A Controlled Study of Medial Arterial Calcification of Legs

Implications for Diabetic Polyneuropathy

Joon-Shik Moon, MD, PhD; Vicki M. Clark; John W. Beabout, MD; Ronald G. Swee, MD; Peter James Dyck, MD

Background: Diabetes mellitus (DM) is associated with an increased prevalence of peripheral arterial disease and medial arterial calcification (MAC), possibly related to prevalence and severity of diabetic polyneuropathy (DPN).

Objective: To assess the prevalence, risk covariates, and implication of MAC in a controlled study of healthy subjects and patients with DM.

Design: Masked evaluation of radiographs.

Setting: Olmsted County, Minnesota.

Patients: Ambulatory volunteers with DM from the Rochester Diabetic Neuropathy Study cohort (n=260) and matched healthy subjects from the Rochester Diabetic Neuropathy Study–Healthy Subject cohort (n=221).

Methods: Patients and controls underwent standard radiographs of distal legs and feet from January 1, 1995, through December 31, 2002. The radiographs were independently read by masked, experienced radiologists for vessel calcification. Medial arterial calcification prevalence, risk covariates, correlation with peripheral arterial disease, and implication for distal, length-dependent sensorimotor polyneuropathy (DSPN) were studied.

Results: Of 481 study participants, MAC was found in 66 (13.7%): 55 of 260 (21.2%) in patients with DM and 11 of 221 (5.0%) in healthy subjects (P<.001). Inter-rater agreement of MAC was 94.1% (κ coefficient of 0.7). Medial arterial calcification was significantly associated with DSPN (P<.001). In stepwise logistic regression analysis, the significant risk covariates for MAC were advancing age, male sex, DM, and stage of microvessel disease (retinopathy).

Conclusions: Medial arterial calcification of legs was approximately 4 times as prevalent in population-representative ambulatory persons with DM as in healthy subjects. Advancing age, male sex, DM, and retinopathy were the significant risk covariates for MAC of legs. Medial arterial calcification of legs, although significantly associated with DSPN, was not a useful surrogate marker of DSPN. Also, MAC was not shown to be a risk covariate for late worsening of DSPN, although other lines of evidence suggest that peripheral arterial disease may worsen DSPN.

Arch Neurol. 2011;68(10):1290-1294
were symptomatic PAD can worsen DSPN by causing ischemic damage of nerves. It is known that ischemic gangrene causes distal infarction of all tissues, including that of peripheral nerves.17

Medial arterial calcification (MAC) is frequently observed in patients with DM and is associated with increased risk of nephropathy, retinopathy, limb amputation, coronary artery disease, and mortality.18,19 Many metabolic and humoral factors as well as activation of the RANKL (receptor activator of nuclear factor κ-B ligand)/osteoprotegerin signaling pathway may be involved in the pathogenesis of MAC, which in patients with DM is thought to be associated with distal symmetric polyneuropathy.20

To explore the possible role of MAC of legs as a surrogate marker of DSPN and its possible role in late progression of DSPN, we assessed the risk covariates for MAC in our established cohort studies of persons with DM (Rochester Diabetic Neuropathy Study [RDNS]) and those without DM (RDNS-HS).

METHODS

EVALUATION OF MAC IN RDNS AND RDNS-HS COHORTS

Anterior-posterior and lateral radiographs of the feet and distal legs were obtained from volunteers in the Olmsted County cohort of inhabitants with DM (RDNS) in Olmsted County, Minnesota,21-23 and age- and sex-matched volunteers from the HS cohort from the same county (RDNS-HS).24,25 Letters requesting participation were approved by institutional review board committees of Mayo Clinic and Olmsted Medical Center. The RDNS and RDNS-HS cohorts are mainly of northern European extraction.

We considered obtaining blood flow measures of the foot and/or functional computed tomography but deferred such studies for 2 reasons. First, patients were already being extensively screened yearly for diabetic and other microvessel complications and many might not have agreed to functional tomography. Second, in any case, we did not have the research funding to do such studies. We began with screening radiographs, which might have been followed by functional computed tomography studies if results had been sufficiently promising.

MEASURES OF CHRONIC GLYCEMIA AND METABOLIC DERANGEMENT

Cross-sectional and longitudinal data available for the RDNS cohort to the date of the present study were as follows: age of onset of DM, duration of DM, type of DM (based on C-peptide response to glucagon stimulation), fasting plasma glucose, and glycated hemoglobin (hemoglobin A1c [HbA1c])—the latter 2 obtained every 3 months and averaged among years. Also available for this study was a model of chronic glycemic exposure that included statistically significant variables correlating and predicting the severity of diabetic complications of polyneuropathy, retinopathy, and nephropathy. In this model, exponent of HbA1c, duration of DM (years), and age of onset of DM (years) or type of DM correlated and predicted the severity of complications.26 Additional risk covariates studied were change in anthropomorphic variables; tobacco and alcohol use; blood pressure; kidney function; measures of caloric intake and energy expenditure; proteinuria; and plasma concentrations of creatinine, cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, and lipoproteins including lipoprotein(a). The same measurements were obtained on 1 occasion at the time of study for the matched RDNS-HS cohort.

ASSESSMENT OF MICROVESSEL COMPLICATIONS

Diagnostic and scored criteria for microvessel complications were described in an earlier publication.22 Severity of DSPN was assessed by 2 approaches: a staged and a continuous measurement approach. The staged approach used was described previously.26 For the continuous measurement of DSPN severity, we used a scored assessment of neuropathy signs (Neuropathy Impairment Score of Lower Limb) plus a composite normal deviate (nd) score (from percentiles corrected for applicable variables) of 5 attributes of nerve conduction (Σ 5 NC nds).27

Stages of DSPN were as follows: stage 0 was no abnormality of NC, ie, Σ 5 NC nds was 97.5 or greater. Stage 1a was confirmed DSPN with Σ 5 NC nds of 97.5 or greater but no signs or symptoms. Stage 1b was abnormality with Σ 5 NC nds of 97.5 or greater and neurologic signs. Stage 2a was abnormality of NC and neuropathy symptoms with or without signs. Stage 2b was abnormality of NC, neuropathy symptoms, and more severe signs (≥50% weakness of ankle dorsiflexion).

ASSESSMENT OF MAC

Standard anterior-posterior and lateral radiographs of the feet and distal leg of patients were obtained from January 1, 1995, through December 31, 2002. They were read independently by 2 radiologists (J.W.B. and R.G.S.) without biographic or clinical information available to them. Medial arterial calcification was defined as continuous parallel lines of calcification at sites of leg and foot arteries.

STATISTICAL ANALYSIS

Differences among measurements between the DM and the HS cohorts, between patients with and without MAC of legs, and between patients with and without complications were evaluated using standard statistical tests. In testing for associations between putative risk covariates and severity of microvessel complication or MAC of legs, both baseline and averaged values (calculating the mean value per year and then the mean of the annual values) were used. Associations between complications and risk covariates were evaluated univariately with the t test or the Wilcoxon rank sum test for continuous variables and with the χ2 test or the Fisher exact test for discontinuous variables. Stepwise (stepping up) multiple logistic regression tests were used for the multivariate analysis, and the criterion for inclusion of a variable in the model was P < .05.

VALIDITY AND REPRODUCIBILITY OF IDENTIFICATION OF MAC OF LEGS

The interrater agreement between the 2 radiologists’ identification of MAC of legs was 94.1%, with a κ coefficient of 0.7, indicating good agreement.

BIOGRAPHIC AND DEMOGRAPHIC CHARACTERISTICS OF STUDY COHORTS

Healthy subjects were not significantly different from patients with DM by the criteria of age, sex, and height.
(Table 1). However, as expected, patients with DM had significantly higher weight, body mass index, and body surface area. Also, patients with DM smoked more and had higher systolic blood pressure. Diastolic blood pressure was significantly lower in patients with DM than in HS, possibly related to more frequent use of antihypertensive drugs in patients with DM (P = .001). Mean values of fasting plasma glucose, HbA1c, and triglycerides were significantly higher in patients with DM; but cholesterol, high-density lipoprotein, low-density lipoprotein, and lipoprotein\(a\) were significantly lower in patients with DM. Conceivably this latter finding could be related to increased use of lipid-lowering drugs in patients with DM compared with such use in HS, but statistical significance was not reached (P = .28).

A history of previous large vessel disease such as coronary artery disease and PAD was more frequent in patients with DM (P = .03 for coronary artery disease; P = .15 for cerebrovascular disease; and P = .002 for peripheral vascular disease), but this did not reach statistical significance (P = .54 for coronary artery disease, .63 for cerebrovascular disease, and .98 for PAD). As expected, patients with DM had a significantly higher frequency of microvascular complications, ie, neuropathy, retinopathy, and nephropathy (Table 1).

### Table 1. Disease Characteristics of the DM Cohort vs the Healthy Subject Cohort in Olmsted County
d

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients With DM (n=260)</th>
<th>Healthy Subjects (n=221)</th>
<th>P Valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>56 (15)</td>
<td>55 (15)</td>
<td>.31</td>
</tr>
<tr>
<td>Sex, No.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>140</td>
<td>109</td>
<td>.32</td>
</tr>
<tr>
<td>Female</td>
<td>120</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>168.3 (9.6)</td>
<td>169.0 (9.7)</td>
<td>.46</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>87.5 (22.2)</td>
<td>79.6 (17.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BMI</td>
<td>30.8 (7.1)</td>
<td>27.8 (5.4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BSA, m²</td>
<td>1.96 (0.26)</td>
<td>1.90 (0.23)</td>
<td>.002</td>
</tr>
<tr>
<td>Smoking, median (range), pack-years</td>
<td>0 (0-105)</td>
<td>0 (0-94)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hemoglobin A(1c)</td>
<td>7.9 (1.5)</td>
<td>5.3 (0.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>130 (17)</td>
<td>126 (16)</td>
<td>.02</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>75 (10)</td>
<td>79 (9)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HDL, median (range), mg/dL</td>
<td>40 (19-99)</td>
<td>45 (17-124)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Cholesterol, median (range), mg/dL</td>
<td>180 (99-372)</td>
<td>197 (118-311)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Triglyceride, median (range), mg/dL</td>
<td>138 (37-1398)</td>
<td>130 (34-371)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LDL, median (range), mg/dL</td>
<td>104 (0-260)</td>
<td>120 (42-212)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Lipoprotein (a), median (range), mg/dL</td>
<td>13 (4.8-110)</td>
<td>17 (4.8-169)</td>
<td>.03</td>
</tr>
<tr>
<td>Microvascular complication, median</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuropathy stage</td>
<td>0</td>
<td>0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Retinopathy stage, worse side</td>
<td>1</td>
<td>0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Retinopathy scale, worse side</td>
<td>2</td>
<td>0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Nephropathy stage</td>
<td>0</td>
<td>0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hx of macrovascular complications, No.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>53</td>
<td>28</td>
<td>.03</td>
</tr>
<tr>
<td>Transient ischemic attack/stroke</td>
<td>22</td>
<td>11</td>
<td>.15</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>17</td>
<td>2</td>
<td>.002</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); BSA, body surface area; DM, diabetes mellitus; HDL, high-density lipoprotein; Hx, history; LDL, low-density lipoprotein.

SI conversion factors: To convert hemoglobin A\(1c\), to proportion of total hemoglobin, multiply by 0.01; cholesterol, HDL, and LDL to millimole per liter, by 0.0259; triglyceride to millimole per liter, by 0.0113; and lipoprotein(a) to micromole per liter, by 0.0357.

a Data are given as mean (SD) unless otherwise indicated.

b Using t test, Wilcoxon rank sum test, \(\chi^2\) test, or Fisher exact test.

### PREVALENCE OF MAC OF LEGS

Considering the entire cohort of 481 persons (DM and HS groups combined), 66 (13.7%) had MAC of legs. Of 260 patients with DM, 55 (21.2%) had MAC, whereas only 11 of the 221 HS (5.0%) had it (P < .001). It was approximately 4 times more frequent in patients with DM than in HS.

### RISK COVARIATES FOR MAC OF LEGS

The multivariate risk covariates for MAC of legs are shown in Table 2; they are age, male sex, DM, and retinopathy. It was of some interest that many of the cardiovascular risk covariates (use of tobacco, hypertension, cholesterol, low-density lipoprotein, lipoprotein\(a\), triglycerides, renal dysfunction, and others) found to relate to MAC of legs in univariate analysis were not risk covariates in multivariate analysis.

### ASSOCIATION BETWEEN MAC AND DSPN AND RISK COVARIATES FOR DSPN

The occurrence of MAC and DSPN were statistically associated (P < .001). However, the association between the two was not close enough to use MAC as a surrogate...
The present study addressing MAC, its putative risk covariates, and its implications for DSPN is unique in the following respects: it was a prospective, population-based, controlled study, and it used masked assessment and extensive assessment of risk covariates studied cross-sectionally and longitudinally. In addition, DSPN was measured using objective and quantitative end points validated in other studies. Our study was ancillary to our prospective and longitudinal assessment of diabetic polyneuropathies of Olmsted County persons with DM (RDNS; 502 persons followed up for \( \geq \) 18 years and supported by grant NS36797 from the National Institute of Neurological Disorders and Stroke). The volunteers with DM and their matched controls without DM were not significantly different by the criteria of age, sex, and height but were different in important respects, eg, weight, body mass index, HbA\(_1c\), plasma lipids, and diabetic microvascular complications (Table 1). The studied groups are therefore comparable by site of study, ethnic background (mainly of northern European extraction), medical care (from Mayo Clinic and Olmsted Medical Center), and selection (volunteers from population-based community lists). Masked evaluations were performed for the major end points (MAC and presence and severity of DSPN). Objective measurement was also used for DSPN, with reference values obtained from the same population.

In the present study, MAC was approximately 4 times as prevalent in patients with DM as in HS. In addition, the risk factors for MAC were advancing age, male sex, DM, and microvessel disease (as represented by retinopathy). The results are in keeping with those of earlier studies.\(^{18,20-31}\)

Other risk covariates may have been operative but not expressed because they were expressed by other significant covariates. This may have been the case for smoking. Smoking was not identified as a separate risk covariate in the present study but conceivably could have been represented by men who smoked much more (mean [SD], 15 [24] pack-years) than women (7 [15] pack-years) (\( P < .001 \)). On the other hand, factors other than smoking that are associated with male sex might explain the male risk covariate role in MAC.

To test whether MAC was a risk factor for symptomatic PAD, we assessed occurrence of MAC against the previous history of cardiovascular disease, cerebrovascular disease, or PAD to the time of the study. Although these events were more common in patients with DM, statistical significance was not achieved. Previous studies, however, have shown that MAC does relate to symptomatic atherosclerotic complications.\(^{32,33}\) Our failure to show a significant correlation could relate to the ambulatory nature of our cross-sectional studies.

An additional important focus of our study was whether MAC of legs might be a surrogate marker for DSPN and whether it could be shown to be a risk covariate for DSPN. In previous studies, MAC was shown to correlate with such microvascular complications as nephropathy.\(^{34}\) Although there is a statistical association between MAC of legs and DSPN, we did not find that MAC of legs was a strong indicator of symptomatic PAD or of DSPN. Therefore, although our data do not rule out the possibility that PAD might be involved in additional nerve damage in established DSPN, we have not found evidence for it either.

In our study, such cardiovascular risk factors as cholesterol, low-density lipoprotein, hypertension, triglycerides, and renal dysfunction were not shown to be risk covariates for MAC, in contrast to previous studies.\(^{18,30,35,36}\) It is possible that they are not measurable as covariates because of treatment effects (antihypertensive or lipid-lowering drugs).

Autonomic symptoms and deficits are common in patients with DM,\(^{37}\) but in our study were not shown to be risk covariates for MAC or DSPN.

**Accepted for Publication:** February 17, 2011.

**Correspondence:** Peter James Dyck, MD, Peripheral Neurology Research Laboratory, Mayo Clinic, 200 First St SW, Rochester, MN 55905 (dyck.peter@mayo.edu).

---

**Table 2. Stepwise Logistic Regression Analysis of the Risk Covariates Predicting Medial Arterial Calcification**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Calcification, OR (95% CI)(^a)</th>
<th>( P ) Value(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Set 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.51 (1.18-3.80)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>5.03 (2.54-10.68)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2.31 (1.03-5.59)</td>
<td>.02</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>3.81 (2.28-6.60)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Set 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>1.51 (1.18-3.80)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>5.03 (2.54-10.68)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Neuropathy, NISLL7N</td>
<td>1.46 (1.05-2.01)</td>
<td>.003</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>3.81 (2.28-6.60)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; NISLL7N, Neuropathy Impairment Score of Lower Limb (summed normal deviate score of peroneal motor nerve amplitude, velocity, and distal latency, tibial motor nerve distal latency, and sural nerve amplitude plus vibration detection threshold for a great toe and those of heartbeat variation with deep breathing); OR, odds ratio.

\(^{a}\)The OR and associated CIs are reported per decade for age and per SD for the other quantitative variables.

\(^{b}\)The threshold of significance was set at \( P < .05 \).

**Table 3. Stepwise Logistic Regression Analysis of the Risk Covariates Predicting Diabetic Sensorimotor Polyneuropathy**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio (95% CI)</th>
<th>( P ) Value(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycemic index II(^b)</td>
<td>2.39 (1.65-3.58)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BSA, m(^2)</td>
<td>1.83 (1.34-2.57)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>2.19 (1.33-3.68)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Medial arterial calcification</td>
<td>1.17 (0.54-2.60)</td>
<td>.57</td>
</tr>
</tbody>
</table>

Abbreviations: BSA, body surface area; CI, confidence interval.

\(^{a}\)The threshold of significance was set at \( P < .05 \).

\(^{b}\)Glycemic index II = \(-2.79 + 3.8 \times (\text{hemoglobin} A\(_1c\))^{14} + 0.75 \times \text{duration of diabetes mellitus in years}^{14} - 0.07 \times \text{age at the time of diabetes mellitus diagnosis}^{14}\). See Dyck et al.\(^{29}\)

marker and predictor of DSPN. The glycemic index (a model combining exponents of average HbA\(_1c\), duration of DM, and age of onset of DM), body surface area, and retinopathy were found to relate to MAC (Table 3).
Author Contributions: Acquisition of data: Clark and Beabout. Analysis and interpretation of data: Moon, Swee, and Dyck. Drafting of the manuscript: Moon and Beabout. Critical revision of the manuscript for important intellectual content: Moon, Clark, Swee, and Dyck. Statistical analysis: Moon and Dyck. Obtained funding: Dyck. Administrative, technical, and material support: Beabout and Swee.

Financial Disclosure: None reported.

Funding/Support: This study was supported in part by grant NINDS 36797 from the National Institute of Neurologic Disorders and Stroke and grant U54RR 24150-5 from the Mayo Clinic Center for Translational Science Activities.

REFERENCES


©2011 American Medical Association. All rights reserved.

Downloaded From: http://archneur.jamanetwork.com/pdfaccess.ashx?url=/data/journals/neur/22522/ on 06/18/2017