

Inflammation and Microvascular and Macrovascular Endothelial Dysfunction in Rheumatoid Arthritis: Effect of Treatment

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ABSTRACT. *Objective.* To determine whether abnormalities in microvascular and macrovascular function in rheumatoid arthritis (RA) are associated with plasma markers [von Willebrand factor (VWF)] of endothelial dysfunction and inflammation [C-reactive protein (CRP)] and whether the abnormalities would be altered by treatment. Endothelial dysfunction and inflammation in RA may contribute to adverse cardiovascular events. Although endothelial dysfunction in RA has been demonstrated by altered plasma markers, the relationships with macrovascular and microvascular function are relatively unexplored.

Methods. We recruited 66 patients with chronic RA, 48 community controls (CC), and 25 patients with diabetes and hypertension as a disease control group (DC). Subjects had venous blood sampled for plasma markers, and underwent laser Doppler perfusion imaging of forearm skin (to assess microvascular circulation) following acetylcholine and sodium nitroprusside iontophoresis, to assess endothelium-dependent and endothelium-independent responses, respectively. Brachial artery flow-mediated dilatation assessed endothelial dysfunction in a macrovascular bed. A subgroup of 29 patients with RA were assessed pretherapy and after 2–4 weeks of antirheumatic therapy.

Results. As expected, patients with RA had higher CRP, erythrocyte sedimentation rate (ESR), and VWF. Endothelium-independent vasoreactivity was abnormal in RA, and this correlated negatively with CRP. All aspects of microvascular function were abnormal in the DC compared to the CC. Macrovascular function was preserved in RA but was abnormal in the DC group. Four weeks of anti-inflammatory therapy reduced CRP and ESR but had no effect on any vascular function index in the patients with RA.

Conclusion. Patients with RA have abnormal endothelium-independent microvascular function that correlates with inflammation but is not altered by short-term antiinflammatory therapy. (First Release Feb 15 2010; J Rheumatol 2010;37:711–6; doi:10.3899/jrheum.090699)

Key Indexing Terms:

RHEUMATOID ARTHRITIS ENDOTHELIUM VON WILLEBRAND FACTOR
LASER DOPPLER MICROVASCULAR FUNCTION MACROVASCULAR FUNCTION

Rheumatoid arthritis (RA) is associated with increased cardiovascular mortality and morbidity, possibly related to inflammation and endothelial damage/dysfunction, a pathology that predicts those at increased risk of adverse cardiovascular events and that may also be used as a surrogate marker for measuring beneficial effects of interventions^{1,2}. Indeed, the “vascular” hypothesis of RA is long established and is supported by abnormal plasma markers of the endothelium, including von Willebrand factor (VWF)^{3,4}.

More recently, changes to vascular reactivity in RA have been reported, such as abnormal large artery flow-mediated dilatation (FMD) and arterial stiffness, both of which support the vascular hypothesis^{5,6}. However, others have failed to find abnormal FMD in RA⁷.

Tools enabling examination of microvascular function have been developed⁸. This can be assessed noninvasively using laser Doppler perfusion imaging of skin microcirculation in a defined area, typically of the nondominant forearm. Vasoactive substances can be delivered to the skin microcirculation using iontophoresis (a small electric current to drive charged particles). Acetylcholine (ACh) can be delivered to the vessels to bring about endothelium-dependent vasodilatation (by nitric oxide release from endothelial cells). As the endothelium is an essential part of the vasodilatory response, abnormalities in endothelial function will impair the response. Sodium nitroprusside (SNP) acts as a nitric oxide donor and consequently acts directly on the smooth muscle of the vessel wall, therefore causing vasodi-

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lation without requiring endothelial cell stimulation. Thus SNP responses are endothelium-independent. Using this technique, Datta, *et al*⁹, in a small study of 8 patients with RA and 8 controls, reported that vascular function was substantially and significantly lower, while Galarraga, *et al*¹⁰ showed that microvascular dysfunction was associated with inflammation [as defined by C-reactive peptide (CRP)].

Despite the acknowledged abnormalities in vascular function defined by plasma markers, macrovascular disease, and microvascular disease, all 3 have yet to be reported in a single study. We tested the hypothesis that all 3 abnormalities of endothelial function are present in RA, and are linked, in that such abnormalities correlate with an established laboratory marker of inflammation, i.e., CRP. In our simple cross-sectional study, we compared patients with RA with 2 control groups: patients attending outpatient clinics for the risk factors for atherosclerosis [i.e., diabetes and concurrent hypertension; disease controls (DC)] and community controls (CC), some of whom had mild disease not requiring hospital outpatient care. We also studied whether antiinflammatory therapy would improve endothelial function.

MATERIALS AND METHODS

We recruited 66 consecutive patients with RA from our rheumatology outpatient clinics at a single UK hospital, 48 CC from hospital staff and relatives or friends of patients, and 25 DC¹¹. The diagnosis of RA was made according to the standard American College of Rheumatology criteria¹². Mean disease duration was 10.7 years. Exclusion criteria were diabetes for the CC and any connective tissue disease for the DC and CC. Exclusion criteria for all subjects were atrial fibrillation, significant valvular heart disease, previous coronary artery bypass surgery, primary angioplasty for acute ST elevation myocardial infarction, infection or pyrexial illness, recent (< 3 months) ischemic stroke, and chronic and systemic illnesses including renal failure (on supportive therapy), hepatic impairment, and/or hormone replacement therapy. After providing informed consent, subjects were invited to attend the hospital on a separate occasion for venepuncture and to have vascular function measured. Subjects were asked to omit their normal medications for 24 h before the first day of the study, and to avoid cigarettes and caffeine-containing drinks for 2 h before the assessments. Subjects were assessed in a quiet room. Demographic details collected from all subjects included age, sex, cigarette smoking status, medical history, family history, and medications. A brief clinical examination was carried out including measurement of resting blood pressure.

A subgroup of 29 patients with RA was started on additional antirheumatic treatment as required. These treatments were infliximab (3 mg/kg) in 5 patients (17%), etanercept (50 mg/twice weekly) in 17 patients (60%), adalimumab (40 mg alternate weeks) in 3 patients (10%), methotrexate (7.5–20 mg/week) in 1 patient (3%), and high-dose steroids (500 mg methylprednisolone) in a further 3 patients (10%). Patients continued parallel therapy, e.g., nonsteroidal antiinflammatory drugs (NSAID), as clinical judgment required. Venous blood was obtained before treatment, a week after treatment had started (15 patients), and then 4 weeks after starting treatment (24 patients). The Research Ethics Committee of Sandwell and West Birmingham National Health Service (NHS) Trust approved the study and each subject gave written informed consent.

Blood samples were taken for routine laboratory biochemistry, hematology, and for serum CRP and VWF. For the latter, samples were allowed to clot and then centrifuged at 3000 rpm for 20 min before aliquoting into suitable volumes for storage at –70°C and later batch analysis. VWF was assessed by an established method¹³ using commercial antisera (Dako-Patts, Ely, UK). CRP was measured by the routine Hospital

Pathology Service using nephelometry with a sensitivity of 5 mg/l. The interassay and intraassay coefficients of variation were < 10% and < 5%, respectively.

Microvascular vasoreactivity. Microvascular endothelial function was assessed using laser Doppler perfusion imaging with the Perimed system (Bury St. Edmunds, Suffolk, UK) with iontophoresis of ACh for endothelium-specific vasodilatation and SNP for the endothelium-independent response. The technology relies on differences between sent and returned wavelengths that reflect changes in blood flow in a unit area of subcutaneous tissue. Aliquots of 1% ACh and 0.1% SNP were prepared in sterile filtered distilled water (Sigma-Aldrich, Poole, Dorset, UK) and about 1 ml of aliquots were stored at –70°C in the dark (foil packaging). Samples were defrosted for 20 min at room temperature prior to each assay. For the assessment, the patient sat comfortably in a constant-temperature room for 20 min before the start of measurements. A hairless area of forearm skin in the nondominant arm was selected and the iontophoresis chamber was placed over this skin after cleaning with a 40% ethanol swab. The other electrode was placed over the volar aspect of the ipsilateral wrist. The chamber was filled with SNP, then covered with a glass slip and clipped in place ensuring that no air bubbles were visible below the glass cover slip. The cathode was attached to this and the anode to the other (wrist) electrode. Iontophoresis was not commenced until 2 complete baseline readings had been taken.

The laser scanner computer was programmed to scan the area of the iontophoresis chamber with settings that generated about 150 datapoints of perfusion inside the chamber within 25 s. Readings were repeated after 5 s, to a total of 20 measurements, such that each experiment had readings at 30-s intervals for a total of 10 min including the baseline scans. After the second reading (i.e., after 2 baseline readings) the iontophoresis driver was started to deliver 0.1 mA over 2 min (total charge delivered 12 mC) as a continuous current. The patient then rested if required before proceeding to ACh measurement. The chamber used for SNP was removed and a clean iontophoresis electrode placed about 4 cm from the other electrode (so that the iontophoresis electrode did not lie within the path of the current from the previous recording). The same procedure was used for ACh except that the electrode polarity was reversed and the current used was 0.2 mA (total charge delivered 24 mC). The dedicated software produces a table of values for each timepoint that is used to determine the dilation peak response (in terms of absolute perfusion units, and also percentage change from baseline) over the 10-min experiment.

Macrovascular vasoreactivity. FMD has been described in detail¹⁴. High-resolution ultrasound was used to assess changes in the diameter of the brachial artery. Measurements were taken after the patient had rested in a supine position for 20 min in a quiet room. High-quality ultrasound scans of the brachial artery were obtained using a 10-MHz vascular ultrasound probe. The artery was identified by the pulsatile nature of flow using color Doppler imaging. A longitudinal section of the brachial artery was identified 5 cm above the antecubital fossa. Careful attention was paid to avoiding altering the vessel diameter by minimizing the pressure applied with the probe. Vessel diameter was measured on a frozen image of the vessel using electronic callipers in 2-D imaging at the upstroke of the R wave on the electrocardiographic trace. Five measurements were taken of vessel diameter over a 1-cm segment of the artery and the mean value calculated. After a baseline scan, a standard pneumatic sphygmomanometer cuff was placed at the middle forearm and inflated to 250 mm Hg for 4.5 min, and then rapidly deflated. Sixty seconds after cuff release the second reading was taken in the same way; care was taken to use the same segment of artery for this scan.

The patient then relaxed in the same position for 20 min, waiting for the vessel changes to be reversed. The scans were then repeated in a similar way before and after the administration of 400 µg sublingual glyceryl trinitrate (delivered as a spray using 2 puffs from a metered dose delivery device) in order to assess the endothelium-independent response. The results were expressed as a percentage change in vessel diameter from the baseline reading in each case. Images were saved to a disk so they could be

blindly reinterpreted to assess interobserver and intraobserver variation, which was < 10%.

Data analysis. Continuous data were subjected to the Shapiro-Wilks test to determine distribution and, if normal, are presented as mean with SD and groups compared by ANOVA. Data distributed non-normally are presented as median with interquartile range and are analyzed by the Kruskal-Wallis test. Differences between groups were sought using the Tukey-Kramer test, after log transformation, if necessary. As the logarithm of zero does not exist, when this value was obtained it was reassigned to be the value midway between zero and the lowest point of sensitivity of the particular assay. Categorical data were compared using the chi-squared test. Correlations were sought using Spearman's method. Data at different timepoints were analyzed by a series of Wilcoxon signed-rank tests. We initially hypothesized significant differences between microvascular function and macrovascular function in RA compared to the CC and DC. Taking as the test statistic⁴ the gold standard plasma marker (VWF), which has a relatively large variance (SD perhaps 30% of the mean), we modeled an increase of 0.75 of SD (e.g., from mean 100 IU/dl in the CC to mean 115 IU/dl in the RA and the DC, pooled SD = 20)^{4,15,16}. To achieve this for α ANOVA $p < 0.01$ and $1\beta = 0.8$, $n = 25$ per group are required. For additional confidence, we recruited in excess of this sample size in view of the planned correlation analysis. We subsequently hypothesized significant relationships between the endothelial markers (VWF, microvascular function, macrovascular function) and the inflammatory marker CRP. Given the clinical and pathological heterogeneity of RA, we recruited more extensively and considered a correlation coefficient (r) of < 0.25 to be scientifically weak, < 0.3 to be modest, < 0.4 to be good, and < 0.5 to be highly significant. P value < 0.05 was considered statistically significant. All analyses, power calculations, and modeling were performed on Minitab release 15 (Minitab Inc., State College, PA, USA).

RESULTS

Baseline data were similar for the RA patient and CC groups with no significant differences in age, sex ratio, or blood pressure (Table 1). CC had higher systolic blood pressure, were older, and had a higher proportion of men. Smoking was more common in patients with RA than in CC and DC.

Serum cholesterol was lower in patients with RA than in CC. This may in part reflect the higher proportion of patients with RA who were taking a statin. Patients with RA had a raised erythrocyte sedimentation rate (ESR).

Table 2 summarizes the research indices in our study and confirms the finding of abnormal VWF at a level similar to that seen in DC¹⁵⁻¹⁷, as well as the expected finding of elevated CRP in RA¹⁸. This result also justified the power calculation for the sample size. The high vascular risk DC group had significant abnormalities of all endothelium-dependent markers of vascular function (both of the microvascular measures as well as FMD), while none of the endothelium-dependent markers were abnormal in the RA group. The endothelium-independent vasoreactivity was also impaired in all cases for the DC apart from the peak response to SNP, and in the RA group only the percentage change to SNP was abnormal.

Overall, there were few correlations between VWF, CRP, and macrovascular and microvascular markers of endothelial function in the 3 groups. CRP correlated significantly with peak response to SNP in the CC ($r = -0.356$, $p = 0.019$) and also in the RA group ($r = -0.356$, $p = 0.004$). In the RA group, CRP also correlated with the percentage increase in SNP ($r = -0.329$, $p = 0.007$), and VWF correlated with the peak SNP response ($r = 0.312$, $p = 0.011$). No correlations were found in the DC group, and no correlations were found with the endothelium-dependent tests of vascular function.

There were no significant correlations between microvascular function and macrovascular function in the study populations, with the exception that microvascular endothelium-dependent response (defined by percentage change in ACh response) correlated modestly and positive-

Table 1. Clinical, pharmacotherapy, and demographic data for the study population. Significance is expressed as the p-value using 1-way ANOVA or the Kruskal-Wallis test (ESR) comparing community controls, rheumatoid arthritis, and hospital controls with diabetes and/or hypertension. Categorical indices were analyzed using the chi-squared test. Data expressed as mean (single-number SD), median (2-number interquartile range), percentage, or actual number.

Characteristics	Community Controls (n = 48)	Rheumatoid Arthritis (n = 66)	Disease Controls (n = 25)	p
Age, yrs	54.0 (10)	58.5 (14)	66 (9)	< 0.001
Sex	17 M, 34 F	25 M, 41 F	19 M, 6 F	0.001
Systolic blood pressure	135 (22)	136 (22)	150 (17)	0.009
Diastolic blood pressure	83 (11)	79 (12)	80 (11)	0.205
Treated hypertension, %	16.7	24.2	95.7	< 0.001
Diabetes, %	0	4.5	100	< 0.001
Active smokers, %	8.3	31.8 ^a	8.0	0.002
Statin-treated, %	2.3	9.2	73.9	< 0.001
Cholesterol, mmol/l	5.7 (1.1)	4.9 (1.1)	4.3 (0.8)	< 0.001
ESR, mm/h	5 (3-29)	23 (8-50)	13 (4-24)	< 0.001
NSAID-treated, %	6.25	62.1	0	< 0.001
Methotrexate-treated, %	0	66.7	0	< 0.001
Steroid-treated, %	0	33.3	0	< 0.001

ESR: erythrocyte sedimentation rate; NSAID: nonsteroidal antiinflammatory drug.

Table 2. Markers of endothelial function and CRP in community controls, rheumatoid arthritis, and disease controls (diabetic hypertension). All data expressed as median with interquartile range.

Marker	Community Controls, n = 48	Rheumatoid Arthritis, n = 66	Disease Controls, n = 25	p
Plasma				
VWF, IU/dl	111 (91–123)	127 (108–137) ^b	123 (101–140) ^c	0.001
CRP, mg/l	0 (0–0)	13 (0–38) ^b	0 (0–5) ^d	< 0.0001
Microvascular function				
ACh peak (APU)	1.76 (1.43–2.07)	1.71 (1.47–1.99)	1.55 (1.36–1.82) ^a	0.021
ACh, % change	326 (258–448)	288 (216–312)	175 (137–231) ^b	< 0.0001
SNP peak (APU)	1.47 (1.22–1.84)	1.49 (1.26–1.79)	1.39 (1.09–1.53)	0.117
SNP, % change	362 (244–485)	261 (180–385) ^a	187 (141–220) ^b	0.001
Macrovascular function				
FMD, % change	3.68 (2.37–5.98)	3.56 (1.11–6.72)	1.56 (–0.39–2.67) ^b	0.017
GTN, % change	17.9 (12.8–22.8)	18.4 (14.5–23.4)	9.8 (6.9–13.0) ^b	< 0.0001

VWF: von Willebrand factor; CRP: C-reactive protein; microvascular: skin microcirculation responses to vasoactive substances; ACh peak: perfusion response to iontophoresis of acetylcholine (endothelium-dependent) in absolute perfusion units (APU); ACh %: percentage change in skin microcirculation flow as a percentage of baseline flow (= 100%) after iontophoresis of acetylcholine (endothelium-dependent); SNP (sodium nitroprus-side) peak: peak perfusion response to iontophoresis of SNP (endothelium-independent) in APU; SNP %: percentage change in skin microcirculation flow as a percentage of baseline flow (= 100%) after iontophoresis of SNP. Overall p value was obtained from the Kruskal-Wallis test. If applicable (i.e., if $p < 0.05$), intergroup differences were sought on log-transformed data using Tukey-Kramer test, which found: ^a $p < 0.025$ compared to CC, ^b $p < 0.001$ compared to CC, ^c $p < 0.05$ compared to CC, ^d $p < 0.001$ compared to RA. FMD: flow-mediated dilatation; GTN: glyceryl trinitrate.

ly with macrovascular endothelium-dependent response in RA, defined by FMD (Spearman $r = 0.36$, $p = 0.023$; Figure 1). The correlation between ACh peak response and FMD only just failed to reach significance ($r = 0.30$, $p = 0.059$).

Table 3 shows the effects of treatment in a subgroup of the patients with RA. Although 29 patients were recruited, a sample at the first timepoint (2 weeks) was obtained from only 15 patients and a sample from the second timepoint (4 weeks) in 24 patients. Although there was an improvement in the only index to be abnormal at baseline (microvascular response to SNP as a percentage) by 6% within 2 weeks and by 11% at 4 weeks, this failed to reach statistical signifi-

cance. The small improvement in levels of VWF of 8% was also not significant. However, treatment significantly improved both CRP and ESR.

DISCUSSION

We assessed endothelial function and markers of inflammation (ESR, CRP) in a small group of patients with RA as well as in CC and hospital DC. The degree of VWF abnormality was similar in RA to that seen in the high-risk DC (suggesting a similarly elevated cardiovascular risk for the RA group^{15–17}), while CRP was also raised only in the RA group¹⁸. Like Van Doornum, *et al*⁷, we did not find abnormal FMD in RA, but unexpectedly^{9,10}, we also did not find evidence of endothelial-dependent microvascular dysfunction.

In the RA group we found microvascular abnormalities, but only in the endothelium-independent response, and they were related to inflammation. As expected, the DC demonstrated abnormal endothelium-dependent and independent microvascular function, confirming that the method is effective. We conclude that vasodilation dysfunction is present in RA, as is the presence of abnormal plasma VWF, and note that the 2 correlate significantly. However, analysis of 2 vascular beds (forearm skin microcirculation and the brachial artery) in patients with RA did not detect any endothelial abnormality. At least 2 mechanisms may explain the disparity between endothelium-dependent and endothelium-independent responses. Perhaps chronically elevated inflammatory markers in RA reflect abnormalities of vascular function that are mediated predominantly at the level of smooth muscle; the overlying

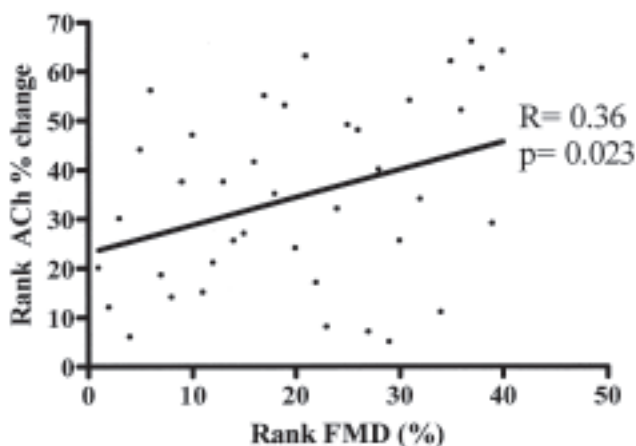


Figure 1. Correlation between percentage change in acetylcholine (ACh) response and flow-mediated dilatation (FMD) in patients with RA. P derived from Spearman's test of correlation.

Table 3. Effect of antirheumatic therapy on microvascular and macrovascular endothelial function. Data are median (interquartile range). Data analyzed by Wilcoxon's method. p^{0-1} is the difference in 15 patients measured at baseline (before treatment) and at the first timepoint (2 weeks). p^{0-2} is the difference in 24 patients measured at baseline (before treatment) and at the second timepoint (4 weeks).

	Baseline, n = 29	After 2 Weeks, n = 15	p^{0-1}	After 4 Weeks, n = 24	p^{0-2}
Microvascular function					
ACh peak	1.55 (1.36–1.88)	1.70 (1.28–1.79)	0.887	1.56 (1.25–1.73)	0.134
ACh % change	308 (210–364)	264 (206–353)	0.98	263 (205–335)	0.31
SNP peak	1.4 (1.19–1.69)	1.34 (0.99–1.64)	0.191	1.27 (1.10–1.56)	0.458
SNP % change	247 (175–386)	261 (181–332)	0.132	273 (221–418)	0.466
Macrovascular function					
FMD, %	3.3 (0.07–4.34)	3.1 (–1.95–4.82)	0.407	2.03 (0.54–4.79)	0.298
GTN, %	18.37 (14.95–23.6)	13.14 (9.23–19.74)	0.294	16.13 (13.02–21.58)	0.897
Plasma markers					
CRP, mg/dl	17 (5–49)	5 (0–29)	0.035	7 (0–31)	0.028
ESR, mm/h	33 (7–59)	13.5 (5–67)	0.052	14 (6–45.5)	0.002
VWF-IU/dl	127 (105–142)	117 (95–131)	0.442	118 (90–132)	0.475

ACh: acetylcholine; SNP: sodium nitroprusside; FMD: flow-mediated dilatation; GTN: glyceryl trinitrate; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; VWF: von Willebrand factor.

endothelium is relatively spared this pathological process. Alternatively, the vascular abnormalities in RA are endothelial, as evidenced by elevated VWF, but the abnormalities are not generalized, i.e., they are confined to specific vascular beds (for example, in the inflamed synovium).

It is possible that parts of both of these explanations apply, as raised VWF levels suggest an endothelium under attack and being damaged², and the abnormalities of endothelium-independent function were detectable in skin where the endothelium appeared functional. We have also previously shown that inflammation (as raised CRP) is not necessarily related directly to VWF levels in RA¹⁹, while we did find an association between endothelium-independent function and inflammatory markers, suggesting 2 different processes affecting the 2 different measures of vascular function. However, in a study of only 8 patients, abnormal microvascular function and CRP both improved upon immunosuppressive treatment⁹. In a larger study¹⁰, CRP correlated inversely with endothelial-dependent (ACh) microvascular function. We could not confirm this, but we did find the same correlation with endothelial-independent microvascular function.

Some studies²⁰⁻²² have found abnormal brachial artery responses in RA. Bergholm, *et al*²⁰ reported that both endothelium-dependent and endothelium-independent responses to brachial artery infusions of vasoactive substances were suppressed in newly diagnosed RA, and the endothelium-dependent response improved significantly with successful treatment. Hürlimann, *et al*²¹ investigated patients with high disease activity despite treatment (mean CRP 38 mg/l), and found an improvement in the endothelium-dependent response with treatment (when the mean CRP had fallen to 15 mg/l). Our study investigated patients with RA of relatively long duration, with mean CRP 13 mg/l

at outset, and therefore our study population was closer to the populations after treatment in these 2 other studies, when endothelium-dependent vasoreactivity had improved. Stamatiopoulos, *et al*²², in an observational study of similar design and size, reported reduced FMD in RA, and also more adverse arterial stiffness, carotid intima-media thickness (IMT), and the presence of carotid plaques. Notably, all these indices were as adverse in the patients with RA as in a DC cohort of patients with diabetes. Their data therefore differ from ours, where we did not find reduced FMD in RA, possibly because of differences in age (our patients being younger, albeit with a longer disease duration), disease status and severity, and the effects of medications.

The finding that VWF is abnormal in RA even in the absence of abnormalities of other markers of endothelial function raises the question of the prognostic significance of VWF in RA. While VWF has prognostic significance in other high-risk groups (such as those with diabetes, hypertension, and cigarette smokers^{2,4}), these groups also have marked abnormalities of other aspects of endothelial function, such as FMD. However, in a group in which abnormalities of FMD were not found, it is plausible that the endothelial abnormalities are confined to vascular beds that do not confer cardiovascular risk, i.e., noncoronary and noncerebral circulations. Recent work¹⁷ investigating carotid IMT in RA has shown a correlation with VWF levels, suggesting that VWF is likely to carry prognostic significance; it has been associated with worse outcomes in apparently healthy individuals²³. But data confirming an adverse cardiovascular outcome in patients with RA with elevated VWF are lacking. Future studies should address this important issue.

We note several limitations. Although the serial study was flawed by incomplete data collection, it demonstrated the effectiveness of antiinflammatory treatment on ESR and

CRP, but not on endothelial markers, for which the treatment period of 4 weeks may be insufficient. This is in contrast to the work of Raza, *et al*²⁴, who reported improved endothelial function in a group with more severe disease (systemic necrotizing vasculitis). In another study, Raza, *et al*²⁵ showed that anti-tumor necrosis factor- α treatment, but not cyclophosphamide and methylprednisolone, causes a rapid (24 h) but not a 14-day improvement in endothelial-dependent vasodilatation in systemic vasculitis. Perhaps, therefore, the endothelium was not sufficiently functionally abnormal in our patients with RA, as it appears to be in systemic vasculitis (and as it is when defined by abnormal VWF¹⁵), making it amenable to antiinflammatory therapy. We are also unable to discount a possible confounding effect of NSAID (and possibly, statins) on the endothelium in different vascular beds. This cannot be corrected for in analysis and therefore remains a caveat, especially in as heterogeneous a group as patients with RA.

Despite general endothelial perturbation (abnormal VWF) in a group of outpatients with RA, we found no abnormalities of endothelium-dependent microvascular or macrovascular reactivity. However, there was evidence of impaired microvascular endothelium-independent vasoreactivity that may be related to inflammation. These data add to the concern regarding the clinical value of these measurements in patients with RA.

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