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BRIEF REPORT

Microvascular function is preserved in newly diagnosed rheumatoid arthritis and low systemic inflammatory activity

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Abstract Rheumatoid arthritis (RA) is associated with increased cardiovascular morbidity and mortality. Microvascular function has been linked to several risk factors for cardiovascular disease and may be affected in RA. It is, however, presently unknown at what point in the disease course the abnormalities in microvascular function occur. We determined whether microvascular function is already disturbed in early disease-modifying antirheumatic drugs (DMARD)-naive RA patients with low systemic inflammation. Fifteen consecutive RA patients with a median symptom duration of 5 months, a C-reactive protein level of ≤20 mg/l and without a history of cardiovascular disease, and age 15 and sex-matched healthy controls were recruited. Endothelium-dependent and endothelium-independent vasodilatation in skin was evaluated with laser Doppler fluxmetry after iontophoresis of acetylcholine and sodium nitroprusside, respectively. Videomicroscopy was used to measure recruitment of skin capillaries after arterial occlusion. CRP and ESR levels were mildly, but significantly elevated in patients compared to controls. No differences in both endothelium-dependent vasodilatation and capillary recruitment were observed between groups [709% (95% CI, 457–961%) vs 797% (95% CI, 556–1,037%), P = 0.59 and 37% (95% CI, 26–47%) vs 41% (95% CI, 31–50%), P=0.59, respectively]. Skin microvascular function is preserved in early, DMARD-naive RA patients with moderately active RA but low systemic inflammatory activity. Both the extent of the systemic inflammation and disease duration, therefore, may be important determinants of microvascular dysfunction and subsequent increased risk for cardiovascular disease.

Keywords Cardiovascular disease · Inflammation · Iontophoresis · Rheumatoid arthritis · Vascular function

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Introduction

Cardiovascular disease (CVD) has been recognized as the major cause of excess morbidity and mortality in patients with rheumatoid arthritis (RA) [1–3]. This increased cardiovascular risk in RA may partly be due to traditional cardiovascular risk factors, i.e., an atherogenic lipid profile and hypertension [4], but chronic inflammation is also thought to be important [5–9].

Inflammation-induced vascular dysfunction of the macrocirculation and microcirculation, in particular impaired endothelium-dependent vasodilatation, may predispose RA patients to CVD [4, 10, 11]. Macrovascular endothelial dysfunction precedes and initiates atherosclerosis and is a predictor of long-term cardiovascular risk [12]. Microvascular endothelial dysfunction, on the other

hand, is important not only in the development of target organ damage in the heart and kidney, but also in the development of cardiovascular risk factors such as hypertension and insulin resistance [13–15]. Progressive impairment of microvascular endothelium-dependent vasodilatation of the skin is associated with an increasing coronary heart disease risk [16].

In longstanding RA, microvascular endothelium-dependent vasodilatation is impaired and associated with increased C-reactive protein (CRP) levels [17–19]. Moreover, anti-inflammatory therapy improves both peripheral (cutaneous) and myocardial microvascular dysfunction in RA patients [20, 21]. This underlines the fact that the atherogenic process is not limited to plaque formation in large conduit vessels but is associated also with impaired microvascular function that plays an important role in regulating tissue perfusion, including myocardial perfusion and associated ischemia.

An intriguing question is at what point in the inflammatory disease course abnormalities in (micro)vascular function occur. Impaired microvascular endothelium-dependent vasodilatation can be demonstrated in patients with longstanding RA with low disease activity [20]. In addition, a flare of inflammatory activity in RA patients impairs microvascular endothelium-dependent vasodilatation even more, whereas inflammatory suppression does not restore vasoreactivity completely to normal [21]. In newly diagnosed RA patients and high systemic inflammation, both endothelium-dependent and endothelium-independent vasodilatation of the resistance vessels are impaired as assessed with venous occlusion plethysmography [10].

It is presently unknown whether microvascular dysfunction is already present in early DMARD-naive RA patients with moderate disease but low systemic inflammatory activity. The aim of the study was to establish whether microvascular endothelium-dependent vasodilatation and capillary recruitment is impaired in patients with very early, DMARD-naive RA with low systemic inflammation compared to healthy controls.

Patients and methods

Subjects

Early untreated RA

Fifteen consecutive eligible patients (12 females) with RA were studied. Diagnosis was confirmed using the 1987 ACR criteria, [22] and low systemic inflammatory activity was defined as a CRP level ≤20 mg/l. The vascular function study was performed <2 weeks after the diagnosis of RA and prior to treatment with disease-modifying anti-rheumatic drugs (DMARDs) or oral corticosteroids. A total of 15 age- and sex-matched healthy subjects were studied as a control group. Clinical and biochemical characteristics of the study groups are shown in Table 1. Exclusion criteria were diabetes mellitus, hypertension, history of cardiovascular disease, Raynaud's syndrome, scleroderma, concurrent infection, thyroid dysfunction, and current or recent medication which might affect vascular function, except non-steroidal anti-inflammatory drugs.

All participants gave written informed consent and the study protocol was approved by the Medical Ethics Committee of the Slotervaart Hospital, Jan van Breemen Institute and BovenIJ Hospital.

Study design

Microvascular measurements were conducted in a quiet, temperature-controlled room (T=23.4±0.4°C) after 20–30 min of acclimatization, with the subjects in the sitting position and the investigated non-dominant hand at heart level. Nailfold capillary studies and iontophoresis studies were performed on the same day by a single experienced investigator (IvE). Subjects were asked to refrain from beverages other than water (especially no caffeine or alcohol), smoking, medication except acetaminophen if necessary, and meals from midnight at the testing day. Nailfold capillaries in the dorsal skin of the third finger were visualized by a capillary microscope as previously described [23, 24]. Nailfold capillaries were recorded on videotape before and after 4 min of arterial occlusion with a digital cuff. This procedure was performed twice, and the mean of both measurements was used for analyses. In addition, venous congestion, with the digital cuff inflated to 60 mmHg for 60 s, was applied to expose a maximal number of non-perfused capillaries. Capillaries were counted by a single observer using the naked eye from a freeze-framed reproduction of the videotape and from the running videotape, when it was uncertain whether a capillary was present or not. Baseline capillary density was defined as the number of continuously erythrocyte-perfused capillaries during a 15-s period. Intermittently perfused capillaries were also visible and are proposed to form an important functional reserve that can be recruited during post-occlusive hyperemia. The maximum number of capillaries visible directly after cuff release was counted for 30 s. Post-occlusive capillary recruitment was calculated by dividing the increase in density by the baseline density. The day to day coefficient of variation (CV) of the capillary density in resting state was 2.3±1.8%. The CV of the

Table 1 Baseline characteristics of the RA patients and controls

Characteristics	Patients with arthritis (n=15)	Healthy controls (n=15)	P value
Age, years	48±10	48±10	0.92
Female, n (%)	12 (80)	12 (80)	N/A
Symptom duration, months	5 (4–12)	N/A	N/A
Rheumatoid factor (RF) positive, n (%)	11 (73)	N/A	N/A
ACPA positive, n (%)	13 (87)	N/A	N/A
Erosive, n (%)	3 (20)	N/A	N/A
Disease activity score (DAS28)	4.7 (0.95)	N/A	N/A
ESR, mm/h	18 (11–35)	5 (3–8)	<0.001
CRP, mg/l	3 (2–17)	1 (1–2)	0.002
Fasting glucose	4.8 (4.2–5.3)	4.5 (4.2–4.7)	0.38
Total cholesterol	4.7 (4.0–5.3)	5.4 (4.1–5.7)	0.42
HDL-c	1.52 (1.31–1.87)	1.42 (1.27–1.88)	0.78
LDL-c	2.70 (2.11–3.37)	2.72 (2.01–3.88)	0.68
Triglycerides	1.01 (0.78–1.78)	0.99 (0.58–1.31)	0.26
Systolic blood pressure, mmHg	126±15	118±7	0.1
Diastolic blood pressure, mmHg	80±8	79±6	1.0
Smoking (%)	2 (13)	1 (7)	0.5
NSAID use, n (%)	10 (67)	N/A	N/A
Body mass index (BMI)	24.5 (22.4–27.4)	24.4 (22.1–25.2)	0.42

Data are mean +/−SD or median (interquartile range); pulse pressure = systolic blood pressure − diastolic blood pressure

ACPA anti-citrullinated protein/peptide antibodies, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *HDL-c* high-density lipoprotein cholesterol, *LDL-c* low-density lipoprotein cholesterol, *NSAID* non-steroidal anti-inflammatory drug, *N/A* not applicable

percentage capillary recruitment and absolute capillary recruitment during post-occlusive hyperemia were 8.3±4.9% and 6.2±4.3%, respectively.

Endothelium-(in)dependent vasodilatation of forearm skin microcirculation was evaluated by iontophoresis of acetylcholine and sodium nitroprusside in combination with laser Doppler fluxmetry as previously described in more detail [23, 25]. Acetylcholine (1%, Miochol-E, Théa Pharma, Zoetermeer, the Netherlands) was delivered with an anodal current; seven doses (0.1 mA for 20 s) were delivered, with a 60-s interval between each dose. Sodium nitroprusside (0.01%, Haagse ziekenhuis apotheek) was delivered with a cathodal current; nine doses (0.2 mA for 20 s) were delivered, with a 90-s interval between each dose. Acetylcholine-dependent laser Doppler flux was measured on the non-dominant forearm, whereas nitroprusside-dependent laser Doppler flux was measured at the same spot on the opposite forearm, with approximately 15 min elapsed between the two measurements. The day to day CV of the percentage increase from baseline to the final 2 min of the plateau phase was 9.8±5.6% for acetylcholine and 8.3±5.4% for sodium nitroprusside.

Assessment of inflammatory parameters

Disease activity was measured by calculating the disease activity score of 28 joints (DAS28) [26]. CRP levels were determined using the Roche/Hitachi cobas 6000 analyzer, based on the principle of particle-enhanced immunological agglutination (Roche Diagnostics GmbH, D-68298, Mannheim, Germany). Values are expressed in milligrams/litre. A CRP below 10 mg/L was considered to be normal. ESR was determined with local measurement techniques (Westergren method) and expressed in millimeter per hour.

Statistical analyses

Data are expressed as mean (SD) or median (range) as appropriate. The distribution of variables was tested for normality and transformed if necessary. Student's t test was used to compare continuous normally distributed variables within patients and matched controls. We used non-parametric Mann–Whitney U tests when appropriate. For dichotomous variables, Pearson chi-square test was used. Correlations between variables were analysed by using Pearson correlation or Spearman's rho tests when appropriate. A two-tailed probability value of $P<0.05$ was considered (statistically) significant.

Power calculations based on repeat microvascular endothelium-dependent responses measured at two-time points 3 months apart in 23 individuals [27], suggest that it is possible to detect a difference of 270% points with 85% power at $P<0.05$ change using a paired comparison in 15 patients.

Table 2 Microvascular measurement

	RA patients	Healthy controls	*P* value
Ach-mediated vasodilatation	*n*=15	*n*=15	
Skin temperature, °C	30.3±0.9	29.9±0.7	0.12
Baseline skin perfusion, PU	8.5 (3.7–14.5)	6.8 (4.6–8.0)	0.58
Ach-mediated vasodilatation, %	709±454	797±435	0.59
SNP-mediated vasodilatation			
Skin temperature, °C	30.0±0.9	29.8±0.8	0.47
Baseline skin perfusion, PU	5.3 (3.6–7.3)	6.7 (5.1–9.6)	0.25
SNP-mediated vasodilatation, %	1,292±772	1,094±638	0.45
Capillary recruitment	*n*=14	*n*=15	
Skin temperature, °C	30.2±1.4	29.7±1.3	0.24
Baseline capillary density, number/mm²	49±11	46±12	0.57
Peak capillary density, number/mm²	66±15	64±16	0.80
Venous occlusion, number/mm^2	72±16	68±18	0.56
Absolute increase, number/mm²	17±8	18±8	0.79
Capillary recruitment, %	37±18	41±18	0.56

Peak capillary density was defined as the maximum number of capillaries visible directly after arterial occlusion during post-ischemic hyperaemia. Venous occlusion represents the maximal number of non-perfused capillaries exposed after venous congestion. Data are mean +/− SD or median (interquartile range). Variables were tested using Student's *t* test or Mann–Whitney *U* test

RA rheumatoid arthritis, *Ach* aceltycholine, *SNP* sodium nitroprusside

Results

Characteristics

Baseline, demographic, and clinical characteristics of patients are shown in Table 1. The mean DAS28 score was 4.7 and CRP and ESR levels were significantly higher in patients compared to controls. The median symptom duration was 5 months.

Microvascular function is not disturbed in very early untreated arthritis

Endothelial (in)dependent vasodilatation

Microvascular vasodilatation in response to acetylcholine (endothelium dependent) in RA patients was comparable to controls [709% (95% CI, 457–961%) vs 797% (95% CI, 556–1,037%), *P*=0.59; Table 2 and Fig. 1]. The response to sodium nitroprusside (endothelium independent) also did not differ significantly between patients and controls [1,292% (95% CI, 865–1,720%) vs 1,094% (95% CI, 741–1,448%), *P*=0.45].

Capillary density and recruitment

Baseline capillary density was similar in both groups, 49/mm^2 in patients vs 46/mm^2 in controls. The absolute and relative post- ischemic capillary recruitment did not differ between patients and controls (17 vs 18 mm^2, respectively for absolute increase, *P*=0.79 and 37% (95% CI, 26–47%) vs 41% (95% CI, 31–50%), respectively for relative increase, *P*=0.56). The total number of anatomically present capillaries visible after venous occlusion was 72 in patients vs 68 in controls, which was not different (*P*=0.56; Table 2, Fig. 2).

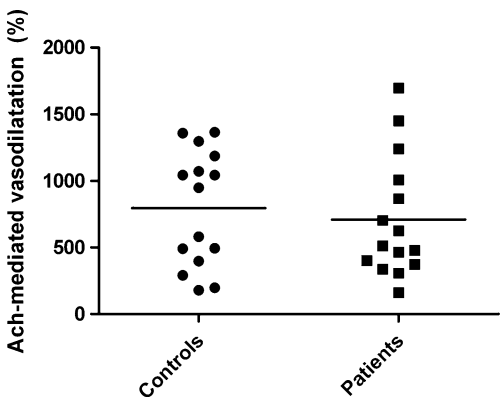

Fig. 1 Acetylcholine-mediated vasodilatation in controls and RA patients. The mean of vasodilatation, indicated by the *horizontal line*, is comparable in patients and controls. *ACh* acetylcholine

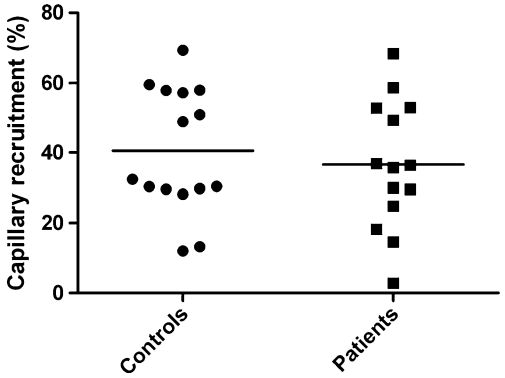

Fig. 2 Capillary recruitment depicted as the percentual increase of visible capillary numbers between baseline and after post-occlusive hyperaemia

No correlations between microvascular function and disease activity markers and symptom duration

We did not find significant correlations between CRP or ESR or DAS28 and endothelium-dependent vasodilatation or capillary recruitment. The endothelium-dependent vasodilatation and capillary recruitment did not correlate significantly with symptom duration.

Discussion

In the present study, we observed a preserved microvascular endothelium-dependent vasodilatation and capillary recruitment during reactive hyperemia in DMARD-naive patients with newly diagnosed RA and low systemic inflammatory activity. Peripheral and coronary microvascular dysfunction is considered important in the development of cardiovascular disease [14, 28]. Previous studies demonstrated that coronary and peripheral microvascular dysfunction is apparent in longstanding established RA with high, but also low inflammatory activity [11, 18, 19]. Moreover, anti-inflammatory therapy improves microvascular function, but does not completely restore it [18, 24]. This persisting microvascular dysfunction may be caused by cumulative effects of inflammation, which in the long run lead to irreversible vascular damage. The finding of an inverse association between coronary microvascular function and disease duration in RA is compatible with such an explanation [20]. On the other hand, microvascular dysfunction may already be present at the time of diagnosis because subtle increased levels sometimes within the normal range of C-reactive protein can already be detected years before the onset of clinical RA [29]. Our findings, however, do not support this, as we found preserved microvascular function in patients with newly diagnosed, moderately severe RA with low systemic inflammatory activity. Interestingly, the CRP levels in our newly diagnosed RA patients are comparable to the CRP levels (4.2 mg/L) of patients with longstanding RA demonstrating impaired coronary microvascular function [20], suggesting that disease duration is an independent determinant of microvascular function. Nevertheless, newly diagnosed patients with RA do exhibit impaired endothelium-dependent and endothelium-independent vasodilatation of small arteries and resistance vessels if systemic inflammatory activity is more pronounced (i.e., CRP 29±10 mg/L) [10]. Moreover, suppression of inflammatory activity seems to restore vasodilatory function [10]. These findings, together with our finding, suggest that systemic inflammatory activity is necessary to cause microvascular dysfunction, which is reversible early in the disease course, but becomes irreversible after a longer disease duration.

Obviously, with relatively low patient numbers, a type 2 error may occur more easily. Although we cannot completely exclude a difference in endothelium-dependent vasodilatation between RA patients and control subjects, it should be realized that the observed difference was very small (88 percentage point difference). To put this difference in perspective, in patients with longstanding RA, hypertension, obesity, or individuals at increased coronary heart disease risk, the difference in endothelium-dependent vasodilatation, as compared with healthy controls, exceeds 300 percentage points [16, 18, 25, 30]. In conclusion, the present study showed that skin microvascular function is not impaired in very early DMARD-naive RA patients with low systemic inflammation, compared to healthy controls.

Disclosures None

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