactivity was evaluated using a laser speckle contrast imaging (LSCI) system with a laser wavelength of 785 nm (PeriCam PSI system, Perimed, Järfälla, Sweden), as previously described (Cordovil et al. 2012). LSCI was used in combination with the iontophoresis of acetylcholine (ACh) or sodium nitroprusside (SNP) for the noninvasive, continuous measurement of cutaneous microvascular perfusion changes inarbitrary perfusion units (APU). The images were analyzed using the manufacturer's software (PIMSoft, Perimed, Järfälla, Sweden). One skin site on the ventral surface of the forearm was randomly chosen for the recordings. Hair, broken skin, areas of skin pigmentation and visible veins were avoided, and a single drug-delivery electrode was installed using adhesive discs (LI 611, Perimed, Järfälla, Sweden). A vacuum cushion (AB Germa, Kristianstad, Sweden) was used to reduce the recording artifacts generated by arm movements. The iontophoresis of ACh 2% *w/v* or SNP 2% w/v (Sigma Chemical CO, USA) was performed using a microphamacology system (PF 751 PeriIont USB Power Supply, Perimed, Sweden) with increasing anodal currents of 30, 60, 90, 120, 150 and 180 μA for 10-s intervals that are spaced 1 min apart, and the total charges were 0.3, 0.6, 0.9, 1.2, 1.5 and 1.8 mC. The dispersive electrode was attached approximately 15 cm away from the iontophoresis chamber. The results of the pharmacological tests were expressed both as peak values (representing the maximal vasodilation observed following the highest dose of ACh or SNP) and as the area under the curve of vasodilation. The measurements of skin blood flow were divided by mean arterial pressure values to provide the cutaneous vascular conductance (CVC) in APUs/mmHg. Microvascular reactivity was also evaluated using a physiological test known as post-occlusive reactive hyperemia (PORH). During the PORH test, arterial occlusion was achieved with supra-systolic pressure (50 mmHg above the systolic arterial pressure) using a sphygmomanometer for three minutes. Following the release of pressure, maximum flux was measured. PORH was not performed in four patients due to technical reasons.

### 2.7. Capillaroscopy by intravital video-microscopy

The dorsum of the non-dominant middle phalanx was used for image acquisition, while keeping the patient sitting comfortably. 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 spontaneously perfused capillary loops per square millimeter of skin area, was assessed by high-resolution intra-vital color microscopy (Moritex, Cambridge, UK), as previously described and validated (Francischetti et al. 2011; Kaiser et al. 2013; Tibirica et al. 2007). We used a video-microscopy system with an epi-illuminated fiber optic 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 (Saisam, Microvision Instruments, Evry, France). 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. The mean number of skin spontaneously perfused capillaries at rest is considered to represent the functional capillary density, as previously described (Tibirica et al. 2015). The features of the study subjects were not available to the investigator during capillary counting. Reproducibility was assessed by examining an identical area of skin. Intra-observer repeatability of data analysis was assessed by reading the same images blindly on two separate occasions (n = 15, coefficient of variability 4.3%).

### 2.8. Statistical analysis

The results were presented as the mean ± SD. For values that did not follow a Gaussian distribution, the medians (25th–75th percentiles) were presented (Shapiro- Wilk normality test). The results were analyzed using either two-tailed unpaired Student's *t*-tests or repeated measures ANOVAs when appropriate. The independent (unpaired) *t*-test was used because we compared two unrelated groups, in which the participants (healthy individuals or patients with IE) in each group are different. $P$ values $< 0.05$ were considered statistically significant. Clinical and laboratory data were shown descriptively. The correlations between the intervention study results (microvascular reactivity) and features of the disease, such as the number of days of presentation, presence of embolic complications and etiological agent, were determined using Pearson's test if the data are found to be of normal distribution (parametric). If the distribution was not normal (non-parametric), Spearman's test was used for the analysis. The identification of potential outliers was performed using the ROUT method (robust regression and outlier removal), which is based on the False Discovery Rate (FDR), with a specified value of Q = 1%.The statistical package used for the statistical analyses was Prism version 6.0 (GraphPad Software Inc. La Jolla, CA, USA) and the R version 3.1.0.

## 3. Results

We included 22 patients with IE; their clinical features, as well as those of the reference group are shown in Table 1. Ten of the 22 patients with IE (45.5%) had arterial hypertension, five (22.7%) had a chronic renal failure, three (13.65%) of whom were hemodialysis dependent, and only two (9%) smoked, though both had stopped more than three months prior to the study protocol. None of the patients presented features of the systemic inflammatory response syndrome (Bone et al. 1992)at the time of intervention. Regarding medications, eight (36.4%) patients were on ACEi or ARB, 10 (45.4%) were on beta-blockers and three (13.6%) used statins. Cardiac surgery was performed in 13 (59.6%) of patients; for one patient, surgery was performed within less than two weeks of antibiotic therapy and for 12, more than two weeks after antibiotics.

Regarding hematocrit levels, patients with IE presented median values of 33% with interquartile interval of 28 to 38%. They were divided into two groups, using the median, defined as with low (< 33%) or high (> 33%) hematocrit levels. There were no differences between the basal microvascular flow of patients with low (34 ± 11 APU) or high (30 ± 12 APU, $P = 0.3746$) hematocrit levels.

### 3.1. Evaluation of skin microvascular flow and reactivity

The baseline values of CVC were significantly higher in patients with IE, compared to healthy subjects (0.36 ± 0.13 vs. 0.21 ± 0.08 APU/mmHg; $P < 0.0001$; Fig. 1C). On the other hand, both endothelial-dependent and -independent microvascular vasodilation were reduced in patients with IE, compared to healthy subjects. The increase in CVC induced by skin ACh iontophoresis was 0.21 ± 0.17 APU/mmHg in patients and 0.37 ± 0.14 APU/mmHg in healthy subjects ($P = 0.0012$; Fig. 1D). Moreover, increase in CVC induced by SNP was 0.18 ± 0.14 APU/mmHg in patients and 0.29 ± 0.15 APU/mmHg in healthy subjects ($P = 0.0140$; Fig. 1E). The area under the curve of microvascular vasodilation induced by skin ACh iontophoresis was not significantly different between patients (20,092 ± 6850 APU/s) and healthy subjects (18,539 ± 6033 APU/s; $P = 0.4182$; Fig. 1A). The area under the curve of microvascular

**Table 1**
Clinical and microbiological characteristics of patients with IE and healthy volunteers.

| Variables | Patients with IE (n = 22) | Healthy volunteers (n = 30) | *P* value |
|---|---|---|---|
| Age (years) | 45.5 ± 17.3 | 42.1 ± 0.6 | 0.0901 |
| Sex | 15 M/7F | 15 M/15F | 0.2587 |
| Affected valve [n (%)] | 9 (40.9) Aortic<br>12 (54.5) Mitral<br>1(4.6) Tricuspid | – | – |
| Type of acquisition [n (%)] | 19 (86.3) Community<br>3 (13.6) Hospital | – | – |
| Predisposing factors for IE[n (%)] | 2 (9.1) Previous IE<br>2 (9.1)Rheumatic valvopathy<br>5 (22.7) CHD<br>2 (9.1) IV drug use<br>2 (9.1) Valve prosthesis<br>2 (9.1) Pacemaker | – | – |
| Disease duration | | – | – |
| ≤1 month | 3 (13.6) | | |
| > 1 month | 19 (86.3) | | |
| *Viridans* groupstreptococci[n (%)] | 7 (31.8) | – | – |
| Blood culture-negative | 3 (13.6) | | |
| Aggressive staphylococci [n (%)] | 4 (18.2) | | |
| Indolent negative coagulase staphylococci [n (%)] | 4 (18.2) | | |
| Enterococci [n (%)] | 4 (18.2) | | |
| < 15 days of antibiotic [n (%)] | 6 (27.3) | – | – |
| > 15 days of antibiotic [n (%)] | 16 (72.7) | | |
| Hypertensive | 10 (45.5) | – | – |
| Previous LV systolic dysfunction | 1 (4.5) | – | – |
| Renal failure on HD on conservative treatment | 3 (13.6)<br>2 (9.1) | – | – |
| Embolization [n (%)] | 9 (40.9) | – | – |
| LV systolic dysfunction complicating IE [n (%)] | 3 (13.6) | – | – |

IE, infective endocarditis; M, male; F, female; CHD, congenital heart disease; IV, intravenous; LV, left ventricle; HD, hemodialysis.

vasodilation induced by skin SNP iontophoresis was also not significantly different between patients [16,663 (14,396–21,000) APU/s] and healthy subjects ([14,628 (11,152–20,733) APU/s]; $P = 0.0930$; Fig. 1B). Finally, the increase in CVC induced by PORH was not significantly different between patients (0.45 ± 0.2 APU/mmHg) and healthy subjects (0.46 ± 0.17 APU/mmHg; $P = 0.9202$). We identified only one outlier in the data regarding skin microvascular flow and reactivity (one value of area under the curve of vasodilation induced by SNP iontophoresis). This value was excluded from the final analyses.

Considering all study parameters, the area under the curve of microvascular vasodilation induced by skin SNP iontophoresis was significantly correlated only with the time of use of antibiotics. The patients that used antibiotics for < 15 days had mean values of area under the curve of 6714 ± 2867 APU/s, compared to patients treated during > 15 days (2828 ± 1749 APU/s; $P = 0.0009$). The area under the curve of microvascular vasodilation induced by skin ACh iontophoresis was not correlated with any study variable.

The peak values of CVC obtained during skin ACh iontophoresis were correlated both with the time of use of antibiotics and with the mean values of PASP. The patients that used antibiotics for < 15 days had mean values of 0.76 ± 0.21 APU/mmHg, compared to patients treated during > 15 days (0.50 ± 0.22 APU/mmHg; $P = 0.02$). The peak values of CVC during skin ACh iontophoresis in patients with PASP ≥40 mmHg were of 0.70 ± 0.16 APU/mmHg and in patients with PASP < 40 mmHg were of 0.50 ± 0.25 APU/mmHg ($P = 0.05$). On the other hand, the increase of CVC induced by skin ACh iontophoresis was not correlated with any study parameter.

The peak values of CVC obtained during skin SNP iontophoresis were correlated only with the time of use of antibiotics by patients. The patients who used antibiotics for < 15 days had median values of 0.79 (0.62–0.94) APU/mmHg, compared to patients treated during > 15 days [0.42 (0.36–0.54) APU/mmHg; $P = 0.003$]. The increase of CVC induced by skin SNP iontophoresis was correlated with the time of use of antibiotics. The patients who used antibiotics for < 15 days had mean values of 0.31 ± 0.14 APU/mmHg, compared with patients treated during > 15 days (0.13 ± 0.11 APU/mmHg; $P = 0.004$). Finally, there were no correlations between changes in CVC induced by PORH and any study variable.

For complete data describing microvascular characteristics of patients see Tibirica et al. 2018.

### 3.2. Evaluation of skin capillary density

Video-capillaroscopy was performed in only nine patients with IE, due to technical reasons. Representative video capillaroscopy images of spontaneously perfused capillaries of the skin of the finger are presented in Fig. 2 A (healthy control subject) and 2B (patient presenting with IE). The basal mean skin capillary density of patients (135 ± 24 capillaries/mm$^2$) was significantly higher, compared with healthy controls (97 ± 21 capillaries/mm$^2$; $P < 0.0001$; Fig. 2C).We did not identify any outlier in the data regarding skin capillary density.

## 4. Discussion

Infective endocarditis remains a disease of high mortality (approximately 15 to 20% among patients who undergo surgery) and morbidity despite improvements in diagnosis and timely surgical intervention (Baddour et al. 2015; Habib et al. 2015). It is associated with persistent bacteremia or fungaemia and consequent sepsis. Studies evaluating the microcirculation of patients with endocarditis are scarce in the literature. Only one French study, in which nailfold capillary microscopy was used to study twenty-six patients with IE, showed significant correlations of the number of capillary abnormalities with systemic involvement and immunological disturbances (Piette et al. 1989). However, the authors concluded that due to the lack of specificity, nailfold capillary microscopy could not be regarded as a useful tool in the diagnosis of IE. More recently, a report was published in which side stream dark field microvasculature imaging was done in a case of vancomycin-resistant enterococcal endocarditis complicated by heparin-induced thrombocytopenia in a critically ill patient (Bechar et al. 2016). In this complex scenario, the authors showed that sublingual microcirculation, probably reflecting splanchnic microcirculation, was compromised while cardiac output and mean arterial pressure measures were reasonably good. This has been shown previously in critically ill patients and suggests that microvascular monitoring in these severely ill patients has benefits and is partially independent of global hemodynamic changes (De Backer et al. 2010). Another important issue to consider is the relatively frequent occurrence of severe valvular regurgitation leading to acute heart failure in left-sided IE, which may further compromise the microcirculation and tissue perfusion, possibly modifying the response of the microvascular endothelium to sepsis.

Several studies have demonstrated the central role of microcirculation in the delivery of nutrients and oxygen to tissue cells and, therefore, in determining adequate organ perfusion (Ince et al. 2016; Muller et al. 2016). In sepsis and septic shock, macro and microcirculatory disturbances both contribute to the development of organ failure. Macro and microcirculatory mismatch in septic patients may lead to inappropriate treatment measures and higher mortality

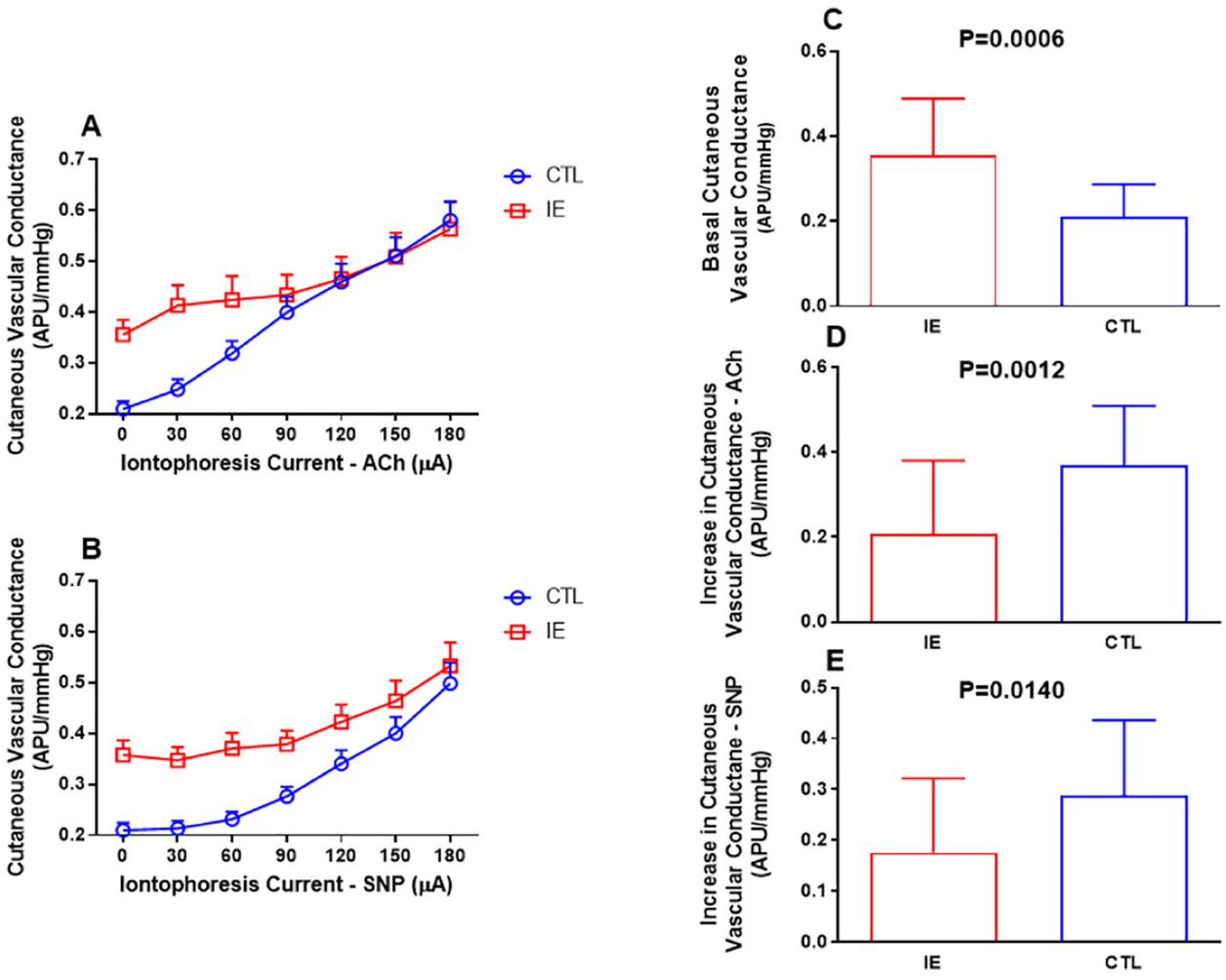


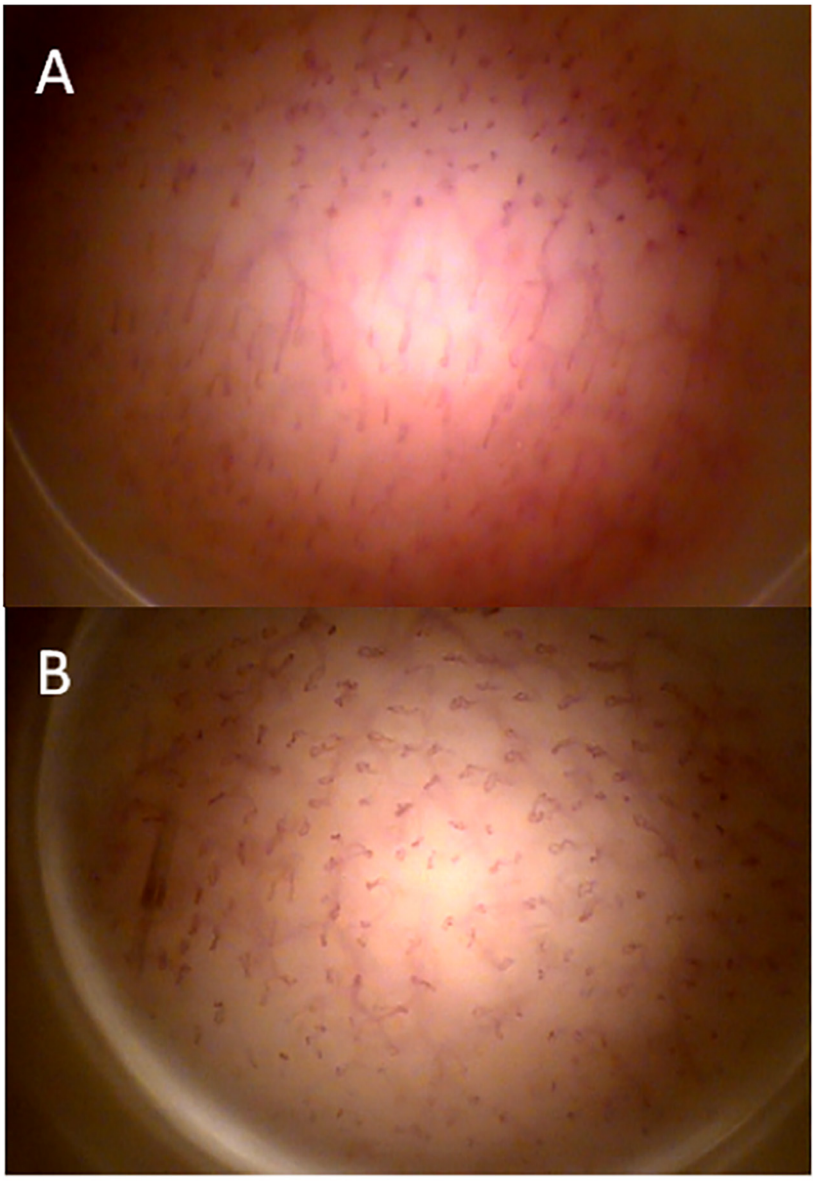

**Fig. 1.** Cumulative effects of acetylcholine (ACh) (A) and sodium nitroprusside (SNP) (B) iontophoresis on the systemic microvascular reactivity of healthy volunteers (CTL, n = 30) and patients with infective endocarditis (IE, n = 22). Baseline cutaneous microvascular conductance (C) and effects of cutaneous acetylcholine (D) and sodium nitroprusside (E) iontophoresis on systemic microvascular reactivity of healthy volunteers and patients with infective endocarditis. Cutaneous microvascular conductance is expressed as arbitrary perfusion units (APU) divided by mean arterial pressure, in mmHg.
Values represent means ± SDs.

(Doerschug et al. 2007). The systemic microcirculation and its regulation are a focus of present and future research that aims to improve diagnostic and treatment strategies. Moreover, it has recently been demonstrated that treatment at an early stage of microvascular dysfunction may be most effective for delaying or reversing the disease processes, thus improving the outcome and survival of patients at risk for pulmonary vascular disease (Gaskill et al. 2017). The authors also suggested that this knowledge may be applied toward microvascular dysfunction observed on the skin (Gaskill et al. 2017). In sepsis, microvascular blood flow is disturbed by endothelial dysfunction, which leads to a reduction in flow or no flow at all to some capillary beds, while other beds have above-normal flow, even surpassing tissue metabolic demands. Consequently, the microvascular perfusion heterogeneity observed during sepsis results in tissue hypoxia.

In our study, patients with definite IE of various causes, and who were clinically well, presented with greater basal microvascular vasodilation and a reduction of the endothelium-dependent and -independent microvascular reactivity, when compared with healthy volunteers. This probably reflects a systemic effect of endovascular infection on microvessels with a limited response of the vascular tone to nitric oxide (NO). NO has a fundamental role in maintaining the flow in microvessels by mediating vascular tone, leukocyte adhesion, platelet aggregation, microthrombi formation, and microvascular permeability. Although systemic NO production is up-regulated in sepsis, NO production may be heterogeneous in different tissues. Variation in the extent of expression of inducible nitric oxide synthase (iNOS) and consumption of NO by reactive oxygen radicals in ischemic tissues may lead to a relative paucity of NO in microcirculatory beds despite a total-body excess. This may result in pathologic shunting of oxygenated blood from tissues, which characterizes the heterogeneous tissue perfusion of sepsis. One mechanism affecting this diversion is the opening of arteriovenous shunts in capillary beds (Ince et al. 2016; Lundy and Trzeciak, 2011).

Additionally, in the present study, functional skin capillary density of patients presenting with IE showed to be markedly and significantly increased, compared to healthy volunteers. The increase of functional capillary density associated with a higher basal microvascular blood flow and a reduction of endothelial-dependent vasodilation in patients with IE, indicates the presence of arteriolar vasodilation in the periphery, accompanied by reduced capillary reserve, undoubtedly because a few capillaries are shut down at baseline. Taken together, these results indicate that patients with IE have increased baseline peripheral microvascular perfusion, in contrast to patients with sepsis or septic shock (Vincent and Taccone, 2016).

Regarding the correlation of our study variables with results of microcirculatory reactivity, patients who had < 15 days of treatment with systemic antibiotics presented a greater vasodilatory effect, both endothelium-dependent and -independent, compared to those patients




(caption on next page)

**Fig. 2.** Representative images of spontaneously perfused capillaries of the skin of the finger, obtained with high-resolution intra-vital color video-microscopy shown at a final magnification of 200×. For each patient, the mean capillary density was calculated as the arithmetic mean of the number of perfused capillaries in three contiguous microscopic fields of 1 $mm^2$ each. (A) Image from a healthy control subject and (B) image from a patient presenting with infective endocarditis. (C) Functional capillary density in healthy control subjects (CTL, n = 30) and in patients presenting with infective endocarditis (ENDO, n = 9).
Values represent means ± SDs.

who had been treated with antibiotics for > 15 days, suggesting a greater microvascular reactivity. However, we believe these data derive from the fact that only one patient with < 15 days of antibiotic therapy had been submitted to cardiac surgery, compared to 12 patients who had surgery of the 15 patients with > 15 days of antibiotics. Undoubtedly, microcirculatory reactivity was reduced in the group of patients with longer antibiotic treatment because these patients had a much higher rate of cardiac surgery. Actually, the marked systemic inflammatory response that follows cardiac surgery under cardiopulmonary bypass is known to induce extensive microvascular dysfunction (Gomes et al. 2014).

When ACh-mediated (that is, endothelium-dependent) vasodilatation was evaluated, a better microvascular reactivity (as measured by peak microvascular conductance following ACh ionthophoresis) was seen in patients with < 15 days of antibiotic therapy. This was also seen in those patients with higher PASP (PASP ≥40 mmHg). These unexpected results may have been found due to the small sample size of our study, as well as its heterogeneity regarding causative agents and surgical vs. and non-surgical treatment.

Although patients with acute staphylococcal endocarditis frequently present with systemic inflammation and disease severity (Baddour et al. 2015), our study did not show differences between results of microvascular reactivity in these patients compared to those with IE caused by less aggressive pathogens. This is probably because our patients, at the time of the study intervention, were clinically stable (none presented with SIRS), had been on antibiotics for some time and some had even had valve replacement surgery.

Some medications are known to improve endothelial function, such as ACE inhibitors and angiotensin II receptor antagonists (Arcaro et al. 1999; Mancini et al. 1996). This is justified by the equilibrium of angiotensin II and bradykinin levels in the endothelium, and to captopril's sulfhydryl group donating ability (which liberates NO) (Arcaro et al. 1999). Statins also improve vascular parameters, due to their stimulation of endothelial NO synthase (eNOS) (Galyfos et al. 2017). However, in our study no differences were seen in patients who used these drugs and those who did not, regarding endothelial-dependent and -independent microcirculatory function. We believe this is a consequence of our small study group.

## 5. Conclusions

Patients with infective endocarditis present greater baseline skin microvascular conductance compared with healthy individuals, as well as a reduced endothelial-dependent and -independent vasodilatory response, as evaluated by laser speckle contrast imaging associated with iontophoresis with acetylcholine and nitroprusside. They also have an increased functional capillary density and a reduced capillary reserve, as seen by skin video capillaroscopy. These techniques may prove useful for the study of the systemic microvasculature in patients with IE who are not critically ill.

## 6. Limitations and strengths of the study

The limitations to this study must be considered. Firstly, the restricted number of participants due to limitations regarding the inclusion of critically ill patients with a diagnosis of infective endocarditis; secondly, the heterogeneity of patients presenting with IE, which is due to several parameters, including underlying diseases, etiologic agents and type of structure involved, the type of treatment received by the patient (surgical vs. non-surgical); thirdly, the duration of treatment at the time of the study protocol. The major strength of the present study is the demonstration of the usefulness of laser-based noninvasive techniques for the evaluation of systemic microcirculation in the scenario of a complex systemic infectious disease involving the cardiovascular system, and its possible role in the early diagnosis of infective endocarditis and of its complications.

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## Competing interests

The authors declare that they have no competing interests.

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