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The effect of statin therapy on vessel wall properties in type 2 diabetes without manifest cardiovascular disease

Beishuizen, E.D.

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Chapter 6

The impact of metabolic syndrome and CRP on vascular phenotype in type 2 diabetes mellitus

Members of the DALI Study Group:
Erasmus Medical Centre Rotterdam,
Department of Internal Medicine
(I. Berk-Planken, N. Hoogerbrugge,
H. Jansen); Erasmus University
Rotterdam, Departments of
Biochemistry and Clinical Chemistry
(H. Jansen); Gaubius Laboratory
TNO-PG, Leiden (H. M. G. Princen);
Leiden University Medical Center
(M. V. Huisman, M. A. van de Ree);
University Medical Centre Utrecht,
Julius Centre for General Practice
and Patient Oriented Research
(R. P. Stolk, F. V. van Venrooij);
University Medical Centre Utrecht,
Division of Internal Medicine
(J. D. Banga, G. Dallinga-Thie,
F.V. van Venrooij).

R Alizadeh Dehnavi¹, ED Beishuizen¹,
MA van de Ree², S Le Cessie³, MV Huisman¹,
C Kluft⁴, HMG Princen⁴, JT Tamsma¹

¹ Department of General Internal Medicine and
Endocrinology, Leiden University Medical
Center

² Department of Internal Medicine,
Diaconessenhuis, Utrecht

³ Department of Medical Statistics and Bio-
Informatics, Leiden University Medical Center

⁴ Gaubius Laboratory TNO-QoL, Leiden

The Netherlands

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ABSTRACT

Objective

The burden of cardiovascular disease in diabetes mellitus type 2 (DM2) patients is variable. We hypothesize that metabolic syndrome (MS) and low-grade systemic inflammation modify the extent of atherosclerosis in DM2.

Research Design and Methods

Vascular phenotype was determined using the following endothelium-related, hemostatic, and sonographic endpoints in 62 DM2 patients with mild dyslipidemia: sVCAM, sE-selectin, von Willebrand factor (VWF), fibrinogen, s-thrombomodulin (sTM), tissue type Plasminogen Activator (tPA), Plasminogen Activator Inhibitor-1 (PAI-1), flow mediated dilation (FMD), and intima-media thickness (IMT). The impact of MS load (number of criteria present), MS components, and CRP on these parameters was assessed.

Results

Serum sVCAM, sTM, and tPA levels significantly increased with increasing MS load. IMT also significantly increased from 0.602 ± 0.034 (one MS criterion) to 0.843 ± 0.145 (four MS criteria, $p = 0.007$). LogCRP significantly correlated with fibrinogen, PAI-1, and IMT. In a multiple regression model with age and gender as covariates, MS load predicted sVCAM and sTM; CRP predicted PAI-1 and fibrinogen; MS load and CRP simultaneously predicted tPA and IMT. For each MS criterion present, IMT significantly increased by 0.04 mm. An increase in CRP from 1 to 3 mg/L resulted in a significant increase of 0.04 mm. Patients with four MS criteria and inflammation ($\text{CRP} \geq 3 \text{ mg/L}$) are predicted to have a 0.21 mm thicker IMT than those without. A second stepwise multiple regression analysis based on gender, traditional risk factors, diabetes-related parameters, renal function, individual MS criteria, and LogCRP as explanatory variables showed a significant effect of systolic and diastolic blood pressure, HDL, and LogCRP on IMT ($r^2 = 0.36$, $p < 0.001$).

Conclusions

MS and low-grade chronic inflammation have an independent impact on vascular phenotype including IMT in DM2.

INTRODUCTION

Patients with diabetes mellitus type 2 (DM2) are known to suffer from increased rates of cardiovascular disease¹⁻⁴. DM2 has even been regarded as a cardiovascular risk equivalent⁵. However, the burden of cardiovascular disease can vary considerably within this population. The metabolic syndrome (MS) is often present in DM2 patients and frequently coexists with elevated C-reactive protein (CRP) levels, a measure of chronic, low-grade inflammation⁶. Variability in MS load (a rough measure for the extent of metabolic dysregulation), defined as the number of MS criteria present, and the level of CRP has also been shown in DM2 patients. MS and CRP are both associated with increased cardiovascular morbidity and mortality but are not interchangeable and may even have additional predictive value⁷. CRP has been related to atherosclerotic vessel wall structural changes such as intima-media thickness (IMT) in several non-diabetic cohorts⁸⁻¹². Moreover, the regulation of CRP has been argued to be independent of MS and is thought to be mostly due to inherited traits¹³. Thus, low-grade systemic inflammation could have a potential independent impact on vascular phenotype in DM2 patients. On the other hand, MS load has been directly related to coronary calcium scores, reflecting total body atherosclerotic burden in non-diabetic subjects¹⁴. MS also modified coronary heart disease prevalence in DM2 patients in an epidemiological study⁴. We hypothesize that MS and low-grade systemic inflammation, as measured by CRP, modify the extent of atherosclerotic disease burden in DM2 patients. We set out to study the impact of MS components, MS load, and CRP levels on endothelial, hemostatic, and ultrasonographically assessed vascular wall parameters in DM2.

RESEARCH DESIGN AND METHODS

Study design

This study was a single-center sub-study of the previously reported DALI study¹⁵. The study was carried out in accordance with the principles of the Declaration of Helsinki. The local medical ethics committee approved the study and all patients gave informed consent.

Patients

Male and female patients aged 45–75 years with DM2 (according to the American Diabetes Association classification) of at least 1-year duration were included in the study. Inclusion criteria were: fasting triglycerides between 1.5 and 6.0 mmol/L, total cholesterol between 4.0 and 8.0 mmol/L, and HbA1c < 10%. Patients were assessed after a washout period of lipid-lowering medication of at least 8 weeks. Patients with manifest or previous cardiovascular disease were excluded from the study. Pre-menopausal women and patients with acute liver disease, hepatic dysfunction, or impaired renal function (plasma creatinine > 150 µmol/L)

were excluded. Patients consuming more than four alcoholic drinks per day or on systemic steroids, androgens, cyclosporine, other immunosuppressive drugs, erythromycin, or mibefradil were also excluded.

Definition of MS criteria cut-off values and low-grade chronic systemic inflammation

The WHO cut-off values were used for the assessment of MS load¹⁶. The criteria were defined as follows: hypertension: systolic/diastolic $\geq 140/90$ (mm Hg); triglyceride levels ≥ 1.7 mmol/L; and HDL cholesterol < 1.0 mmol/L (women) and < 0.9 mmol/L (men). Furthermore, waist circumference was used as a measure for obesity with a cut-off value of ≥ 94 cm in men and ≥ 80 cm in women. MS load was defined by the number of factors present exceeding the thresholds defined above, in addition to DM2. Five groups with respectively 0, 1, 2, 3, or 4 additional MS criteria could be defined. As for CRP, patients with levels over 15 mg/L were excluded from the evaluations on the suspicion of being a temporary outlier not representing the continuous habitual level¹⁷.

Endpoints

The impact of MS and CRP on vascular phenotype in DM2 was assessed using sonographic vascular parameters, IMT, and flow mediated dilatation (FMD), as well as the following endothelium-related and hemostatic factors: fibrinogen, s-thrombomodulin (sTM), tissue type plasminogen-activator (tPA), plasminogen-activator inhibitor-1 (PAI-1), sVCAM, sE-selectin, and von Willebrand factor (VWF).

Laboratory investigations

Blood sampling and plasma lipid measurements, according to standard protocols, have been described previously¹⁵. CRP was measured in citrate, theophylline, adenosine, dipyridamol (CTAD) plasma with an enzyme immunoassay (EIA) using polyclonal antibodies (Dako, Copenhagen, Denmark) showing an intra- and inter-assay coefficient of variation around 2 mg/L of 2.7 and 4.3%, respectively¹⁷. Functional fibrinogen was measured in citrated plasma using a clotting rate method, essentially according to Clauss¹⁸. PAI-1 antigen was determined in CTAD plasma using an EIA (Innotest PAI-1, Innogenetics, Temse, Belgium)¹⁹. VWF antigen was measured in CTAD plasma with an in-house EIA using polyclonal rabbit antibodies to human VWF (DAKO, Copenhagen, Denmark)²⁰. tPA antigen was determined in citrated plasma using an EIA (Immulyse™; Biopool, Umea, Sweden). An EIA was also used to measure sVCAM (Quantikine human sVCAM-1 from R&D systems, Abingdon, UK), sTM (Asserachrom s-Trombomodulin, from Diagnostica Stago, Asnières, France), and sE-selectin (Quantikine human sE-selectin from R&D systems, Abingdon, UK).

Flow mediated dilatation (FMD) and intima-media thickness (IMT)

These measurements were performed as described previously²¹. Patients were in a fasting state, did not use tobacco on the morning of the evaluation, and rested in the supine position for 10 min before the study. FMD of the brachial artery was obtained using three baseline images frozen on the R-wave of the electrocardiogram. After the induction of ischemia, R-wave-triggered brachial artery images were frozen and recorded every 15 s for 5 min (reactive hyperemic period). All images were analyzed off-line in a standardized and blinded manner.

For the measurement of IMT, patients were examined in the supine position with the neck slightly extended and rotated in the opposite direction. A 7.5-MHz linear array transducer was used (Aloka SD1400). A three-lead ECG was attached for R-wave triggering. Subsequently, the right and left distal 10 mm of the common carotid artery (CCA) were visualized under the angle with the clearest image of near and far walls. Three images were frozen on the R-wave of the ECG (end-diastole) and stored on S-VHS videotapes for off-line IMT analysis. Images from the videotape were displayed on a personal computer and the optimal images digitized. The mean carotid IMT of the distal 10 mm of the CCA was measured using the Artery Measurement System, which semi-automatically traced the trailing edges on the near wall and the leading edges of the far wall to provide for near and far wall IMT. The measurements of near walls and far walls, left and right, were averaged to provide an individual IMT.

Statistical analysis

Means between the groups were analyzed using ANOVA including an analysis for a linear trend. Percentages were compared using the chi-square test for trend. Since the distribution of CRP was skewed, a logarithmic transformation (log 10) was performed and used in correlation analysis and multiple regression analysis. Pearson's correlations were calculated for LogCRP and fibrinogen, sVCAM, sTM, tPA, PAI-1, sE-selectin, VWF, FMD, and IMT. A multiple regression analysis was performed with MS load and LogCRP as independent variables, age and gender as covariates, and the abovementioned parameters as dependent variables. In the case of sTM, renal clearance (calculated using the Cockcroft formula) was also added as a covariate as its plasma levels are known to be dependent on renal function²²⁻²⁴. The concept of MS load results in a binary response due to the fixed thresholds used for each parameter. Furthermore, the impact of traditional risk factors, diabetes-related factors, and renal function should be taken into account with regard to vascular phenotype. Therefore, a second stepwise multiple regression analysis was performed to examine the impact of the MS criteria used as continuous variables taking diabetes and cardiovascular risk factors into account. The following variables were used: traditional risk factors (age, gender, and cholesterol), waist circumference, systolic/diastolic blood pressure, HDL, triglycerides, fasting blood glucose, inflammation (LogCRP), DM2-related parameters (duration of DM2 and HbA1c) and, finally, renal function (Cockcroft clearance). Possible interactions were systematically assessed. As no relevant interactions were observed, none was taken into account in the final models. The

level of significance was set at $p < 0.05$. All analyses were performed using SPSS for Windows software, version 11.0.1.

RESULTS

Patient characteristics

Patient characteristics are given in Table 1. There were no patients without additional MS criteria. In addition to DM2, one other MS criterion was observed in 4 patients; two additional MS criteria were observed in 12 patients; three in 30 patients; and four in 16 patients. Hypertension, body mass index (BMI), waist circumference, weight, HDL cholesterol, and triglycerides significantly differed between the groups with different MS loads. A trend to differences was observed for fasting blood glucose. Renal function was similar for the groups.

Effect of MS load and CRP on vascular parameters (Table 2)

Mean sVCAM, fibrinogen, sTM, and tPA levels differed between the groups. The plasma levels of sVCAM, sTM, and tPA significantly increased with increasing MS load. LogCRP correlated with fibrinogen and PAI-1. Endothelial function as assessed by FMD was not significantly influenced by MS load or by inflammation. Imaging of the carotid artery yielded a mean IMT

Table 1 Patient characteristics and laboratory results.

	All patients (n=62)	DM + 1 MS criterion (n=4)	DM + 2 MS criteria (n=12)	DM + 3 MS criteria (n=30)	DM + 4 MS criteria (n=16)	P value
Age (years)	59.6±6.98	55.5±3.70	59.8±6.86	59.7±6.66	60.4±8.34	0.66
Male/Female (n/n)	38/24	4/0	7/5	16/14	11/5	0.30
Current smoking (%)	21.0	0.0	41.7	23.3	6.3	0.72
Blood pressure (mmHg)						
Systolic	143.5±19.3	112.3±16.2	133.6±13.3	145.5±13.6	154.4±22.5	<0.001
Diastolic	84.2±9.1	76.0±9.8	77.1±10.5	85.8±7.1	87.9±7.9	0.002
Waist (cm)	105.4	86.0	104.3	105.2	111.4	0.004
BMI (kg/m ²)	30.8±5.2	22.86±1.8	31.1±6.4	30.9±4.0	32.5±5.4	0.007
Weight (kg)	90.9±16.5	68.8±8.0	91.2±19.9	89.6±12.3	98.8±17.5	0.008
HbA1c (%)	9.2±1.4	7.8±1.2	9.2±1.4	9.4±1.5	9.2±1.3	0.24
Glucose (mmol/L)	10.7±2.9	7.1±1.5	11.1±3.1	11.1±3.0	10.6±2.4	0.06
Cockcroft clearance (ml/min)	97.96±30	79.63±18	103.30±39	97.72±29	98.98±28	0.61
Total Cholesterol (mmol/L)	6.01±0.93	5.93±0.61	6.48±0.72	5.88±1.05	5.96±0.84	0.29
HDL cholesterol (mmol/L)	1.04±0.22	1.18±0.25	1.20±0.22	1.06±0.18	0.83±0.10	<0.001
Triglycerides (mmol/L)	2.50±0.88	2.22±0.68	1.98±0.56	2.32±0.65	3.31±0.99	<0.001

Data are means ± SD, except for gender and current smoking which are given as percentage.

Table 2 MS load and CRP vs vascular parameters

Factor	DM+1 MS criterion	DM+2 MS criteria	DM+3 MS criteria	DM+4 MS criteria	p-value difference between groups	p-value linearity	Correlation of factors with logCRP
sVCAM (µg/L)	427.9 (63.3)	389.5 (74.4)	453.8 (130.9)	510.5 (89.7)	0.04	0.01	NS
sE-selectin (µg/L)	54.1 (16.0)	59.9 (23.6)	59.8 (26.3)	66.7 (20.6)	NS	NS	NS
VWF-ag (%)	105.3 (38.3)	122.0 (52.3)	128.9 (53.4)	136.4 (47.7)	NS	NS	NS
fibrinogen (g/L)	2.91 (0.22)	3.90 (0.79)	3.72 (0.56)	3.91 (0.57)	0.03	NS(0.07)	r=0.62 p<0.001
sTM (µg/L)	27.85 (7.25)	28.31 (9.13)	25.98 (11.45)	38.70 (13.75)	0.008	0.03	NS
tPA (µg/L)	9.55 (1.92)	12.25 (2.34)	12.70 (2.80)	15.01 (4.37)	0.01	0.002	NS
PAI-1 (µg/L)	69.1 (28.8)	115.2 (81.9)	123.7 (73.1)	145.9 (83.3)	NS	NS(0.08)	r=0.37 p=0.003
Sonography							
FMD (%)	1.37 (1.88)	3.29 (1.31)	3.28 (5.29)	4.30 (3.43)	NS	NS	NS
IMT (mm)	0.602 (0.034)	0.794 (0.086)	0.800 (0.134)	0.843 (0.145)	0.01	0.007	r=0.29 p=0.02

Data are means (SD). NS = not significant

of 0.797 ± 0.135 mm. IMT was different between the groups and significantly increased with increasing MS load. Furthermore, IMT significantly correlated with LogCRP (Table 2).

Multiple regression analysis

1. Model including age, gender, MS load, and CRP (Table 3, model 1)

A multiple regression analysis was performed to assess the impact of MS load and LogCRP on the endpoints of the study using age and gender as covariates. The significant models are summarized in Table 3, model 1. MS load was a significant explanatory variable in the model for: sVCAM (β : 48.56[16.76, 80.37]) and sTM (β : 4.39[1.13, 7.64]). CRP significantly predicted plasma levels of fibrinogen (β : 0.86[0.50, 1.22]) and PAI-1 (β : 65.67[14.94, 116.40]). MS and CRP predicted tPA and IMT. For each MS criterion present, IMT significantly increased by 0.04 mm (SE 0.02, $p = 0.03$). In addition, an increase in CRP from 1 mg/L (log 1 = 0) to 3 mg/L (log 3 = 0.47) resulted in an increase of IMT by 0.04 mm ($0.47 * 0.095$). Thus, patients with four MS criteria and a CRP level of 3 mg/L are predicted to have a 0.21 mm thicker IMT than DM2 patients without MS and a CRP level of 1 mg/L. This implies a higher risk of reaching the upper limit of normal IMT (0.90 mm) in these patients²⁵.

2. Model based on gender, traditional risk factors, renal function, MS criteria, inflammation, and diabetes-related parameters (Table 3, model 2)

A stepwise, multiple regression approach was used including traditional risk factors, diabetes-related factors, and renal function as independent factors in addition to the individual MS criteria and LogCRP. Significant models were observed for all variables except FMD ($p = 0.05$). The predictive power was comparable to model 1 for sVCAM, fibrinogen, and tPA. The model improved for PAI-1 (explanatory variables: LogCRP, diabetes duration and HbA1c) and IMT. LogCRP (β : 0.09[0.007, 0.164]) and the following MS criteria were the significant determinants of IMT: systolic (β : 0.003[0.001, 0.005]) and diastolic (β : -0.007[-0.011, -0.003]) blood pressure and HDL (β : -0.27[-0.44, -0.11]); triglycerides contributed non-significantly: (β : -0.03)[-0.068, 0.013]). The model explained 36% ($p < 0.001$) of IMT. A decrease of 0.1 mmol/L HDL cholesterol increased IMT by 0.27 mm. The impact of CRP on IMT is very similar in model 1 and model 2 (β : 0.095 vs 0.09, respectively). Traditional risk factors and diabetes-related factors did not significantly contribute to the model explaining IMT in these patients.

Table 3. Multiple regression models

Dependent variable	Model 1 r^2 (p-value)	Model 2 r^2 (p-value)	Explanatory variables model 2
sVCAM ($\mu\text{g/L}$)	0.25 (0.002)	0.20 (0.03)	Gender ^a , age, cholesterol, diastolic blood pressure, and LogCRP
sE-Selectin ($\mu\text{g/L}$)	NS	0.30 (0.003)	HbA1c ^a , gender, DM2 duration, systolic blood pressure, Triglycerides, Cockcroft clearance
VWF-ag (%)	NS	0.13 (0.02)	HbA1c ^a , LogCRP
Fibrinogen (g/L)	0.41 (< 0.001)	0.46 (< 0.001)	Cholesterol ^a , LogCRP ^a , and systolic blood pressure
sTM ($\mu\text{g/L}$)	0.35 ^b (< 0.001)	0.47 (< 0.001)	Gender ^a , waist circumference ^a , age, cholesterol, HbA1c, and Cockcroft clearance
tPA ($\mu\text{g/L}$)	0.26 (0.001)	0.29 (0.001)	Gender ^a , Triglycerides ^a , LogCRP ^a , age, and cholesterol
PAI-1 ($\mu\text{g/L}$)	0.16 (0.04)	0.30 (< 0.001)	LogCRP ^a , DM2 duration ^a , HbA1c ^a , and HDL
FMD (%)	NS	0.10 (0.05)	Triglycerides ^a , and gender
IMT (mm)	0.24 (0.003)	0.36 (< 0.001)	Systolic ^a /diastolic ^a blood pressure, HDL ^a , LogCRP ^a , and Triglycerides

Model 1: Age, gender, MS load, LogCRP.

Model 2: Stepwise model based on gender, traditional risk factors, renal function, MS criteria, inflammation, and diabetes-related parameters.

^a: Significant explanatory variable; the other factors listed were found to have high betas, thus contributing to the model, but they did not reach the predefined level of significance.

^b: Model using renal function as covariate, as described in text.

CONCLUSIONS

In this study, we observed that MS load, the presence of the individual defining criteria, and chronic low-grade inflammation independently contributed to vascular phenotype in this

DM2 population. The data support the hypothesis that, although partly interrelated, metabolic and inflammatory pathways both modify vascular phenotype.

MS is estimated to be present in 75–80% of DM2 patients and has been shown to influence cardiovascular risk^{4,26} and fibrinolytic function^{27,28}. In patients with MS but without diabetes, increased measures of atherosclerosis^{14,29–31} and a higher incidence of cardiovascular events have been observed^{27,32}. This has partly been attributed to impaired fibrinolytic function²⁷ due to altered regulation of PAI-1, tPA, and fibrinogen^{28,33,34}. In patients with MS, it has been shown that most procoagulant features are associated with anthropometry²⁸, while fibrinogen is associated with inflammation²⁸. High tPA and PAI-1 levels have previously been reported in DM2³⁵. In line with these studies, we observed that tPA levels depended on both MS load and CRP, while fibrinogen and PAI-1 levels were solely explained by CRP. For tPA, model 2 (Table 3) points to triglycerides as the most relevant MS-related predicting factor. Diabetes-related factors were significant predictors for PAI-1 in addition to CRP, while none of the MS-defining criteria seemed to contribute to fibrinogen and PAI-1 levels. Thus, the main inhibitor of fibrinolysis was found to be severely influenced by the state of diabetes *per se* in alliance with low-grade systemic inflammation as measured by CRP.

An increased IMT was observed with increasing MS load, compatible with an increased burden of atherosclerosis (Table 3, model 1). Model 2 revealed that systolic and diastolic blood pressure and HDL were the MS criteria significantly defining IMT. HDL cholesterol levels had a strong inverse relationship with IMT, underlining the potential importance of HDL and reverse cholesterol transport pathways in mildly dyslipidemic DM2 patients. Previous studies reported on the positive relation between MS and IMT: in healthy children without any other risk factors⁸; in adult male dyslipidemic patients⁹; and in non-diabetic adults with cardiovascular disease or at high-risk of cardiovascular disease^{10–12}. Our findings extend these observations to mildly dyslipidemic patients with DM2. Furthermore, our data support previously published reports showing that the presence of MS is strongly associated with the prevalence of cardiovascular disease in subjects with DM2⁴.

MS has been found to be associated with low-grade systemic inflammation as assessed by CRP. CRP is thought to provide additional predictive information regarding cardiovascular risk in patients with MS⁷. The current study shows that, in the setting of DM2, CRP is directly related to vascular wall abnormalities and the abovementioned adverse changes in the fibrinolytic system. We observed that low-grade chronic systemic inflammation contributed to IMT in addition to the effects of MS and that, together, they could predict 36% of IMT ($p < 0.001$).

All parameters studied could have been influenced by the state of DM2 *per se*. This was indeed found to be the case for PAI-1 (Table 3, model 2). However, the state of diabetes *per se* is also likely to be relevant for FMD. We and others^{15,36–38} have consistently observed low FMD in DM2. Endothelial dysfunction is most likely an early feature in the pathogenesis of atherosclerosis in patients with diabetes. If diabetes is present, the low FMD cannot deterio-

rate further by increasing MS load or low-grade inflammation. This hypothesis is, however, at variance with one other previously reported observation³⁹.

In summary, both MS and low-grade systemic inflammation modify vascular phenotype in patients with diabetes. Their impact is not mutually exclusive, nor is it synergistic for all parameters. Some hemostatic variables are especially influenced by inflammation (e.g., fibrinogen, PAI-1) while others are predicted by MS (e.g., sVCAM). Together, MS and inflammation significantly predict IMT, a direct measurement of the vascular wall and established surrogate marker for future cardiovascular events. Our study showed that, although MS load is a useful rough estimate for clinical practice, the power of the model to predict IMT improved when the MS criteria were used as individual, continuous variables. The use of individual MS factors, however, did not exclude systemic low-grade inflammation as an independent, contributing variable explaining vascular phenotype in DM2 patients.

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