Plasma Vascular Endothelial Growth Factor, Angiopoietin-1, and Angiopoietin-2 in Diabetes

Implications for cardiovascular risk and effects of multifactorial intervention

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OBJECTIVE — Vascular endothelial growth factor (VEGF) and angiopoietin (Ang)-1 and Ang-2 are mediators of angiogenesis. More recent data suggest that the balance between these growth factors may affect vascular endothelial integrity. Because diabetes is closely associated with endothelial perturbation, we studied plasma levels of these angiogenic growth factors in patients with diabetes; their relationship with glycemia, inflammation, and endothelial damage/dysfunction; and the effect of intensified cardiovascular risk management.

RESEARCH DESIGN AND METHODS — We measured plasma VEGF, Ang-1, and Ang-2 alongside plasma von Willebrand factor (vWf) and urine albumin–to–creatinine ratio (marking endothelial damage/dysfunction) and interleukin (IL)-6 in 94 patients (38 with overt cardiovascular disease [CVD]) with diabetes and 34 normal control subjects.

RESULTS — Plasma vWf ($P = 0.009$), IL-6 ($P < 0.001$), VEGF ($P = 0.001$), and Ang-2 ($P = 0.001$), but not Ang-1 ($P = 0.635$), were higher in diabetic patients with and without CVD than in control subjects. On multivariate analysis, HbA_{1c} was an independent predictor of plasma VEGF ($P = 0.032$) and Ang-2 ($P = 0.015$). Of the 94 patients, a subgroup of 33 patients with and 31 patients without CVD participated in a year of intensified cardiovascular risk management. HbA_{1c} and LDL cholesterol reduced significantly with treatment, along with associated reductions in plasma vWf and VEGF in both groups ($P < 0.001$). Ang-2 decreased ($P < 0.001$) only in patients without CVD. There were no significant changes in plasma IL-6 levels in both groups.

CONCLUSIONS — Plasma Ang-2 (but not Ang-1), like VEGF levels, are selectively elevated in patients with diabetes and are associated with indexes of endothelial damage/dysfunction, regardless of vascular disease. Intensive multifactorial intervention is associated with reductions in plasma VEGF, vWf, and (in patients without CVD) Ang-2 levels, possibly reflecting an improved vascular profile with treatment.

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Vascular endothelial growth factor (VEGF) and the angiopoietins are two families of growth factors believed to act predominantly on vascular endothelial cells. VEGF is mitogenic for endothelial cells, acting early and at most points in the angiogenic cascade (1). Increasing evidence suggests a role for VEGF in the pathophysiology of cardiovascular disease (CVD) (2). Elevated plasma VEGF has been shown in patients with hypertension (3) and diabetes (4,5), with levels correlating with measures of endothelial damage/dysfunction and overall cardiovascular risk in hypertensive patients (3). Furthermore, VEGF has independent prognostic significance in patients with acute coronary syndromes (6).

In contrast to VEGF, the angiopoietins have little effect on endothelial proliferation (7). Angiopoietin (Ang)-1 promotes endothelial cell survival, stabilizes endothelial interactions with supporting cells, and limits the permeability-inducing effects of VEGF (8–12). Ang-2, on the other hand, has been proposed as a natural antagonist of Ang-1, promoting vessel regression in the absence of VEGF (13), but facilitates endothelial cell migration and proliferation with VEGF (13,14). More recent data suggest that the angiopoietins may also be involved in the regulation endothelial integrity and inflammation (15), with Ang-1 having anti-inflammatory and potentially atheroprotective properties (16). Hence, selective increase in plasma VEGF and Ang-2, but not Ang-1, may favor aberrant neovascularization and endothelial abnormalities. These abnormalities are closely linked to the pathophysiology of microvascular and atherosclerotic vascular complications in type 2 diabetes (17). However, there are no data on plasma angiopoietins and the relationship with inflammation and endothelial damage/dysfunction in patients with type 2 diabetes, with and without CVD.

In this study, we hypothesized that plasma Ang-2 and VEGF (but not Ang-1) are *1*) selectively elevated in diabetes, *2*) higher in patients with clinically overt CVD, and *3*) related to the degree of glycemia, inflammation (as indicated by levels of the inflammatory cytokine, interleukin [IL]-6), and endothelial damage/dysfunction, as indicated by the plasma von Willebrand factor (vWf) (also known to be increased in diabetes [18]) and urinary albumin–to–creatinine ratio (UACr)

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Abbreviations: Ang, angiopoietin; CVD, cardiovascular disease; IL, interleukin; UACr, urinary albumin–to–creatinine ratio; VEGF, vascular endothelial growth factor; vWf, von Willebrand factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

(an index of renal microvascular damage). We tested our hypothesis in a cross-sectional study of healthy individuals compared with patients with diabetes with and without clinically overt CVD. In addition, we hypothesized that the treatment of multiple risk factors, shown to improve clinical outcome in diabetes (19), would reduce plasma VEGF and Ang-2. We tested this hypothesis in an intervention study using a "package of care" of intensified diabetes and cardiovascular risk management.

RESEARCH DESIGN AND METHODS

— Study patients were recruited from general practices across West Birmingham, U.K. The contact details of patients with established diagnosis of type 2 diabetes (20) were obtained from general practices (with general practitioner support for the study). These patients were contacted, and responders were invited to participate in this study. Patients with acute vascular events or hospitalizations (defined as stroke, myocardial infarction, unstable angina, or coronary or peripheral revascularization within the last 3 months) were excluded. Other exclusion criteria included patients with evidence of neoplastic, inflammatory, hepatic, and significant renal disease (requiring dialysis) established by careful history, examination, and routine laboratory tests. Patients with a prior history (over 3 months) of CVD (previous myocardial ischemia/infarction, stroke/transient ischemic attack, or coronary or peripheral revascularization) were defined as patients with overt CVD. Data from diabetic patients were compared with data from healthy control subjects recruited from healthy hospital staff, relatives, and friends of patients. These subjects were normotensive and were determined "healthy" by careful clinical history, examination, and routine laboratory tests.

Intervention and follow-up

Patients who attended the initial appointment were then offered 3 monthly consultations. Patients who were unable or unwilling to attend these consultations were excluded from this intervention aspect of the study. Patients with well-controlled glycemia, LDL cholesterol, and blood pressure (i.e., all within treatment targets as detailed below) were also excluded. During these consultations, we provided lifestyle (e.g., recommending at least three 30-min sessions of light-to-moderate exercise per week) and dietary advice (e.g., carbohydrates from whole grains, fruits, and vegetables, together with monounsaturated fat, should provide 60–70% of daily caloric intake). In addition, their oral hypoglycemic therapy was adjusted according to regular monitoring of HbA_{1c}, with an HbA_{1c} target of 6.5%. Metformin was started in overweight patients (defined as BMI of >25 kg/m^2) and added as a second-line agent in lean patients, who received gliclazide MR as a first-line treatment (maximum dose of 120 mg). Gliclazide MR was added in overweight patients if hyperglycemia remained uncontrolled on metformin. Insulin therapy was recommended for patients whose HbA_{1c} remained over 7.0% on maximal doses of oral agents. Blood pressure management followed a similar stepwise approach to a target of 130/80 mmHg. All patients received ACE inhibitor therapy. A diuretic, calcium-channel blocker, or β-blocker was added as an additional agent. Lipid-lowering (statin) therapy was recommended to all patients, with the aim of reducing LDL cholesterol to a target of ≤2.5 mmol/l. Similarly, low-dose aspirin was recommended to all patients.

Blood pressure, HbA_{1c}, and research indexes were measured at baseline, 6 months, and 12 months. Lipid profile was measured at baseline and 12 months. Venous blood was obtained by atraumatic venepuncture into sodium citrate and was immediately centrifuged at 1,000*g* and 4°C for 20 min. Plasma was aliquoted and stored at −70°C for batch analysis.

Laboratory

Ang-1, Ang-2, VEGF, IL-6, and vWf were measured by enzyme-linked immunosorbent assay using commercial kits and reagents (R&D Systems, Abingdon, Oxon, U.K., and DakoPatts, Ely, Cambs, U.K.) as described fully elsewhere (3,4,18,21). All assays have intra- and interassay coefficients of variation of <5% and <10%, respectively. HbA_{1c} was measured by liquid chromatography (BioRad Variant 2; BioRad, Hertfordshire, U.K.). UACr was measured by immunoturbidimetry (I-Lab 600 Clinical Chemistry System; Instrumentation Laboratory, Warrington, U.K.) from single-void urine samples taken on two separate occasions at least 2 weeks apart. The intra- and interassay coefficients of variation of the latter assays were also <5% and <10%, respectively.

Power calculations

Because there are no data on plasma Ang-1 or Ang-2 levels in patients with diabetes or metabolic syndrome, we based our power calculation on our previous studies (3,4), hypothesizing differences in levels of Ang-1 and Ang-2 of similar magnitude to that of VEGF, i.e., at least half a standard deviation in logged data between patients with diabetes and control subjects. To achieve this, we needed data from a minimum of 34 subjects in each of the three groups for a two-sided $P < 0.05$ and $1\text{-}\beta = 0.90$. Serial pairwise measurements in 30 subjects provides the power to detect a change of half of a standard deviation at two-sided $P < 0.05$ and $1\text{-}\beta = 0.80$.

Statistical analysis

Continuous data were subjected to the Anderson-Darling test to determine their distribution. Non-normal data, presented as median and interquartile range, were analyzed by the Kruskal-Wallis test. Normally distributed data are presented as mean and SD and analyzed by ANOVA with Tukey's post hoc test. ANOVA was performed on log-transformed data for indexes that were not normally distributed. Categorical data were analyzed by the χ^2 test. Correlations within each group were sought using Spearman's method. In patients undergoing intensified diabetes and cardiovascular risk management, changes in indexes over the three time points (baseline, 6 months, and 12 months) were evaluated with Friedman's two-way repeated-measures ANOVA. Correlation coefficients were computed to assess the association between the change in VEGF and Ang-2 observed over the period of follow-up with changes in clinical and metabolic parameters. A stepwise multiple regression analysis was performed to assess the impact on research indexes of clinical variables in our patients with diabetes (including age, systolic and diastolic blood pressure, BMI, HbA_{1c}, triglyceride, total and HDL cholesterol, etc.). A multivariate analysis was also performed to determine whether changes in clinical or metabolic parameters (i.e., weight, systolic and diastolic blood pressure, HbA_{1c}, serum triglyceride, total and HDL cholesterol, etc.) were predictors of the changes in research indexes with intervention. Analyses and power calculations were performed using Minitab 13 (Minitab, State College, PA).

Table 1—*Clinical characteristics and research indexes in the study groups*

	Control subjects	Diabetic subjects without CVD	Diabetic subjects with CVD	*P*
n	34	56	38	—
Age (years)	66 ± 8	69 ± 6	68 ± 6	0.136
Males	19 (56)	31 (55)	24 (63)	0.451
Current smokers	—	7 (12.5)	8 (21.1)	0.266
BMI (kg/m^2)	25 (24–28)	29 (26–31)	25 (24–27)	0.001*
Systolic blood pressure (mmHg)	129 ± 16	137 ± 15	135 ± 19	0.064
Diastolic blood pressure (mmHg)	76 ± 8	78 ± 8	74 ± 11	0.222
HbA_{1c} (%)	5.4 (5.3–5.6)	7.3 (6.4–8.3)	7.6 (6.8–8.7)	<0.001†
LDL cholesterol (mmol/l)	3.1 ± 0.9	2.8 ± 1.0	2.5 ± 0.8	0.051
Triglyceride (mmol/l)	1.9 (1.1–3.1)	1.6 (1.2–2.7)	1.8 (1.2–2.6)	0.984
HDL cholesterol (mmol/l)	1.6 (1.3–2.0)	1.3 (1.1–1.4)	1.3 (1.0–1.6)	0.001†
UACr (mg/mmol)	—	1.4 (0.5–3.1)	1.3 (0.6–6.3)	0.590
Statins	—	12 (21)	10 (26)	0.583
Additional antihypertensive therapy	—	36 (64)	28 (74)	0.337
vWf (IU/dl)	120 ± 60	164 ± 80	176 ± 76	0.009†
IL-6 (pg/ml)	1.0 (1.0–2.5)	1.0 (1.0–5.7)	2.8 (1.0–19.3)	<0.001‡
VEGF (pg/ml)	90 (10–230)	180 (120–420)	200 (130–825)	0.001†
Ang-1 (ng/ml)	6.0 (2.3–24.0)	5.9 (2.9–19.0)	5.0 (1.5–15)	0.635
Ang-2 (ng/ml)	4.0 (2.5–5.0)	6 (3.9–8.9)	5.6 (3.5–7.6)	0.001†

Data are means ± SD, *n* (%), or medians (interquartile range) unless otherwise indicated. *Difference between patients without overt CVD and control subjects and patients with overt CVD but not between the control subjects and patients with overt CVD. †Significant between control subjects and each patient group but not between patient groups. ‡Higher in patients compared with control subjects but no significant difference between patient groups (Tukey's post hoc analysis of log-transformed data).

RESULTS — A total of 94 patients with diabetes (38 with and 56 without clinically overt CVD) and 34 age- and sex-comparable healthy control subjects were recruited (Table 1). As expected, HbA_{1c} was higher and HDL cholesterol was lower in the diabetic patients. There were no significant differences in lipid profile, use of statins and antihypertensive therapy, blood pressure, HbA_{1c}, or UACr between patients with or without established CVD. As expected, vWf (18) and VEGF (4) were higher in the diabetic patients (Table 1). Plasma Ang-2, but not Ang-1, was significantly higher in the diabetic patients.

Correlations and multivariate analysis

In the 56 patients free of overt CVD (Table 2), plasma Ang-2 correlated with vWf, UACr, and VEGF. Greaves et al. (18) previously reported a correlation between vWf and albumin excretion rate in diabetic subjects, and our data are consistent with this finding. VEGF ($r = 0.338$, $P = 0.012$; Fig. 1*A*) and Ang-2 ($r = 0.356$, $P = 0.007$; Fig. 1*B*) correlated with HbA_{1c}.

In contrast, Ang-2 failed to correlate with plasma vWf, UACr, and HbA_{1c} in the 38 patients with overt CVD (Table 2). As in patients without overt CVD, plasma vWf correlated with UACr and VEGF with HbA_{1c} ($r = 0.367$, $P = 0.024$; Fig. 1*C*). BMI and systolic and diastolic blood pressure did not correlate significantly with plasma VEGF and Ang-2 in both patient groups.

In a stepwise multiple regression analysis incorporating clinical variables

Table 2—*Spearman correlations between plasma vWf, VEGF, and Ang-2 levels, with HbA_{1c} and UACr*

	UACr	HbA_{1c}	VEGF	Ang-2	vWf
In 56 patients without overt CVD					
HbA_{1c}	$r = -0.024$ $P = 0.862$				
VEGF	$r = 0.230$ $P = 0.092$	$r = 0.338$ $P = 0.012$			
Ang-2	$r = 0.307$ $P = 0.021$	$r = 0.356$ $P = 0.007$	$r = 0.693$ $P = 0.001$		
vWf	$r = 0.403$ $P = 0.005$	$r = -0.008$ $P = 0.957$	$r = 0.271$ $P = 0.065$	$r = 0.398$ $P = 0.005$	
IL-6	$r = -0.157$ $P = 0.248$	$r = 0.028$ $P = 0.840$	$r = 0.230$ $P = 0.094$	$r = 0.119$ $P = 0.387$	$r = -0.127$ $P = 0.356$
In 38 patients with overt CVD					
HbA_{1c}	$r = -0.378$ $P = 0.019$				
VEGF	$r = 0.258$ $P = 0.117$	$r = 0.367$ $P = 0.024$			
Ang-2	$r = 0.189$ $P = 0.256$	$r = 0.173$ $P = 0.299$	$r = 0.309$ $P = 0.059$		
vWf	$r = 0.444$ $P = 0.014$	$r = -0.307$ $P = 0.026$	$r = 0.451$ $P = 0.012$	$r = 0.269$ $P = 0.151$	
IL-6	$r = -0.080$ $P = 0.632$	$r = -0.093$ $P = 0.580$	$r = 0.054$ $P = 0.749$	$r = 0.174$ $P = 0.297$	$r = 0.082$ $P = 0.629$

r, Spearman's correlation coefficient.

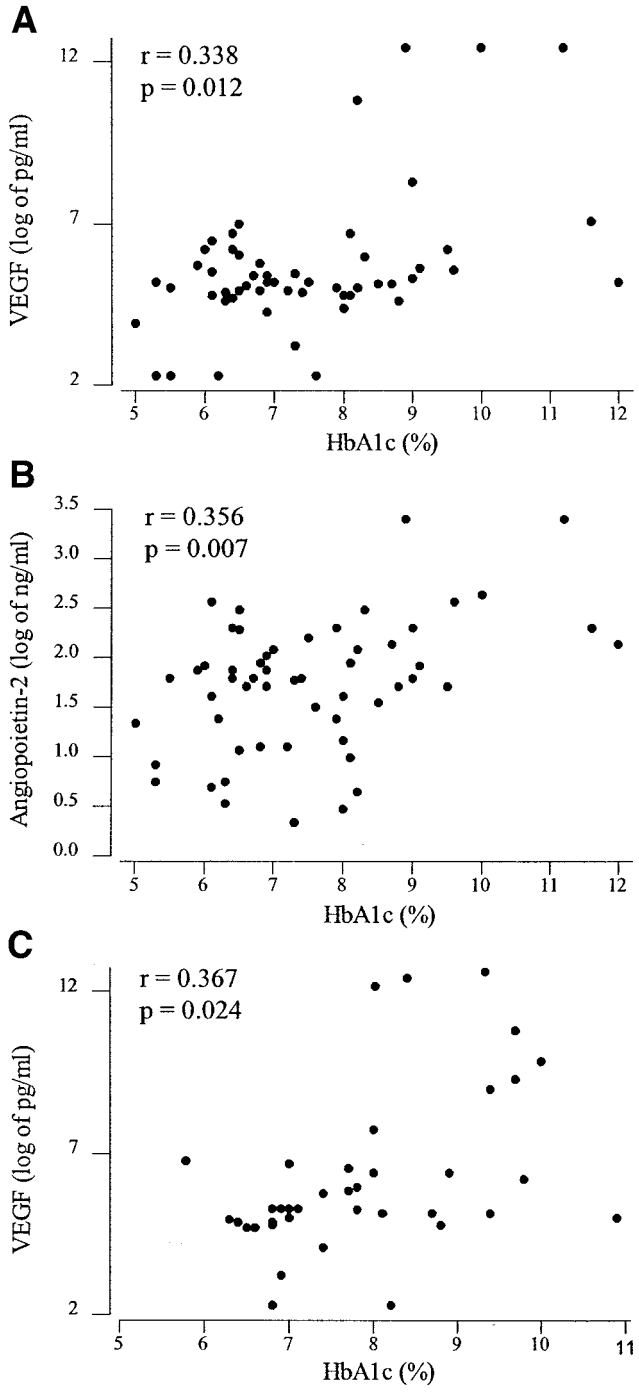

Figure 1—A*: Correlation between plasma VEGF and HbA_{1c} in patients without overt CVD* (n = *56).* B*: Correlation between Ang-2 and HbA_{1c} in patients without overt CVD* (n = *56).* C*: Correlation between plasma VEGF and HbA_{1c} in patients with overt CVD* (n = *38).*

only, HbA_{1c} was the only significant predictor of plasma VEGF ($P = 0.032$), whereas HbA_{1c} ($P = 0.015$) and HDL cholesterol ($P = 0.024$) were independent predictors of Ang-2 levels ($P = 0.015$). However, when the stepwise multiple regression analysis for Ang-2 levels also incorporated UACr, plasma vWf, IL-6, and VEGF into the statistical model, plasma VEGF was the only significant predictor of plasma Ang-2 levels ($P < 0.001$). Similarly, for VEGF levels, a model that also incorporated UACr, plasma vWf, IL-6, and Ang-2 found that plasma Ang-2 ($P < 0.001$), HbA_{1c} ($P = 0.009$), and HDL cholesterol ($P = 0.042$) independently predicted plasma VEGF levels.

Effects of intervention

A total of 33 patients with and 31 patients without overt CVD participated in the intervention study over 1 year. Statin use increased significantly in both groups, which was associated with significant reductions in total and LDL cholesterol. HbA_{1c} fell significantly from baseline to 12 months in both groups. Aspirin use increased significantly in patients with CVD. Blood pressure was generally well controlled, although there was no significant change from baseline. There was also no significant weight change.

In the subgroup of 31 patients without overt CVD (Table 3), the improvement in these metabolic parameters was associated with significant reductions in vWf, median plasma VEGF, and Ang-2 levels from baseline to 12 months, although the intermediate changes were not significant.

In the 33 patients with overt CVD (Table 4), plasma vWf and VEGF fell significantly, but there was no significant change in Ang-2 levels. The changes in VEGF and Ang-2 from baseline to 6 months or from 6 to 12 months were not statistically significant. There was no significant change in plasma IL-6 levels in patients with or without overt CVD.

Using a multivariate analysis, the reduction in plasma VEGF levels in patients without overt CVD was independently related to changes in HbA_{1c} ($P < 0.001$) and serum triglyceride levels ($P = 0.002$), but not to total cholesterol ($P = 0.276$) and weight change ($P = 0.097$). The reduction in Ang-2 levels was only related to change in HbA_{1c} ($P = 0.008$). In patients with overt CVD, a reduction in VEGF levels was only related to the decrease in HbA_{1c} levels ($P = 0.004$).

CONCLUSIONS — In this study, we have shown that in patients with type 2 diabetes, *1*) there is a profile of raised plasma VEGF and Ang-2 but not Ang-1; *2*) plasma levels of VEGF and Ang-2 are related to indexes of metabolic control and endothelial damage/dysfunction; and *3*) intensified multifactorial intervention is associated with reductions in plasma VEGF and Ang-2 in patients without CVD, but only VEGF fell in patients with overt CVD, suggesting an altered relation-

Table 3—*Effects of intensified diabetes and cardiovascular risk management in the subgroup of 31 patients without overt CVD*

	Baseline	6 months	12 months	*P*
Systolic blood pressure (mmHg)	136 ± 13	135 ± 10	135 ± 9	0.900
Diastolic blood pressure (mmHg)	76 ± 11	75 ± 8	77 ± 8	0.800
Weight (kg)	89 ± 15	—	88 ± 15	0.288
HbA_{1c} (%)	7.7 ± 1.2	7.3 ± 1.0	6.9 ± 0.9	<0.001*
Total cholesterol (mmol/l)	5.3 ± 1.2	—	4.1 ± 0.5	<0.001†
LDL cholesterol (mmol/l)	3.1 ± 1.0	—	2.1 ± 0.5	<0.001†
Triglycerides (mmol/l)	1.8 (1.2–3.0)	—	1.4 (1.1–2.0)	0.039†
HDL cholesterol (mmol/l)	1.23 ± 0.22	—	1.28 ± 0.24	0.480
Smokers (*n*)	5/31	—	3/31	0.449
Statin use (*n*)	4/31	—	22/31	<0.001
Aspirin use (*n*)	11/31	—	16/31	0.200
Metformin use (*n*)	6/31	—	22/31	<0.001
VEGF (pg/ml)	300 (150–250,000)	200 (100–800)	130 (60–240)	<0.001‡
Ang-2 (ng/ml)	6.0 (3–10)	3.7 (2.4–8.5)	4.4 (2.4–5.9)	0.001‡
vWf (IU/ml)	162 ± 66	—	86 ± 23	<0.001†
IL-6 (pg/ml)	1.0 (1.0–4.2)	—	1.0 (1.0–3.6)	0.784

Data are means ± SD or medians (interquartile range) unless otherwise indicated. *Significant difference at baseline 6 months, baseline 12 months, and 6–12 months. †Significant difference baseline 12 months. ‡Significant difference baseline 6 months and baseline 12 months, but no significant difference 6–12 months.

ship between growth factors and metabolic control by overt disease.

Our data implicate raised blood glucose (among the clinical and metabolic variables) as the primary determinant of circulating VEGF and Ang-2 levels. Raised blood glucose in diabetes exerts toxic effects on the endothelium through a number of mechanisms. By increasing substrate flux through the sorbitol pathway, elevated blood glucose can induce a hyperglycemic pseudo-hypoxic state that may induce VEGF production (22). The accelerated formation and accumulation of glycation products associated with raised blood glucose may also upregulate VEGF and Ang-2 mRNA in vitro (23). Therefore, our finding of selective elevation of plasma VEGF and Ang-2 and their relationship with HbA_{1c} supports these in vitro observations.

Experimental studies suggest that VEGF may stimulate the expression of adhesion molecules by endothelial cells and promote vascular inflammation (24,25). Ang-1 has been shown to inhibit leukocyte adhesion in vitro through the suppression of VEGF-induced upregulation of adhesion molecules (24). Ang-2 as an antagonist of Ang-1 (14) may accentuate the effects of VEGF on endothelial cells. Hence, selective increase in VEGF and Ang-2 may reflect more adverse vascular endothelial perturbations. In this study, plasma VEGF and Ang-2 correlated with indexes of endothelial damage/dysfunction, but not inflammation, on univariate analysis. This discordance may reflect treatment with a combination of antihyperglycemic, antihypertensive, and lipid-lowering therapy, which may have variable impacts on different aspects of vascular biology in different patients (as shown by the different effects of treatment on plasma VEGF, Ang-2, vWf, and IL-6 in this study), hence altering the relationship between these circulating indexes.

Furthermore, the relationship between VEGF and Ang-2 with endothelial damage/dysfunction, although significant on univariate analysis, became nonsignificant on multivariate analysis (when the statistical model included VEGF for Ang-2 and vice versa). These data suggest that the relationship between VEGF and Ang-2 with endothelial damage/dysfunction may be at least partly mediated by Ang-2 and VEGF, respectively. Indeed, this supports an interaction between VEGF and Ang-2 in the relationship between angiogenesis and endothelial damage/dysfunction.

Intensive multifactorial intervention is associated with significantly improved cardiovascular outcome (19). Our findings are consistent with these clinical observations, and it is tempting to speculate that improvements in cardiovascular outcomes may be related to reductions in these angiogenic growth factors and associated improvement in endothelial abnormalities. Of note, plasma Ang-2 was not reduced significantly with treatment in patients with overt CVD (unlike patients free of overt CVD). These data suggest that the relationship between Ang-2, glycemia, and cardiovascular risk may be altered by the presence of overt disease and

Table 4—*Effects of intensified diabetes and cardiovascular risk management in the subgroup of 33 patients with established CVD*

	Baseline	6 months	12 months	*P*
Systolic blood pressure (mmHg)	137 ± 18	136 ± 16	136 ± 15	0.945
Diastolic blood pressure (mmHg)	75 ± 11	74 ± 9	75 ± 8	0.858
Weight (kg)	79 ± 13	—	80 ± 13	0.163
HbA_{1c} (%)	7.7 (7.1–8.5)	7.5 (6.7–8.5)	7.1 (6.6–7.5)	<0.001*
Total cholesterol (mmol/l)	4.9 ± 0.9	—	4.4 ± 1.0	0.014†
LDL cholesterol (mmol/l)	2.7 ± 0.8	—	2.3 ± 0.9	0.002†
Triglycerides (mmol/l)	1.6 (1.2–2.6)	—	1.6 (1.0–2.7)	0.814
HDL cholesterol (mmol/l)	1.26 ± 0.35	—	1.24 ± 0.30	0.862
Smokers (*n*)	6/33	—	3/33	0.282
Statin use (*n*)	8/33	—	22/33	0.001†
Aspirin use (*n*)	19/33	—	28/33	0.014
Metformin use (*n*)	18/33	—	25/33	0.071
VEGF (pg/ml)	560 (300–34,500)	400 (210–3,250)	300 (170–1,500)	<0.001*
Ang-2 (ng/ml)	5.3 (2.2–7.3)	5.3 (2.9–6.9)	4.2 (2.2–6.4)	0.599
vWf (IU/ml)	161 ± 64	—	120 ± 60	0.003†
IL-6 (pg/ml)	1.0 (1.0–10.0)	—	1.0 (1.0–9.0)	0.576

Data are means ± SD or medians (interquartile range) unless otherwise indicated. *Significant difference baseline 1 year but not baseline 6 months or 6–12 months. †Significant difference baseline 12 months.

emphasizes the benefits of early intensive intervention.

In this study, glycemic control, but not cholesterol-lowering per se, appeared to be the primary determinant of reductions in plasma VEGF and Ang-2, although lipid-lowering was previously shown to be associated with reductions in vWf (26) and VEGF (27). However, different study populations (with lower preintervention cholesterol levels in our study) may have contributed to these different findings. Furthermore, our study was not designed (and may not be adequately powered) to assess the contributions of the individual components of this intervention strategy.

Study limitations

Our study has limitations that merit consideration. First, our study did not include a control group not receiving the "package of care" to compare treatment effect. However, ethical concerns precluded such a comparison, with the current weight of evidence in support of multifactorial risk management in these high-risk patients (28,29). Second, our study was designed to evaluate the effect of a currently recommended "package of care" intervention, and, therefore, we cannot confirm the individual component(s) responsible for the changes in our research indexes. Third, our study relied on the willing participation of the patients, which may have contributed to patient selection bias, as reflected by (for instance) the relatively good blood pressure control. Fourth, we did not define or quantify CVD by objective measures, such as coronary, peripheral, or carotid angiography, because it would not be ethical to subject asymptomatic patients to such invasive procedures. Therefore, we were unable to define the relationship between our research indexes and the extent of atherosclerotic disease or evaluate asymptomatic/subclinical disease in patients without overt CVD. Finally, the strength of our study depends on clinical efficacy in the treatment groups. The blood pressure, glycemic, and cholesterol reductions achieved in this study were at least comparable if not better than that of recent studies in patients with diabetes (30–34), although we do accept that this remains short of current recommended targets (28,35).

In conclusion, plasma VEGF and Ang-2 (but not Ang-1) are selectively elevated in patients with diabetes regardless of CVD. Intensified multifactorial intervention is associated with reductions in plasma VEGF and (in patients without overt CVD) Ang-2 levels, suggesting improvement in vascular profile with this treatment strategy, particularly before the development of clinically overt vascular disease.

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