Increased Pregnancy-Associated Plasma Protein-A as a Marker for Peripheral Atherosclerosis: Results from the Linz Peripheral Arterial Disease Study

Thomas Mueller,¹* Benjamin Dieplinger,¹ Werner Poelz,² and Meinhard Haltmayer¹,³

Background: The aim of the present investigation was to test the hypothesis that pregnancy-associated plasma protein-A (PAPP-A), a zinc-binding metalloproteinase implicated in acute coronary syndrome, is associated with atherosclerotic peripheral arterial disease (PAD).

Methods: The study comprised 433 patients with symptomatic atherosclerotic PAD (i.e., chronic limb ischemia) and 433 controls matched to the patients with PAD in a 1:1 design by sex, age (±2 years), and diabetes mellitus status. Serum PAPP-A concentrations were measured with an enzymatically amplified 2-step sandwich-type immunoassay.

Results: The entire study sample included 612 male and 254 female patients with a median age of 68 years. The median PAPP-A value was higher in the patients with PAD than in the referents (0.81 vs 0.64 mU/L; \( P < 0.001 \)). After we adjusted for several possible confounding variables with multivariable logistic regression, odds ratios for PAD were 1.59 (95% confidence interval, 1.00–2.52; \( P = 0.049 \)), 2.28 (95% confidence interval, 1.45–3.61; \( P < 0.001 \)), and 2.86 (95% confidence interval, 1.78–4.59; \( P < 0.001 \)) in the 2nd, 3rd, and 4th quartiles of serum PAPP-A concentrations compared with the first quartile. In the present study, PAPP-A added to the predictive value of other markers commonly in use.

Conclusions: PAPP-A was associated with atherosclerotic PAD in the elderly sample studied. Because atherosclerotic disease in elderly patients, the present results indicate that circulating PAPP-A may be a marker for systemic atherosclerotic disease.

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Pregnancy-associated plasma protein A (PAPP-A)⁴ is a high–molecular-mass glycoprotein first identified in sera of pregnant women (1). PAPP-A is also present in unstable atherosclerotic coronary plaques (2). Its circulating concentrations are higher in patients with acute coronary syndrome than in patients with chronic stable angina and in healthy persons (2–4), and PAPP-A concentrations are associated with the presence and extent of stable coronary heart disease (5). Furthermore, increased serum PAPP-A concentrations are predictive of future ischemic cardiac events and the need for percutaneous coronary intervention or coronary artery bypass graft surgery (6, 7).

PAPP-A is a zinc-binding metalloproteinase (8) that specifically degrades insulin-like growth factor (IGF)-binding proteins (9, 10), thereby allowing unbound/active IGF to bind to cell-surface IGF receptors (11, 12). Both unbound IGF and metalloproteinases are thought to be mediators of atherosclerosis (11, 13). In contrast to this proposed proatherogenic role of IGF and, subsequently, of the PAPP-A molecule, an increasing body of evidence indicates that IGF provides protection against ischemic vascular diseases by preventing plaque formation and disruption (14–16). Because IGF may be cardioprotective, increased PAPP-A should also offer cardiovascular protection. Thus, increased PAPP-A may react to vascular damage as part of a compensatory, IGF-mediated, reparative process.

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⁴ Nonstandard abbreviations: PAPP-A, pregnancy-associated plasma protein-A; IGF, insulin-like growth factor; proMBP, proform of eosinophil major basic protein; PAD, peripheral arterial disease; tHcy, total homocysteine; (hs-)CRP, (high-sensitivity) C-reactive protein; and eGFR, estimated glomerular filtration rate.
ative process. PAPP-A may be involved not only in plaque destabilization and/or rupture, but also in the development of atherosclerotic lesions (5, 10). Atherosclerosis-related PAPP-A is different from pregnancy-related PAPP-A and is thought to circulate as a homodimeric active form, uncomplexed with the proform of eosinophil major basic protein (proMBP) (9, 17).

In the present study we addressed the association of atherosclerotic peripheral arterial disease (PAD), an important manifestation of systemic atherosclerosis (18, 19), with serum PAPP-A concentrations.

**Materials and Methods**

**STUDY SAMPLE AND STUDY DESIGN**

To test the hypothesis that PAPP-A is associated with atherosclerotic PAD, we used data from the Linz Peripheral Arterial Disease (LIPAD) study (20), which was designed to evaluate possible phenotypic and genotypic risk factors for atherosclerotic PAD. The study protocol was approved by the local ethics committee in accordance with the Declaration of Helsinki, and all study participants gave informed consent. The LIPAD study objectives, recruitment procedures, and characteristics have been described previously (20). The study sample comprised 433 consecutive patients with symptomatic atherosclerotic PAD admitted to the St. John of God Hospital in Linz, Austria, for inpatient diagnostics and treatment of chronic limb ischemia, and 433 controls matched to the PAD patients with a 1:1 design by sex, age (±2 years), and diabetes mellitus status (i.e., for each PAD patient enrolled in the study, an appropriate referent was recruited by the investigators). This series of study participants underwent evaluation for the presence of risk factors for atherosclerosis and comorbid conditions, as recommended by Rutherford et al. (21).

PAD was defined as chronic atherosclerotic disease of the lower extremities associated with typical symptoms, such as claudication or leg pain on exertion, rest pain, or minor or major tissue loss, and was verified by interview; physical examination; Doppler segmental blood pressure measurement of the lower limbs, including continuous-wave spectral analysis and resting ankle-brachial index measurements; and intraarterial aortofemoral angiography. Patients with PAD were included in the present study on the basis of the final clinical diagnosis established by the attending vascular surgeons. All cases with acute ischemia (i.e., peripheral arterial thrombosis of a native artery, popliteal artery aneurysm, or acutely thrombosed peripheral bypass grafts) were excluded. Additional exclusion criteria were PAD attributable to nonatherosclerotic causes (cardioembolic disease, thromboangiitis obliterans, vasculitis, or congenital or metabolic vascular disease) and a history or presence of any malignancy. Controls were free of manifest or previous atherosclerotic disease (i.e., coronary artery disease, cerebrovascular disease, or PAD). All controls had an ankle-brachial index ≥1.0, no pathologic pattern of pulse waves in lower limbs by continuous-wave spectral analysis, no stenosis of the internal carotid artery ≥50% by color duplex ultrasound scans, and no history or presence of any malignancy. None of the controls received a lipid-lowering medication, and took folate supplement or B vitamin by study design. The referents were patients of our hospital who had been admitted for treatment of minor health problems, such as cataract surgery, vertebrogenic pain, or nonvascular surgery (e.g., hemiomyotomy or varicose vein removal) and were recruited without knowledge of their laboratory data.

Coronary artery disease was defined as remote myocardial infarction by history, occult myocardial infarction by electrocardiography, previous coronary bypass surgery or percutaneous transluminal coronary angioplasty, and stable or unstable angina and acute coronary syndrome (cardiac troponin positive or negative). Cerebrovascular disease was defined as transient or temporary stroke, completed stroke with permanent neurologic deficit, or acute stroke. Arterial hypertension, diabetes mellitus, and smoking were classified according to recommended standards (21).

**BIOCHEMICAL ANALYSES**

Blood was collected at venipuncture in Vacutette polyethylene terephthalate glycol clot activator tubes (Greiner Bio-One) after the patient had fasted overnight. Creatinine, fasting glucose, hemoglobin A1c, total cholesterol, triglycerides, apolipoprotein A1, apolipoprotein B, and lipoprotein(a) were analyzed by standard assays on a COBAS Integra analyzer (Roche Diagnostics). For determination of HDL- and LDL-cholesterol, we used quantitative electrophoresis with enzymatic staining (Helena BioSciences Europe). Total homocysteine (tHcy), folate, and vitamin B12 assays were performed on an AxSYM analyzer (Abbott Diagnostics). The concentration of C-reactive protein (CRP) was measured by a high-sensitivity assay (N High Sensitivity CRP) on a BN ProSpec analyzer (Dade Behring) with polystyrene particles coated with monoclonal mouse antibodies to CRP. Estimated glomerular filtration rate (eGFR) was calculated as recommended recently (22).

**PAPP-A MEASUREMENTS**

Serum samples for PAPP-A measurements were immediately frozen and stored at −80 °C until assessment. Frozen samples were thawed and mixed thoroughly before use. PAPP-A was then quantified automatically by the commercially available Active® Ultra-Sensitive PAPP-A ELISA (Diagnostic System Laboratories Inc.) on a BEP® 2000 instrument (Dade Behring). This assay is an enzymatically amplified 2-step sandwich type immunoassay with a lower detection limit of 0.06 mU/L, and the principles of the assay have been described in detail previously (3). Of note, both monoclonal antibodies used for this assay are directed against the PAPP-A molecule but not against the proMBP subunit. The whole procedure
for PAPP-A determination was performed according to the manufacturer’s recommendations. Controls provided by the manufacturer were used to accept or reject individual runs. Samples from matched patients with PAD and controls were assayed in duplicate on the same microwell plates.

To evaluate the precision of the PAPP-A assay in our laboratory, we performed a replication study according to Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) guideline EP5-A (23). A high and a low control (provided by the manufacturer) and 1 pooled patient serum sample (aliquoted into twenty 1.5-mL plastic tubes and frozen at −80°C) were used for the experiment. We analyzed these samples in duplicate in 1 run per day for 20 days on the BEP 2000 instrument. Within-run and total imprecision (CV) were calculated with the CLSI single-run precision evaluation test (23). The PAPP-A assay had a within-run CV of 4.0% and a total CV of 4.7% at a mean concentration of 11.33 mU/L (high control), a within-run CV of 3.5% and a total CV of 7.1% at a mean concentration of 2.53 mU/L (low control), and a within-run CV of 14% and a total CV of 20% at a mean concentration of 0.24 mU/L (pooled serum sample).

**Statistical Methods**

Statistical analyses were performed with the SPSS (Ver. 13.0) software (SPSS Inc.) and the MedCalc 8.0.0.0 package (MedCalc Software). Dichotomous variables are given as prevalence in number, and continuous data are expressed as the median (25th–75th percentiles). Univariate comparisons of risk factors and other dichotomous variables between the 2 study groups were calculated with the Fisher exact test, and continuous variables were evaluated with the nonparametric Mann–Whitney U-test (respective P values were not adjusted for multiple comparisons and are therefore descriptive only). To determine whether the serum PAPP-A concentration was a predictor for PAD and to calculate multivariable odds ratios, we performed logistic regression analyses without variable selection techniques (possible confounding risk factors were included simultaneously in the regression models). Dichotomous risk factors were coded with an indicator variable of 1 for having the condition and 0 for its absence. Variable selection was based on significant univariate differences and clinical relevance. We calculated the Spearman coefficient of rank correlation (r_s) to assess the relationship of continuous data in the study sample. We also used logistic regression to explore whether PAPP-A increased the predictive value of other commonly used markers. Probabilities were two-tailed, and P <0.05 was regarded as statistically significant.

**Results**

Study patients with atherosclerotic PAD (i.e., chronic limb ischemia; n = 433) were admitted because of mild to severe claudication or leg pain on exertion (n = 359; 83%), ischemic rest pain (n = 15; 3%), or minor or major tissue loss (n = 59; 14%). Of the 433 patients with PAD, 131 (30%) had concomitant coronary artery disease and 80 (18%) had concomitant cerebrovascular disease. Furthermore, 101 patients with PAD had ≥50% carotid stenosis. Eighteen additional patients with PAD were classified as having stenosis ≥50% as well because they had undergone previous carotid surgery to treat stenosis. Per definition, none of the 433 controls had coronary artery disease, cerebrovascular disease, or internal carotid stenosis ≥50%, but many (n = 365) had carotid plaques, indicating mild but not clinically relevant atherosclerosis.

The clinical and biochemical characteristics of all individuals included in the study are described in Table 1. Current smoking, arterial hypertension, and renal dysfunction were significantly more prevalent in the PAD group than in the control group. Among other biochemical markers, the median serum PAPP-A concentrations were significantly higher in the 433 patients with PAD (0.81 mU/L) than in the 433 controls (0.64 mU/L; P <0.001). Thus, the median serum concentration of PAPP-A for the patients with PAD was ~1.3-fold higher than that in the referents.

The odds ratios for symptomatic PAD in the sample studied, calculated without variable selection technique, are shown in Table 2. We found an increasing and constant relationship of PAPP-A and PAD for all 4 statistical models across the quartiles of PAPP-A. In the final multivariable model (model 4), PAPP-A was a significant predictor of symptomatic PAD. After adjustment with multivariable logistic regression for age, sex, diabetes mellitus status, smoking, arterial hypertension, renal dysfunction, body mass index, fasting glucose, LDL- and HDL-cholesterol, triglycerides, high-sensitivity CRP (hs-CRP), and tHcy, the odds ratios for PAD were 1.59 (95% confidence interval, 1.00–2.52; P = 0.049), 2.28 (95% confidence interval, 1.45–3.61; P <0.001), and 2.86 (95% confidence interval, 1.78–4.59; P <0.001) in the 2nd, 3rd, and 4th quartiles of serum PAPP-A concentrations compared with the first quartile.

To assess PAPP-A independently of concomitant coronary artery disease and cerebrovascular disease in the PAD patients, we performed an exploratory subgroup analysis. Of the 433 patients, 253 had symptomatic PAD without clinically manifest concomitant coronary artery disease or cerebrovascular disease. These 253 patients were compared with the corresponding 253 controls, and the median PAPP-A value was higher in the patients with PAD than in the matched controls (0.82 vs 0.62 mU/L; P <0.001). When we used the same multivariable approach as in model 4 in Table 2, but with slightly modified cut points of PAPP-A for the quartile approach [1st quartile <0.439 mU/L (n = 126); 2nd quartile 0.439–0.716 mU/L (n = 127); 3rd quartile 0.717–1.163 mU/L (n = 127); and 4th quartile >1.163 mU/L (n = 126)], the analyte was also independently related to the presence of PAD in this subgroup of 506 study participants, displaying odds ra-
Table 1. Clinical and biochemical data of patients with PAD and controls.\( ^a \)

<table>
<thead>
<tr>
<th></th>
<th>PAD group</th>
<th>Control group</th>
<th>( p^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. in group</td>
<td>433</td>
<td>433</td>
<td>( &gt;0.99^c )</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>306 (71)</td>
<td>306 (71)</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>68 (59–75)</td>
<td>68 (60–75)</td>
<td>0.69( ^c )</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
<td>26 (24–29)</td>
<td>26 (24–29)</td>
<td>0.21</td>
</tr>
<tr>
<td>Current smoking,( ^d ) n (%)</td>
<td>193 (45)</td>
<td>51 (12)</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Arterial hypertension, n (%)</td>
<td>251 (58)</td>
<td>178 (41)</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>115 (27)</td>
<td>115 (27)</td>
<td>( &gt;0.99^c )</td>
</tr>
<tr>
<td>Renal dysfunction,( ^e ) n (%)</td>
<td>90 (21)</td>
<td>40 (9)</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Coronary artery disease, n (%)</td>
<td>131 (30)</td>
<td>0</td>
<td>( &lt;0.001^f )</td>
</tr>
<tr>
<td>Cerebrovascular disease, n (%)</td>
<td>80 (18)</td>
<td>0</td>
<td>( &lt;0.001^f )</td>
</tr>
<tr>
<td>Carotid stenosis ( \geq 50%), n (%)</td>
<td>119 (27)</td>
<td>0</td>
<td>( &lt;0.001^f )</td>
</tr>
<tr>
<td>ABI,( ^g ) mmHg/mmHg</td>
<td>0.63 (0.47–0.79)</td>
<td>1.18 (1.09–1.29)</td>
<td>( &lt;0.001^f )</td>
</tr>
</tbody>
</table>

Relevant medication

<table>
<thead>
<tr>
<th>Antihypertensive treatment, n (%)</th>
<th>Single medication</th>
<th>Two-fold combination</th>
<th>Three-fold combination</th>
<th>Aspirin therapy, n (%)</th>
<th>Oral anticoagulation, n (%)</th>
<th>Lipid-lowering medication, n (%)</th>
<th>Folate supplement, n (%)</th>
<th>B-Vitamin supplement, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>86 (20)</td>
<td>105 (24)</td>
<td>60 (14)</td>
<td>200 (46)</td>
<td>49 (11)</td>
<td>102 (24)</td>
<td>10 (2)</td>
<td>18 (4)</td>
</tr>
</tbody>
</table>

Biochemical markers

<table>
<thead>
<tr>
<th></th>
<th>PAD group</th>
<th>Control group</th>
<th>( p^b )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine ( \mu)mol/L</td>
<td>88.4 (79.6–97.2)</td>
<td>79.6 (70.7–88.4)</td>
<td></td>
</tr>
<tr>
<td>eGFR, mL ( \times ) ( 1) min ( \times ) ( 1) ( m^2)</td>
<td>1.0 (0.9–1.1)</td>
<td>0.9 (0.8–1.0)</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Total-cholesterol ( \mu)mol/L</td>
<td>5.93 (5.05–6.68)</td>
<td>5.57 (4.71–6.29)</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>LDL-cholesterol ( \mu)mol/L</td>
<td>229 (195–258)</td>
<td>215 (182–243)</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>HDL-cholesterol ( \mu)mol/L</td>
<td>3.88 (3.13–4.61)</td>
<td>3.52 (2.80–4.09)</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Triglycerides ( \mu)mol/L</td>
<td>150 (121–178)</td>
<td>136 (108–158)</td>
<td></td>
</tr>
<tr>
<td>Apolipoprotein B,( ^i ) g/L</td>
<td>0.97 (0.80–1.16)</td>
<td>0.84 (0.70–0.99)</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Apolipoprotein A1,( ^i ) g/L</td>
<td>1.47 (1.27–1.67)</td>
<td>1.44 (1.26–1.59)</td>
<td>0.14</td>
</tr>
<tr>
<td>Fasting glucose( ^j ) mmol/L</td>
<td>5.33 (4.88–5.77)</td>
<td>5.16 (4.77–5.66)</td>
<td></td>
</tr>
<tr>
<td>Lipoprotein(a),( ^i ) mg/L</td>
<td>147 (81–396)</td>
<td>81 (81–223)</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>PAPP-A, mU/L</td>
<td>0.81 (0.53–1.26)</td>
<td>0.64 (0.40–1.04)</td>
<td>( &lt;0.001 )</td>
</tr>
</tbody>
</table>

\( ^a \) Age, body mass index, resting ankle brachial index, and biochemical markers are given as the median (25th–75th percentiles).

\( ^b \) Nonparametric Mann–Whitney \( U \)-test or Fisher exact test as appropriate.

\( ^c \) Matched variables.

\( ^d \) Current smoking was defined as any amount of tobacco use, including abstinence less than 1 year (21).

\( ^e \) Renal dysfunction was defined as eGFR \( <60 \) mL \( \times \) \( 1\) min \( \times \) \( 1\) \( m^2\).

\( ^f \) Differences by study design.

\( ^g \) ABI, resting ankle brachial index; Hb \( A_{\text{tot}} \), hemoglobin \( A_{\text{tot}} \).

\( ^i \) Apolipoprotein A1, apolipoprotein B, and lipoprotein(a) were measured in 300 patients with PAD and 300 matched controls.

\( ^j \) Persons with diabetes mellitus were excluded.
Table 2. Results of logistic regression analysis of the capability of PAPP-A for predicting PAD independently of possible confounding variables.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Quartile of PAPP-A</th>
<th>1st quartile (&lt;0.458 mU/L) (n = 216)</th>
<th>2nd quartile (0.458–0.726 mU/L) (n = 217)</th>
<th>3rd quartile (0.727–1.183 mU/L) (n = 217)</th>
<th>4th quartile (&gt;1.183 mU/L) (n = 216)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odds ratios 1\textsuperscript{a}</td>
<td>1.00</td>
<td>1.48 (1.01–2.17)</td>
<td>2.06 (1.40–3.03)</td>
<td>2.52 (1.71–3.71)</td>
</tr>
<tr>
<td>( P )</td>
<td>0.046</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Odds ratios 2\textsuperscript{a}</td>
<td>1.00</td>
<td>1.47 (1.00–2.16)</td>
<td>2.10 (1.43–3.10)</td>
<td>2.57 (1.72–3.82)</td>
</tr>
<tr>
<td>( P )</td>
<td>0.048</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Odds ratios 3\textsuperscript{a}</td>
<td>1.00</td>
<td>1.51 (0.98–2.33)</td>
<td>2.07 (1.34–3.19)</td>
<td>2.53 (1.63–3.93)</td>
</tr>
<tr>
<td>( P )</td>
<td>0.062</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Odds ratios 4\textsuperscript{a}</td>
<td>1.00</td>
<td>1.59 (1.00–2.52)</td>
<td>2.28 (1.45–3.61)</td>
<td>2.86 (1.78–4.59)</td>
</tr>
<tr>
<td>( P )</td>
<td>0.049</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Data are expressed as odds ratio (95% confidence interval); multivariate odds ratios were calculated by logistic regression analysis without variable selection technique (all independent variables were included simultaneously into the model). Current smoking was defined as any amount of tobacco use, including abstinence less than 1 year (21). Renal dysfunction was defined as eGFR <60 mL·min\(^{-1}\)·(1.73 m\(^2\))\(^{-1}\).

\textsuperscript{b} Univariate analysis, not adjusted for possible confounders.

\textsuperscript{c} Multivariate analysis adjusted for age, sex, diabetes mellitus status, current smoking, arterial hypertension, and renal dysfunction.

\textsuperscript{d} Multivariate analysis adjusted for age, sex, diabetes mellitus status, current smoking, arterial hypertension, renal dysfunction, body mass index, LDL- and HDL-cholesterol, triglycerides, fasting glucose, hs-CRP, and tHcy.

To explore whether PAPP-A added to the predictive value of other markers commonly in use, we computed the odds ratios of symptomatic PAD by logistic regression analyses in which the study participants (n = 866) were stratified into 9 groups according to terciles of LDL-cholesterol [1st tercile <3.21 mmol/L (<124 mg/dL); 2nd tercile, 3.21–3.95 mmol/L (124–158 mg/dL); 3rd tercile >3.95 mmol/L (>158 mg/dL)], hs-CRP (1st tercile <0.57 mg/L; 2nd tercile, 0.57–1.84 mg/L; 3rd tercile >1.84 mg/L), tHcy (1st tercile <13.3 μmol/L; 2nd tercile, 13.3–17.6 μmol/L; 3rd tercile >17.6 μmol/L), eGFR [1st tercile >88 mL·min\(^{-1}\)·(1.73 m\(^2\))\(^{-1}\); 2nd tercile, 88–71 mL·min\(^{-1}\)·(1.73 m\(^2\))\(^{-1}\); 3rd tercile <71 mL·min\(^{-1}\)·(1.73 m\(^2\))\(^{-1}\)], and PAPP-A serum concentrations (1st tercile <0.540 mL/L; 2nd tercile, 0.540–0.984 mL/L; 3rd tercile >0.984 mL/L), respectively. For each marker included in this analysis, the odds ratio of symptomatic PAD was lowest among participants with low serum PAPP-A concentrations and low concentrations of LDL-cholesterol, hs-CRP, and tHcy as well as high eGFR (Fig. 1). In contrast, odds ratios appeared to be highest among participants with high serum PAPP-A concentrations and high concentrations of LDL-cholesterol, hs-CRP, and tHcy as well as diminished eGFR. For example, in the top tercile of LDL-cholesterol, odds ratios of symptomatic PAD were 2.77, 3.89, and 6.42 for the 1st, 2nd, and 3rd terciles of serum PAPP-A concentrations (Fig 1A). Similar results were obtained for the top tercile of hs-CRP, with odds ratios of symptomatic PAD increasing from 4.96 to 7.53 and 7.36 for the 1st, 2nd, and 3rd terciles of PAPP-A (Fig. 1B); for the top tercile of tHcy, with odds ratios of symptomatic PAD increasing from 1.78 to 2.99 and 3.99 for the 1st, 2nd, and 3rd terciles of PAPP-A (Fig. 1C); and for the lowest tercile of eGFR, with odds ratios of symptomatic PAD increasing from 3.47 to 4.49 and 6.72 for the 1st, 2nd, and 3rd terciles of PAPP-A (Fig. 1D).
The present study provides evidence that increased serum concentrations of the metalloproteinase PAPP-A are associated with symptomatic atherosclerotic PAD. In addition, the association between PAD and PAPP-A appeared to be independent of concomitant clinically manifest coronary artery disease and cerebrovascular disease, as demonstrated by subgroup analyses. Furthermore, measurement of serum PAPP-A concentrations appeared to increase the predictive value of other markers commonly in use, such as LDL-cholesterol, hs-CRP, tHcy, and eGFR.

Chronic ischemia of the lower limbs is the consequence of the progression of atherosclerosis with the occurrence of hemodynamically relevant stenoses and occlusions of the arterial tree, but without clinically relevant thromboembolic or atherothrombotic events (24, 25). In the population older than 55 years, atherosclerotic PAD is an acknowledged indicator of systemic atherosclerotic disease (19, 25); therefore, the association between increased PAPP-A and symptomatic PAD demonstrated in our study suggests that circulating PAPP-A may be a marker not only for plaque destabilization and/or plaque rupture in the coronary vasculature, but also for systemic atherosclerotic disease. Indeed, it has been proposed that increased PAPP-A concentrations may not be limited to patients with acute coronary syndrome but could also reflect earlier stages of atherosclerotic lesions, even in the absence of clinical signs of atherosclerosis (10). In addi-

**Discussion**

The present study provides evidence that increased serum concentrations of the metalloproteinase PAPP-A are associated with symptomatic atherosclerotic PAD. In addition, the association between PAD and PAPP-A appeared to be independent of concomitant clinically manifest coronary artery disease and cerebrovascular disease, as demonstrated by subgroup analyses. Furthermore, measurement of serum PAPP-A concentrations appeared to increase the predictive value of other markers commonly in use, such as LDL-cholesterol, hs-CRP, tHcy, and eGFR.

Chronic ischemia of the lower limbs is the consequence of the progression of atherosclerosis with the occurrence of hemodynamically relevant stenoses and occlusions of the arterial tree, but without clinically relevant thromboembolic or atherothrombotic events (24, 25). In the population older than 55 years, atherosclerotic PAD is an acknowledged indicator of systemic atherosclerotic disease (19, 25); therefore, the association between increased PAPP-A and symptomatic PAD demonstrated in our study suggests that circulating PAPP-A may be a marker not only for plaque destabilization and/or plaque rupture in the coronary vasculature, but also for systemic atherosclerotic disease. Indeed, it has been proposed that increased PAPP-A concentrations may not be limited to patients with acute coronary syndrome but could also reflect earlier stages of atherosclerotic lesions, even in the absence of clinical signs of atherosclerosis (10). In addi-
tion to the relationship of PAPP-A with stable and unstable coronary artery disease (2–7, 10), PAPP-A concentrations are considered potential markers of echogenic carotid atherosclerotic lesions in asymptomatic hyperlipidemic patients (26). A second study showed weak but significant associations of PAPP-A concentrations with carotid intima-media wall thickness and toe-brachial index in patients with diabetes mellitus (27). The results of these studies provide evidence for PAPP-A being a marker of atherosclerotic disease in vascular territories other than the coronary arteries, in accordance with our findings that PAPP-A may be significantly related to the presence of systemic atherosclerosis.

Divergent results on the associations between serum PAPP-A concentrations and several other markers of atherosclerotic disease have been demonstrated. A positive association between PAPP-A and hs-CRP concentrations has been shown in some studies (2, 7, 26) but was not confirmed by others (5, 6, 27). Preliminary findings indicated that in patients with renal failure, serum PAPP-A concentrations might be related to renal function and to dialysis modalities (28); however, another study did not find an association of PAPP-A and creatinine clearance in patients with type 2 diabetes (27). There are also conflicting results related to an association of PAPP-A and LDL-cholesterol (26, 27), with reported associations shown predominantly in smaller samples. Our case–control study of 866 participants showed no significant associations with respect to PAPP-A vs hs-CRP, eGFR, and LDL-cholesterol, indicating that there is no substantial degree of overlap between serum concentrations of PAPP-A and the these 3 markers.

Circulating PAPP-A in acute coronary syndrome has been suggested to differ from that found in the serum of pregnant women (17). In particular, PAPP-A in serum of patients with atherosclerotic disease appears to be present as a homodimer, making it difficult to measure PAPP-A as a marker for coronary syndromes/atherosclerosis by immunoassays that are designed to detect molecules in pregnancy serum via a sandwich formed by 2 antibodies, one specific for PAPP-A and the other specific for proMBP (10, 17). The commercially available ultrasensitive PAPP-A assay used in our study was specifically developed for measuring PAPP-A as a cardiac marker (3). Each manufacturer’s assay uses different sets of antibodies for the detection of atherosclerosis-related PAPP-A; thus, the absolute concentration in a serum sample varies depending on the assay used. The absolute PAPP-A serum concentrations obtained in our study sample may therefore not be directly comparable to the PAPP-A concentrations reported by other authors for distinct atherosclerotic conditions.

Our study has several potential limitations. First, as with any cross-sectional study, our study cannot establish causality. Hence, the issue of whether the association between PAPP-A and vascular damage is causative or merely reactive (14) cannot be addressed by our study. Therefore, prospective studies on the relationship between PAPP-A and atherosclerosis are needed to address the proposed proatherogenic role of PAPP-A. Second, although we tried to demonstrate that the association between PAPP-A and PAPP-A was independent of concomitant clinically manifest coronary artery disease and cerebrovascular disease, it is impossible to completely disentangle PAD (as a marker of systemic atherosclerotic disease) and coronary artery disease and cerebrovascular disease, respectively, because the latter 2 were defined as clinically manifest diseases in our study. Thus, our study design precludes exclusion of concomitant early atherosclerotic disease, especially in the coronary vasculature. Third, the study sample was a selected subgroup of the overall population of PAD group (patients admitted for inpatient diagnostics and treatment of atherosclerotic PAD). Thus, the findings cannot be generalized to asymptomatic patients with PAD or patients who do not meet criteria for hospitalization. Because the association between atherosclerotic PAD and increased PAPP-A concentrations might be most easily detected in the most severely diseased patients (i.e., the symptomatic patients admitted to the hospital), further studies should clarify the role of PAPP-A as a potential risk marker for asymptomatic PAD.

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References