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Sublingual functional capillary rarefaction in chronic heart failure

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Abstract

Background and objective: Microcirculatory changes contribute to clinical symptoms and disease progression in chronic heart failure (CHF). A depression of coronary flow reserve is associated with a lower myocardial capillary density in biopsies. We hypothesized that changes in cardiac microcirculation might also be reflected by a systemic reduction in capillaries and visualized by sublingual videomicroscopy. The aim was to study in vivo capillary density and glycocalyx dimensions in patients with CHF vs healthy controls.

Methods: Fifty patients with ischaemic and nonischaemic CHF and standard treatment were compared to 35 healthy age-matched subjects in a prospective cross-sectional study. Sublingual microcirculation was visualized using a side-stream darkfield videomicroscope. Functional and perfused total capillary densities were compared between patients and controls. A reduced glycocalyx thickness was measured by an increased perfused boundary region (PBR).

Results: Median functional and total perfused capillary densities were 30% and 45% lower in patients with CHF (both $P < .001$). Intake of oral vitamin K antagonists was associated with significantly lower capillary densities ($P < .05$), but not independent of NT-proBNP. Dimensions of the glycocalyx were marginally lower in CHF patients than in healthy controls (<7% difference). However, PBR correlated significantly with inflammation markers (fibrinogen: $r = .58$; C-reactive protein: $r = .42$), platelet counts ($r = .36$) and inversely with measures of liver/renal function such as bilirubin ($r = -.38$) or estimated glomerular filtration rate ($r = -.34$) in CHF patients.

Conclusion: CHF patients have got a markedly lower functional and total perfused capillary density in sublingual microvasculature when compared to controls, indicating a systemic decrease in microcirculation.

KEYWORDS
capillaries, glycocalyx, heart failure, microcirculation, perfused boundary region

1 | INTRODUCTION

Impairment of the microcirculatory system remains a clinical challenge contributing to oedema, micro- and macrovascular atherogenesis and heart failure.[1,2] Myocardial tissue oedema may develop due to glycocalyx disintegration[1] and adversely affects myocardial function.[3,4] In addition, decreased capillary density may promote

pathophysiological processes of chronic heart failure (CHF) at least in patients with idiopathic dilated heart failure.[5] This is also supported by previous reports using a 3-compartment model comprising arterial, capillary and venous resistance during rest and hyperaemia, concluding that capillaries are major regulators of coronary blood flow and thus coronary flow reserve.[6] Skeletal muscle capillary density is diminished in patients with CHF and inversely correlates with maximal oxygen consumption.[7] This might contribute to exercise intolerance and probably negatively impact the pathogenesis and progression of CHF.[7]

To date, no in vivo measurements of perfused capillary density in CHF patients have been performed. On the basis of biopsy studies showing a rarefaction of myocardial capillary density in CHF patients[5] and reduced capillaries in skeletal muscle biopsies in such patients,[7] we hypothesized that these changes might be systemic and may also be reflected by in vivo sublingual videomicroscopic measurements.

2 | PATIENTS AND METHODS

The study was performed in accordance with the Declaration of Helsinki and approved by the local ethics committee of the Medical University of Vienna. All participants signed a written informed consent. Reporting of the study conforms to STROBE statement along with references to STROBE statement and the broader EQUATOR guidelines.[8]

2.1 | Study population

A single-centre prospective cross-sectional cohort study was performed in patients with CHF and pharmacological treatment. Recruitment period was from February to November 2015.

Fifty outpatients with a history of severe systolic CHF were consecutively included. Inclusion was based on at least one NT-proBNP level above 2000 pg/mL in 1 of the preceding clinical visits, irrespective of the current NYHA class because NT-proBNP levels are established as prognostic markers in CHF.[9] Heart failure with reduced ejection fraction (HFrEF) was confirmed in all patients, when patients presented for the first time at our outpatient unit. It was defined as reduced left ventricular ejection fraction (LVEF), below 40%, accompanied with signs and symptoms of heart failure in accordance with the European Guidelines for Heart failure.[10] LVEF was not measured regularly at index time based on its interobserver variability, the difficulty to quantify LVEF correctly, especially in ischaemic heart failure and its inferiority to predict outcome compared to NT-proBNP.[9] Prescribed medication was continued, and patients were measured after an overnight fast. Patients' medication (CHF therapy, anticoagulation) was given according to guideline recommendations.[10]

Seventeen patients with nonischaemic and 33 with ischaemic heart failure were included. The group of nonischaemic heart failure consisted of 16 patients with dilated heart failure, as well as 1 patient with hypertensive heart failure. CHF patients were compared to 35 healthy controls. These subjects participated in various studies and passed the screening examination including medical history, clinical status and laboratory investigation. None of them had signs or symptoms suggestive of coronary artery disease or CHF.

We recorded current medication, age and weight/height and obtained routine laboratory parameters in all study participants.

Laboratory measures were obtained on the same day as the sublingual videomicroscopy in both groups.

2.2 | Microscope imaging

In vivo measurements of the sublingual vasculature were performed using a sidestream darkfield videomicroscope (CapiScope HVCS Handheld Video Capillary Microscope; KK Technology, Honiton, England) by one person to avoid interobserver variability. The camera is provided with light-emitting diodes using a wavelength of 525 nm to detect the haemoglobin of circulating red blood cells. The standard lens of the microscope enables a 0.92 μm/pixel magnification in 752 × 480 pixels (field of view: 692 × 442). The software for acquisition and calculation of the perfused boundary region (PBR) is supplied by GlycoCheck BV (Maastricht, the Netherlands), and detailed methodology was described by Lee et al[11]. In short, the camera is placed under the tongue near the frenulum and the software identifies microvessels below 30 μm of thickness due to the contrast of red blood cells (RBC). RBC column widths are measured in at least 3000 vessel segments. The PBR is the most luminal part of the glycocalyx, which allows for limited penetration of the RBCs.[12] It is located at both sides of the RBC column; to determine its properties, the distance between the median RBC column width (P50) and the outer edge of the RBC-perfused luminal part of the glycocalyx (= perfused diameter) is calculated using the following equation: (perfused diameter-median RBC column width)/2. The increase in PBR reflects glycocalyx destruction.[11-13] The average PBR of microvessels between 5 and 25 μm diameter was used for statistical analyses. Method validation of PBR measurements was described by Dane et al[13]: it was shown that changes in PBR dimensions are reflected by RBC column size and independent of vascular diameter; PBR is inversely proportional to the glycocalyx.[13]

To assess capillary density, the software recognizes all microvessels below 30 μm of thickness by determination of the red blood cells against the background. Vascular segments (line markers) are placed every 10 μm of the vessel length. The recording process continues until a minimum of 3000 vascular segments. After the acquisition, on the first frame of each recording session a total of 21 line markers are placed every 0.5 μm of the vascular segments. Only those vessels with an appropriate contrast of more than 60% of all 21 line markers are considered as functional (=valid perfused) vessels. All perfused vessels are referred to as total capillary density. RBC filling percentage is calculated by determining the percentage of vessels with RBCs present during the recording session (corresponding to 40 frames per session).[11] RBC filling percentage and perfused capillary density are regarded as estimates of microcirculatory perfusion.[11]

2.3 | Laboratory measurements

Markers of CHF (NT-proBNP in patients), liver malfunction (as consequence of increased central venous pressure or impaired perfusion due to decreased cardiac output[14]: total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase, gamma-glutamyltransferase and albumin), renal impairment (serum creatinine, estimated glomerular filtration rate (eGFR) according to the MDRD formula (eGFR = 186 × Serum creatinine $(mg/dL)^{-1.154}$ x age $(years)^{-0.203}$ × 0.742 (if female)) and inflammation (fibrinogen, C-reactive protein (CRP), platelet count and leucocytes) were obtained. All laboratory measurements were conducted routinely as follows:

NT-proBNP: cobas e 601 immunoassay analyser, Roche Diagnostics GmbH, Germany.

aPTT, international normalized ratio (INR), prothrombin time, thrombin time, D-dimer and fibrinogen: STA-R Evolution analyser, Diagnostica Stago, Asnières sur Seine, France.

Hemogram: Sysmex Xe-5000 System, Sysmex Corporation, Kobe, Japan.

Aspartate aminotransferase, ALT, gamma-glutamyl transferase, total bilirubin, albumin, CRP, serum creatinine: cobas e701/702 analyser, Roche Diagnostics GmbH, Germany.

2.4 | Sample size calculations and statistics

On the basis of a previous publication, we hypothesized a minimum 18% difference in capillary density[5] between CHF and controls. On the basis of a preliminary assessment of the variability of capillary density in normal controls (CV=24%), we estimated that we would need to include a total of 88 participants to demonstrate an 18% difference in capillary density with an alpha error of ≤0.016 and a beta of 0.85.

Data were tested for normal distribution using the Kolmogorov-Smirnov test. Groups were compared with independent *t* tests, Mann-Whitney *U* tests or χ^2 analyses, as appropriate. Continuous normally distributed variables are presented as mean ± standard deviation (SD), unless otherwise specified.

Vascular parameters (PBR, RBC filling percentage, perfused and total capillary density) are described as median and interquartile range and differences analysed with the nonparametric Mann-Whitney U test. Correlations between PBR and laboratory parameters were calculated using Spearman's rank correlation. Multiple regression analyses were performed to assess the associations of PBR with biomarkers (total bilirubin, ALT, serum creatinine and fibrinogen).

Elevated NT-proBNP levels with a cut-off above 1500 pg/ml are proposed as prognostic markers for 1-year mortality[15] and were used to stratify patients into two subgroups with regard to disease severity. Analyses of covariance were used to exclude the influence of age or disease severity in subgroup analyses.

Two-sided *P*-values < .05 were considered statistically significant.

Statistical analyses were performed using IBM SPSS Statistics for Macintosh, version 21.0. (IBM Corp. Armonk, NY, Released 2012). The figure was created with OriginPro 2015 (Northampton, MA, USA).

3 | RESULTS

3.1 | Clinical characteristics

Detailed characteristics of all study participants are depicted in Table 1. Medication of CHF patients is described in the Table S1. Patients were aged between 25 and 88 years and healthy subjects between 25 and 80 years, which was not significantly different. Several disease-related variables were different between groups: body mass index, distribution of sex, serum creatinine, CRP, fibrinogen, leucocytes, total bilirubin, D-dimer and INR. Clinically, 15% of the patients were stage NYHA I, 32% stage NYHA II and 53% stage NYHA III.

3.2 | Decreased total and perfused capillary density in chronic heart failure

This study investigated whether capillary density is decreased systemically and can therefore be visualized sublingually in CHF patients. Indeed, the number of perfused and total capillaries per mm^2 was markedly lower in CHF patients (30% for perfused, 45% for total capillary density, both $P < .001$; Table 2, Figure 1). Multivariate adjustment for possible confounding factors (variables with significant

TABLE 1 Clinical characteristics of study participants

	Patients (n = 50)	Healthy controls (n = 35)	*P*
Age, years	68 ± 13	66 ± 10	.419
Male sex, n	44 (88%)	23 (66%)	.017
Body mass index	28 ± 5	26 ± 3	.029
Serum creatinine (μmol/L)	120.2 (IQR: 95.5-194.6)	80.4 (IQR: 70.7-90.2)	<.001*
Estimated glomerular filtration rate (ml/min)	52.7 ± 24.8	82.4 ± 18.4	<.001
C-reactive protein (mg/L)	4.6 (IQR: 2.2-8.7)	1.6 (IQR: 0.9-2.6)	<.001*
Fibrinogen (g/L)	4.17 ± 0.94	3.38 ± 0.56	<.001
Leucocytes ($*10^9$/L)	7.9 ± 2.1	6.6 ± 1.6	.003
Platelets ($*10^9$/L)	223 ± 73	233 ± 42	.445
Total bilirubin (μmol/L)	14.1 ± 8.6	7.7 ± 3.1	<.001
Alanine aminotransferase (μmol/s·L)	0.38 ± 0.19	0.42 ± 0.2	.383
International normalized ratio	1.85 (IQR: 1.00-2.83)	1.00 (IQR: 0.09-1.00)	<.001*
D-dimer (μg/mL)	0.51 (IQR: 0.27-1.44)	0.37 (IQR: 0.27-0.56)	.045 *
NT-proBNP (ng/L)	3156 (range 180-35000)		

Data are presented as mean ± SD unless otherwise indicated.
*Denotes Mann-Whitney *U* test due to skewed distribution.

TABLE 2 Capillary densities in patients suffering from chronic heart failure and controls

	Chronic heart failure (n = 50)	Healthy controls (n = 35)
Functional capillary density (n/mm^2)	245 (207-284)	352 (310-406)*
Total capillary density (n/mm^2)	354 (302-462)	646 (526-763)*
Ratio (%) (Functional/ total capillary density)	73 (57-77)	57 (52-62)*

Data are presented as median and IQR.
*Denotes $P < .001$.

difference between groups) showed CHF as single independent risk factor for loss of perfused and total capillary densities ($P < .001$). Furthermore, there was no association between capillary density and hypertension or tobacco smoke (data not shown). The ratio of perfused capillary density/total capillary density was higher in patients (73%) than in healthy subjects (57%) (Table 2). There were no differences between ischaemic and nonischaemic heart failure.

3.3 | Similar glycocalyx thickness in chronic heart failure patients and healthy volunteers

The PBR was similar between patients and healthy volunteers: 1.93 μm (range 1.70-2.06 μm) in CHF patients and 1.83 μm (range 1.75-2.00 μm) in controls ($P = .391$). There was a high negative correlation between PBR and RBC filling (patient cohort: $r = -.92$, $P < .001$, n = 50, healthy subjects: $r = -.77$, $P < .001$, n = 35) indicating an association between PBR and microcirculatory perfusion.[11]

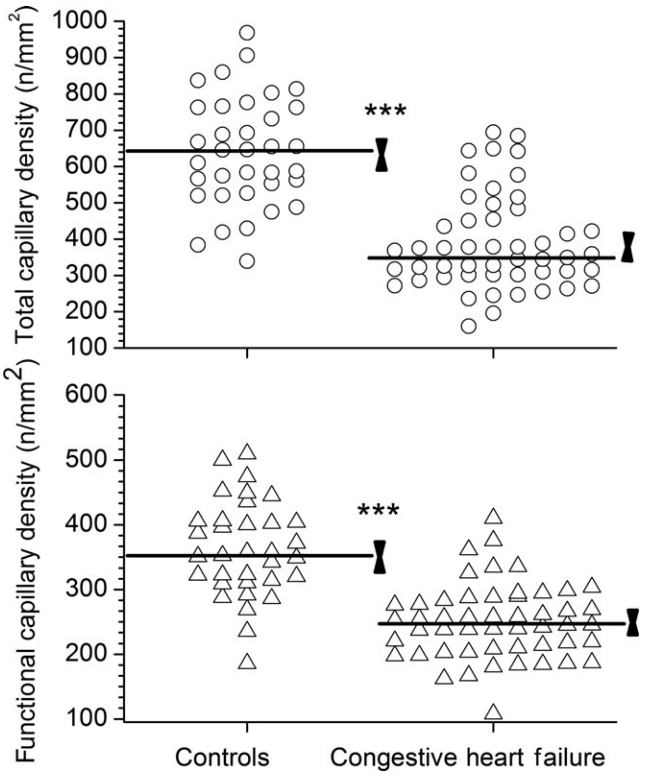

FIGURE 1 Reduced capillary density in CHF patients compared to healthy controls. Median total capillary density was 45% and perfused capillary density 30% lower in the patients with chronic heart failure. (Symbols: *** denotes $P < .001$; whiskers denote 95% confidence intervals)

3.4 | Organ manifestation of chronic heart failure and inflammation

To clarify whether microvascular changes correlate with markers of inflammation or organ damage, an exploratory correlation analysis was performed in the patient group.

Correlations between microcirculatory parameters and disease-related biomarkers are depicted in Table 3. Multiple regression analysis taking into account total bilirubin, ALT, serum creatinine and fibrinogen as covariates showed a weak independent correlation between fibrinogen ($\beta = 0.102$; CI (95%) = 0.030-0.174, $P = .007$) and ALT ($\beta = -0.006$; CI (95%) = −0.012 to −0.000, $P = .043$) and elevated PBR, ie reduced glycocalyx thickness.

3.5 | Comparison of microvascular parameters between men and women

No sex differences were seen in either cohort (Table S2).

3.6 | Coagulation and sublingual microcirculation

To examine whether anticoagulation or antiplatelet therapy could alter microcirculation, we performed a subgroup analysis for patients with and without vitamin K antagonists (VKAs) or antiplatelet drugs. Both perfused and total capillary densities were about 20% lower in patients treated with VKAs ($P < .05$; Table S3), and consistently, there was a correlation with INR ($r = -.35$, $P = .024$ and $r = -.31$, $P = .043$, respectively). However, NT-proBNP levels were higher in patients on VKAs (4123 pg/mL (IQR: 2371-6862 pg/mL) vs 2144 pg/mL (IQR: 1382-4193 pg/mL), $P = .024$). To assess the confounding effect of disease severity, analysis of covariance was performed taking into account perfused or total capillary density as dependent variable and NT-proBNP levels as covariate. Intake of neither VKAs nor NT-proBNP levels emerged as an independent risk factor.

TABLE 3 Correlations between vascular parameters with organ manifestation of chronic heart failure including liver/renal insufficiency and inflammation

	Perfused boundary region (*r*)	Functional capillary density (*r*)	Total capillary density (*r*)
NT-proBNP	**.33***	−.22	−.23
Total bilirubin	**−.38***	−.00	−.04
Alanine aminotransferase	−.28	.11	.03
Aspartate aminotransferase	−.24	.14	.13
Gamma-glutamyltransferase	−.13	−.08	−.21
Albumin	−.30	−.09	−.08
Serum creatinine	**.34***	−.05	.01
Estimated glomerular filtration rate	**−.34***	.05	−.00
Fibrinogen	**.58****	.05	.28
C-reactive protein	**.42****	−.13	.00
Platelets	**.36***	.12	.21
Leucocytes	.02	.04	−.10

Correlations are presented by the Spearman rank coefficient. Note that an increased perfused boundary region reflects a decreased glycocalyx.
*Denotes $P < .05$.
**Denotes $P < .001$.

3.7 | Association of microcirculation with disease severity

Patients were stratified into two subgroups with NT-proBNP levels below/above 1500 pg/mL because this cut-off was predictive of 1-year mortality.[15] Patients with high NT-proBNP levels (>1500 pg/mL; n = 38) had lower total perfused capillary density (328/mm^2, IQR: 300-417/mm^2 vs 460, IQR: 356-581, $P = .039$) and a trend towards lower functional capillary density (243/mm^2, IQR: 207-271/mm^2 vs 274, IQR: 223-332, $P = .172$) than those with low NT-proBNP levels (<1500 pg/mL, n = 8). Because there was an unequal age distribution in the two subgroups ($P = .079$), analysis of covariance was performed taking into account perfused or total capillary density as dependent variable and age as covariate. NT-proBNP levels above 1500 pg/mL emerged as an independent risk factor for loss of total perfused capillary density ($P = .030$; age=n.s.). However, there was only a trend-wise correlation between NT-proBNP and capillary density, possibly related to the skewed distribution of NT-proBNP levels.

4 | DISCUSSION

This study provides the first in vivo measurements of total and perfused capillary density in CHF patients. Sublingual total and perfused capillary densities were markedly lower in patients with CHF compared to healthy controls.

4.1 | Capillary density

Exercise tolerance is compromised in heart failure and cannot be attributed to a reduction in left ventricular ejection fraction or an increase in pulmonary capillary wedge pressure during exercise.[7,16] Patients with hypertrophic heart failure have got reduced myocardial perfusion.[17] Decreased myocardial capillary density may contribute to the depressed coronary flow reserve hence compromising exercise capacity in CHF patients.[5] In patients with acute heart failure,

sublingual microvascular blood flow was altered and perfused capillary density was diminished.[18,19] Previous studies in patients with CHF characterized capillary density invasively, mainly by obtaining skeletal muscle biopsies from patients.[7,20,21] The current study shows for the first time a rarefaction of systemic functional and total capillaries in CHF measured sublingually with an in vivo microscopic technique. While the local reduction in capillary density in the myocardium and skeletal muscle may be explained by CHF and lack of muscle training, the sublingual rarefaction hints towards a true systemic phenomenon. In addition, loss of capillary density might also be associated with disease progression as NT-proBNP levels above 1500 pg/mL are associated with a pronounced loss of total perfused capillary density. Further studies are indicated to clarify if this is due to an endocrinologic process and if it can be targeted by specific drug therapy. However, successful pre-clinical approaches to improve angiogenesis by vascular endothelial growth factor in cardiovascular diseases have failed to exhibit a clear benefit in clinical studies so far.[22]

The elevated perfused/total capillary ratio in CHF patients possibly reflects the body's partial compensation and corresponds to previous observations of perfused capillary recruitment in persons with a higher cardiovascular risk profile.[23]

4.2 | Glycocalyx

Previous studies reported the loss of glycocalyx in a small subgroup of dialysis patients with concomitant cardiovascular disease.[24] Additionally, patients with premature coronary artery disease and their healthy first-degree relatives may have microvascular dysfunction.[25] In the present study, there was only a small trend towards an increase in the PBR in CHF patients, measured by an indirect analysis of intravascular spatial dimensions of the RBC column width compared to healthy controls.

The endothelium has a crucial role in the regulation of inflammatory processes, which might be mainly influenced by the glycocalyx constitution.[26] Inflammation may have a negative impact on the glycocalyx,[26] which would be consistent with the observed correlation between PBR and fibrinogen or CRP. Furthermore, fibrinogen alters viscoelastic blood properties[27] and might induce conformational and structural changes in glycosaminoglycans contributing to the modulation of blood flow resistance by the endothelial surface layer.[28]

Patients with kidney failure and decreased eGFR[13] have a slightly increased PBR, which is in line with the observed correlation between PBR and eGFR in our patients. These observations suggest that the glycocalyx is possibly modulated in a multifactorial manner by CHF and related organ damage.

4.3 | Anticoagulation

From in vitro experiments, it is known that thrombin decreases heparan sulphate accumulation on endothelial cells.[29] As oral VKAs reduce the in vivo thrombin generation,[30] we performed a subgroup analysis with regard to anticoagulation. In patients on oral VKAs, there was only a minimal trend towards higher glycocalyx dimensions. Interestingly, however, perfused and total capillary densities were significantly lower, and a weak correlation between INR and perfused as well as total capillary density could be observed. Although these results may be a chance finding or due to a selection bias because of more severe CHF as indicated by higher NT-pro-BNP levels in this subgroup, oral VKAs may interfere with angiogenesis.[31] The present subgroup analysis might indicate a modulation of angiogenesis and microvascular perfusion in CHF patients. This should be the subject of further investigations, because it may have a clinical impact for those CHF patients who are currently treated with oral VKAs.

4.4 | Study limitations

Correlation and subgroup analyses are of exploratory nature, must be interpreted with caution and require confirmation in larger populations to identify independent predictors of capillary rarefaction. Residual confounding due to differences in demographic characteristics is possible. Our population was solely defined by the fact that the patients had a history of significant HF with reduced ejection fraction defined by a NT-proBNP>2000 pg/mL in the preceding clinical visit, as NT-proBNP is an established diagnostic and prognostic marker for patients with heart failure.[9] We therefore investigated heterogenous population; however, the achieved result can be more generalized.

In our population, there were no differences in capillary density or glycocalyx dimensions between men and women and we have not found literature, which would support this hypothesis. The study was of observational character, with a hypothesis-generating aspect. The mechanisms of altered microvascular function and its putative pharmacological modulation warrant further studies.

5 | CONCLUSION

Patients suffering from CHF have got substantially lower in vivo capillary densities than controls as measured by videomicroscopy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. All authors contributed substantially, read and approved the manuscript.

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SUPPORTING INFORMATION

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