Serum Plasma Pregnancy-Associated Protein A
A Potential Marker of Echogenic Carotid Atherosclerotic Plaques in Asymptomatic Hyperlipidemic Subjects at High Cardiovascular Risk


Objective—The proteolytic activity of metalloproteinases, proinflammatory enzymes that degrade extracellular matrix, is elevated in lipid-rich atherosclerotic plaques, thereby contributing to plaque fragility and rupture. Pregnancy-associated plasma protein (PAPP-A) is a metalloproteinase, expressed in unstable atherosclerotic plaques, whose circulating levels are elevated in acute coronary syndromes. We evaluated serum PAPP-A levels as a marker of the premature development of atherosclerosis in hyperlipidemic subjects at elevated cardiovascular risk.

Methods and Results—Serum PAPP-A levels were determined in asymptomatic hyperlipidemic male subjects (n=64; mean±SD age, 51±7 years) in whom intima-media thickness (IMT) and lesion status in the carotid artery were evaluated by noninvasive ultrasonography and compared with those of a normolipidemic control group (n=25). No difference was observed in circulating PAPP-A levels between hyperlipidemic subjects and controls (8.99±2.93 and 8.03±2.75 mIU/L, respectively; mean±SD) nor between hyperlipidemic subjects who presented with a luminal obstruction of the carotid artery (9.26±2.53 mIU/L) and those who did not (8.85±3.29 mIU/L). By contrast, in patients with atheromatous carotid plaques, a positive association between serum levels of PAPP-A and C-reactive protein was observed (P<0.05); moreover, subjects exhibiting hyperechoic or isoechoic, echogenic lesions had significantly higher PAPP-A levels compared with those with hypoechoic lesions (10.32±2.72 vs 8.27±2.18 mIU/L, P<0.05) and with normolipidemic controls (P<0.05).

Conclusions—Elevated serum PAPP-A levels represent a potential marker of the degree of echogenicity of carotid atherosclerotic plaques in asymptomatic hyperlipidemic patients at high cardiovascular risk and equally of an enhanced local inflammatory state involving remodeling of subendothelial extracellular matrix. (Arterioscler Thromb Vasc Biol. 2003;23:●●●●●●.)

Key Words: pregnancy-associated plasma protein A ■ metalloproteinase ■ hyperlipidemia ■ carotid atherosclerosis

Inflammation plays a key role in the initiation, progression, and rupture of the atherosclerotic plaque.1,2 Several systemic proteins have been proposed as markers of a chronic inflammatory state in the arterial wall, including high-sensitive C-reactive protein (hsCRP), serum amyloid A protein, and soluble adhesion molecules.3–5 Elevated levels of hsCRP were shown to be predictive of peripheral or coronary atherosclerosis1 and to constitute an independent predictor of peripheral or coronary events, thereby contributing to plaque fragility and rupture.7

Pregnancy-associated plasma protein A (PAPP-A) is a high-molecular-weight, zinc-binding metalloproteinase that is typically measured during pregnancy in maternal blood for the fetal diagnosis of Down syndrome. However, circulating PAPP-A is physiologically present in both men and women; moreover, it is abundantly expressed in advanced atherosclerotic lesions and constitutes a specific activator of insulin-like growth factor, a mediator of atherosclerosis.8 Peripheral blood levels of PAPP-A have been recently proposed as a biological marker of acute coronary syndromes.9 To evaluate PAPP-A as a potential marker of the presence and echogenicity of atheromatous carotid plaques in asymptomatic hyperlipidemic subjects, we evaluated the relationship between circulating PAPP-A levels and carotid intima-media thickness (IMT) in atherogenic dyslipidemia; equally, PAPP-A levels were compared with those of CRP, an
Clinical and Biological Characteristics of Asymptomatic Hyperlipidemic Subjects With or Without Atheromatous Lesions and of Normolipidemic Control Subjects

### Hyperlipidemic Subjects

<table>
<thead>
<tr>
<th></th>
<th>With Atherosclerotic Lesions</th>
<th>Without Atherosclerotic Lesions</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>34</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>Age, y</td>
<td>52±7 (40–70)</td>
<td>50±7 (40–64)</td>
<td>48±7 (37–64)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.6±3.6 (19.5–36.1)</td>
<td>26.5±3.0 (20.8–34.6)</td>
<td>24.3±2.5 (18.2–29.5)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>134±14 (105–160)</td>
<td>132±15 (110–180)</td>
<td>118±14 (105–140)</td>
</tr>
<tr>
<td>Diastolic blood pressure, m Hg</td>
<td>82±6 (70–90)</td>
<td>84±10 (70–110)</td>
<td>78±6 (70–90)</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>6.67±1.65§ (4.75–13.02)</td>
<td>6.49±0.88† (5.09–8.11)</td>
<td>4.56±0.47 (3.25–5.10)</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.69±1.19§ (0.27–6.42)</td>
<td>2.11±2.22‡ (0.42–12.70)</td>
<td>1.20±0.40 (0.45–1.70)</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/L</td>
<td>5.30±2.02† (3.07–8.90)</td>
<td>4.50±0.86† (2.95–5.97)</td>
<td>2.55±0.61 (1.80–3.05)</td>
</tr>
<tr>
<td>HDLC, mmol/L</td>
<td>1.37±0.31† (0.85–2.38)</td>
<td>1.34±0.28† (0.83–1.96)</td>
<td>1.82±0.31 (1.25–2.36)</td>
</tr>
<tr>
<td>TC/HDLC ratio</td>
<td>4.8±1.3† (2.5–7.79)</td>
<td>5.0±1.1‡ (3.2–7.9)</td>
<td>2.9±0.6 (1.9–4.3)</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>1.50±0.95* (0.20–3.90)</td>
<td>1.18±0.53* (0.20–2.30)</td>
<td>1.14±0.58 (0.28–2.95)</td>
</tr>
<tr>
<td>PAPP-A, mIU/L</td>
<td>9.26±2.53 (4.93–15.79)</td>
<td>8.85±3.29 (4.00–20.39)</td>
<td>8.03±2.75 (4.12–13.56)</td>
</tr>
</tbody>
</table>

Values are mean±SD (minimum–maximum). TC indicates total cholesterol; HDLC, HDL-cholesterol.

*P<0.01, †P<0.001 vs control group.

§Calculated when triglycerides levels were <4 mmol/L.

### Methods

#### Patients

Sixty-four male patients (mean±SD age, 51±7 years) who were referred to the Outpatient Clinic at Hôpital de la Pitié (Paris) for hyperlipidemia (serum total cholesterol ≥5.2 mmol/L and/or triglycerides ≥1.7 mmol/L) were included in the study. Patients with diabetes, thyroid dysfunction, acute or chronic hepatic disease, a serum creatinine level >120 μmol/L, or a previous history of cardiovascular disease (myocardial infarction, angina, or ischemic stroke) were excluded. Subjects who presented with an acute or chronic inflammatory disease, as defined by a plasma CRP concentration ≥10 mg/L, and those taking aspirin or any other nonsteroidal anti-inflammatory agents were also excluded. All patients systematically underwent ultrasonography of the extracranial carotid arteries on a Duplex system (Acuson Sequoia 512) that was performed in a single laboratory. IMT was measured as previously reported. Carotid lesions were classified according to their echogenic characteristics: an isoechoic plaque was defined as having the echogenicity of a normal intima-media complex, whereas a hyperechoic, echogenic plaque was brighter than an isoechoic plaque, and a hypoechoic plaque was not as bright as an isoechoic plaque. Ultrasonographic determinations and analysis were done in a blinded fashion. A control group of 25 age-matched, normolipidemic, healthy male subjects (mean±SD age, 48±7 years) served as a reference for biological parameters.

#### Laboratory Analysis

Blood samples were taken by venipuncture after a 12-hour overnight fast. Serum lipids and lipoproteins were analyzed by standard analytical procedures, as previously described. Insulin-like growth factor binding protein 14 (sIGFBP14) levels were measured with an immunonephelometric method on a BNII analyzer (Dade-Behring); the detection limit of this assay was 0.20 mg/L. Serum PAPP-A values were assayed with a time-resolved fluorimunoassay on a Kryptor analyzer (Brahms); this assay used monoclonal antibodies that recognized the circulating form of PAPP-A, i.e., the complex PAPP-A/pro-major basic protein. The detection limit was 4 mIU/L. The assay was calibrated against the World Health Organization’s international reference standard 78/610, which is the standard for pregnancy-associated proteins.

### Results

The clinical and biological characteristics of asymptomatic hyperlipidemic subjects and normolipidemic controls are given in Table 1. On the basis of ultrasonographic analysis of carotid arteries, hyperlipidemic subjects were classified into 2 groups: the first (n=30) consisted of patients without evident carotid atherosclerotic lesions (no detectable luminal obstruction), whereas the second group (n=34) consisted of patients with a luminal obstruction of the carotid artery representing 20% or more (up to 40%) of the total lumen. The mean value for IMT of carotid arteries in the latter group (0.67±0.17 mm) was significantly elevated compared with that of hyperlipidemic patients lacking carotid plaques and those free of atherosclerosis or controls (Figure 1). Multiple regression models showed that serum PAPP-A levels were not associated with age, risk factors, or lipid parameters in either group. In the same way, circulating levels of PAPP-A were not correlated with IMT values in

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Statistical Analysis

Data are expressed as mean±SD values. Because a normal distribution of serum PAPP-A levels was not established in a hyperlipidemic population, statistical analysis was performed by the nonparametric Mann-Whitney U test. Differences among the groups were evaluated by the Kruskal-Wallis test. Associations between serum PAPP-A, levels, serum CRP levels, and IMT were assessed by Spearman’s rank correlation test. Statistical significance was assumed at a level of P<0.05. All statistical analyses were performed with Statview 5.1 for PC Windows (SAS).
asymptomatic hyperlipidemic patients in whom plaques were present as well as in the group lacking plaques. By contrast, we observed a significant association between serum PAPP-A concentrations and CRP levels in hyperlipidemic subjects with carotid lesions (Spearman’s rho = 0.36, P < 0.05). Moreover, patients who exhibited hyperechoic or isoechoic, echogenic plaques (n = 16) displayed significantly higher serum PAPP-A levels compared with patients with hypoechoic plaques (n = 11; 10.32 ± 2.72 vs 8.27 ± 2.19 mIU/L, P < 0.05) and with normolipidemic controls (P < 0.05).

Discussion
The present study provides evidence for the first time that circulating levels of the metalloproteinase PAPP-A are associated not only with the echogenicity of atherosclerotic carotid lesions but also with an enhanced inflammatory state in asymptomatic hyperlipidemic subjects.

The progression of atheromatous plaques involves major changes in the structure of the arterial wall. The occurrence of a local inflammatory state is well established, as revealed by inflammatory markers such as CRP.3 MMPs are also potential indicators of arterial inflammation, and by degrading extracellular matrix, they contribute to the fragility of the lipid-rich, atherosclerotic plaque and finally to its rupture. As previously described for several other MMPs (MMP-1, MMP-3, MMP-12, or MMP-13),10,11 PAPP-A was recently found to be abundantly expressed in both eroded and ruptured plaques but in contrast, is only moderately expressed in stable plaques.9 Our results are consistent with this observation, because patients with hyperechoic or isoechoic plaques, corresponding to type V (or greater) lesions according to the American Heart Association–recommended classification,12 exhibited significantly higher PAPP-A levels than those with hypoechoic early lesions (type III or IV in the American Heart Association classification). The production of PAPP-A by activated cells and its release into the extracellular matrix appear to be strongly linked to the local inflammatory process occurring within the arterial wall, as indicated by the significant positive correlation observed between CRP and PAPP-A levels. A similar correlation was previously observed in patients with acute coronary syndromes.8 To complement our original findings, studies are in progress to determine whether serum CRP levels are associated with the echogenicity of carotid atherosclerotic plaques in asymptomatic hyperlipidemic subjects (authors’ unpublished observations).

It is noteworthy that serum PAPP-A levels were not correlated with plasma lipid levels, including the total cholesterol to HDL cholesterol ratio, which was recently described to be the strongest lipid predictor of risk for systemic atherosclerosis.13 Such a lack of correlation was equally observed for circulating levels of another metalloproteinase, MMP-9, in normolipidemic subjects apparently free of atherosclerosis14 and in patients with premature coronary atherosclerosis.15

Thus, the report of Bayes-Genis et al9 and our present studies are concordant in identifying elevated serum PAPP-A levels as a marker of unstable or advanced hyper/isoechoic carotid atherosclerotic lesions, because early hypoechoic plaques are not associated with an elevation in circulating PAPP-A levels.

Conclusions
The present study indicates that circulating PAPP-A levels are significantly associated with those of CRP in asymptomatic hyperlipidemic patients exhibiting carotid plaques. In addition, patients displaying hyperechoic or isoechoic, echogenic carotid lesions exhibited significantly higher serum PAPP-A levels compared with patients with hypoechoic lesions, as well as with normolipidemic controls. Circulating PAPP-A levels therefore represent a potential marker not only of an enhanced local inflammatory state but also of echogenic carotid atherosclerotic lesions in asymptomatic hyperlipidemic patients at elevated cardiovascular risk.

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References

Figure 1. Serum levels of hsCRP (*) and PAPP-A (●) in hyperlipidemic patients with or without atherosclerotic lesions (as defined by ultrasonography; see Methods). *P < 0.05 vs control group and vs hyperlipidemic patients without carotid atherosclerotic plaques.
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