Skin microcirculation dynamics are impaired in patients with rheumatoid arthritis and no cardiovascular comorbidities

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Abstract

Objective

Rheumatoid arthritis (RA) is associated with increased cardiovascular disease (CVD) risk. Microvascular endothelial dysfunction contributes to the development of vascular injury and subsequent CVD. We hypothesised that RA patients exhibit blunted microvascular reactivity regardless of CVD risk factors and investigated potential associations with coronary microvascular perfusion and surrogate markers of CVD.

Methods

This case-control study recruited RA patients and non-RA individuals in the absence of cardiovascular comorbidities. Skin microvascular reactivity was dynamically assessed using laser speckle contrast imaging coupled with post-occlusive reactive hyperaemia protocol. Applanation tonometry was applied to assess subendocardial viability ratio, an index of myocardial microvascular perfusion, and central arterial stiffness [carotid-femoral pulse wave velocity (PWV), augmentation index]. Peripheral arterial stiffness (carotid PWV, β-stiffness index) and carotid atherosclerosis (intima-media thickness) were assessed with carotid ultrasound software.

Results

Skin microvascular responses before and following reperfusion [baseline flux, occlusion flux, time-to-peak, peak magnitude, peak-to-baseline magnitude, baseline cutaneous vascular conductance (CVC), and percentage increase in CVC] were significantly impaired in RA patients (n=35) compared to controls (n=35). Presence of RA independently predicted altered microvascular reactivity in multivariate analysis. Skin microcirculation dynamics significantly correlated with coronary microvascular perfusion and peripheral arterial stiffness, yet not carotid atherosclerosis, even after adjustment for CVD risk factors.

Conclusion

Patients with RA present impaired microvascular reactivity regardless of CVD risk factors at a preclinical stage preceding CVD. Assessment of skin microvascular dysfunction may reflect a state of generalised vasculopathy, including myocardial microvascular abnormalities, and serve as a non-invasive surrogate indicator of CVD risk in RA.

Key words

rheumatoid arthritis, laser speckle contrast imaging, microcirculation, myocardial perfusion, arterial stiffness, cardiovascular disease

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Introduction

Rheumatoid arthritis (RA) is the most prevalent autoimmune inflammatory rheumatic disease (1), characterised by increased cardiovascular mortality by as much as 50% compared to the general population (2). Accelerated atherosclerosis is regarded as an extra-articular manifestation of the disease, as a result of the complex interactions between chronic inflammation and increased prevalence of CVD risk factors (3,4). Microvascular endothelial dysfunction has been consistently documented in RA and is presumed to play a major role in the development of subclinical vascular injury and subsequently, clinically evident cardiovascular manifestations (5, 6). It could be reasonably hypothesised that direct, *in vivo* visualisation of the microcirculation in patients with RA at an early, pre-symptomatic stage would provide valuable information regarding cardiovascular health.

Skin microvascular network is an easily accessible vascular bed, implemented as a widely applied model of generalised microvascular function. Laser Speckle Contrast Imaging (LSCI) has emerged as a novel, non-interventional and highly reproducible technique for the direct, *in vivo* mapping of the skin microvascular perfusion (7, 8). Compared with conventional laser Doppler flowmetry (LDF), LSCI presents improved spatial and temporal reproducibility when coupled with reactivity tests such as post-occlusive reactive hyperaemia (PORH) and local thermal hyperaemia (9, 10). LSCI has been applied in the field of cardiovascular research to reveal altered microcirculation responses in patients with high CVD risk, including essential and secondary hypertension, diabetes mellitus, end-stage kidney disease and coronary artery disease (11-15). It has been used to assess skin microvascular pathology in rheumatic populations, mainly those with pronounced microvascular injury and skin manifestations such as individuals with systemic sclerosis, Raynaud's phenomenon, and systemic lupus erythematosus (16-18), whilst relevant data in RA remain far too limited.

The present study aimed to examine skin microvascular responses using LSCI in RA patients free from established CVDs and comorbidities, and investigate whether these changes mirror increased CVD burden at an early, preclinical stage. We further assessed potential interactions between microvascular reactivity and (i) coronary microvascular perfusion, (ii) surrogate markers of CVD, *i.e.* arterial stiffness and carotid atherosclerosis.

Methods

Study population

This was an observational, case-control study. Consecutive patients with RA (19) were recruited from the Rheumatology Outpatient Unit, provided they were free from hypertension, diabetes mellitus, established heart disease, arrythmias, acute or past cardiovascular or cerebrovascular events. Other exclusion criteria were moderate to severe renal or hepatic dysfunction, active infection or other concurrent inflammatory conditions, pregnancy and malignancy. Cardiovascular events were defined as stroke, angina, and myocardial infarction based on self-report and ascertained by a medical record and medication review. The control group consisted of healthy individuals free from any known health problems, who were matched for traditional CVD risk factors [age, body mass index (BMI), smoking] and were recruited from both the community and the Outpatient Clinics of the 3rd Department of Internal Medicine, Papageorgiou General Hospital, Thessaloniki, Greece. All participants were adults and gave written informed consent before inclusion in the study. The study was approved by the institutional ethics committee (approval no.: 377/27072017) and conducted in accordance with the Helsinki Declaration.

Clinical assessment

Medical history, anthropometric characteristics, and medication use were recorded. BMI was calculated in kg/m^2. Systolic/diastolic blood pressure (BP) was measured according to the guidelines with a validated oscillometric device (Microlife Exact BP, Microlife AG, Widnau, Switzerland) (20). Mean BP was estimated as [(systolic BP) + 2*(diastolic BP)]/3. Disease activity

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was assessed by the treating rheumatologist based on the Disease Activity Score-28 (DAS28) with erythrocyte sedimentation rate (ESR) (21). Fasting blood samples were collected at the end of the procedures for routine biochemical measurements (lipids, glucose, renal function). C-reactive protein (CRP) and ESR and were additionally measured in patients' samples. Participants were instructed to abstain from smoking, coffee, tea or alcohol consumption, and intense physical activity for >2 hours before the procedures. All vascular measurements were conducted in the same temperature-controlled room (23±1°C) with ambient light, after a 15-minute acclimatisation period with participants lying in the supine position.

Assessment of microvascular perfusion with LSCI

The main principle of LSCI (PeriCam PSI NR System, Perimed, Järfälla, Sweden) is the visualisation of a wide skin tissue area with a coherent laser beam (penetration wavelength of 785 nm), which creates an instant image (speckle pattern) of the reflected light from that area. The speckle pattern is dynamic due to the continuous movement of the red blood cells in the illuminated area, that backscatters the laser beam and generates intensity fluctuations. Based on measurements of the intensity fluctuations in several frames of the speckle pattern acquired within a certain time interval, LSCI creates two-dimensional maps of skin microvascular perfusion (flux) in real time, with very high spatial and temporal resolution (22, 23).

At the beginning of the procedure, the right arm of each participant was immobilised by a vacuum cushion to reduce moving artifacts. A sphygmomanometer cuff was placed above the elbow. The laser head was fixed at a working distance of 15±1 cm from the ventral surface of the forearm of each participant, and two skin sites (circular Regions of Interest, ROIs) >10 mm^2 were selected (24). Images were recorded at a frame rate of 21 images/s and a resolution of 0.41–0.46 mm. LSCI was coupled with PORH as the reactivity test of choice. As previously described, the PORH protocol consisted of a 2-min baseline recording period; a 5-min occlusion period where blood flow was obstructed by inflating the sphygmomanometer cuff at suprasystolic levels of 250 mmHg, and finally, a 5-min post-occlusive period (7, 16). The latter commenced immediately after the deflation of the cuff and included the peak blood flow, coinciding with the maximum skin microvascular response, and the return to the baseline condition. Cutaneous perfusion recorded schematically with LSCI in a patient with RA before and following occlusion is presented in Figure 1. The average blood perfusion of the two ROIs per testing period was documented, and values were expressed in perfusion units (PU).

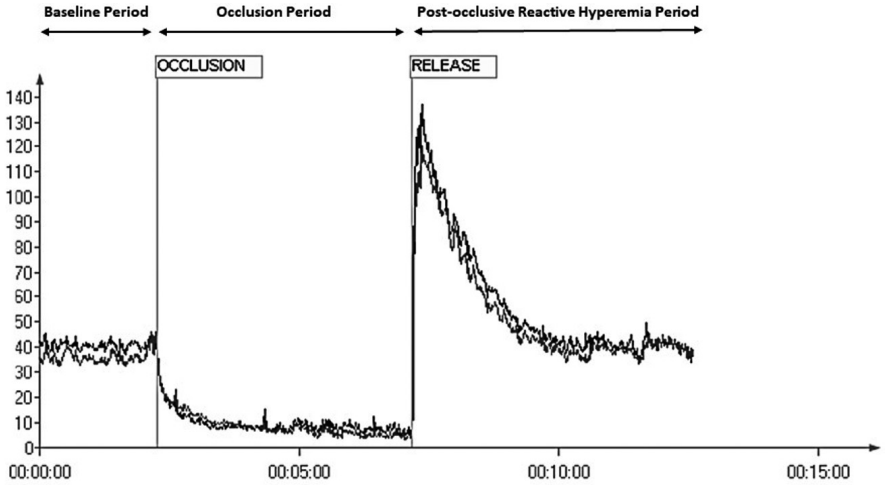

Fig. 1. Schematic presentation of cutaneous microvascular perfusion recorded with LSCI coupled with post-occlusive reactive hyperaemia (PORH). Relative flux changes correspond to the regions of interest (ROIs), which represent two randomly selected skin sites where recording of the skin microvascular flux is performed. Blood flow recording during the first couple of minutes corresponds to a baseline recording period. Recording from 2 to 7 minutes corresponds to the occlusion period, starting with inflation of the sphygmomanometer cuff at suprasystolic levels of 250 mmHg. Recording from 7 to 12 minutes corresponds to the post-occlusive period, commencing immediately after the deflation of the cuff. The latter enables the recording of the peak blood flow that coincides with the maximum skin microvascular response. Subsequently, blood flow returns to the baseline condition.

Analysis of the results was performed using the manufacturer's software (PIMSoft, Perimed, Järfälla, Sweden). The traces were analysed by an operator blinded to the presence of RA (P.D.). Variables obtained by LSCI, indicative of skin microvascular responses, are the following: baseline flux, corresponding to the mean flux during the baseline period expressed in arbitrary perfusion units (Laser Speckle Perfusion Units, LSPUs); occlusion flux, corresponding to the mean flux during the occlusion period (LSPUs); peak time, which represents the time taken since the sphygmomanometer cuff deflation to the moment of maximal post-occlusion flux value (sec); peak flux, referring to the highest flux value at the post-occlusive period (LSPUs); base to peak flux, expressed as the percentage increase of flux from baseline to the maximal post-occlusive response (%); PORH amplitude, calculated as the difference between peak and baseline cutaneous vascular conductance (CVC), and percentage increase in CVC, estimated as (PORH amplitude/baseline CVC)*100%. CVC was calculated as the ratio of mean flux in each relevant period divided by mean BP (in LSPUs/mmHg).

Assessment of coronary microvascular perfusion

Subendocardial viability ratio (SEVR), also known as the Buckberg index, has been introduced as a functional index of microvascular myocardial perfusion. SEVR correlates with the ratio of subendocardial to subepicardial blood flow, with lower values of SEVR indicative of poorer perfusion of the subendocardium (25). SEVR was non-invasively estimated by applanation tonometry in the radial artery with the SphygmoCor device (AtCorMedical, Sydney, Australia) as previously described (26, 27), from the ratio of diastolic pressure time

index to tension time index, which correspond to myocardial supply and demand, respectively.

Assessment of arterial stiffness

Carotid-femoral pulse wave velocity (cfPWV) and augmentation index (AIx) were assessed with applanation tonometry as the most widely applied, validated markers of arterial stiffness, which has been acknowledged as a surrogate marker of CVD (28). Briefly, cfPWV was calculated using the Sphygmocor device (AtCor Medical, Sydney, Australia) as the distance traveled by the arterial wave between the carotid and the femoral sampling site, divided by time (PWV=$\Delta d/\Delta t$), according to a standard methodology (29, 30). For the assessment of AIx, the aortic pressure waveform was analysed from the radial pressure waveform using the same device as previously described (30, 31). To exclude the influence of heart rate on the AIx, AIx was automatically corrected for the mean heart rate of 75 bpm.

Peripheral arterial stiffness was measured locally in the carotid arteries with carotid PWV and carotid β-stiffness index. Measurements were performed with a high-definition echo-tracking system (Aloka Pro Sound A7, Ultrasound System, Tokyo, Japan) equipped with a 7-12 mHz linear array transducer and a vessel wall moving detector. The Aloka ultrasound system provides a valid on-line one-point measurement of local PWV, while the β-stiffness index was calculated as the ratio of the natural logarithm of systolic/diastolic BP, to the relative change (Δ) in diameter, according to a previously described standard methodology (32).

Evaluation of carotid atherosclerosis

Subclinical atherosclerosis was evaluated with carotid intima-media thickness (cIMT), which has been acknowledged as a surrogate measure of atherosclerotic CVD. Mean cIMT was calculated in the far wall of the distal 10 mm of each artery with ultrasound (Aloka Pro Sound A7, Ultrasound System, Tokyo, Japan), as previously described (33, 34).

Statistical analysis

Data analysis was performed using SPSS (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA) software, version 22. Results were expressed as frequencies for qualitative variables, and as mean±standard deviation (m±SD) or median (interquartile range) for continuous variables. Comparison of frequencies was

Table I. Baseline characteristics and cardiovascular risk factors in the study population.

	RA patients (n=35)	Controls (n=35)	*p*-value
Age (years)	55.6 ± 11.2	51.2 ± 7.5	0.056
Male gender, n (%)	5 (14.3)	11 (31.4)	0.088
Disease duration (years)	12 (11.5)	NA	NA
ESR (mm/hr)	22 (16)	NA	NA
CRP (mg/L)	0.39 (0.45)	NA	NA
DAS28 score	2.1 (1.9)	NA	NA
Conventional DMARDs (%)	75	NA	NA
Biologic DMARDs (%)	58.3	NA	NA
Corticosteroids (%)	35.7	NA	NA
Office SBP (mmHg)	120.2 ± 16.2	116.6 ± 13.4	0.307
Office DBP (mmHg)	75.1 ± 9.0	74.5 ± 9.0	0.788
Heart rate (/min)	70.9 ± 9.7	72.9 ± 9.0	0.352
BMI (kg/m^2)	26.4 ± 4.0	27.1 ± 4.3	0.499
Smoking, %	23.5	32.0	0.470
Total cholesterol (mg/dl)	204.5 (67)	208 (28)	0.886
LDL-C (mg/dl)	131 (59)	138.5 (26.5)	0.610
HDL-C (mg/dl)	52.9 ± 9.3	52.0 ± 7.2	0.734
Triglycerides (mg/dl)	111 (77)	93 (52)	0.577
Glucose (mg/dl)	91.0 ± 8.8	85.0 ± 7.9	0.019
Urea (mg/dl)	27.0 ± 7.1	29.2 ± 5.6	0.247
Creatinine (mg/dl)	0.76 (0.17)	0.75 (0.13)	0.587

RA: rheumatoid arthritis; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; DAS28: Disease Activity Score in 28 joints; DMARDs: disease-modifying anti-rheumatic drugs; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: Body Mass Index; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; NA: Not applicable.
Continuous variables are presented as mean ± SD or median (interquartile range) according to normality tests.

Table II. Vascular measurements in the study population.

A. Microvascular endothelial dysfunction assessed by Laser Speckle Contrast Imaging.

	RA patients (n=35)	Controls (n=35)	*p*-value
Baseline flux (LSPUs)	46.8 (18.9)	36.4 (13.9)	**<0.001**
Occlusion flux (LSPUs)	12.4 (13.5)	7.0 (3.6)	**0.001**
Time to peak (s)	7.0 (0.4)	11.0 (8)	**<0.001**
Peak magnitude (LSPUs)	113.1 (25.4)	99.1 (32.5)	**0.017**
Peak to baseline magnitude (%)	136.5 (75.2)	172.1 (65.9)	**0.014**
Baseline CVC (LSPUs/mmHg)	0.54 ± 0.15	0.44 ± 0.14	**0.006**
Peak CVC (LSPUs/mmHg)	1.35 ± 0.38	1.20 ± 0.28	0.069
PORH amplitude (LSPUs/mmHg)	0.77 (0.29)	0.75 (0.24)	0.741
Increase in CVC (%)	139.5 (75.3)	172.1 (67.3)	**0.011**

B. Coronary microvascular perfusion and surrogate markers of cardiovascular disease.

Coronary microvascular perfusion			
SEVR (%)	142.5 ± 21.2	157.4 ± 23.2	**0.041**
Carotid atherosclerosis			
Carotid IMT (mm)	0.64 ± 0.12	0.58 ± 0.10	**0.022**
Arterial stiffness			
Carotid-femoral PWV (m/s)	7.8 ± 1.6	7.2 ± 1.0	0.095
AIx (%)	28 (16)	20 (15)	**0.009**
Carotid PWV (m/s)	5.8 (1.6)	6.0 (1.5)	0.790
Carotid β-stiffness index (%)	7.2 ± 2.4	6.0 ± 1.3	**0.028**

RA: rheumatoid arthritis; CVC: cutaneous vascular conductance; PORH: post-occlusive reactive hyperaemia; SEVR: subendocardial viability ratio; IMT: intima media thickness; PWV: pulse wave velocity; AIx: augmentation index.
Continuous variables are presented as mean ± SD or median (interquartile range) according to normality tests.

performed by Pearson chi-square test. Student's t-test or Mann-Whitney test was used to estimate differences between mean values. Correlations between LSCI parameters and the main continuous variables were assessed using the parametric Pearson or the non-parametric Spearman's Rho correlation coefficient. Partial correlation analysis adjusting for presence of RA and CVD risk factors was performed to estimate the strength of the univariate associations between LSCI indices and vascular markers. Multiple linear regression analysis with the "Enter" method was used to identify the statistically significant predicting factors of LSCI indices after adjustment for other variables. Sample size calculation was based on a previous study of LSCI in patients with systemic lupus erythematosus compared to controls (16). With a 5% level of significance and 90% power, the estimated sample size was 26 individuals per group. A probability value of $p \leq 0.05$ was considered statistically significant.

Results

Study population

A total of 70 individuals were studied, whose baseline characteristics are presented in Table I. Non-significant differences were observed in traditional CVD risk factors between groups. RA patients (n=35) were characterised by long-term disease, low levels of inflammation, and all patients were under treatment.

Dynamic assessment of microcirculation with LSCI

Table IIA presents the results from the dynamic assessment of microvascular endothelial dysfunction assessed with LSCI. Most LSCI indices were altered in patients with RA compared to controls. Microvascular responses following PORH in RA patients and controls are further presented in Figure 2.

Multiple regression analysis was further performed for the LSCI indices that were significantly impaired in RA patients compared to controls. After adjustment for traditional CVD risk factors (age, gender, BP, BMI), presence of RA remained an independent predictor of baseline flux, occlusion flux, time to peak, baseline CVC, and increase in CVC, as presented in Table III.

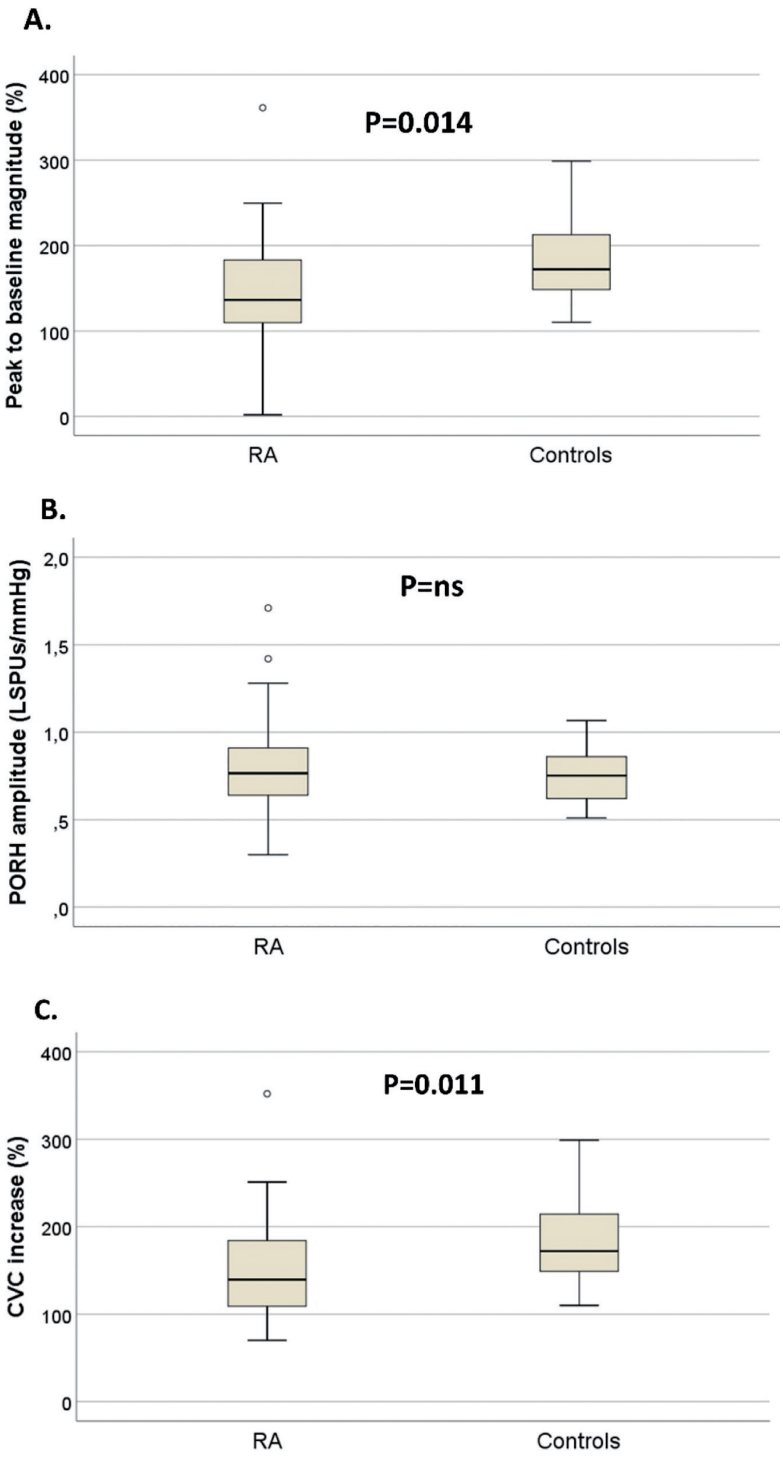

Fig. 2. Microvascular responses in patients with rheumatoid arthritis and controls following post-occlusive reactive hyperaemia.
A. Peak to baseline magnitude, **B**. Post-occlusive reactive hyperaemia amplitude (PORH), **C**. Percentage increase in CVC.

Coronary microvascular dysfunction and surrogate markers of CVD

Comparison of coronary microvascular perfusion and markers of atherosclerosis, central and peripheral arterial stiffness between groups is presented in Table IIB. Patients with RA presented significantly lower SEVR, and increased cIMT, AIx and carotid β-stiffness index compared to controls.

Table III. Multiple regression analysis for microvascular endothelial dysfunction assessed with LSCI in the study population.

Variable	Dependent variable: **Baseline flux** $p<0.001$, $R^2=0.464$, adjusted $R^2=0.411$		Dependent variable: **Occlusion flux** $p<0.001$, $R^2=0.415$, adjusted $R^2=0.357$		Dependent variable: **Time to peak** $p<0.001$, $R^2=0.432$, adjusted $R^2=0.376$		Dependent variable **Peak magnitude** $p=0.031$, $R^2=0.208$, adjusted $R^2=0.131$		Dependent variable **Peak to baseline magnitude** $p=0.259$, $R^2=0.117$, adjusted $R^2=0.030$		Dependent variable: **Baseline CVC**, $p<0.001$, $R^2=0.387$, adjusted $R^2=0.327$		Dependent variable: **Increase in CVC** $p=0.160$, $R^2=0.140$, adjusted $R^2=0.056$	
	Beta	*p*-value	Beta	*p*-value	Beta	*p*-value	Beta	*p*-value	Beta	*p*-value	Beta	*p*-value	Beta	*p*-value
Age (years)	0.199	0.114	0.044	0.738	-0.244	0.061	0.173	0.255	-0.127	0.428	0.174	0.195	-0.084	0.593
Gender (f)	0.225	**0.050**	0.374	**0.002**	0.071	0.542	0.207	0.136	-0.104	0.474	0.168	0.167	-0.104	0.466
Mean blood pressure (mmHg)	-0.023	0.857	0.269	**0.045**	-0.014	0.914	-0.133	0.387	0.021	0.897	-0.394	**0.005**	-0.033	0.835
BMI (kg/m^2)	0.209	0.066	0.087	0.459	0.057	0.619	0.183	0.182	-0.018	0.900	0.214	0.078	0.001	0.997
Rheumatoid arthritis	-0.485	**<0.001**	-0.336	**0.004**	0.565	**<0.001**	-0.259	0.054	0.242	0.086	-0.435	**<0.001**	0.295	**0.036**

CVC: cutaneous vascular conductance; BMI: Body Mass Index

Association of microvascular endothelial dysfunction with traditional CVD risk factors and disease-related parameters

Univariate associations were sought in the study population between LSCI indices that were significantly impaired in the RA group and traditional CVD risk factors (age, sex, BP, BMI, lipids, smoking). Age was significantly associated with baseline flux (r=0.378, *p*=0.001), occlusion flux (r=0.376, *p*=0.001), time to peak (r=-0.343, *p*=0.004), peak magnitude (r=0.325, *p*=0.006). Analysis stratified by gender showed that females presented significantly increased baseline flux (45.9±11.5 *vs*. 36.3±12.8 LSPUs, *p*=0.005), occlusion flux [10.7 (8.8) *vs*. 5.6 (4.0) LSPUs, *p*<0.001], peak magnitude [112.4 (29.5) *vs*. 90.5 (23.1) LSPUs, *p*=0.002], and baseline CVC (0.51±0.13 *vs*. 0.40±0.17 LSPUs/mmHg, *p*=0.009) compared to males. Baseline CVC additionally correlated with diastolic BP (r=-0.249, *p*=0.039). No other associations of LSCI indices with traditional CVD risk factors were observed.

Further correlation analysis was performed in the RA group to explore associations between LSCI parameters and disease-related parameters. Disease duration positively correlated with time to peak (r=0.385, *p*=0.039), and inversely with peak to baseline magnitude (r=-0.452, *p*=0.014) and increase in CVC (r=-0.465, *p*=0.013). No associations of LSCI indices were observed with DAS28, ESR or CRP, with the only exception of the latter which significantly correlated with occlusion flux (r=-0.645, *p*=0.009).

Association of microvascular endothelial dysfunction with coronary microvascular perfusion and surrogate markers of CVD

Univariate associations of LSCI indices with coronary microvascular perfusion in the study population are presented in Table IVa. SEVR strongly and significantly correlated with nearly all LSCI indices.

Regarding large artery stiffness, cf-PWV was significantly associated with baseline flux (r=0.301, *p*=0.021), occlusion flux (r=0.327, *p*=0.012) and time to peak (r=-0.300, *p*=0.021). AIx significantly correlated with baseline flux (r=0.340, *p*=0.007), occlusion flux (r=0.420, p=0.001) and peak magnitude (r=0.371, *p*=0.003). Carotid PWV inversely correlated with peak CVC (r=-0.399, *p*=0.007) and PORH amplitude (r=-0.476, *p*=0.001). Likewise, negative associations were found between β-stiffness index and peak CVC (r=-0.399, *p*=0.007), PORH amplitude (r=-0.476), as well as time to peak (r=-0.303, *p*=0.031). By contrast, non-significant associations were observed between cIMT and LSCI parameters.

Multivariate associations of LASCA indices with vascular measurements

Taking into account the univariate associations of LASCA indices with vascular markers in the study population, partial correlation analysis was performed to adjust further for presence of RA and CVD risk factors (age, gender, mean BP levels) that strongly influence the above micro- and macrovascular measurements. As presented in Table IVb, most of the observed univariate associations between SEVR and LSCI indices (baseline flux, peak magnitude, baseline CVC and peak CVC) remained statistically significant even after adjustment for the above parameters.

Regarding the univariate associations with arterial stiffness, the association between carotid PWV and peak CVC remained statistically significant (r=-0.353, *p*=0.024) in partial correlation analysis similarly accounting for age, gender, BP and presence of RA. The same was observed for the association between carotid β-stiffness index with both peak CVC (r=-0.475, *p*=0.001) and PORH amplitude (r=-0.430, *p*=0.003). By contrast, the observed associations of cfPWV and AIx with LSCI indices no longer remained significant after adjustment in partial correlation analysis.

Discussion

The main study finding is the demonstration of pronounced impairment of microcirculatory blood flow responses assessed by LSCI, in a well-characterised sample of RA individuals free from CVD. Furthermore, we found an independent association of impaired skin microvascular reactivity with coronary microvascular perfusion and surrogate markers of CVD such as arterial stiffness. These findings suggest a potential role for microcirculation dynamics as an index of systemic microangiopathy, including myocardial microvascular abnormalities, as well as macrovascular injury in RA patients without overt CVD.

The significance of these results is strengthened by the characteristics of our study population. First, although infrequently encountered in clinical

Table IV. Results of correlation analysis of skin microvascular perfusion with coronary microvascular perfusion in the study population.

Variable	Correlation analysis for SEVR			
	(**a**) Univariate analysis		(**b**) Partial correlation analysis	
	r	*p*-value	r*	*p*-value
Baseline flux (LSPUs)	-0.526	**<0.001**	-0.437	**0.005**
Occlusion flux (LSPUs)	-0.551	**<0.001**	-0.293	0.070
Time to peak (s)	0.237	0.117	0.221	0.171
Peak magnitude (LSPUs)	-0.294	**0.050**	-0.321	**0.043**
Peak to baseline magnitude (%)	0.407	**0.006**	0.276	0.089
Baseline CVC (LSPUs/mmHg)	-0.448	**0.002**	-0.402	**0.010**
Peak CVC (LSPUs/mmHg)	-0.294	0.053	-0.322	**0.043**
PORH amplitude (LSPUs/mmHg)	-0.067	0.667	-0.186	0.252
Increase in CVC (%)	0.380	**0.012**	0.252	0.122

*Adjusted for age, gender, mean blood pressure and rheumatoid arthritis.
CVC: cutaneous vascular conductance; PORH: post-occlusive reactive hyperaemia.

practice, RA patients were meticulously selected to exclude not only established CVD, but also concomitant hypertension and diabetes, which may severely compromise microvascular function. Importantly, the distribution of other traditional CVD risk factors was similar between patients and controls, minimising the influence of these confounders. Secondly, RA patients were characterised by long-term disease with low disease activity and low systemic inflammatory load at the time of the examination. These characteristics combined denote that impairment of microvascular function is inherent in RA, reflecting the cumulative effects of systemic inflammation on vascular architecture during the course of the disease. It could be further assumed that patients with flares and high inflammatory load might present even more pronounced impairment of microcirculation dynamics. As pharmacological treatments for RA are constantly evolving (35), their impact on microcirculation dynamics needs to be delineated in larger studies.

Although microvascular injury is traditionally associated with CVD, the mechanistic model of endothelial dysfunction in RA is considered secondary to vasculodestructive processes potentiated by chronic inflammation and autoimmune dysregulation (6). Accumulating evidence shows impaired functional and morphological markers of microvascular dysfunction in divergent vascular beds (5, 26, 36, 37), in relationship with increased levels of circulating biomarkers of thromboinflammation and endothelial dysfunction (38). Our study adds to this growing body of evidence by use of a novel, non-invasive method for the evaluation of cutaneous microcirculation dynamics. Indeed, most indices of microvascular reactivity were altered in patients with RA throughout the whole LSCI examination, from baseline flux to reperfusion following induced ischemia. Regarding the former, a pattern of increased baseline perfusion has been consistently described with LSCI in other patient groups, possibly as an effort to recruit more functional vessels in order to compensate the early microvascular impairment (16, 39, 40). It can be further hypothesised that even the higher number of recruited vessels are not enough to respond to the ischemic period, leading to a significantly lower microvascular reactivity during reperfusion. The observed significant associations with disease duration concur with previous studies showing substantial detrimental effects in CVD risk from the cumulative exposure to RA-related inflammation and disease severity (41). The lack of association between altered microvascular responses and disease activity or inflammation in the current study, can be attributed to the low inflammatory load at the time of examination which may not adequately reflect the underlying status of the microvasculature in RA.

Besides cutaneous microcirculation dynamics, patients with RA presented impaired myocardial microvascular perfusion, higher degree of carotid atherosclerosis and impaired markers of central and peripheral arterial stiffness compared to controls, as previously described (5,42,43). One of the most important study findings is the association of LSCI indices with coronary microvascular dysfunction, the generalisability of which, however, should be addressed with circumspection, as it was demonstrated in a RA population, though comorbidities free. However, this association further supports the hypothesis that apart from peripheral microangiopathy, skin microcirculation may also mirror the global functional status of the microvasculature, which is being currently under investigation (44, 45). It needs to be further highlighted that "classical", routinely used SEVR estimated by radial applanation tonometry results in an overestimation of the SEVR values and has been recently challenged by an integrated SEVR estimated by carotid applanation tonometry (accounting for the intra-ventricular diastolic pressure and proper allocation to systole and diastole of left ventricular isometric contraction and relaxation). This novel, non-interventional SEVR provided more reliable SEVR values when both were compared with those evaluated invasively by cardiac catheterisation (46).

On the other hand, the observed association with markers of arterial stiffness in this CVD free population suggests a cross-talk between small and large vessels in RA, that has been described in early stages of microvascular dysfunction in other high CVD risk populations (30, 47). By contrast, atherosclerotic burden as measured by cIMT did not correlate with skin microcirculation dynamics, in line with a previous report by Sandoo *et al*. applying conventional laser Doppler imaging (48). While investigation of pathophysiological aspects was beyond the scope of our study, it may be considered that cIMT reflects a morphological abnormality that develops independently of functional microcirculation abnormalities in the progression of atherosclerosis, and may therefore not be representative of the functional status of the microvasculature. Although an association with circulating biomarkers of endothelial dysfunction has been previously documented in RA

(49), microvascular vasodilatory function evaluated with laser Doppler imaging with iontophoresis was not associated with either endothelial-dependent (flow-mediated dilation) or endothelial-independent (glyceryl trinitrate-mediated dilation) macrovascular vasodilatory function in a former study by Sandoo *et al*. (50). Further studies are needed to clarify the complex interactions, if any, between micro- and macrocirculation in RA and their clinical interpretation, taking into account different assessments of vascular function and morphology in distant vascular beds and their diversity in terms of underlying pathophysiological processes.

To date, few case-control studies have applied older laser Doppler techniques in patients with RA with divergent results, potentially attributed to divergent methodological approaches (51-53). A single study has applied the newest LSCI technique in RA patients in a finger joint with and without active synovitis, with no differences in estimated peripheral blood perfusion compared to controls (54). Using the same LSCI methodology as of the present study, our group has previously shown impaired microvascular reactivity in patients with systemic lupus erythematosus with and without CVD risk factors (16), but also in other high CVD risk groups of patients (11, 14, 15, 40), thus providing further evidence of the sensitivity of this technique.

The study is limited by its observational nature, which does not allow for causality assumptions, mechanistic explanations or prognostic implications of the main findings. The relatively small sample size may have hindered further associations or risk factors. Proinflammatory cytokines such as inteleukin-6 were not assessed as measures of systemic inflammation. Nevertheless, we meticulously recruited a population of RA individuals free from established CVD and cardiovascular comorbidities, and matched controls, providing novel data on early functional impairment of the microvasculature and its potential clinical significance in the course of the disease.

In conclusion, skin microcirculation dynamics appear impaired in RA patients regardless of CVD risk factors and presumably long before the establishment of overt CVD manifestations. In our study, the direct association with measures of coronary microvascular perfusion and arterial stiffness independently of traditional CVD risk factors suggests that assessment of skin microvascular dysfunction may be a useful, non-invasive surrogate indicator of CVD risk in RA, inclusive of myocardial microvascular abnormalities. Additional prospective studies are needed to address the prognostic potential of LSCI in terms of CVD risk in RA.

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