Enhanced Endothelium-Dependent Microvascular Responses in Patients with Wegener's Granulomatosis

HANS L.A. NIENHUIS, KARINA de LEEUW, ANDRIES J. SMIT, JOHAN BIJZET, COEN A. STEGEMAN, CEES G.M. KALLENBERG, and MARC BIJL

ABSTRACT. ***Objective.*** To assess endothelial cell (EC) function of the cutaneous microcirculation in patients with Wegener's granulomatosis (WG) and to relate EC function to EC activation and presence of atherosclerosis.

Methods. We studied 28 WG patients with inactive disease and 28 age and sex matched controls. Common carotid intima-media thickness (IMT), as a measure of atherosclerosis, was determined by ultrasonography. EC function of microcirculation in the fingers was assessed using laser Doppler fluxmetry in combination with iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP), which are endothelium-dependent and endothelium-independent vasodilators, respectively. In addition to vascular responses, traditional cardiovascular risk factors were recorded, and EC activation was assessed by serological measures.

Results. WG patients had increased IMT compared to controls (0.71 mm vs 0.66 mm; $p < 0.05$). In WG patients IMT correlated positively with age and body mass index (BMI), and negatively with duration of prednisolone use and cumulative prednisolone dose. Levels of von Willebrand factor and C-reactive protein were increased in patients with WG ($p < 0.05$). ACh-induced but not SNP-induced vasodilatation was enhanced in WG patients compared to controls. When patients and controls with increased IMT were excluded, the difference in relative response to ACh became significant (median 567% vs 334%; $p = 0.007$). The response to ACh correlated negatively with age.

Conclusion. We confirmed that patients with WG have accelerated atherosclerosis as measured by IMT. EC activation and disturbed microvascular endothelium-dependent vasodilatation were present in the microcirculation of WG patients with inactive disease and without signs of atherosclerosis, indicating and contributing to a proatherogenic state. (First Release July 15 2007; J Rheumatol 2007;34:1875–81)

Key Indexing Terms:

ENDOTHELIAL FUNCTION | ENDOTHELIAL ACTIVATION | ATHEROSCLEROSIS
MICROCIRCULATION | ACETYLCHOLINE | INFLAMMATION
RISK FACTORS | WEGENER'S GRANULOMATOSIS

Atherosclerosis is considered to reflect an inflammatory process[1,2], and various large prospective epidemiological studies have demonstrated that increased levels of inflammatory markers are predictive of future cardiovascular disease (CVD)[3,4].

Inflammation is also one of the hallmarks of systemic autoimmune diseases. Wegener's granulomatosis (WG) is a chronic systemic autoimmune disease that usually begins as a localized granulomatous inflammation of upper and/or lower respiratory tract mucosa and may progress into generalized necrotizing vasculitis and glomerulonephritis. Antineutrophilic cytoplasmic antibodies (ANCA) are almost invariably present[5]. Levels of C-reactive protein (CRP), reflecting the inflammatory expression of this disorder, are often increased and correlate with disease activity[6]. Systemic autoimmune diseases, including WG, are indeed associated with an increased prevalence of CVD[7-13]. Accelerated atherosclerosis cannot be fully explained by the presence of traditional cardiovascular risk factors. Therefore, nontraditional risk factors, disease-related factors in particular, are probably involved and might include increased levels of autoantibodies, systemic inflammation, renal impairment, and use of medication such as corticosteroids. The presence of early atherosclerosis can be assessed by measuring the intima-media thickness (IMT) by ultrasound[14].

Atherogenesis is associated with endothelial cell (EC) activation and dysfunction indicated by increased expression of adhesion molecules, which leads to leukocyte adhesion and

From the Department of Internal Medicine, Division of Rheumatology and Clinical Immunology, Division of Vascular Diseases, and Division of Nephrology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

H.L.A. Nienhuis, BSc; K. de Leeuw, MD; J. Bijzet, BSc; C.G.M. Kallenberg, MD, PhD; M. Bijl MD, PhD, Division of Rheumatology and Clinical Immunology; A.J. Smit, MD, PhD, Division of Vascular Diseases; C.A. Stegeman, MD, PhD, Division of Nephrology, University Medical Center Groningen.

Address reprint requests to Dr. H. Nienhuis, Department of Internal Medicine, Division of Rheumatology and Clinical Immunology, University Medical Center Groningen, University of Groningen, Hanzeplein 1, 9713 GZ, PO Box 30.001, 9700 RB Groningen, The Netherlands. E-mail: h.l.a.nienhuis@int.umcg.nl

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migration of these cells into the vessel wall. EC dysfunction leads to dysregulation of the vascular tone and can be detected by several techniques. Flow-mediated dilation (FMD), measuring the hyperemic response to ischemia, is most commonly used[15,16]. Indeed, impaired FMD of the brachial artery was reported in several systemic autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus and in patients with primary systemic vasculitis[17-19]. Laser Doppler fluxmetry (LDF) combined with iontophoresis of vasoactive agents is another noninvasive method to assess endothelial function. Using this method, local vasodilation via endothelium-dependent and endothelium-independent pathways can be assessed in the microcirculation.

EC activation and dysfunction are early and reversible events in the pathogenesis of atherosclerosis[20]. Therefore, evaluation of endothelial function may be of clinical relevance, because it offers the possibility to intervene early in the process of atherosclerosis. We assessed early atherosclerosis and EC function in patients with WG compared to controls, and investigated whether endothelial function is related to the presence of early atherosclerosis and to traditional and nontraditional risk factors for CVD.

MATERIALS AND METHODS

Thirty consecutive patients fulfilling the American College of Rheumatology criteria for WG[21] and attending our outpatient clinic at University Medical Center Groningen were studied. Pregnancy and active disease were exclusion criteria. Disease activity was assessed by the Birmingham Vasculitis Activity Score (BVAS), and active disease was defined as BVAS > 1[22]. Healthy age and sex matched volunteers served as controls. The local research ethics committee gave approval for the study, and informed consent was obtained from each participant. Information was obtained from all subjects with respect to traditional risk factors for CVD, including blood pressure, body mass index (BMI), lipid levels, smoking status, diabetes, family history of CVD (considered positive if first-degree relatives had CVD before 60 years of age), and manifest CVD. Hypertension was defined as mean systolic blood pressure > 140 mm Hg and/or mean diastolic pressure > 90 mm Hg, or use of antihypertensive drugs prescribed to reduce blood pressure. Disease-related factors that might influence the development of atherosclerosis were also assessed. Cumulative BVAS as a measure of overall disease burden was calculated by adding the BVAS scores of each exacerbation. In addition, we recorded duration of prednisolone use, cumulative prednisolone dose, and creatinine clearance.

Blood analyses. Plasma lipid concentrations (cholesterol, high density lipoprotein, low density lipoprotein, and triglycerides) were measured by routine techniques. Additionally, serum and plasma samples were stored at –20°C for determination of levels of markers of EC activation and inflammation. Serum levels of vascular cell adhesion molecule-1 (R&D Systems, Abingdon, UK) and thrombomodulin (Diaclone, Besancon, France) were measured according to the manufacturer's instructions. Von Willebrand factor and CRP were determined using in-house ELISA as described[8].

Measurement of intima-media thickness. The method used in this study has been described[14]. In brief, IMT was determined on the far wall of the left common carotid artery roughly 1 cm proximal to the bulbus at 3 different positions using an Acuson 128XP device with 7 MHz linear array transducers (Acuson, Mountain View, CA, USA). A B-mode image was obtained of the common carotid artery, then the probe was positioned perpendicular to the far wall, showing an intima-media complex over about 1 cm. Mean IMT was calculated (m-IMT; the mean value over the last 1-cm segment before the bulb, averaged over 3 measurements). IMT was considered to be increased when m-IMT exceeded 0.8 mm before the age of 50 years and 0.9 mm when age was over 50 years[23].

Laser Doppler fluxmetry in combination with iontophoresis. Skin perfusion was measured with a Periflux 4000 laser Doppler system in combination with a Periflux tissue heater set to 31°C (PF4005, Peritemp; all equipment from Perimed, Stockholm, Sweden). Vasoactive drugs were administered using iontophoresis. Iontophoresis allows charged substances to cross the skin by means of a small electrical current. Acetylcholine (ACh, 1%, Miochol; IOL AB, Bournonville Pharma, The Hague, The Netherlands) was used to induce an endothelium-dependent vasodilation. In contrast, sodium nitroprusside (SNP, 0.1%, dissolved in NaCl 0.9%) was used to induce an endothelium-independent vasodilation, as SNP acts as a NO donor, bypassing the endothelium. Subjects were asked to refrain from caffeine, alcohol, and smoking for 12 h preceding the test. We followed the same protocol as described[24,25]. In 10 subjects studied twice, this protocol gave intraindividual coefficients of variation for maximal responses of 16% for ACh and 18% for SNP. Two of the 30 patients were excluded from further analysis because they did not show a response because of technical failures. One control was excluded for the same reason; another control was found to use a diuretic and was also excluded as he did not fulfil the requirements for a healthy control.

Statistical methods. Values are expressed as mean (SD) when variables were normally distributed. In case of a non-normal distribution, data are reported as median (25th to 75th centile). Comparisons between patients and controls were made by independent-samples t tests or Mann-Whitney U tests for continuous variables, and by chi-squared analysis for categorical variables. The univariate correlation between IMT or relative change to ACh or SNP and other categorical variables was assessed by Pearson correlation coefficient when variables were normally distributed. Otherwise, Spearman correlation coefficient was used. Stepwise linear regression analysis was used to assess the influence of demographic and clinical measures on microvascular responses. A 2-sided p value $\leq$ 0.05 was considered significant.

RESULTS

Risk factors of patients and controls. Diastolic blood pressure, lipid levels, smoking habit, family history for CVD, manifest CVD, and diabetes were comparable. Only BMI and systolic blood pressure were significantly increased in WG patients (26.8 ± 3.4 kg/m^2, 130 ± 15 mm Hg, respectively) compared to controls (24.2 ± 2.2 kg/m^2 and 118 ± 9 mm Hg, respectively; $p < 0.05$). Eleven patients (39%) used one or more antihypertensive drugs. Two patients used 3-hydroxy-3-methylglutaryl coenzyme A inhibitors (Table 1). To study the relation between disease characteristics and EC function, several disease related factors were recorded as shown in Table 2.

Intima-media thickness. Mean IMT (m-IMT), measured over an arterial segment of 1 cm in the common carotid artery, was increased in WG patients compared to controls ($p < 0.05$; Table 1). Univariate analyses performed using data from all subjects showed a positive correlation between m-IMT and age ($r = 0.275$, $p = 0.049$), BMI ($r = 0.431$, $p = 0.036$), total cholesterol ($r = 0.407$, $p = 0.003$), LDL-cholesterol ($r = 0.361$, $p = 0.009$), and smoking ($r = 0.365$, $p = 0.008$). Among WG patients m-IMT was correlated positively with age ($r = 0.450$, $p = 0.028$), BMI ($r = 0.431$, $p = 0.036$), and total cholesterol ($r = 0.412$, $p = 0.046$), and negatively with duration of prednisolone use ($r = –0.449$, $p = 0.024$) and cumulative prednisolone dose ($r = –0.399$, $p = 0.048$).

Markers of EC activation. Levels of von Willebrand factor

Table 1. Characteristics of patients and controls.

	Controls, n = 28	WG Patients, n = 28
Age, yrs	50 ± 9	49 ± 9
Sex	11 female	11 female
Manifest CVD, n (%)	0 (0)	1 (4)
BMI, kg/m^2	24.2 ± 2.2	26.8 ± 3.4*
Blood pressure, mm Hg		
Diastolic	77 ± 7	81 ± 10
Systolic	118 ± 9	130 ± 15*
Hypertension, n (%)	0 (0)	13 (46)*
Antihypertensive drugs	0 (0)	11 (39)*
Increased blood pressure	0 (0)	5 (18)*
Lipid levels, mmol/l		
Cholesterol	5.51 ± 1.04	5.81 ± 0.96
HDL	1.66 ± 0.52	1.58 ± 0.37
LDL	3.09 ± 0.95	3.41 ± 0.95
Triglycerides	1.60 ± 0.98	1.85 ± 1.06
Smokers, n (%)	3 (11)	5 (18)
Family history for CVD, n (%)	8 (26)	12 (39)
Diabetes, n (%)	0 (0)	2 (6)
Antihypertensive agents, n (%)		
Beta-blockers	0 (0)	5 (18)*
ACE inhibitors	0 (0)	6 (21)*
Calcium antagonists	0 (0)	1 (4)
AT1 antagonists	0 (0)	3 (11)
Diuretics	0 (0)	3 (11)
HMG-CoA inhibitors, n (%)	0 (0)	2 (7)
m-IMT, mm	0.66 ± 0.10	0.71 ± 0.18*

Unless otherwise indicated, data are expressed as mean ± standard deviation. BMI: body mass index, HDL: high density lipoprotein, LDL: low density lipoprotein, CVD: cardiovascular disease, ACE: angiotensin-converting enzyme, AT1: angiotensin II type 1 receptor, HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme A; IMT: intima-media thickness, m-IMT: mean IMT. * $p < 0.05$ compared to controls.

Table 2. Disease-related factors.

Characteristics	
Disease duration, mo	91 (60–124)
Creatinine clearance, ml/min	78 ± 21
Cumulative BVAS	32 ± 17
Prednisolone use, mo	24 (8–54)
Cumulative prednisolone dose, g	14 (6–26)
Number of exacerbations, n	3 (1–5)

Data are expressed as mean ± standard deviation when normally distributed and as median (25%-75%) when non-normally distributed. BVAS: Birmingham vasculitis activity score.

were significantly elevated in WG patients. Levels of thrombomodulin were slightly increased and levels of vascular cell adhesion molecule-1 were slightly decreased in patients with WG, although not significantly (Table 3, Figure 1).

Microvascular function. Data for microvascular measurements are presented in Table 4. No significant differences for baseline flux, plateau flux, and absolute change between WG patients and controls were found. The relative change in flow to ACh was slightly increased in WG patients ($p = 0.06$; Figure 2). In this study, one patient with WG already had CVD, as she had had a cerebral infarction. Assuming a negative effect of manifest CVD on ACh response, we excluded this patient. After exclusion the patients' relative response to ACh was significantly increased ($p = 0.045$). Based on this finding it might be hypothesized that patients with manifest atherosclerosis exhibit decreased responses and that inclusion of these patients masks increased responses possibly present in patients without atherosclerosis. Therefore, we subdivided patients and controls into those with normal and those with increased IMT.

Table 3. Markers of endothelial cell activation.

	Controls, n = 28	WG Patients, n = 28
TM, ng/ml	3.0 (1.8–4.7)	4.3 (2.7–6.8)
VCAM-1, ng/ml	247 (224–272)	221 (179–264)
vWF, %	42 (22–58)	79 (37–235)*
CRP, mg/l	1.3 (0.59–2.6)	5.6 (3.4–17.3)*

Data are expressed as median (25%–75%). CRP: C-reactive protein, TM: thrombomodulin, VCAM-1: vascular cell adhesion molecule-1, vWF: von Willebrand factor. * $p < 0.05$ compared to controls.

When patients without increased IMT were compared to controls, the difference in the relative response to ACh was even more pronounced (median 567% vs 334%; $p = 0.007$). No differences were found between patients with increased IMT and controls (Figure 3). Next, we related ACh response to clinical and biochemical measures. The response to ACh was found to be negatively correlated with age ($r = –0.261$, $p = 0.05$).

Influence of demographic and clinical measures on microvascular responses. Differences in BMI and prevalence of hypertension, which includes increased blood pressure and the use of antihypertensive drugs, could have influenced our results. Further, vascular responses are known to be related to age. Therefore, we investigated the relations between microvascular responses and these variables. Because BMI is related to the presence of hypertension, we entered an interaction term in the regression model. Stepwise regression analysis revealed that WG and age are independent predictors of the microvascular response (Table 5).

DISCUSSION

We evaluated EC function and the presence of EC activation in relation to early atherosclerosis in patients with WG. First, we confirmed our previous findings that accelerated atherosclerosis and EC activation are present in WG patients[8]. Second, unexpectedly, we observed that the microvascular vasodilator response was increased in WG patients, especially after exclusion of those patients with manifest atherosclerosis

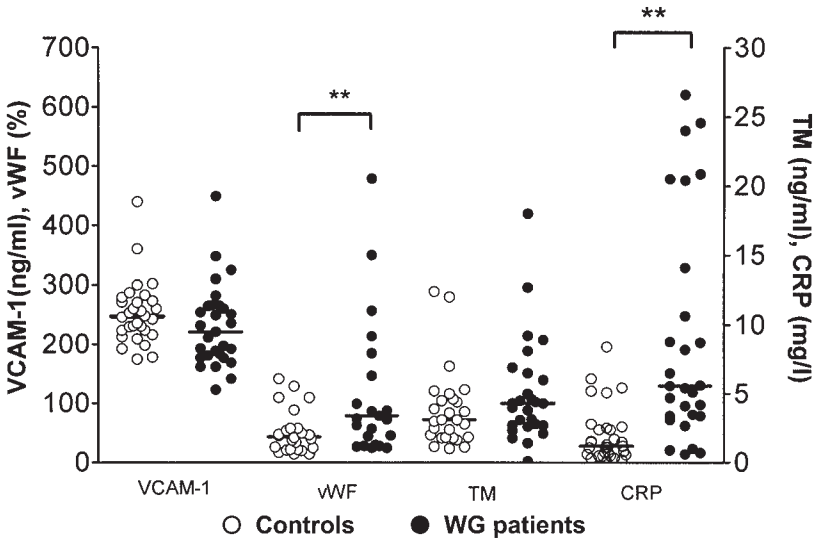

Figure 1. Markers of endothelial activation in WG patients and controls. VCAM-1: vascular cell adhesion molecule-1, vWF: von Willebrand factor, TM: thrombomodulin, CRP: C-reactive protein. **p < 0.01.

Table 4. Results of microvascular measurements.

	Controls, n = 28	WG Patients, n = 28
Acetylcholine		
Skin temperature, °C	31.7 ± 0.68	31.7 ± 0.81
Baseline flux, PU	30 (22–41)	23 (16–33)
Plateau flux, PU	141 (109–171)	148 (110–205)
Absolute change, PU	109 (83–134)	116 (84–171)
Relative change, %	345 (273–465)	440 (327–862)
Sodium nitroprusside		
Skin temperature, °C	31.8 ± 0.79	31.6 ± 0.66
Baseline flux, PU	30 (19–42)	22 (15–30)
Plateau flux, PU	114 (83–145)	99 (69–144)
Absolute change, PU	91 (43–103)	79 (51–104)
Relative change, %	266 (154–560)	355 (184–658)

Data are expressed as mean ± standard deviation when normally distributed and as median (25%–75%) when non-normally distributed. Mann-Whitney tests were used to compare the different groups. PU: arbitrary perfusion units.

or those with an increased IMT. This indicates abnormal EC function of the microcirculation in WG patients.

Our results are unexpected, as data are discordant with some other studies in which decreased vasodilator responses were observed in primary vasculitis[17,26-28]. Discrepancies between the present study and other studies might relate to methodological differences. Most studies used FMD to assess EC function. FMD measures the response to reactive hyperemia in the brachial artery, whereas LDF measures the response to ACh and SNP in the microcirculation. In addition, ACh-mediated vasodilatation involves NO, prostanoids, and endothelium-derived hyperpolarizing factor[29,30], whereas FMD results from shear stress-induced NO production alone. In contrast to our results, Filer, *et al* showed a decreased vasodilator response in patients with ANCA-associated systemic vasculitis and polyarteritis nodosa using LDF of the microcirculation[17]. Filer, *et al* included patients with inactive disease as well as active disease, whereas we excluded patients with active disease in order to focus on the underlying condition, excluding influences of temporary disease activity. Although Filer, *et al* found no correlation between vascular responses and BVAS it cannot be ruled out that differences in disease activity explain the contrasting results, especially because study groups were rather small.

Use of medication might have influenced our results. Beta-blockers, angiotensin-converting enzyme inhibitors, and angiotensin type-1 receptor antagonists, for example, are known to influence EC function[31-33]. Antihypertensive agents were used by our patients and not by controls. However, when we compared the responses to ACh of patients using antihypertensive agents with those of patients not using these drugs, we found no differences. Also, when we excluded patients with hypertension the differences between patients and controls remained significant. Comorbidity might be a confounder as well. Diabetes is known to impair vascular function; however, exclusion of 2 patients with diabetes did not influence our results (data not shown).

The increased response to ACh in our patients is consistent with the results of a small study on 10 patients (7 with ANCA-associated systemic vasculitis) in which an enhanced vasodilator response to ACh in resistance vessels using forearm plethysmography was observed[34]. As well, in other conditions associated with an increased risk of atherosclerosis, such as preeclampsia, we and others have reported an increased microvascular vasodilatory response[24,35,36]. Therefore, it might be hypothesized that EC dysfunction is expressed differently in resistance or microvascular vessels

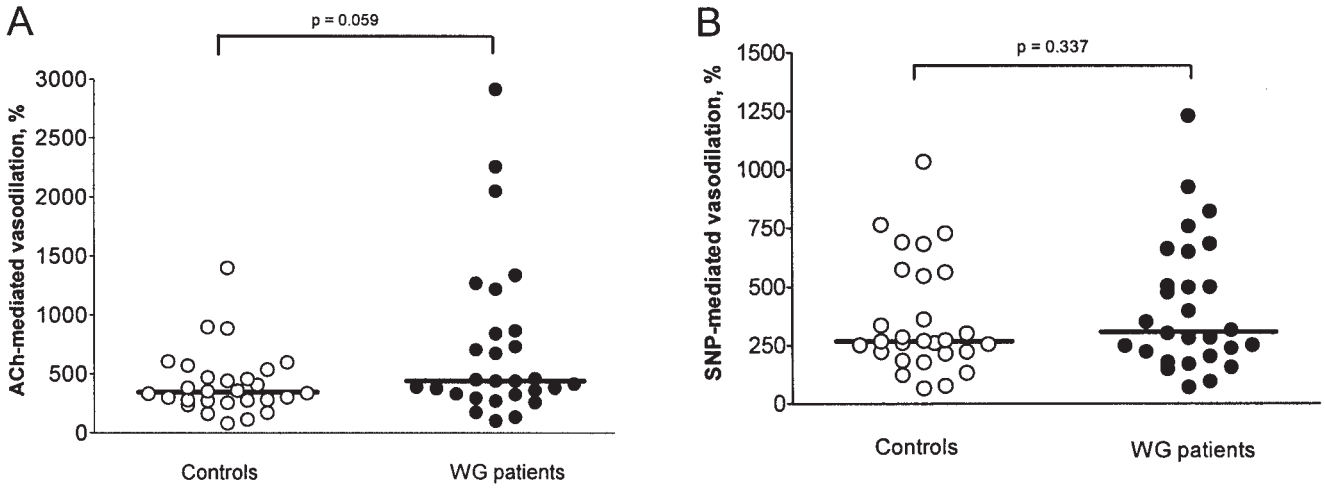

Figure 2. A. Acetylcholine (ACh)-mediated vasodilation in controls and WG patients. Median vasodilation (horizontal line) is slightly increased in WG patients compared to controls (p = 0.059). B. Sodium nitroprusside (SNP)-mediated vasodilation. Median vasodilation (horizontal line) is not significantly different in WG patients compared to controls (p = 0.337).

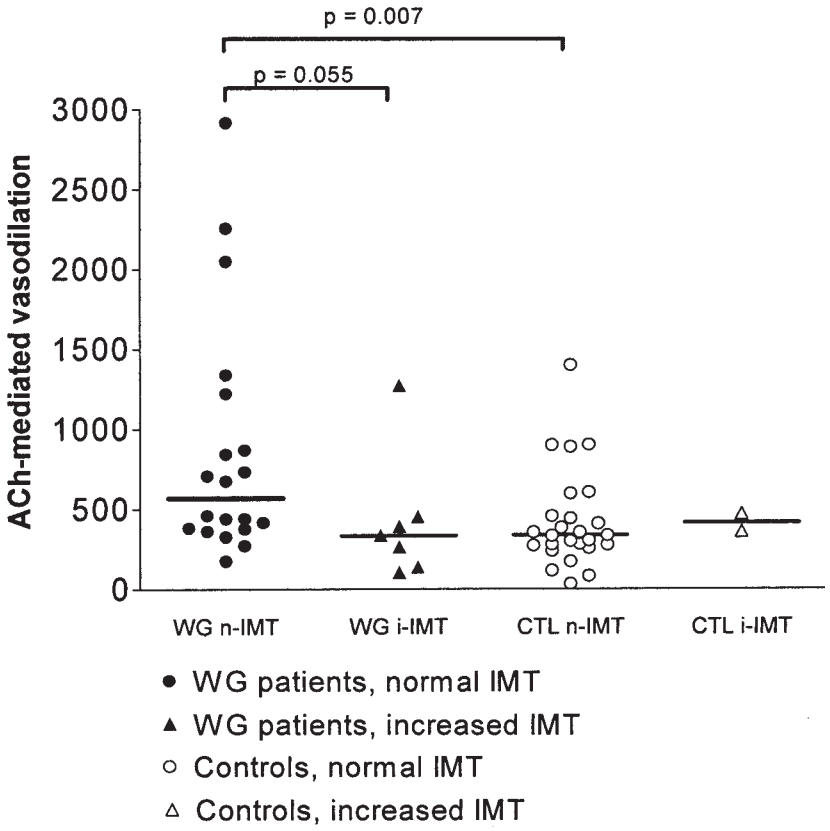

Figure 3. Acetylcholine (ACh)-mediated vasodilation in controls (CTL) and WG patients subdivided for intima-media thickness (IMT). Median vasodilation (horizontal line) is increased in WG patients with normal IMT (n-IMT) compared to controls with normal IMT, and also compared to WG patients with increased IMT (i-IMT).

than in large vessels such as the brachial artery. Another possible explanation could be that periods of inactive disease and low-grade inflammation are characterized by a relative overproduction of endothelial-derived vasodilatory substances, which results in an enhanced vasodilator response to ACh. Although our patients were inactive on clinical grounds, increased levels of CRP suggest low-grade inflammation. CRP has been found to be not only a biomarker, but also an

Table 5. Regression analysis of determinants of acetylcholine-mediated vasodilation in all subjects (n = 56).

Variable	Univariate Analyses		Multivariate Analyses (R^2 = 0.398)	
	r	p	Beta	p
Age	–0.26	0.05	–0.261	0.05
BMI	0.16	0.24		
Hypertension*	0.10	0.49		
BMI × hypertension	0.41	< 0.01		
Vasculitis**	0.30	0.03	0.30	0.02

* Mean systolic blood pressure > 140 mm Hg and/or mean diastolic blood pressure > 90 mm Hg, or use of antihypertensive drugs, prescribed with the aim to reduce blood pressure. ** WG patients defined as 0, controls as 1.

active mediator in the pathogenesis of atherosclerosis[1]. Further, increased levels of CRP were found to be associated with an increased risk of CVD[4,37]. Levels of CRP found in these studies were several times lower than levels in our patient group.

Several studies using FMD have shown that atherosclerosis, considered the underlying cause of CVD, is associated with decreased vasodilator responses[38,39]. The effect of established atherosclerosis on vasodilator responses of the microcirculation is not known. Therefore, we subdivided patients and controls into those with normal and those with increased IMT. Patients with a normal IMT showed an increased response compared to controls with a normal IMT, whereas patients with an increased IMT did not differ in response from controls. Based on these findings we propose that the increased vasodilator response in patients without established atherosclerosis could be masked by a decreased response in patients with increased IMT.

The negative correlation between duration of prednisolone use, cumulative prednisolone dose, and IMT might suggest that more vigorous therapy diminishes the development of atherosclerosis. This is in agreement with a large study showing that aggressive immunosuppressive therapy might decrease the likelihood and burden of atherosclerosis in patients with systemic lupus erythematosus[11].

Our study confirms the presence of accelerated atherosclerosis and EC activation in patients with WG. In addition, the endothelium-dependent vasodilator response is increased in patients with WG compared to controls, in particular in patients without established atherosclerosis. This abnormal endothelium-dependent response is not fully explained by differences in traditional cardiovascular risk factors; therefore disease-related factors are probably involved. Whether EC dysfunction is expressed differently in resistance vessels versus large vessels remains speculative and requires further investigation. Further, our data suggest that the presence of atherosclerosis should be taken into account when data on EC function in the microcirculation are interpreted.

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