Aortic Stiffness and Vitamin D are Independent Markers of Aortic Calcification in Patients with Peripheral Arterial Disease and in Healthy Subjects

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KEYWORDS
Peripheral arterial disease; Arterial stiffness; Aortic calcification; Vitamin D

Abstract
Objective: Arterial stiffness is a significant determinant of cardiovascular risk and is related to vascular calcification. Vitamin D may regulate arterial calcification and has been associated with cardiovascular survival benefits. However, data about the relationship between arterial stiffness, aortic calcification and vitamin D levels in patients with peripheral arterial disease (PAD) and in healthy subjects are limited. We examined the potential association between aortic calcification, arterial stiffness and vitamin D levels in patients with symptomatic PAD and in healthy individuals.

Methods: We studied 78 men with PAD (aged 63 ± 7 years) and 74 healthy men (aged 61 ± 10 years). Aortic pulse wave velocity (aPWV) was determined by applanation tonometry using the SphygmoCor device. Aortic calcification score (ACS) was quantified by computed tomography. Serum 25-hydroxyvitamin D (25(OH)D) levels were measured using a radioimmune assay.

Results: ACS (4.9(2.3–8.9) vs. 0.2(0.03–1.6) (cm^2); p < 0.01), aPWV (9.8 ± 2.4 vs. 8.2 ± 1.6 (m s^-1); p < 0.01) and 25(OH)D (15.1 ± 5.4 vs. 19.0 ± 5.9 (ng ml^-1); p < 0.01) were different in the patients compared with the controls. In multivariate analysis, ACS was independently determined by 25(OH)D, aPWV, calcium and age in patients with PAD (R^2 = 0.49; p < 0.001) and by 25(OH)D, aPWV, cholesterol/high-density lipoprotein (HDL) and age in the control group.

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Arterial calcification is an independent predictor of vascular morbidity and mortality in the general population and is also a marker of sub-clinical atherosclerotic disease. There is evidence that vascular calcification might contribute to the increase in arterial stiffness and vice versa. In chronic kidney disease, a strong cross-sectional relationship has been demonstrated between arterial calcifications and stiffness of the aorta. Aortic pulse wave velocity (aPWV), the current gold-standard measure of aortic stiffness, is related to aortic calcification in patients with peripheral arterial disease (PAD), isolated systolic hypertension and in the general population. Vitamin D is closely related to vascular calcification. Serum 25-hydroxyvitamin D (25(OH)D) correlates with calcification of the iliac and femoral arteries. Injection of activated vitamin D is associated with better survival in haemodialysis patients. Moreover, recent data indicate that 1,25-dihydroxyvitamin D (1,25(OH)2D) reduces pro-inflammatory cytokine secretion and down-regulates the renin–angiotensin system (RAS).

Calcified atherosclerotic arteries, increased arterial stiffness and vitamin D deficiency might influence the clinical course of PAD. However, data about the association between these parameters in patients with PAD are limited. The aim of this study was to evaluate the relationship between aortic calcification, arterial stiffness and 25(OH)D in patients with symptomatic PAD and in clinically healthy men. In addition, we examined the association of 25(OH)D with peripheral and central blood pressure (BP).

Methods

Subjects

A total of 78 patients with documented PAD participated in this study. The patients, fulfilling the inclusion criteria, were recruited from the Department of Vascular Surgery, University of Tartu. The diagnosis of PAD required (1) clinical symptoms of PAD, (2) ankle–brachial pressure index (ABPI) <0.9 and (3) significant stenoses or occlusions of arteries confirmed by angiography. The stage of the disease was determined according to the Fontaine classification: stage II = intermittent claudication, stage III = leg pain at rest and stage IV = tissue loss due to ischaemic ulcer or gangrene. The exclusion criteria were myocardial infarction, coronary revascularisation, or cerebrovascular events during the previous 6 months, earlier revascularisation procedures at the lower limb, upper limb occlusive arterial disease, cardiac arrhythmias or valve pathologies, diabetes mellitus, malignancies, renal failure (estimated glomerular filtration rate (eGFR) <60 ml min⁻¹ 1.73 m⁻²), known inflammatory conditions or use of vitamin D supplements. In total, 30 (38.5%) patients with hypertension and 12 (15.4%) patients with coronary artery disease as the co-morbidity were included in the study.

A total of 74 clinically healthy men were recruited by a family physician and by a specialist of sports medicine. To be eligible as controls, the subjects had to be free from any acute or chronic inflammatory disease, coronary artery disease, cardiac arrhythmias or valve pathologies, cerebral or peripheral atherosclerotic disease, diabetes mellitus, malignancies, renal failure (eGFR <60 ml min⁻¹ 1.73 m⁻²) or use of vitamin D supplements. The control subjects did not use any medications on a regular basis. All participants provided written informed consent and the study was approved by the Ethics Committee of the University of Tartu.

Haemodynamic measurements

Brachial BP and heart rate were measured supine in the left arm using an automated digital oscillometric BP monitor (OMRON M4-I; Omon Healthcare Europe, Hoofdorp, the Netherlands). Radial artery waveforms were recorded with a high-fidelity micromanometer (SPT-301B; Millar Instruments, Houston, TX, USA) from the wrist of the left hand. Pulse wave analysis (SCOR Px, 7.0; AtCor Medical, Sydney, Australia) was used to generate radial artery waveforms. A generalised validated transfer function was then used to generate a corresponding central (ascending aortic) waveform. Aortic augmentation index (Alx) was measured as the height of the late systolic peak divided by aortic pulse pressure. The Alx was adjusted to a heart rate of 75 beats min⁻¹ (Alx@75), as calculated by the Sphygmocor software. Mean arterial pressure (MAP) was calculated from the integration of the radial artery waveform. PWV was determined by the foot-to-foot method, using the Sphygmocor device. Electrocardiogram-gated femoral and carotid artery waveforms were sequentially recorded to measure aPWV. Brachial PWV (bPWV) was determined from carotid and radial waveforms. Haemodynamic measurements were performed by two experienced investigators. All measurements were made in duplicate, and mean values were used in subsequent analysis. The within- and between-observer measurement reproducibility values for aPWV and Alx were consistent with previously published data.

The ABPI was measured using the Bidirectional Doppler MD 6 (D.E. Hokanson, Bellevue, WA, USA). Systolic BP was measured bilaterally over the brachial, tibialis posterior and dorsalis pedis arteries. The higher systolic BP of the
dorsalis pedis or the posterior tibial artery was used for calculation of the ABPI. Two readings of the ABPI were performed and the mean was calculated. The lower ABPI of the two legs was included in statistical analysis.

**Computed tomography**

Computed tomography (CT) scans of the aorta were performed in 52 patients with PAD and in 60 clinically healthy men. All study participants were informed about the purpose, methods, radiation dose and risks associated with radiation exposure. We could not perform CT scans in all of the subjects due to the lack of consent and considering also the fact that some patients had several CT scans in their previous medical history. The entire aorta (from the aortic valve to bifurcation) was visualised by obtaining 5-mm-thick slices through the thorax, abdomen and pelvis with non-contrast helical CT (GE LightSpeed 16, General Electrical Medical Systems, Milwaukee, WI, USA; total dose: <8 mSv) (Fig. 1). Analysis was conducted using a Siemens Syngo Multimodality Workplace workstation. Aortic calcification score (ACS) was measured by an independent observer. The degree of calcification was determined by the volume scoring method. The number of voxels of 130 or greater Hounsfield units within the wall of the aorta yielded a calcification score in cubic centimetres. This is a validated and accurate technique that compares favourably with electron beam CT.

**Laboratory analysis**

Blood was collected after an overnight fast on the morning of CT assessment. Serum (25(OH)D) level was measured using a radioimmune assay (25-Hydroxyvitamin D, 125I Ria Kit, DiaSorin Corporation, Minnesota, USA). The plasma level of soluble intercellular adhesion molecule-1 (sICAM) was measured by an enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (Human soluble intercellular adhesion molecule-1 Immunoassay; R&D Systems; Minneapolis, MN, USA). Serum levels of interleukin (IL)-6 were determined using a chemiluminescent immunoassay (Immulite; Diagnostic Products Corporation, Los Angeles, CA, USA). Plasma cholesterol, triglyceride, high-density lipoprotein, glucose, creatinine, high-sensitivity C-reactive protein (hsCRP) and calcium concentrations were measured by standard laboratory methods using certified assays in the local clinical laboratory. The eGFR was determined using the Modification of Diet in Renal Disease formula, equation MDRD 1. All determination procedures were performed in accordance with the manufacturer’s recommendation.

**Protocol**

The subjects were studied in the morning between 8 AM and 10 AM. They had abstained from smoking and intake of caffeine-containing food and beverages for the previous 12 h. Studies were conducted in a quiet temperature-controlled room. First, height and weight were assessed and, next, body mass index was calculated. After the subjects had spent 15 min resting in the supine position, brachial BP and radial artery waveforms were recorded. Further, aPWV, bPWV, AIx@75 and ABPI were measured. Twenty millilitres of blood were drawn from the antecubital fossa into plain tubes. The subjects then underwent CT.

**Statistical analysis**

Statistical analysis was performed using the software STATISTICA (version 9.1 for Windows; StatSoft, Tulsa, Oklahoma, USA). All variables included in the analysis were verified for normality using the Kolmogorov-Smirnov test. Because distribution of ACS was significantly skewed, the calcification scores were log10-transformed for subsequent analyses. As there was significant difference in BP between the study groups, aPWV and bPWV have been adjusted for MAP before analysis. The results are presented as means ± standard deviations for normally distributed data. Variables with a non-normal distribution are presented as medians and interquartile ranges. Comparisons between the patients and the controls were performed using the unpaired t-tests for parametric data and the Mann–Whitney U-tests for non-parametrically distributed data. Comparison between multiple unpaired groups was performed using the one-way analysis of variance (ANOVA). Multiple regression analysis was performed to investigate the independent determinants of ACS. Values of *p* < 0.05 were considered statistically significant.

**Results**

**Participant characteristics**

The characteristics of the study population are summarised in Table 1. The patients with PAD had higher ACS, aPWV and peripheral and central systolic BP, compared with the controls. By contrast, 25(OH)D levels were significantly lower in the patients with PAD, compared with the healthy controls. No significant association was found between ACS and body mass index or age.

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**Figure 1** A cross-section of the abdomen illustrating (A) non-calcified aortic wall, (B) calcification within the aortic wall.
higher among the healthy participants. Biomarkers of inflammation, such as hsCRP, sICAM and IL-6, were higher in the patient group. There were no differences between the groups regarding bPWV, eGFR, plasma glucose and HDL concentrations. Differences in the use of medication were observed between the study groups: 31 (39.7%) patients were on pentoxifylline treatment, 20 (25.6%) patients received aspirin, 9 (11.5%) patients were on statin therapy, 15 (19.2%) patients received angiotensin-converting enzyme inhibitors, 14 (17.9%) patients were on calcium channel blocker treatment, 7 (9%) patients received angiotensin-receptor blockers, 5 (6.4%) patients were on beta-blocker therapy and 3 (3.8%) patients received diuretics, whereas the control subjects did not use any medications on a regular basis.

### Relationship between aortic calcification, arterial stiffness and vitamin D

Log-ACS was significantly correlated with aPWV in PAD patients ($r = 0.28; p = 0.03$) and in the controls ($r = 0.57; p < 0.001$) (Fig. 2(A)). The log-ACS showed positive correlation with 25(OH)D levels in PAD patients ($r = 0.33; p = 0.01$) and negative correlation in the control subjects ($r = -0.47; p < 0.001$) (Fig. 2(A)). Log-ACS was positively correlated with log-hsCRP ($r = 0.29, p = 0.03$) and log-IL-6 ($r = 0.28, p = 0.03$) only in PAD patients. Multivariate analysis revealed that log-ACS was independently associated with 25(OH)D, aPWV, eGFR, calcium and age ($R^2 = 0.49; p < 0.001$) in PAD patients and with 25(OH)D, aPWV, cholesterol/HDL ratio and age ($R^2 = 0.55; p < 0.001$) in the clinically healthy men (Table 2). Adjustment for seasonal variation of vitamin D, anti-hypertensive and vasodilator treatment, systolic, diastolic and pulse pressure, glucose, LDL, triglycerides, height and weight did not alter the associations (data not shown). Log-ACS correlated with both peripheral and central pulse pressure in PAD patients ($r = 0.31, p = 0.01$ and $r = 0.29, p = 0.02$, respectively) and in the control subjects ($r = 0.37, p = 0.004$ and $r = 0.46, p < 0.001$, respectively). It was significantly correlated with Alx@75 ($r = 0.48, p < 0.001$) only in the control subjects. However, this correlation was not significant after adjustment for confounders.

Vitamin D levels were negatively correlated with peripheral systolic blood pressure (PSBP) ($r = -0.3, p = 0.01$), peripheral diastolic blood pressure (PDBP) ($r = -0.29, p = 0.01$), central systolic blood pressure (CSBP) ($r = -0.3, p = 0.01$), central diastolic blood pressure (CDBP) ($r = -0.28, p = 0.02$), peripheral pulse pressure (PPP) ($r = -0.24, p = 0.04$) and MAP ($r = -0.3, p = 0.01$) only in PAD patients. Vitamin D correlated significantly with Alx@75 in the controls ($r = -0.26; p = 0.03$). There was a trend towards correlation between 25(OH)D concentrations and aPWV ($r = -0.23, p = 0.057$) only in the control subjects.

### Determinants of severity grade of PAD

We tested the hypothesis about whether increased arterial stiffness and decreased vitamin D levels are associated with more advanced atherosclerotic disease. In univariate analysis, there was borderline correlation between ABPI and 25(OH)D ($r = 0.26, p = 0.051$). We evaluated the relationship between ABPI and the composite measure of aPWV and 25(OH)D. Patients were divided into three

### Table 1 Baseline characteristics of the participants (mean ± s.d.)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PAD patients (n = 78)</th>
<th>Controls (n = 74)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63 ± 7</td>
<td>61 ± 10</td>
<td>0.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.4 ± 3.8</td>
<td>27.0 ± 3.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ABPI (mmHg)</td>
<td>0.43 ± 0.3</td>
<td>1.2 ± 0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ACS (cm³)</td>
<td>4.9 (2.3–8.9)</td>
<td>0.2 (0.03–1.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>66.6 ± 12.1</td>
<td>58.9 ± 9.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>103.9 ± 13.8</td>
<td>97.0 ± 11.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PSBP (mmHg)</td>
<td>148.4 ± 20.8</td>
<td>131.9 ± 15.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PDBP (mmHg)</td>
<td>81.3 ± 10.4</td>
<td>77.9 ± 8.6</td>
<td>0.03</td>
</tr>
<tr>
<td>PPP (mmHg)</td>
<td>66.1 ± 14.6</td>
<td>54.1 ± 10.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CSBP (mmHg)</td>
<td>135.7 ± 19.2</td>
<td>122.3 ± 16.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CDBP (mmHg)</td>
<td>82.3 ± 10.9</td>
<td>79.3 ± 9.3</td>
<td>0.07</td>
</tr>
<tr>
<td>CPP (mmHg)</td>
<td>53.4 ± 12.9</td>
<td>43.0 ± 11.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>aPWV (m/s)</td>
<td>9.8 ± 2.4</td>
<td>8.2 ± 1.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>bPWV (m/s)</td>
<td>8.7 ± 1.3</td>
<td>8.8 ± 1.2</td>
<td>0.73</td>
</tr>
<tr>
<td>Alx@75 (%)</td>
<td>28.2 ± 8.0</td>
<td>17.1 ± 9.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.6 ± 1.0</td>
<td>5.4 ± 0.5</td>
<td>0.13</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.7 ± 1.2</td>
<td>5.3 ± 1.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>4.2 ± 1.1</td>
<td>3.7 ± 1.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.3 ± 0.4</td>
<td>1.3 ± 0.3</td>
<td>0.49</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.7 ± 0.7</td>
<td>1.1 ± 0.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>99.7 ± 25.8</td>
<td>95.1 ± 20.7</td>
<td>0.2</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>4.1 (1.4–8.5)</td>
<td>0.9 (0.5–1.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.4 ± 0.2</td>
<td>2.2 ± 0.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>15.1 ± 5.4</td>
<td>19.0 ± 5.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>sICAM (ng/mL)</td>
<td>265.5 ± 65.5</td>
<td>174.9 ± 36.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL-6 (ng/mL)</td>
<td>4.0 (2.1–7.0)</td>
<td>2.0 (1.9–3.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Current CDBP (mmHg)</td>
<td>78 (100)</td>
<td>18 (24)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Framingham risk score (%)</td>
<td>ND</td>
<td>11.4 ± 7.3</td>
<td>ND</td>
</tr>
</tbody>
</table>

**PAD**, peripheral arterial disease; BMI, body mass index; ABPI, ankle–brachial pressure index; ASC, aortic calcification score; MAP, mean arterial pressure; PSBP, peripheral systolic blood pressure; PDBP, peripheral diastolic blood pressure; PPP, peripheral pulse pressure; CSBP, central systolic blood pressure; CDBP, central diastolic blood pressure; CPP, central pulse pressure; aPWV, aortic pulse wave velocity; bPWV, brachial pulse wave velocity; Alx@75, augmentation index corrected for a heart rate of 75 beats per minute; LDL, low-density lipoprotein; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; 25(OH)D, 25-hydroxyvitamin D; sICAM, soluble intercellular adhesion molecule; IL-6, interleukin-6.

* Indicates medians and interquartile ranges.

* aPWV and bPWV have been adjusted for MAP.

# Risk was not assessed for PAD patients since they had established vascular disease, which indicates high cardiovascular risk.
The principal finding of the present study is that increased aortic stiffness and abnormal vitamin D levels are independently associated with the extent of aortic calcification in patients with symptomatic PAD and in clinically healthy subjects. Furthermore, 25(OH)D and aPWV were related to the severity grade of atherosclerotic disease. To the best of our knowledge, this is the first study that has demonstrated relationship between aortic stiffness, calcification of the aorta and vitamin D levels in patients with angiographically proven PAD.

We have demonstrated that PAD is associated with aortic calcification, which is consistent with the results of previous studies. In subjects with type 2 diabetes, aortic calcification was higher in the patients who developed intermittent claudication than in those who were symptom-free during follow-up. Our finding that aPWV is elevated in patients with PAD is supported by the results of a large cross-sectional study.

In our study, serum 25(OH)D was significantly lower in the patient group as compared with the controls. Similarly, the results of a large epidemiological study indicate that there is strong association between lower levels of 25(OH)D levels and prevalence of PAD. It has been hypothesised that patients with PAD may be less mobile and receive therefore less sun exposure.

The association between arterial stiffness and vascular calcification has been demonstrated in several cross-sectional studies. In dialysis patients, strong relationship has been demonstrated between abdominal aortic calcification and stiffness of the aorta. Moreover, aortic calcification was positively correlated with aPWV and peripheral pulse pressure in healthy individuals. We demonstrated association between aPWV and ACS both in patients with PAD and in clinically healthy men. These associations remained significant after adjustment for several cardiovascular risk factors. Central pulse pressure, which is a well-known measure of aortic stiffness, correlated with aortic calcification in both study groups. These findings suggest that aortic calcification and its stiffening might influence development of PAD and indicate also the presence of sub-clinical atherosclerosis in healthy subjects. By contrast, there was no relationship between ACS and bPWV in the patients with PAD. One potential explanation for the lack of association between ACS and bPWV is that aortic calcification is specifically related to the stiffness of the aorta rather than the brachial artery.

There are a number of possible explanations for the relationship between aortic stiffness and vascular calcification. It has been hypothesised that increased arterial stiffness could lead to vessel wall damage and calcification. Alternatively, vascular calcification could lead to stiffening of the arteries. Finally, it might be that both vascular calcification and arterial stiffness are the consequences of age-related degenerative processes in the vasculature.

The effects of vitamin D on vascular calcification appear to follow a biphasic pattern, with both excess and deficiency promoting its development. Low levels of 25(OH)D are associated with extensive vascular calcification and injection of activated vitamin D increases survival in patients with end-stage renal disease. On the other hand, treatment with vitamin D increases aortic calcification in animal models. In our study, serum levels of 25(OH)D correlated positively with ACS in PAD patients and

**Figure 2** Correlation between aPWV and log-ACS for the patients ($r = 0.28, p = 0.03$) and for the control subjects ($r = 0.57, p < 0.001$) (A). Correlation between 25(OH)D and log-ACS for the patients ($r = 0.33, p = 0.01$) and for the control subjects ($r = -0.47, p < 0.001$) (B). The aPWV has been adjusted for MAP. Filled dots represent patients; empty dots represent controls. Continuous line represents regression line through the patient data; interrupted line represents regression line through the control data.

**A**

![Graph A](image)

**B**

![Graph B](image)
negatively in the control subjects. It might be that different localisations of calcium deposits in the aortic wall are responsible for different patterns of correlation between aortic calcification and vitamin D seen among the study groups. Previous studies indicate that, in patients with atherosclerosis, mostly intimal calcification is seen. By contrast, medial calcification is associated with diabetes mellitus, end-stage renal disease and ageing.6 Based on these observations, we hypothesised that a mixture of intimal and medial calcification occurred in PAD patients, whereas age-related medial calcification was predominant in the healthy men. Thus, serum 25(OH)D levels might be differently associated with adverse morphologic changes in the aorta, depending on the clinical condition of subjects.

Lower circulating 25(OH)D levels are associated with higher prevalence of hypertension.25 Previous studies indicate that vitamin D may modulate vascular smooth muscle tone26 and may also act as an endogenous inhibitor of RAS12 by influencing the concentration of calcium in juxtaglomerular cells. Similarly, we have shown that 25(OH)D levels were negatively correlated with central and peripheral BP in patients with atherosclerosis. Our results support the role of vitamin D in modulation of BP.

The ABPI has been widely adopted for confirmation of the clinical diagnosis of PAD and for determining its severity grade. Having a low ABPI value is an independent risk factor for all-cause and cardiovascular mortality in patients with PAD.27 We have shown that increased aPWV and lower 25(OH)D levels are associated with lower ABPI values. This finding suggests that aPWV and vitamin D status might provide additional information about severity grade of PAD.

There are potential limitations to the present study. First, the study was cross sectional. As in any cross-sectional study, the established associations do not indicate cause-and-effect relationship. Second, we studied only men to avoid the complicating effects of endogenous hormones, considering also that patients with PAD are predominantly men. This makes it difficult to extrapolate our results to women. Third, a substantial proportion of PAD patients used anti-hypertensive, vasodilating and anti-aggregant medications, which might have affected the results of haemodynamic measurements. Fourth, the patients with PAD were older than the control subjects. However, we performed statistical analysis separately in either of the study groups. Moreover, the proportion of smokers was significantly higher in the patient group. Finally, as the study groups were relatively small, our findings should be verified in larger studies.

In conclusion, the present study demonstrated that serum 25(OH)D and aortic stiffness are independently associated with aortic calcification in patients with PAD and in healthy individuals. Furthermore, increased aortic stiffness and lower levels of 25(OH)D are related to higher severity grade of PAD. In patients with atherosclerosis, vitamin D was negatively correlated with peripheral and central BP. Our results suggest that vascular calcification, arterial stiffening and vitamin D may have an important role in the pathogenesis of atherosclerosis. Potentially, assessment of vitamin D status, arterial stiffness and aortic calcification may be used for the risk stratification and optimisation of treatment in patients with atherosclerosis.

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Conflict of Interest

The authors have no conflict of interest to declare.

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