Clinical Improvement in Rheumatoid Arthritis Is Associated with Healthier Microvascular Function in Patients Who Respond to Antirheumatic Therapy

BERNAT GALARRAGA, JILL J.F. BELCH, TOM PULLAR, SIMON OGSTON, and FAISEL KHAN

ABSTRACT. ***Objective.*** Rheumatoid arthritis (RA) is associated with increased cardiovascular (CV) mortality. Microvascular endothelial dysfunction occurs early in the development of CV disease and is worsened by inflammation. The effect of drug treatment for RA on microvascular function has been poorly studied. We assessed the effect of antirheumatic treatment on microvascular endothelial function in patients with RA, particularly to examine responders versus nonresponders to therapy.

Methods. Fifty-one patients with active RA and no previous history of CV disease were assessed at baseline and after 2 and 4 months' therapy with either anti-tumor necrosis factor-α drugs (etanercept, n = 27, adalimumab, n = 3) or methotrexate, n = 21. RA disease activity, inflammatory measures, and skin microvascular responses, measured using laser Doppler imaging after iontophoretic delivery of acetylcholine (ACh) and sodium nitroprusside (SNP), were assessed at each study visit.

Results. Disease Activity Score (DAS28) decreased significantly from baseline to visit 2 and 3 (6.04 ± 1.2, 4.34 ± 1.3, 4 ± 1.3, respectively; $p < 0.0001$). Endothelium-dependent (ACh) and independent (SNP) responses for the whole cohort did not improve significantly after drug treatment ($p = 0.250$, $p = 0.062$, respectively). When patients who responded to antirheumatic therapy (n = 31) were analyzed, there were significant improvements in both ACh ($p = 0.028$) and SNP responses ($p = 0.019$).

Conclusion. Microvascular endothelial function improves in patients who respond to antirheumatic therapy. These results support the importance of effective therapy for RA patients in terms of CV effects, which might extrapolate to reduced CV events in the future. Clinical trial registration no. ISRCTN57761809. (J Rheumatol First Release Jan 15 2010; doi:10.3899/jrheum.090417)

Key Indexing Terms:
RHEUMATOID ARTHRITIS CARDIOVASCULAR DISEASE ETANERCEPT
METHOTREXATE ADALIMUMAB

Cardiovascular (CV) mortality and morbidity is increased in patients with rheumatoid arthritis (RA)[1-3]. Although traditional risk factors contribute to this excess risk, recent evidence suggests that inflammation plays an important role[4]. Endothelial dysfunction occurs early in atherosclerosis and is independently associated with future CV events[5,6]. Several methods have been devised to measure the integrity of the endothelium, particularly by imaging of large and small blood vessels and by measuring biomarkers[7-10].

Numerous studies have demonstrated endothelial dysfunction in the macrocirculation and its correlation with CV risk[11-13]; recent interest has focused on the microvascular bed and its role as a predictor of CV events[14-16]. Indeed, some authors suggest that microvascular involvement may occur earlier than macrovascular dysfunction in the course of atherosclerosis[12,17], making it an interesting early surrogate marker of CV disease (CVD). Given that microvascular dysfunction is present in RA[18,19], and strongly relates to systemic inflammation[20], investigation of its role in response to therapy is warranted.

Epidemiological data suggest that both methotrexate (MTX)[21] and anti-tumor necrosis factor-α (anti-TNF-α) therapy reduce CV mortality in RA[22,23]. Other groups have reported an improvement in surrogate markers of CVD and CV risk factors, such as macrovascular endothelial dysfunc-

From the Vascular and Inflammatory Diseases Research Unit, Centre for Cardiovascular and Lung Biology, Division of Medical Sciences, Ninewells Hospital and Medical School, Dundee; and the Public Health Section, Community Health Science Division, University of Dundee, Dundee, UK.

Supported by Wyeth Pharmaceuticals. Prof. Belch also receives support from the Harkness Bequest and the Sir John Fisher Foundation.

B. Galarraga, LMC, MRCP, PhD, Clinical Lecturer; J.J.F. Belch, MB, ChB, MD, FRCP, Professor; T. Pullar, MB, ChB, MD, FRCP, Consultant Physician, Vascular and Inflammatory Diseases Research Unit, Centre for Cardiovascular and Lung Biology, Division of Medical Sciences, Ninewells Hospital and Medical School; S. Ogston, MSc, PhD, Statistician, Public Health Section, Community Health Science Division, University of Dundee; F. Khan, BSc (Hons), PhD, Reader, Vascular and Inflammatory Diseases Research Unit, Centre for Cardiovascular and Lung Biology, Division of Medical Sciences, Ninewells Hospital and Medical School.

Address correspondence to Dr. B. Galarraga, Vascular and Inflammatory Diseases Research Unit, Centre for Cardiovascular and Lung Biology, Division of Medical Sciences, Ninewells Hospital and Medical School, Dundee, DD1 9SY, Scotland. E-mail: Lauberdoc@hotmail.com

Accepted for publication October 26, 2009.

tion[24,25], arterial stiffness[26], carotid intima-media thickness[27], and insulin resistance[28] after treatment with anti-TNF-α agents (particularly infliximab). It is not clear, however, whether the same results can be obtained in the microvascular circulation, and whether the improvement in vascular surrogate markers occurs in response to decreased inflammation in general or is a direct drug effect, particularly of TNF-α antagonists.

Our aim was to assess whether improvement in microvascular function in patients with RA was related to decreased inflammation and control of RA disease activity, or whether it was an effect of drug therapy per se. To do this we evaluated RA disease activity and microvascular endothelial function in "responders" [those achieving European League Against Rheumatism (EULAR) good response criteria[29]] compared to nonresponders to drug therapy.

MATERIALS AND METHODS

This was an open-label study carried out in Ninewells Hospital and Medical School, Dundee (clinical registration no. ISRCTN57761809). Fifty-one patients with RA meeting the 1987 American College of Rheumatology classification criteria for RA[30] and with moderate or active disease as defined by the EULAR Disease Activity Score in 28 joints (DAS28)[29] were recruited. Recruitment was of sequential patients attending the rheumatology outpatient clinics, who fulfilled the inclusion criteria, were about to start either MTX or an anti-TNF-α agent, and who gave written informed consent. Exclusion criteria included previous history of CVD, diabetes mellitus, uncontrolled hypertension or hypercholesterolemia, and any other inflammatory conditions.

Patients were given either MTX or anti-TNF-α [etanercept (ETA) or adalimumab (ADA)] therapy as part of their standard care after they failed to respond to adequate doses of, or had side effects to, a previous disease-modifying antirheumatic drug (DMARD), and were anti-TNF-naive. Treatment allocation was decided by rheumatologists in charge of patients' care, based on patients' clinical needs. Only when the treatment decision was made were patients invited to take part in the study. Doses of anti-TNF-α drugs were standard (ETA 25 mg twice a week or 50 mg weekly and ADA 40 mg every other week). Titration of MTX dose was decided by the rheumatologist responsible for the patient's care and followed no specific dosing protocol.

The study was approved by the Tayside Committee on Medical Research Ethics and all patients gave written informed consent.

Clinical and laboratory measurements. Patients were assessed at baseline and at 2 and 4 months after starting antirheumatic therapy. During each visit a clinical evaluation was performed comprising measurements of height and weight, blood pressure (mean of 3 measurements taken on the left arm with the patient in seated position after 25 min rest), DAS28 [validated statistically derived disease activity index that combines 28 tender joint count, 28 swollen joint count, C-reactive protein (CRP), and a 100 mm general health visual analog scale (VAS)], and Stanford Health Assessment Questionnaire (HAQ; a validated questionnaire to assess quality of life and function in RA)[31]. Blood samples were taken for assessment of full blood count, CRP, and plasma viscosity (PV).

"Responders" were defined as patients who achieved the EULAR good response criteria (a reduction of DAS28 > 1.2 from baseline to the 4-month visit or a DAS28 ≤ 3.2 at 4 months)[29]. Nonresponders were defined as those who did not achieve this.

Assessment of microvascular function. Measurements of microvascular function were conducted in a laboratory with constant room temperature set at 22 ± 1°C and at the same time of day (1–3 PM). Patients were asked to refrain from smoking and drinking alcohol at least 10 hours and caffeine-containing beverages at least 3 hours before measurements. Subjects were studied in the supine position and were given a 30 min resting period. During measurements, subjects were asked to neither speak nor sleep.

Subjects were asked to lie in a supine position. After a 20 min rest, forearm microvascular function was assessed as described[8,16,32,33], by measuring skin vascular responses to iontophoresis of 1% acetylcholine (ACh; Sigma-Aldrich Co. Ltd., Poole, UK) and sodium nitroprusside (SNP; David Bull Laboratories, Warwick, UK). ACh and SNP were both dissolved in deionized water to a concentration of 10 g/l (1%). Iontophoresis allows noninvasive delivery of drugs across the skin without inducing systemic effects. The volar aspect of the forearm was cleaned gently with adhesive tape, alcohol, and sterile water. The iontophoresis chamber (Moor Instruments, Devon, UK) consisted of a 20 mm internal diameter ring with a wire electrode running around the inner surface. The 2 chambers were fixed to the skin using double-sided adhesive tape and filled with 2 ml solution. The leads from the electrodes were connected to the iontophoresis controller. ACh and SNP were delivered simultaneously to the skin through the 2 separate iontophoretic chambers and using consecutive increases in anodal and cathodal current, respectively: 10, 15, 20, 50, and 100 μA. Two baseline scans of skin perfusion were taken before the iontophoresis protocol was administered. After the baseline scans, each dose was iontophoresed for approximately 200 s and 4 scans (each 50 s) were performed for each dose. Skin perfusion was measured using a laser Doppler imager (moorLDI, Moor Instruments, Axminster, UK). For each scan, the computer builds up a color-coded image termed laser Doppler flux and measured in perfusion units (PU), which represents skin blood flow over the scan area. The average of the last 2 stable scans was taken as the reading for that particular dose. Overall microvascular response for the total drug delivery period was determined by calculating the area under the perfusion × time curve (AUC) over baseline.

The perfusion response to the diluent only was not measured because previously, using large-diameter electrodes such as those in the present study, we did not elicit any nonspecific effect at the anodal electrode and a minimal effect at the cathode (which is negligible in comparison to the SNP response itself)[34]. Moreover, in other studies, where the authors subtracted the vehicle response, they found that this did not alter their conclusions[33]. The reproducibility of this technique in our hands is 11%[16].

Statistical analysis. SPSS v15.0 for Windows was used for all statistical analyses. Results are presented as mean ± standard deviation. Data were checked for normality with the Kolmogorov-Smirnov test and parameters that were not normally distributed were log-transformed where this achieved normality. Analysis of variance (ANOVA) for repeated measures (including iontophoresis current and study visit) was used to compare the microvascular responses before and after treatment, followed by a modified post-hoc paired-sample t test at each dose when a significant difference was found. Independent-samples t tests were used to compare group differences. The relationship between quantitative variables was measured with Pearson's correlation, and stepwise multiple regression analysis was used to determine the independent determinants of ACh area under the curve (AUC) and SNP AUC responses. P values < 0.05 were considered statistically significant. Power calculations were based on repeat microvascular responses measured at 2 timepoints at least 2 months apart in 44 individuals. The standard deviation (SD) of the change for the ACh response was 0.80. It was therefore possible to detect a difference of 0.5 SD (which equates to 13.4% change) with 80% power at $p < 0.05$ change using a paired comparison in 25 patients. A previous study with normal individuals by our group showed that n = 28 is sufficient to demonstrate improvements of 29% before and after intervention[35].

RESULTS

Baseline. Fifty-one patients aged 31–75 years, 43 women and 8 men, were studied (the numbers selected for the study were based on our previous observations of responder and

nonresponder rates to these drug therapies). Thirty-seven (72.5%) were rheumatoid factor (RF)-positive and 32 (63%) had erosive disease. The mean disease duration was 10 (SD 9.5) years. Thirty-nine (76%) patients were taking regular nonsteroidal antiinflammatory drugs (NSAID). Thirty-seven (72.5%) patients were already taking a DMARD at baseline, of which 10 (20%) were on combination therapy. The 2 most common DMARD were sulfasalazine (SSZ) and MTX (these latter were all in the group about to start a TNF-α inhibitor) taken by 23 (45%) and 19 (37%) patients, respectively. Eleven (22%) patients had hypertension that was well controlled with medication and all 4 (8%) patients that had hypercholesterolemia were receiving a statin. After the baseline visit, 27 patients were started on ETA by their rheumatologist, 3 on ADA, and 21 on MTX. Baseline characteristics of all patients are given in Table 1.

As expected at baseline, significant differences were observed in disease duration ($p < 0.0001$), number of tender joints ($p < 0.0001$), DAS28-CRP ($p < 0.0001$), and HAQ ($p < 0.0001$) between the patients about to receive MTX and those starting TNF-α blockade. Twenty-three (74%) TNF patients were taking a DMARD at baseline (15 on MTX alone, 4 in combination with SSZ or hydroxychloroquine, and 4 monotherapy with other DMARD). The average weekly dose of patients' MTX was 18 ± 5 mg. In the MTX group, 15 patients were taking SSZ, 1 combination therapy, and 5 were taking no DMARD. Baseline prednisolone dose was higher in the TNF than the MTX group but this difference was not statistically significant (1.6 ± 2.6 mg vs 0.5 ± 1.5 mg; $p = 0.087$; Table 1).

Baseline vascular responses to ACh and SNP, reflecting better vascular function, were significantly higher in the MTX group (ACh AUC 194,566 ± 83,165 and SNP AUC 139,963 ± 56,404) than in the TNF group (149,751 ± 76,409 and 91,854 ± 66,074) ($p = 0.05$ and $p = 0.009$; Table 1).

After antirheumatic treatment. All patients. By visit 2, the average dose of MTX in the MTX group was 12 ± 3 mg/week. This dose increased further by visit 3 (13 ± 5 mg/week).

For all patients, whether receiving MTX or TNF therapy, disease activity (DAS28-CRP) improved significantly from baseline (6.04 ± 1.18) to visit 2 (4.34 ± 1.34) and this improvement was maintained at visit 3 (4.0 ± 1.27) ($p < 0.0001$, ANOVA).

Reductions in levels of $\log_{10}$CRP (visit one 1.28 ± 0.44 mg/l, visit two 0.97 ± 0.44 mg/l, and visit three 0.92 ± 0.46 mg/l; $p < 0.0001$, ANOVA) and PV (1.8 ± 0.17 mPa.s, 1.72 ± 0.16 mPa.s, 1.75 ± 0.18 mPa.s; $p < 0.0001$, ANOVA) were also observed through the study period.

Although there was a trend toward improvement in the ACh and SNP responses from baseline to visit 2 and 3, this did not reach statistical significance ($p = 0.250$, ANOVA, and $p = 0.062$, ANOVA, respectively).

When the TNF and MTX groups were analyzed separately, although the ACh and SNP responses throughout the study were borderline significant in the TNF group they did not reach significance in either group (TNF ACh $p = 0.122$ and SNP $p = 0.087$; MTX ACh $p = 0.836$ and SNP $p = 0.531$; Figure 1). As shown in Figure 2, DAS28 decreased in

Table 1. Baseline characteristics of study patients.

	All Patients	Anti-TNF-α	Methotrexate	p
No. of patients	51	30	21	
Age, yrs	56 ± 11	57 ± 11	54 ± 11	0.321
Female/male, n	43/8	26/4	17/4	0.86
Disease duration, yrs	10 ± 9.5	14 ± 10	4 ± 3	< 0.0001
No. RF-positive (%)	37 (72.5)	23 (77)	14 (67)	0.635
Erosions, n (%)	32 (63)	20 (67)	15 (71)	0.962
Current smokers, n (%)	11 (21)	6 (20)	5 (24)	1
No. tender joints	17 ± 8	21 ± 6	11 ± 7	< 0.0001
No. swollen joints	9 ± 5	9 ± 4	8 ± 5	0.465
DMARD at baseline, n (%)	37 (72.5)	22 (73)	15 (71)	0.835
Prednisolone dose, mg	1.2 ± 2.2	1.6 ± 2.6	0.5 ± 1.5	0.087
On NSAID, n (%)	39 (76)	21 (70)	18 (86)	0.167
$\log_{10}$CRP, mg/l	1.28 ± 0.44	1.31 ± 0.45	1.24 ± 0.43	0.586
PV, mPa.s	1.8 ± 0.17	1.84 ± 0.18	1.78 ± 0.17	0.250
DAS28	6.04 ± 1.2	6.51 ± 0.68	5.36 ± 1.37	< 0.0001
HAQ	1.71 ± 0.7	1.97 ± 0.52	1.33 ± 0.7	< 0.0001
ACh AUC	168,205 ± 81,542	149,751 ± 76,409	194,566 ± 83,165	0.05
SNP AUC	111,664 ± 66,156	91,854 ± 66,074	139,963 ± 56,404	0.009

Values are mean ± SD. p value represents significance for comparisons between the methotrexate and anti-TNF-α groups. RF: rheumatoid factor; DMARD: disease modifying antirheumatic drug; CRP: C-reactive protein; PV: plasma viscosity; DAS28: Disease Activity Score of 28 joints; HAQ: Stanford Health Assessment Questionnaire; ACh AUC: acetylcholine area under the curve; SNP AUC: sodium nitroprusside area under the curve.

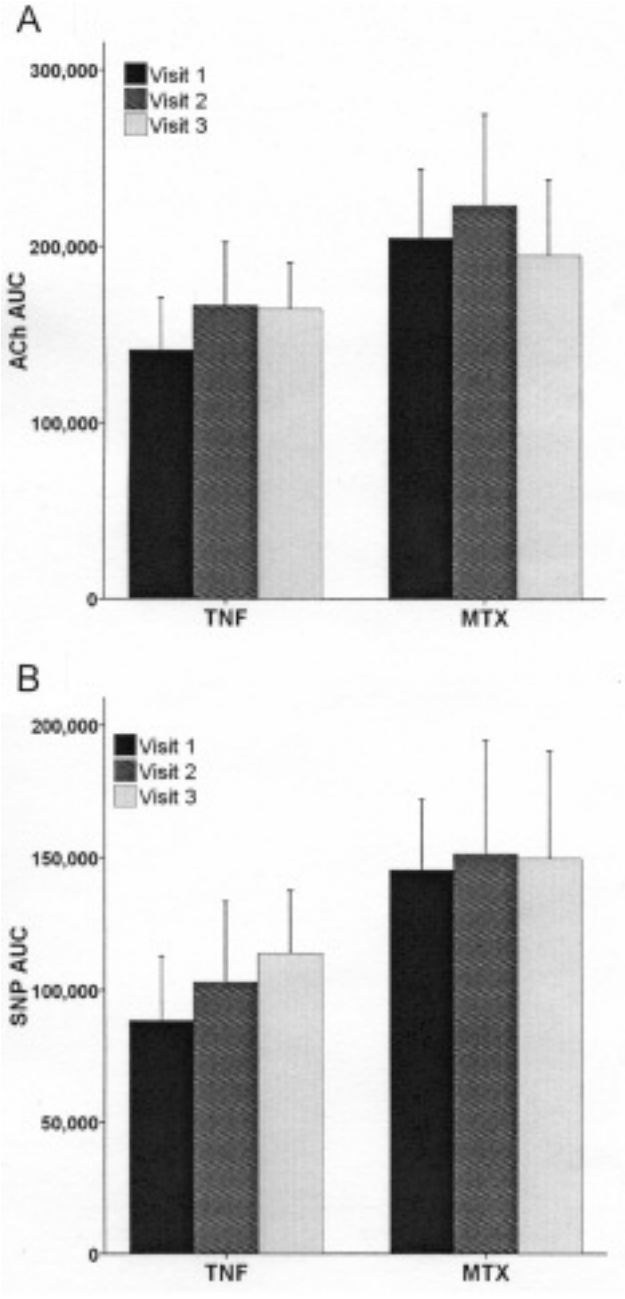

Figure 1. A. ACh AUC in the 30 TNF (p = 0.122) and 21 MTX (p = 0.836) patients throughout the study. B. SNP AUC in the 30 TNF (p = 0.087) and 21 MTX (p = 0.531) patients throughout the study. Data are mean ± 95% CI.

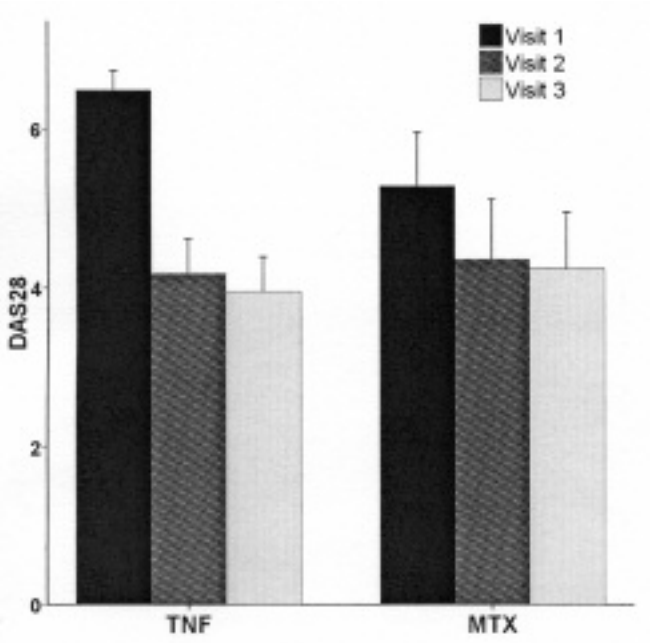

Figure 2. DAS28 in the 30 TNF and 21 MTX patients throughout the study. Data are mean ± 95% CI (TNF, p < 0.0001; MTX, p = 0.004).

the MTX and TNF groups from baseline to visit 3, but the drop in DAS28 score was significantly greater in the TNF group (TNF 2.49 ± 1.12 vs MTX 1.25 ± 1.64; p = 0.003).

DAS28 responders. Thirty-one patients (61%) [22 (81%) patients taking ETA, 2 (66%) of those on ADA, and 7 (33%) of those on MTX] achieved the EULAR response criteria by visit 3. At baseline, significant differences in the number of tender joints, DAS28-CRP, and HAQ score were observed between DAS28 responders and nonresponders — 19 ± 7 vs 12 ± 8 (p = 0.004); 6.46 ± 0.68 vs 5.39 ± 1.51 (p = 0.002); and 1.87 ± 0.52 vs 1.42 ± 0.84 (p = 0.014), respectively (Table 2).

When DAS28 responders were evaluated, there was a significant improvement in both the endothelium-dependent (p = 0.028, ANOVA; Figure 3) and endothelium-independent (p = 0.019, ANOVA; Figure 4) vascular responses from baseline to visit 2 and 3. A significant change in all the disease activity measures and inflammation was also observed from baseline to visit 3, as shown in Table 2.

Despite the statistically significant reduction in the number of swollen joints (p = 0.021) and DAS28-CRP (p = 0.023), no significant differences in endothelium-dependent (p = 0.833, ANOVA) and endothelium-independent (p = 0.915, ANOVA) vascular responses were observed throughout the study in DAS28 nonresponders.

Comparison between the DAS28 responders of the MTX and TNF groups was not possible due to the small numbers of responders in the MTX group. Although an improvement in the dose-dependent ACh and SNP responses of TNF patients was observed from baseline to visit 2 and 3, this did not reach statistical significance (p = 0.107 and p = 0.139, ANOVA, respectively).

Correlations. On univariate analysis at visit 3, ACh AUC and SNP AUC correlated with PV ($r^2 = 0.309$, p = 0.032, and $r^2 = 0.371$, p = 0.011, respectively) and as expected, with age ($r^2 = 0.449$, p = 0.001, and $r^2 = 0.334$, p = 0.020).

The variables tested in the model included age, sex,

Table 2. Disease activity scores and inflammatory mediators throughout the study in DAS28 responders and nonresponders.

	DAS28 Responders				DAS28 Nonresponders			
	Baseline	2 months	4 months	p	Baseline	2 months	4 months	p
No. tender joints	19 ± 7*	7 ± 6	4 ± 4	< 0.0001	12 ± 8*	9 ± 8	11 ± 9	0.135
No. swollen joints	9 ± 4	4 ± 3	2 ± 2	< 0.0001	9 ± 5	6 ± 4	6 ± 3	0.021
DAS28-CRP	6.46 ± 0.68*	4.03 ± 1.15	3.46 ± 0.82	< 0.0001	5.39 ± 1.51*	4.71 ± 1.41	4.99 ± 1.38	0.023
Log_{10}CRP, mg/l	1.30 ± 0.41	0.87 ± 0.38	0.8 ± 0.35	< 0.0001	1.21 ± 0.50	1.09 ± 0.51	1.13 ± 0.55	0.493
PV, mPa.s	1.82 ± 0.18	1.67 ± 0.97	1.74 ± 0.15	0.008	1.81 ± 0.19	1.79 ± 0.22	1.77 ± 0.22	0.438
HAQ	1.87 ± 0.52*	1.67 ± 0.52	1.44 ± 0.76	0.001	1.42 ± 0.84*	1.51 ± 0.70	1.44 ± 0.69	0.224

Values are mean ± SD. * p < 0.05 between DAS28 responders and nonresponders at baseline. DAS28: Disease Activity Score of 28 joints; CRP: C-reactive protein; PV; plasma viscosity; HAQ: Stanford Health Assessment Questionnaire.

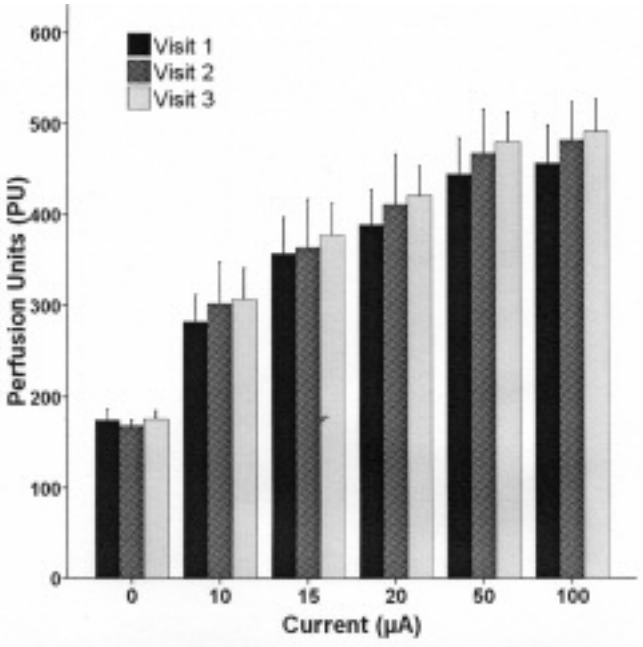

Figure 3. Dose-dependent ACh responses in the 31 DAS28 responders throughout the study. Data are mean ± 95% CI (p = 0.028, ANOVA).

smoking habits, history of hypertension, RF positivity, body mass index, number of tender joints, number of swollen joints, log_{10}CRP, DAS28 score, PV, and systolic and diastolic blood pressure. Forward stepwise multiple regression analysis revealed that PV (ß = –0.298, p = 0.029) and age (ß = –0.457, p = 0.001) were the only independent predictors of ACh AUC response, and that PV (ß = –0.500, p = 0.002) and DAS28 score (ß = 0.363, p = 0.02) independently predicted the SNP AUC response.

Adverse events. Three patients (6%) did not complete the study. All 3 patients were in the TNF group (2 started ETA and 1 ADA) and developed side effects after the 2-month visit. The patient starting ADA died from a combination of pulmonary fibrosis, infection, and pneumonitis that occurred 8 weeks after starting the ADA (added to a MTX regimen). One patient developed probable pulmonary fibrosis 8 weeks after starting ETA. He had concomitant MTX therapy (commenced 4 years earlier); he recovered shortly after stopping both drugs following a course of prednisolone. The third patient had worsening of mild congestive cardiac failure 10 weeks after commencement of ETA, and recovered satisfactorily after discontinuation of the TNF-α blocker and appropriate treatment for heart failure. These 3 patients were included in the baseline but not in the post-treatment analysis.

DISCUSSION

We demonstrated that in active RA, microvascular function is improved in those patients who respond to antirheumatic treatment, using the EULAR response criteria to define responders. This study strengthens the view that inflammation plays a key role in CVD and gives further evidence on the importance of tight and aggressive control of RA disease activity, not only to avoid further joint damage and disability, but to potentially reduce CVD risk.

Microvascular endothelial dysfunction has been implicated in the pathogenesis of several conditions such as diabetes mellitus, metabolic syndrome, and chronic renal failure. Abnormalities in the microvascular bed correlate with CV risk factors[36-38] and established coronary artery disease[39]. Recently we showed a correlation between microvascular function in the forearm and coronary flow reserve in healthy subjects[16]. Indeed, the study of this vascular bed may provide distinct and additional information to that obtained through testing the macrocirculation, as microvascular dysfunction may in fact occur earlier[17]. Although macrovascular function may be a stronger determinant of future CV events, the correlation between microvascular function and the presence of CV risk factors is more robust[12].

Microvascular dysfunction has been reported in RA[18,19]. It is associated with systemic inflammation[40] and Datta, *et al*, in a pilot study, showed that in active RA, vascular function in the microcirculation can be improved with antiinflammatory treatment[19]. However, that study was small (8 patients) and disease activity was not measured, and it had variable timepoints and treatments that may have influenced the results. Further, it was not clear if this benefit was a drug

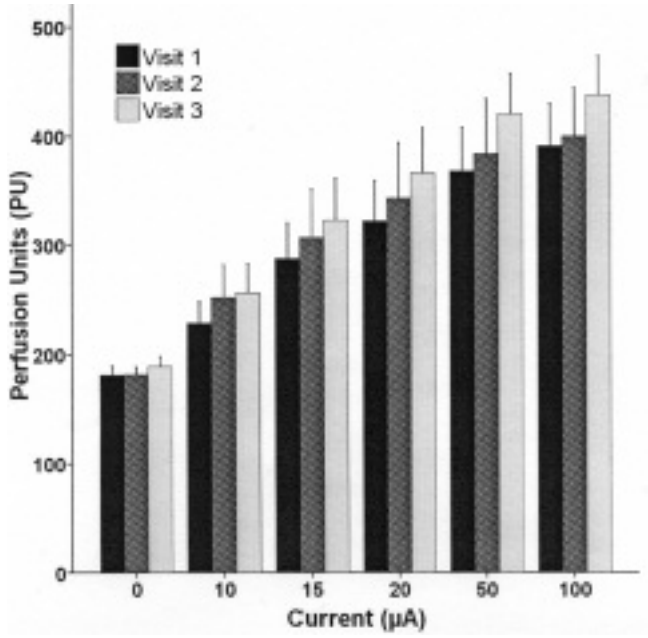

Figure 4. Dose-dependent SNP responses in the 31 DAS28 responders throughout the study. Data are mean ± 95% CI (p = 0.019, ANOVA).

effect or due to decreasing the inflammatory stimuli. Our study was carried out in 51 patients following power calculations to ensure adequate numbers of both responders and nonresponders, patients were treated with 2 different drug types (MTX and TNF-α blockade), assessments were done at 3 preplanned timepoints, and we measured several markers of inflammation and disease activity. Although a trend to improvement in vascular function was seen in the group as a whole, statistical significance was reached only in those that fulfilled the EULAR response criteria.

We found several differences between patients receiving MTX and those receiving anti-TNF-α drugs. At baseline, both groups seemed to represent distinct stages in the disease process, with those starting TNF-α blockade being at the severe end. Anti-TNF-α patients had longer duration of disease and more disease activity, and this was associated with significantly lower microvascular responses compared with MTX patients. Although not statistically significant, there was a trend toward better improvement in vascular function after TNF than after MTX treatment. Indeed, patients receiving TNF-blocking drugs were more likely to have a reduction in disease activity, and this reduction was greater than that observed in patients receiving MTX. Whether anti-TNF-α therapy possesses greater beneficial effects in vascular function than MTX or whether the trend toward better vascular function observed in anti-TNF-α patients was due to their greater antiinflammatory or other distinct properties remains unanswered; however, it is clear that uncorrected inflammation in the nonresponder group was paralleled by lack of improvement in endothelial function. In favor of the greater improvement in vascular function with anti-TNF-α therapy than with MTX would be the fact that TNF-α is a pivotal inflammatory cytokine directly involved in the pathogenesis of atherosclerosis; thus blocking this inflammatory mediator directly may be more effective in improving vascular health[41,42]. On the other hand, improvement in endothelial function has also recently been observed in RA patients refractory to TNF-α antagonists who were treated with rituximab; this was associated with a significant reduction in disease activity and CRP, suggesting that reducing inflammation may be the key factor[43,44].

We have demonstrated a direct and independent correlation between microvascular function and inflammation (PV). This relationship was not found in the 2 previous studies assessing microvascular function in RA[18,19] (probably due to their small numbers), but was in keeping with our findings in a previous study, where we reported CRP was an independent determinant of microvascular function[40]. This not only emphasizes the key role of inflammation in CVD, but also shows the importance that aggressive disease control may have in reducing this risk in patients with RA.

Most studies of macrovascular function in RA have shown evidence of endothelial-dependent but not independent vascular impairment in this group of patients. Moreover, after antiinflammatory treatment, improvement in endothelial-dependent but not independent function has also been observed[18,24,25,45,46]. The results at the level of the microcirculation, however, differ from these, not only because both endothelium-dependent and independent responses were found to be impaired in RA compared to healthy controls[18,47] and because high CRP patients had lower ACh and SNP responses than low CRP patients[20], but also because both in our present study and in the previous pilot study[47], endothelium-dependent and independent responses increased significantly after antiinflammatory treatment. As reduced ACh responses reflect endothelial dysfunction, whereas impairment in SNP suggests a dysfunction at the vascular smooth-muscle level, it is possible that the vascular involvement of the microcirculation is more global (endothelium and vascular smooth-muscle), while in the macrocirculation the endothelium is mostly affected.

A limitation of our study was the open-label design that could have introduced treatment selection bias. This was a result of current UK prescription guidelines that state that only those RA patients with active disease who have failed to respond to 2 previous DMARD, one of which must have been MTX, are eligible to receive TNF-α blockers[48]. Allocation of treatment was, however, decided by the rheumatologist responsible for the patient's care and not by the investigators. Moreover, previous studies on endothelial function used this same study design[24,49].

We demonstrated that microvascular dysfunction is present in patients with active RA, that it correlates with systemic inflammation, and that it can be at least partially

reversed with antirheumatic treatment, particularly in those patients who respond to it. As the anti-TNF-α drugs clearly showed the best antiinflammatory effect, we suggest that such drugs need to be assessed in early RA, as CV risk also occurs soon after diagnosis.

ACKNOWLEDGMENT

We are grateful to Gwen Kiddie for technical and administrative support.

REFERENCES

1. Myllykangas-Luosujarvi R, Aho K, Kautiainen H, Isomaki H. Shortening of life span and causes of excess mortality in a population-based series of subjects with rheumatoid arthritis. Clinical Exp Rheumatol 1995;13:149-53.
2. DeMaria AN. Relative risk of cardiovascular events in patients with rheumatoid arthritis. Am J Cardiol 2002;89 Suppl 1:33-8.
3. Alkaabi JK, Ho M, Levison R, Pullar T, Belch JJ. Rheumatoid arthritis and macrovascular disease. Rheumatology 2003;42:292-7.
4. van Leuven SI, Franssen R, Kastelein JJ, Levi M, Stroes ES, Tak PP. Systemic inflammation as a risk factor for atherothrombosis. Rheumatology 2008;47:3-7.
5. Vita JA, Keaney JF Jr. Endothelial function: a barometer for cardiovascular risk? Circulation 2002;106:640-2.
6. Gonzalez MA, Selwyn AP. Endothelial function, inflammation, and prognosis in cardiovascular disease. Am J Med 2003;115 Suppl 8A:99S-106S.
7. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 1992;340:1111-5.
8. Newton DJ, Khan F, Belch JJ. Assessment of microvascular endothelial function in human skin. Clin Sci (Lond) 2001;101:567-72.
9. Dessein PH, Joffe BI, Singh S. Biomarkers of endothelial dysfunction, cardiovascular risk factors and atherosclerosis in rheumatoid arthritis. Arthritis Res Ther 2005;7:R634-43.
10. Belch JJ, Shaw JW, Kirk G, McLaren M, Robb R, Maple C, et al. The white blood cell adhesion molecule E-selectin predicts restenosis in patients with intermittent claudication undergoing percutaneous transluminal angioplasty. Circulation 1997;95:2027-31.
11. Anderson TJ. Prognostic significance of brachial flow-mediated vasodilation. Circulation 2007;115:2373-5.
12. Mitchell GF, Parise H, Vita JA, Larson MG, Warner E, Keaney JF Jr, et al. Local shear stress and brachial artery flow-mediated dilation: the Framingham Heart Study. Hypertension 2004;44:134-9.
13. Huang AL, Silver AE, Shvenke E, Schopfer DW, Jahangir E, Titas MA, et al. Predictive value of reactive hyperemia for cardiovascular events in patients with peripheral arterial disease undergoing vascular surgery. Arterioscler Thromb Vasc Biol 2007;27:2113-9.
14. Khan F, Green FC, Forsyth JS, Greene SA, Morris AD, Belch JJ. Impaired microvascular function in normal children: effects of adiposity and poor glucose handling. J Physiol 2003;551:705-11.
15. Shamim-Uzzaman QA, Pfenninger D, Kehrer C, Chakrabarti A, Kacirotti N, Rubenfire M, et al. Altered cutaneous microvascular responses to reactive hyperaemia in coronary artery disease: a comparative study with conduit vessel responses. Clin Sci (Lond) 2002;103:267-73.
16. Khan F, Patterson D, Belch JJ, Hirata K, Lang CC. Relationship between peripheral and coronary function using laser Doppler imaging and transthoracic echocardiography. Clin Sci (Lond) 2008;115:295-300.
17. Philpott A, Anderson TJ. Reactive hyperemia and cardiovascular risk. Arterioscler Thromb Vasc Biol 2007;27:2065-7.
18. Arosio E, De Marchi S, Rigoni A, Prior M, Delva P, Lechi A. Forearm haemodynamics, arterial stiffness and microcirculatory reactivity in rheumatoid arthritis. J Hypertens 2007;25:1273-8.
19. Datta D, Ferrell WR, Sturrock RD, Jadhav ST, Sattar N. Inflammatory suppression rapidly attenuates microvascular dysfunction in rheumatoid arthritis. Atherosclerosis 2007;192:391-5.
20. Galarraga B, Khan F, Kumar P, Pullar T, Belch JJ. C-reactive protein: the underlying cause of microvascular dysfunction in rheumatoid arthritis. Rheumatology 2008;47:1780-4.
21. Choi HK, Hernan MA, Seeger JD, Robins JM, Wolfe F. Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study. Lancet 2002;359:1173-7.
22. Dixon WG, Watson KD, Lunt M, Hyrich KL, Silman AJ, Symmons DP. Reduction in the incidence of myocardial infarction in patients with rheumatoid arthritis who respond to anti-tumor necrosis factor alpha therapy: Results from the British Society for Rheumatology Biologics Register. Arthritis Rheum 2007;56:2905-12.
23. Jacobsson LT, Turesson C, Gulfe A, Kapetanovic MC, Petersson IF, Saxne T, et al. Treatment with tumor necrosis factor blockers is associated with a lower incidence of first cardiovascular events in patients with rheumatoid arthritis. J Rheumatol 2005;32:1213-8.
24. Hurlimann D, Forster A, Noll G, Enseleit F, Chenevard R, Distler O, et al. Anti-tumor necrosis factor-alpha treatment improves endothelial function in patients with rheumatoid arthritis. Circulation 2002;106:2184-7.
25. Gonzalez-Juanatey C, Testa A, Garcia-Castelo A, Garcia-Porrua C, Llorca J, Gonzalez-Gay MA. Active but transient improvement of endothelial function in rheumatoid arthritis patients undergoing long-term treatment with anti-tumor necrosis factor alpha antibody. Arthritis Rheum 2004;51:447-50.
26. Maki-Petaja KM, Hall FC, Booth AD, Wallace SM, Yasmin, Bearcroft PW, et al. Rheumatoid arthritis is associated with increased aortic pulse-wave velocity, which is reduced by anti-tumor necrosis factor-alpha therapy. Circulation 2006;114:1185-92.
27. Del Porto F, Lagana B, Nofroni I, Tinti F, Mitterhofer AP, D'Amelio R. Effects of tumour necrosis factor alpha blockade on lipid profile in active rheumatoid arthritis. Rheumatology 2007;46:1626-7.
28. Gonzalez-Gay MA, De Matias JM, Gonzalez-Juanatey C, Garcia-Porrua C, Sanchez-Andrade A, Martin J, et al. Anti-tumor necrosis factor-alpha blockade improves insulin resistance in patients with rheumatoid arthritis. Clin Exp Rheumatol 2006;24:83-6.
29. van Gestel AM, Prevoo ML, van 't Hof MA, van Rijswijk MH, van de Putte LB, van Riel PL. Development and validation of the European League Against Rheumatism response criteria for rheumatoid arthritis. Comparison with the preliminary American College of Rheumatology and the World Health Organization/International League Against Rheumatism Criteria. Arthritis Rheum 1996;39:34-40.
30. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
31. Hagglund KJ, Roth DL, Haley WE, Alarcon GS. Discriminant and convergent validity of self-report measures of affective distress in patients with rheumatoid arthritis. J Rheumatol 1989;16:1428-32.
32. Spence VA, Khan F, Belch JJ. Enhanced sensitivity of the peripheral cholinergic vascular response in patients with chronic fatigue syndrome. Am J Med 2000;108:736-9.
33. Morris SJ, Shore AC, Tooke JE. Responses of the skin

microcirculation to acetylcholine and sodium nitroprusside in patients with NIDDM. Diabetologia 1995;38:1337-44.
34. Khan F, Newton DJ, Smyth EC, Belch JJ. Influence of vehicle resistance on transdermal iontophoretic delivery of acetylcholine and sodium nitroprusside in humans. J Appl Physiol 2004;97:883-7.
35. Khan F, Elherik K, Bolton-Smith C, Barr R, Hill A, Murrie I, et al. The effects of dietary fatty acid supplementation on endothelial function and vascular tone in healthy subjects. Cardiovasc Res 2003;59:955-62.
36. Khan F, Elhadd TA, Greene SA, Belch JJ. Impaired skin microvascular function in children, adolescents, and young adults with type 1 diabetes. Diabetes Care 2000;23:215-20.
37. Farkas K, Kolossvary E, Jarai Z, Nemcsik J, Farsang C. Non-invasive assessment of microvascular endothelial function by laser Doppler flowmetry in patients with essential hypertension. Atherosclerosis 2004;173:97-102.
38. Khan F, Belch JJ. Skin blood flow in patients with systemic sclerosis and Raynaud's phenomenon: effects of oral L-arginine supplementation. J Rheumatol 1999;26:2389-94.
39. Ijzerman RG, de Jongh RT, Beijk MA, van Weissenbruch MM, Delemarre-van de Waal HA, Serne EH, et al. Individuals at increased coronary heart disease risk are characterized by an impaired microvascular function in skin. Eur J Clin Invest 2003;33:536-42.
40. Galarraga B, Khan F, Kumar P, Pullar T, Belch J. Microvascular dysfunction in rheumatoid arthritis [abstract]. Rheumatology 2008;46:ii6.
41. Galarraga B, Khan F, Kumar P, Pullar T, Belch JJ. Etanercept improves inflammation-associated arterial stiffness in rheumatoid arthritis. Rheumatology 2009;48:1418-23.
42. Gonzalez-Gay MA, Garcia-Unzueta MT, De Matias JM, Gonzalez-Juanatey C, Garcia-Porrua C, Sanchez-Andrade A, et al. Influence of anti-TNF-alpha infliximab therapy on adhesion molecules associated with atherogenesis in patients with rheumatoid arthritis. Clin Exp Rheumatol 2006;24:373-9.
43. Gonzalez-Juanatey C, Llorca J, Vazquez-Rodriguez TR, Diaz-Varela N, Garcia-Quiroga H, Gonzalez-Gay MA. Short-term improvement of endothelial function in rituximab-treated rheumatoid arthritis patients refractory to tumor necrosis factor alpha blocker therapy. Arthritis Rheum 2008;59:1821-4.
44. Kerekes G, Soltesz P, Der H, Veres K, Szabo Z, Vegvari A, et al. Effects of rituximab treatment on endothelial dysfunction, carotid atherosclerosis, and lipid profile in rheumatoid arthritis. Clin Rheumatol 2009;28:705-10.
45. Vaudo G, Marchesi S, Gerli R, Allegrucci R, Giordano A, Siepi D, et al. Endothelial dysfunction in young patients with rheumatoid arthritis and low disease activity. Ann Rheum Dis 2004;63:31-5.
46. Hansel S, Lassig G, Pistrosch F, Passauer J. Endothelial dysfunction in young patients with long-term rheumatoid arthritis and low disease activity. Atherosclerosis 2003;170:177-80.
47. Datta D, Ferrell WR, Sturrock RD, Jadhav ST, Sattar N. Inflammatory suppression rapidly attenuates microvascular dysfunction in rheumatoid arthritis. Atherosclerosis 2007;192:391-5.
48. National Institute for Health and Clinical Excellence. Adalimumab, etanercept and infliximab for the treatment of rheumatoid arthritis (final appraisal determination). [Internet. Accessed November 24, 2009.] Available from: http://guidance.nice.org.uk/download.aspx?o=388554
49. Gonzalez-Juanatey C, Llorca J, Sanchez-Andrade A, Garcia-Porrua C, Martin J, Gonzalez-Gay MA. Short-term adalimumab therapy improves endothelial function in patients with rheumatoid arthritis refractory to infliximab. Clin Exp Rheumatol 2006;24:309-12.