For more than a decade, pregnancy-associated plasma protein-A (PAPP-A) has been examined for its relation to acute coronary syndrome (ACS) and the vulnerable plaque. This review summarizes the current knowledge of plasma PAPP-A in relation to nonpregnant individuals focusing on patients with ACS, discusses its use as a possible biomarker for diagnosis and prognosis in ACS, briefly describes the challenges in different assay technologies and describes the effect of heparin administration on PAPP-A concentrations in plasma.

**Keywords:** ACS • heparin • PAPP-A • vulnerable plaque

**Historical aspects of pregnancy-associated plasma protein-A**

Pregnancy-associated plasma protein-A (PAPP-A) was first discovered in the early 1970s by Lin, Gall and Halbert [1,2]. It was identified in serum from pregnant women, and PAPP-A in clinical research has predominantly been focused on gynecology and obstetrics. PAPP-A is synthesized in the cytoplasm of the trophoblast, primarily in the syncytiotrophoblast [3,4]. The concentration of PAPP-A in circulation increases during pregnancy until labor, at first exponentially and later to a lesser extent [5-8]. Interestingly, PAPP-A concentrations are reduced in women pregnant with fetuses having trisomy 21, and PAPP-A is now used as a routine screening tool for trisomy 21 in conjunction with other markers and measures [9,10]. Low PAPP-A concentrations in pregnant women have also been associated with other types of aneuploidy, complications during pregnancy and generally a poor outcome [11-13].

In 2001, the first report of PAPP-A in heart disease was published [14]. The paper described PAPP-A elevation in patients with acute coronary syndrome (ACS) and the proposed source of PAPP-A was the vulnerable plaque. Following this finding the production of papers concerning this issue has been vast, but the findings have been inconsistent. The hopes have been to find a biomarker that could detect a vulnerable plaque and thereby be helpful in improving diagnostic and therapeutic decision-making in patients with ACS.

PAPP-A is a high molecular weight glycoprotein, a homo-dimer with each subunit weighing around 200 kDa [15,16]. In pregnancy PAPP-A primarily circulates in a heterotetramer form where it is bound to pro-MBP in a 2:2 complex [17]. In serum from patients with ACS PAPP-A is primarily present in the unbound form [18,19].

The biological function of PAPP-A is not fully understood. PAPP-A expression seems to be stimulated by various cytokines in different cell types, for example, TNF-α and IL-1β [20]. PAPP-A is a local regulator of the IGF axis. It cleaves IGF-binding protein 4 and 5 [21-23] lowering their affinity to IGF, and thereby frees IGFs making them available for interaction with their receptors. During pregnancy, IGF has mitogenic and anabolic effects, but IGF may also play a role in atherosclerosis by increasing the uptake of cholesterol into atheromatomic plaques and by increasing the release of cytokines [24]. On the other hand, IGF-1 is
also known to have a wide array of favorable cardiovascular effects, and its metabolic ability in coupling vasodilation is suggested to play a key role in its anti-atherogenic function [25]. These contrasting effects make the role of PAPP-A in atherosclerosis is unclear.

Assay technology
The present assays for measuring PAPP-A concentrations in plasma are based on the radio immunoassay. Several assays based on sandwich technology using both monoclonal and polyclonal anti-PAPP-A antibodies as well as a combination of both are commercially available, and these are the foundation for the papers describing the role of PAPP-A in heart disease. A problem when interpreting the results from these commercially available assays is that PAPP-A in pregnancy is present in the complex form and in ACS in the free form [18,19]. Therefore, only assays measuring free or total PAPP-A are useful in ACS.

In this review we report the results as stated in the original articles with their original units; mIU/ml and ng/ml. As per measurement, 1 mIU/ml converts to 4.5 ng/ml. However, it is important to note that the absolute PAPP-A values measured with assays based on different antibodies are not directly comparable.

PAPP-A in healthy controls & patients with nonatherosclerotic disease
Knowing the normal concentration of PAPP-A in plasma from healthy individuals is mandatory when considering the protein as a biomarker. Only one study has estimated the biological variation in healthy men and nonpregnant women by measuring PAPP-A weekly on 11 subjects for 5 subsequent weeks. The weekday and time of day of blood sampling were the same during the 5 weeks. They found a within-subjects biological variation of 12.6% and a between-subjects biological variation of 14.0% [26]. By measuring PAPP-A concentrations in 123 healthy individuals, Coskun et al. [27] found the upper reference limits of PAPP-A in men and nonpregnant women to be 22.9 ng/ml and 33.6 ng/ml, respectively. The median serum PAPP-A levels were significantly higher in men than in nonpregnant women (6.85 vs 3.40 ng/ml).

Iversen et al. [28] examined serum concentrations of PAPP-A in nonpregnant patients admitted to hospital without ACS. PAPP-A was elevated above the 99th percentile in healthy individuals in 19.2% of the total cohort of patients. Excluding patients with known heart disease left the results almost unaltered (17.5% had elevated PAPP-A levels). There was no relation between a specific diagnosis, markers of inflammation or any chronic disease to elevated PAPP-A.

PAPP-A in patients with stable atherosclerotic disease
Diagnostic PAPP-A
In patients with known or presumed atherosclerotic disease the concentration of PAPP-A has been examined in different patient populations and with different assays (Table 1).

PAPP-A has been found to be elevated in patients with type II diabetes compared with controls, and additionally to be correlated with cholesterol [29]. In patients with type I diabetes and diabetic nephropathy PAPP-A levels are higher than in patients without nephropathy [30]. Three studies [31–33] have showed PAPP-A concentration to be higher in patients in dialysis than in healthy controls. Only minor changes were seen in PAPP-A concentration during dialysis [31]. Blood samples were drawn during dialysis, and the patients were likely to be treated with heparin. PAPP-A has also been reported as elevated in renal transplant patients [34]. In patients with carotid stenosis, Heider et al. [35] found PAPP-A concentrations to be slightly higher in asymptomatic than in symptomatic patients (0.11 vs 0.07 µg/ml; p = 0.025). However, Beaudieux et al. [36] found no such difference in PAPP-A concentration between patients with carotid stenosis and healthy controls. Only one paper has examined the concentration of PAPP-A in patients with peripheral arterial disease [37]. This study showed PAPP-A to be slightly higher in patients compared with healthy controls (0.81 vs 0.64 mIU/l; p < 0.001). In patients with heart failure, PAPP-A levels are shown to be higher than in an age-matched control group, and levels increase with the severity of heart failure [38].

Stable angina pectoris
In all but two studies of PAPP-A in stable angina pectoris, patients were recruited from a population of patients undergoing coronary angiography (CAG), and blood samples were probably drawn during angiography with patients heparinized. Danzig et al. [39] made a small study of 21 patients with stable angina pectoris undergoing exercise tests without prior heparin administration. Iversen et al. [40] included 4242 nonhospitalized patients with previous admissions with angina pectoris or myocardial infarction (MI) and previous coronary revascularization and found elevated PAPP-A levels in 12.9%, thus higher than in a population of healthy blood donors [41], but lower than in unselected admitted patients without any sign of heart disease [28]. The results are generally inconsistent, also for studies using the same assay. The four studies with a healthy control group included did not find a significant difference in PAPP-A concentrations between patients and controls [14,39,42,43] — these are however relatively small
### Table 1. Pregnancy-associated plasma protein-A in patients with stable atherosclerotic disease.

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Type of patients</th>
<th>Patients (n)</th>
<th>Mean PAPP-A value</th>
<th>Treated with heparins prior to blood sample</th>
<th>Related to prognosis</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aso et al. (2004)</td>
<td>Type 2 diabetes mellitus</td>
<td>103</td>
<td>7.82 mIU/l</td>
<td>No</td>
<td>NA</td>
<td>[29]</td>
</tr>
<tr>
<td>Astrup et al. (2007)</td>
<td>Albuminuric diabetics</td>
<td>197</td>
<td>3.6 mIU/l</td>
<td>No</td>
<td>Not related to prognosis in multivariate analysis</td>
<td>[30]</td>
</tr>
<tr>
<td>Bayes-Genis et al. (2001)</td>
<td>SAP undergoing CAG</td>
<td>19</td>
<td>8.4 mIU/l</td>
<td>Possible</td>
<td>NA</td>
<td>[14]</td>
</tr>
<tr>
<td>Beaudeux et al. (2003)</td>
<td>Hyperlipidemic with carotid stenosis</td>
<td>34</td>
<td>9.26 mIU/l</td>
<td>No</td>
<td>NA</td>
<td>[36]</td>
</tr>
<tr>
<td>Consuegra-Sanchez et al. (2008)</td>
<td>SAP undergoing CAG</td>
<td>663</td>
<td>5.26 mIU/l (median)</td>
<td>Possible</td>
<td>Related to all-cause mortality</td>
<td>[47]</td>
</tr>
<tr>
<td>Cosin-Sales et al. (2004)</td>
<td>SAP undergoing CAG</td>
<td>396</td>
<td>5.5 mIU/l</td>
<td>Possible</td>
<td>NA</td>
<td>[44]</td>
</tr>
<tr>
<td>Cosin-Sales et al. (2005)</td>
<td>SAP undergoing CAG</td>
<td>643</td>
<td>6.0 mIU/l</td>
<td>Possible</td>
<td>NA</td>
<td>[45]</td>
</tr>
<tr>
<td>Coskun et al. (2007)</td>
<td>Dialysis</td>
<td>113</td>
<td>4.7 mIU/l (hemodialysis); 4.5 mIU/l (peritoneal dialysis)</td>
<td>Possible</td>
<td>PAPP-A was related to other markers of a poor prognosis</td>
<td>[52]</td>
</tr>
<tr>
<td>Coskun et al. (2007)</td>
<td>Renal transplant</td>
<td>78</td>
<td>10.5 mIU/l</td>
<td>No</td>
<td>NA</td>
<td>[34]</td>
</tr>
<tr>
<td>Danzig et al. (2009)</td>
<td>SAP undergoing exercise test</td>
<td>21</td>
<td>8.6 mIU/l</td>
<td>No</td>
<td>NA</td>
<td>[39]</td>
</tr>
<tr>
<td>Dosh et al. (2009)</td>
<td>SAP + UAP undergoing CAG</td>
<td>1096</td>
<td>3.5 mIU/l (median: 1.1 mIU/l)</td>
<td>Possible</td>
<td>Not related to outcome after 1 year</td>
<td>[49]</td>
</tr>
<tr>
<td>Eleber et al. (2006)</td>
<td>SAP undergoing CAG</td>
<td>103</td>
<td>4.0 mIU/l (median)</td>
<td>Possible</td>
<td>Related to all cause mortality but not revascularization</td>
<td>[48]</td>
</tr>
<tr>
<td>Etter et al. (2009)</td>
<td>Hemodialysis</td>
<td>170</td>
<td>21.0 mIU/l (median)</td>
<td>Possible</td>
<td>PAPP-A was higher in nonsurvivors</td>
<td>[33]</td>
</tr>
<tr>
<td>Funayama et al. (2011)</td>
<td>Heart failure</td>
<td>182</td>
<td>8.8 ng/ml; controls: 5.2 ng/ml</td>
<td>Possible</td>
<td>PAPP-A was higher in patients with cardiac events</td>
<td>[38]</td>
</tr>
<tr>
<td>Furenes et al. (2009)</td>
<td>SAP undergoing CAG</td>
<td>10</td>
<td>5.9 ng/ml</td>
<td>Possible</td>
<td>NA</td>
<td>[76]</td>
</tr>
</tbody>
</table>

studies (15–110 patients). Two larger studies have reported PAPP-A concentrations to be lower in patients with normal coronary arteries compared with patients with significant stenosis [44, 45]. The timing of blood sampling is, however, not described in details in most of these studies. It is therefore not possible to determine whether heparin was administered in relation to the CAG prior to the blood sampling. This could well help to explain the heterogeneity of the results.

Prognostic PAPP-A

When following 197 patients with type I diabetes and nephropathy, Astrup and colleagues [30] found excess all-cause mortality in patients with PAPP-A above 10 mIU/l. After adjustment for traditional risk markers the relation, however, disappeared. In a study of patients with kidney disease, PAPP-A was reported to be associated with other markers of poor prognosis [32], and in another study, PAPP-A was found to be higher in nonsurvivor dialysis patients than in survivor dialysis patients [33]. In patients with peripheral arterial disease, a weak but significant association with all-cause mortality has been described (risk ratio [RR] 1.31 in multivariate analysis) [46]. In 182 patients with heart failure, PAPP-A was shown to be an independent risk factor in predicting adverse clinical outcomes within the follow-up of 796 days, and the highest levels of PAPP-A were associated with the highest risk for cardiac events [38].

Stable angina pectoris

In patients with stable coronary artery disease, the prognostic importance of PAPP-A has, except in one study, been investigated only in patients undergoing CAG. In all these studies, procedural heparin could play a role in the interpretation of data. Consuegra-Sanchez [47] found an adjusted hazard ratio (HR) of 1.91 for all-cause mortality in patients having PAPP-A levels above 4.8 mIU/l in a population of 663 patients followed for 8.8 years. Further increase in PAPP-A concentration above the threshold value of 4.8 mIU/l had no prognostic value. Elesber et al. [48] showed PAPP-A to be correlated to all-cause mortality (HR: 5.29 for doubling of PAPP-A) and to a lesser extent to a combination of death and ACS (HR: 3.56) and not to death and revascularization (HR: 1.06). In 1096 patients mainly with stable coronary artery disease planned for percutaneous coronary intervention (PCI), PAPP-A was not associated with outcome after 1 year of follow-up [49]. Li and colleagues [50] found that PAPP-A predicted the risk of angiographic restenoses after PCI, also after adjustment for other risk factors. Iversen et al. [40] found that having elevated PAPP-A was an independent predictor of mortality in

<table>
<thead>
<tr>
<th>Study (year)</th>
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<th>Related to all-cause mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hajek et al. (2008)</td>
<td>SAP undergoing CAG</td>
<td>110</td>
<td>7.91 mIU/l</td>
<td>Possible</td>
<td>NA</td>
</tr>
<tr>
<td>Heider et al. (2010)</td>
<td>Carotid stenosis</td>
<td>66</td>
<td>0.07–0.112 mg/ml</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>Iversen et al. (2010)</td>
<td>Stable ischemic heart disease</td>
<td>4242</td>
<td>7.4 mIU/l (in patients with elevated PAPP-A)</td>
<td>No</td>
<td>Related to all-cause mortality</td>
</tr>
<tr>
<td>Kalousova et al. (2006)</td>
<td>Hemodialysis</td>
<td>20</td>
<td>20.5 mIU/l</td>
<td>Yes</td>
<td>NA</td>
</tr>
<tr>
<td>Li et al. (2008)</td>
<td>SAP undergoing CAG</td>
<td>184</td>
<td>12.5 mIU/l (median)</td>
<td>Possible</td>
<td>NA</td>
</tr>
<tr>
<td>Liu et al. (2008)</td>
<td>SAP undergoing CAG</td>
<td>15</td>
<td>8.5 mIU/l</td>
<td>Possible</td>
<td>NA</td>
</tr>
<tr>
<td>Miedema et al. (2008)</td>
<td>SAP undergoing CAG</td>
<td>51</td>
<td>0.75 mIU/l</td>
<td>No</td>
<td>Related to all-cause mortality</td>
</tr>
<tr>
<td>Mueller et al. (2006)</td>
<td>Peripheral artery disease</td>
<td>433</td>
<td>0.81 mIU/l</td>
<td>No</td>
<td>Related to all-cause mortality</td>
</tr>
</tbody>
</table>


Table 1. Pregnancy-associated plasma protein-A in patients with stable atherosclerotic disease (cont.).

Biomark. Med. (2014) 8(8)
4248 patients with stable atherosclerotic disease followed for 2.8 years. PAPP-A was equally related to cardiovascular and all-cause mortality.

**PAPP-A in patients with non-ST-elevation ACS**

**Diagnostic PAPP-A**

PAPP-A has been investigated in several studies of patients with non-ST-elevation ACS (NSTE-ACS) (Table 2).

In 2001, Bayes-Genis et al. [14] were the first to describe PAPP-A in patients with unstable angina pectoris (UAP) and non-st-elevation myocardial infarction (NSTEMI). They found PAPP-A to be elevated in both patients with MI (20.6 mIU/l) and in patients with UAP (14.9 mIU/l) but found no significant difference between the two groups. Both groups, however, displayed higher PAPP-A concentrations than patients with stable angina pectoris and age-matched controls. Blood samples were drawn during CAG and there is no information on the use of heparin. Similar results have been found by You et al. [51], and Khosravi et al. [52] also showed that PAPP-A was higher in a group of patients with coronary heart disease (CHD) than in healthy controls, and that it was correlated to markers of myocardial necrosis.

Lund and colleagues [53] were the first to describe PAPP-A in a population of troponin-negative patients with chest pain from the emergency department. The median PAPP-A concentration was 2.3 mIU/l at admission. There was no information on the use of heparin. In a multimarker study of 415 patients presenting with chest pain, McCann et al. [54] showed that PAPP-A concentrations were slightly but significantly higher in patients with MI than in patients with normal coronary enzymes (6.7l vs 5.0 ng/ml). However, after correction for other markers, the significant difference disappeared. Similar results were found by Miedema et al. [55] in 35 patients with different types of ACS. In a small study, Liu and colleagues [56] found higher PAPP-A levels in 15 patients with chest pain and ECG changes than in controls. There was no correlation between number of diseased vessels and PAPP-A. In a large group of patients presenting with cardiac chest pain and not treated with heparin, von Haehling et al. [57] found slightly higher serum levels of PAPP-A in patients with confirmed ACS than in patients with stable angina (24.8 vs 24.4 mIU/l; p = 0.01).

In 2008, Hajek et al. [43] found equally elevated PAPP-A in patients with UAP and NSTEMI (19.7 and 21.1 mIU/l, respectively). They found a reasonably high diagnostic accuracy of PAPP-A in diagnosing UAP and NSTEMI in an unselected group of patients presenting with suspected ACS (area under curve [AUC] 0.718 and 0.803, respectively). 4 years later, the same authors showed serum PAPP-A level to be an independent marker of ACS diagnosis (mean PAPP-A levels in ACS patients: 9.01 mIU/l; non-ACS patients: 7.13 mIU/l) with high positive predictive value (95.7% at PAPP-A levels above 9.1 mIU/l) and to contribute to the correct final diagnosis in troponin-negative NSTE-ACS patients in heparin-naïve patients suspected of ACS [58].

In a group of patients suspected of MI not treated with heparin Schaub et al. [59] found a minor but significantly higher concentration of PAPP-A in patients with a final diagnosis of MI than in patients where MI was rejected (4.6 vs 4.0 mIU/l). The diagnostic accuracy was however low (AUC: 0.62). Body et al. [60] examined whether PAPP-A could be used as a mean for early exclusion of MI in an undifferentiated group of patients admitted to the emergency department with cardiac chest pain. Median PAPP-A concentrations were significantly higher in patients diagnosed with MI than patients not diagnosed with MI (4.3 vs 3.4 mIU/l). PAPP-A had an AUC of 0.58, and they concluded that PAPP-A could not be used to facilitate early diagnosis or exclusion of MI. Wlazzl et al. [61] found significantly higher PAPP-A concentrations in patients admitted with their first MI (defined as elevated cardiac troponin I) than in a control group of healthy individuals (11.42 and 7.22 ng/ml).

In 2009, Kavsak and colleagues [62] published a paper describing PAPP-A levels in 320 patients presenting with chest pain. The initial PAPP-A level was low (1.21 mIU/l) but increased in patients treated with heparin. This was not the case for patients not treated with heparin (4.03 and 1.56 mIU/l, respectively). Brügger-Andersen et al. [63] presented PAPP-A concentrations in 298 patients with MI; blood samples were taken 4–6 days after the event, and PAPP-A levels were normal (0.5 mIU/l). There is no information on the use of heparin. In 2009 Iversen et al. [64] conducted a study with serial measurements of PAPP-A in 538 patients admitted with symptoms of NSTE-ACS; they were divided into high risk (n = 123) and low risk (n = 415). Blood samples for PAPP-A analysis were drawn at admission and every 6–8 h until levels of biomarkers of myocardial necrosis was consistently falling. Overall, detectable PAPP-A concentrations (>4 mIU/l) were found in significantly more samples from the high-risk group than the low-risk group (63 vs 49%). The percentage of detectable PAPP-A in samples drawn at admission and/or prior to heparin treatment did however not differ between high- and low-risk NSTE-ACS.

When measuring only the free form of PAPP-A in a group of NSTEMI patients, Lund et al. [65] found...
### Table 2. Pregnancy-associated plasma protein-A in patients with non-ST-elevation acute coronary syndrome.

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Type of patients</th>
<th>Patients (n)</th>
<th>Mean PAPP-A level</th>
<th>Treated with heparins</th>
<th>Prognostic importance</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bayes-Genis et al. (2001)</td>
<td>High-risk ACS</td>
<td>17 with elevated troponin; 20 without</td>
<td>20.6 mIU/l respectively, 14.9 mIU/l</td>
<td>Unknown</td>
<td>NA</td>
<td>[14]</td>
</tr>
<tr>
<td>Bonaca et al. (2012)</td>
<td>High- and low-risk ACS</td>
<td>3782</td>
<td>Optimized cut point from derivation set: 6.0 μIU/ml</td>
<td>Unknown</td>
<td>Yes, cardiovascular death and recurrent ischemic events (short and long term)</td>
<td>[68]</td>
</tr>
<tr>
<td>Brügger-Andersen et al. (2008)</td>
<td>High-risk ACS</td>
<td>298</td>
<td>0.5 mIU/l</td>
<td>Unknown, samples taken 4–6 days after the event</td>
<td>No prognostic importance after 45 months</td>
<td>[63]</td>
</tr>
<tr>
<td>Hajek et al. (2012)</td>
<td>High- and low-risk ACS</td>
<td>27</td>
<td>9.4 mIU/l</td>
<td>No</td>
<td>NA</td>
<td>[58]</td>
</tr>
<tr>
<td>Heeschen et al. (2005)</td>
<td>Population 1: high-risk ACS; Population 2: high + low risk</td>
<td>Population 1: 547; Population 2: 626</td>
<td>Population 1: 14.2 mIU/l; Population 2: 20.9 mIU/l</td>
<td>Population 1: yes; Population 2: no</td>
<td>Yes, death and nonfatal MI after 6 months in both populations; optimal cutoff: 12.1 mIU/l</td>
<td>[66]</td>
</tr>
<tr>
<td>Iversen et al. (2009)</td>
<td>High-risk ACS</td>
<td>123</td>
<td>14.0 mIU/l (peak)</td>
<td>PAPP-A prior and after are available</td>
<td>Not in multivariate analysis</td>
<td>[70]</td>
</tr>
<tr>
<td>Iversen et al. (2010)</td>
<td>Low-risk ACS</td>
<td>415</td>
<td>12.3 mIU/l (peak)</td>
<td>PAPP-A prior and after are available</td>
<td>All-cause mortality not nonfatal MI</td>
<td>[71]</td>
</tr>
<tr>
<td>Kavsak et al. (2009)</td>
<td>High- and low-risk ACS</td>
<td>320</td>
<td>1.21 mIU/l</td>
<td>No</td>
<td>Yes, mortality after 9 years</td>
<td>[62]</td>
</tr>
<tr>
<td>Laterza et al. (2004)</td>
<td>High- and low-risk ACS</td>
<td>Total cohort of chest pain patients: 346, hereof 15 or 53 patients with MI (depending on whether the WHO or the ESC/ACC definition of MI is used)</td>
<td>1.27 mIU/l or 2.14 mIU/l in MI patients depending on definition</td>
<td>Unknown</td>
<td>Single measurement is a modest predictor of adverse events in emergency department patients</td>
<td>[67]</td>
</tr>
<tr>
<td>Liu et al. (2008)</td>
<td>High-risk ACS, all normal troponin</td>
<td>15</td>
<td>15.2 mIU/l</td>
<td>Unknown</td>
<td>NA</td>
<td>[56]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Type of patients</th>
<th>Patients (n)</th>
<th>Mean PAPP-A level</th>
<th>Treated with heparins</th>
<th>Prognostic importance</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lund et al. (2003)</td>
<td>High- and low-risk ACS</td>
<td>136</td>
<td>2.3 mIU/l</td>
<td>Unknown</td>
<td>Yes, cardiovascular death, MI and revascularisation within 6 months</td>
<td>[53]</td>
</tr>
<tr>
<td>Lund et al. (2010)</td>
<td>High- and low-risk ACS</td>
<td>267</td>
<td>1.43 mIU/l</td>
<td>No</td>
<td>Yes, combination of death and nonfatal MI</td>
<td>[65]</td>
</tr>
<tr>
<td>McCann et al. (2008)</td>
<td>High- and low-risk ACS</td>
<td>415</td>
<td>6.7 ng/ml with MI, 5.0 ng/ml without</td>
<td>Unknown</td>
<td>NA</td>
<td>[54]</td>
</tr>
<tr>
<td>Mei et al. (2011)</td>
<td>High- and low-risk ACS</td>
<td>129 (87 UAP; 42 NSTEMI)</td>
<td>29.85 mIU/l (with adverse event), 20.47 mIU/l (without adverse event)</td>
<td>No</td>
<td>Yes, strong predictive value for combined end point for PAPP-A &gt;11.3 mIU/l</td>
<td>[69]</td>
</tr>
<tr>
<td>Miedema et al. (2008)</td>
<td>High- and low-risk ACS</td>
<td>35</td>
<td>2.05 mIU/l</td>
<td>Unknown</td>
<td>NA</td>
<td>[55]</td>
</tr>
<tr>
<td>Mjelva et al. (2013)</td>
<td>High- and low-risk ACS</td>
<td>290</td>
<td>4.8 mIU/l (median for total cohort of chest pain patients)</td>
<td>No</td>
<td>No prognostic importance for total mortality, recurrent troponin-positive events and nonfatal MI after 7 years</td>
<td>[72]</td>
</tr>
<tr>
<td>Schaub et al. (2012)</td>
<td>High-risk ACS</td>
<td>76 MI (STEMI + NSTEMI, based on troponin)</td>
<td>4.6 mIU/l (median MI). No significant difference between STEMI and NSTEMI</td>
<td>No</td>
<td>No prognostic importance on all-cause mortality after 2 years (whole chest pain patient cohort)</td>
<td>[59]</td>
</tr>
<tr>
<td>von Haeahling et al. (2013)</td>
<td>High- and low-risk ACS</td>
<td>1215</td>
<td>24.4 mIU/l</td>
<td>No</td>
<td>Yes, cardiovascular death, MI, ischemic stroke and stent thrombosis within 90 days; best cutoff 34.6 mIU/l</td>
<td>[57]</td>
</tr>
<tr>
<td>You et al. (2010)</td>
<td>High- and low-risk ACS</td>
<td>18 UAP; 37 MI</td>
<td>UAP: 2.33 mIU/l; MI: 2.42 mIU/l</td>
<td>Unknown</td>
<td>NA</td>
<td>[51]</td>
</tr>
</tbody>
</table>

that free PAPP-A was elevated above the 97.5 percentile in 65.9% of patients at admission. Accordingly, none of the patients had received heparin prior to blood sampling.

When examining two patient populations (1: angiographically validated ACS pretreated with heparin; 2: presenting to the emergency department, not treated with heparin) Heeschen and colleagues [66] found mean PAPP-A levels higher in the patients with a final diagnosis of ACS in population 2 than the patients in population 1 (20.9 mIU/l vs 14.6 mIU/l). There was no correlation between PAPP-A concentration and troponin concentration in any of the two groups.

### Prognostic PAPP-A

Several studies have described the prognostic value of PAPP-A in patients with NSTE-ACS.

In patients with ACS but normal markers of myocardial necrosis PAPP-A seems highly correlated to a combination of cardiovascular death, MI and revascularization within 6 months (RR: 4.6 in multivariate analysis) [53]. In high-risk patients with either elevation of troponin or ECG-changes PAPP-A measured in blood drawn 4–6 days after the initial event showed no correlation with any clinical endpoint (long term) [63]. When describing an unselected group of 346 patients presenting to the emergency department with chest pain of possible cardiac origin, Latzer et al. [67] found elevated PAPP-A to be correlated to an increased risk of death, MI and revascularization within 30 days (RR: 4.7 in univariate analysis). Similar mixed populations have been examined in other studies [57,62,66,68], and the results from these studies show that PAPP-A is related to mortality, MI and revascularization both short term and long term. In all but one of the studies [57], the use of heparin in association with the drawing of blood is however very poorly described. In patients admitted for PCI after NSTE-ACS (low risk) PAPP-A levels measured prior to anticoagulant therapy were significantly associated with adverse cardiac events within 20 months [69]. Lund et al. [65] also described the prognostic importance of PAPP-A in a heparin-naive population and found PAPP-A to be an independent predictor of the combined endpoint of death and nonfatal MI. Furthermore, the study suggested that the use of a new principle calculating free PAPP-A by measuring both total PAPP-A and PAPP-A in complex with two different assays could improve the prognostic importance of PAPP-A. Iversen et al. [70] showed that the risk of death and nonfatal MI was greatest in the upper quartile of peak PAPP-A in patients with high-risk ACS. The risk was significantly higher than in the three lower quartiles pooled. When performing multivariate analyses, the relation was however not significant [70]. In a group of patients with low-risk ACS, PAPP-A was correlated with all-cause mortality but not with nonfatal MI [71].

Two recent studies have shown PAPP-A not to have a prognostic importance in NSTE-ACS patients [59,72]. Both were studies on long-term prognosis (at least 2 years) and included patients not treated with heparin.

### PAPP-A in patients with ST-elevation ACS

#### Diagnostic PAPP-A

The role of PAPP-A in patients with NSTE-ACS has been investigated in eight trials with varying designs (Table 3).

In a study of 80 patients not treated with heparin, Dominguez-Rodriguez et al. [73] found no differences in mean PAPP-A concentrations when comparing with 80 healthy controls. Similarly, Brügger-Andersen et al. [74] found low-circulating PAPP-A concentrations in samples drawn prior to medication in NSTE-ACS patients admitted for thrombolysis and treated with unfractionated heparin (UFH) or PCI and treated with enoxaparin (a low-molecular-weight heparin [LMWH]). In samples drawn 90 min after medication and treatment, PAPP-A concentrations were higher. Lund et al. [75] found high admission levels of PAPP-A (8.8 mIU/l) in patients admitted for acute reperfusion therapy rising to peak levels (11.6 mIU/l) after 1 h. PAPP-A remained elevated in the first few hours after admission and then returned to normal. In blood samples drawn 30 min prior to angiography, Liu and colleagues [56] found elevated PAPP-A in 12 patients with NSTE-ACS. PAPP-A has also been demonstrated increased (30.3 mIU/l) during primary PCI [48] and 3 h after primary PCI (7.5 mIU/l) [76]. The latter was however similar to patients with stable coronary disease undergoing the same procedure.

Common for the four last studies is that the timing of medication in relation to the drawing of blood samples is unknown. Iversen et al. [77] measured PAPP-A concentrations in patients with NSTE-ACS referred for primary PCI at admission and then every 6–8 h until biomarkers of myocardial necrosis was consistently falling. All patients were treated with 10,000 IU UFH to blood drawing. The median PAPP-A concentration in the first sample was 12.2 mIU/l (23.7 mIU/l if drawn within 2 h of revascularization) and had fallen to normal levels in the second and third blood samples.

Terkelsen et al. [78] showed PAPP-A to be lower in NSTE-ACS patients not treated with heparin as compared with NSTE-ACS patients treated with heparin prior to blood sampling.
Prognostic PAPP-A

Only two studies have described the prognostic value of PAPP-A in patients with NSTE-ACS. Lund et al. [75] showed that having PAPP-A at admission above 10 mIU/l (the highest tertile) is related to a composite endpoint of cardiovascular death and nonfatal MI. Iversen et al. [70] followed a population of NSTE-ACS patients for 3.02 years. There was no difference in the combined outcome (nonfatal MI or death) between the four quartiles of PAPP-A. Being in the highest quartile was, however, significantly associated with mortality.

The role of heparin in the measurement of PAPP-A concentration

Terkelsen et al. [78] were the first to demonstrate lower levels of PAPP-A in a group of STEMI patients not treated with heparin compared with patients treated with heparin prior to blood sampling. Equal findings have since been made with both UFH and LMWH in patients undergoing dialysis [79,80], patients undergoing CAG [79], patients undergoing different types of vascular surgery [80] and in control groups of patients without angiographic signs of significant atherosclerosis [81], patients without ACS [81], and in healthy volunteers [82]. Bivalirudin, a direct thrombin inhibitor, does not give rise to any increase in PAPP-A levels [80], and angiography without heparin administration does not cause PAPP-A increase either [81].

The route of administration and type of heparin influence the size and duration of the elevation in PAPP-A concentration. The response of subcutaneous LMWH administration is a continuously increasing PAPP-A concentration during the period of administration [83,84] with peak levels 3 h after the last administration [84] followed by normalization after discontinuation of the drug [83]. The response of PAPP-A levels after administration of subcutaneous LMWH is significantly lower [80,83] but more sustained [83] than after intravenous administration of UFH or LMWH. When administering intravenous UFH, peak PAPP-A concentrations appear within 5 min [83,84]. This is followed by a rapid decrease and normalization [82–84]. An extra injection of UFH results in another peak in PAPP-A levels of equal size as the first [83]. The effect of heparin on PAPP-A level increase is directly related to therapeutic dose [81]. If heparin is added to blood in vitro, no PAPP-A elevation is seen [79].

Wittfooth and colleagues [80] extracted PAPP-A from both denaturized vulnerable plaques and tissue from an aortic aneurysm (without plaques) by using LMWH. Most was extracted from the aortic tissue. No control


<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Patients (n)</th>
<th>Mean PAPP-A value</th>
<th>Treated with heparins prior to blood sample</th>
<th>Prognostic importance</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brügger-Andersen et al. (2006)</td>
<td>38 (18 treated with thrombolysis; 20 treated with PCI)</td>
<td>0.62 and 1.03 mIU/l at admission; 4.26 and 8.78 mIU/l 90 min after treatment</td>
<td>Treated with heparin after the first blood sample</td>
<td>NA</td>
<td>[74]</td>
</tr>
<tr>
<td>Dominguez–Rodriguez et al. (2005)</td>
<td>80</td>
<td>1.29 mIU/l</td>
<td>No</td>
<td>NA</td>
<td>[73]</td>
</tr>
<tr>
<td>Furenes et al. (2009)</td>
<td>20</td>
<td>7.5 mIU/l</td>
<td>Unknown</td>
<td>NA</td>
<td>[76]</td>
</tr>
<tr>
<td>Hajek et al. (2008)</td>
<td>66</td>
<td>30.3 mIU/l</td>
<td>Unknown</td>
<td>NA</td>
<td>[43]</td>
</tr>
<tr>
<td>Iversen et al. (2008)</td>
<td>314</td>
<td>12.1 mIU/l (23.7 mIU/l for samples taken within 2 h after intervention)</td>
<td>Yes</td>
<td>All-cause mortality, not nonfatal myocardial infarction</td>
<td>[77]</td>
</tr>
<tr>
<td>Liu et al. (2008)</td>
<td>12</td>
<td>16.9 mIU/l</td>
<td>Unknown</td>
<td>NA</td>
<td>[56]</td>
</tr>
<tr>
<td>Lund et al. (2006)</td>
<td>62</td>
<td>8.0 mIU/l at admission (median)</td>
<td>Unknown</td>
<td>Related to all-cause mortality an nonfatal myocardial infarction in univariate analysis</td>
<td>[75]</td>
</tr>
<tr>
<td>Terkelsen et al. (2010)</td>
<td>98</td>
<td>18.0 mIU/l in heparin treated patients; 5.5 mIU/l in patients not treated with heparin</td>
<td>Some</td>
<td>NA</td>
<td>[78]</td>
</tr>
</tbody>
</table>

tissues were examined. Iversen et al. [82] question the vulnerable plaque as the main source for PAPP-A as they were able to elute significant amounts of PAPP-A from aortic tissue from an autopsy of a patient without cardiovascular disease when incubating it in UFH, LMWH or fondaparinux at different concentrations. No PAPP-A was eluted when aortic tissue was incubated in saline or from control tissue (cardiac muscle, striated muscle, liver, kidney and lung from the same patient) in any of the aforementioned dilutions.

Furthermore Terkelsen et al. [78] found reduced clearance of injected PAPP-A in mice if they were treated with heparin.

**Immunohistochemistry**

In 2001, Bayes-Genis et al. [14] were the first to publish a paper describing the presence of PAPP-A in the vulnerable plaque. They found PAPP-A to be less expressed in stable plaques than in eroded or ruptured plaques. Bayes-Genis et al. [14] found staining of PAPP-A in the inflammatory shoulder region, in areas surrounding the lipid core, in spindleshaped smooth-muscle cells, in the extracellular matrix and in noneroded endothelial cells. In contrast, Sangiorgi and colleagues [85] found expression of PAPP-A mainly in monocytes and macrophages when examining carotid plaques.

The polyclonal antibodies used by Bayes-Genis and colleagues [14] have however in a different study demonstrated false-positive reactions [41].

Another study [86] demonstrated PAPP-A in the extracellular matrix of plaques from STEMI patients undergoing aspiration thrombectomy during PCI, but there was no staining for PAPP-A in the thrombi. The authors suggest the reason for the lack of staining to be administration of UFH during the procedure and thereby detachment of PAPP-A from the vessel wall.

In 2010, Iversen et al. [82] found no PAPP-A staining in plaques with large necrotic lipid cores. They were not able to demonstrate PAPP-A in different types of myocardial tissue either, but positive control tissue (term placenta) showed a strong positive and specific staining reaction within the cytoplasm of the syncytiotrophoblast. They express doubts that a small amount of PAPP-A in a single ruptured plaque could explain the marked and rapid increase in serum levels of PAPP-A.

**Challenges & considerations**

Patients with known or likely stable atherosclerotic disease have increased PAPP-A concentrations compared with controls. The reported results are, however, highly variable and could reflect differences in populations, assay techniques and the use of heparin. The studies suggest that PAPP-A is related to all-cause mortality but the role of PAPP-A as a biochemical predictor for future coronary events or mortality due to CHD is still controversial and the results are very varying. A broad cohort of patients admitted to hospital show equal rises in PAPP-A concentration in patients without known cardiac disease and in patients with stable atherosclerotic disease.

Since 2001, it has been postulated that PAPP-A is released by and is a marker of the vulnerable plaque. The background for this hypothesis was that PAPP-A was found elevated in patients with unstable coronary artery disease and that immunohistochemistry demonstrated the presence of PAPP-A in vulnerable plaques. The hypothesis seems, however, questionable. The increase of plasma PAPP-A in patients with ACS appears to be dependent on heparin administration rather than ACS itself. Heparin administration in healthy individuals elicits responses in the same range as found in ACS patients. Moreover, immunohistochemistry studies are inconsistent and show PAPP-A staining in different cell types and locations within the plaque – or not even present in the plaque – and additional evaluation of normal vascular tissue with heparin showed sign of PAPP-A release. Consequently, some authors have expressed doubts that the release of PAPP-A from a single vulnerable plaque would be able to elicit such significant elevations of PAPP-A in circulation. The fact that PAPP-A concentrations are only weakly related to future coronary events highly emphasizes the doubts that PAPP-A is released mainly from the vulnerable plaque.

As heparin has been shown to release PAPP-A and thereby increase the concentration of PAPP-A in the blood stream, only papers with exact data on heparin administration are truly reliable. When only considering these papers, the results are however the same as described above; patients with CHD have higher PAPP-A concentrations compared with controls, and the results on PAPP-A as a predictor of future coronary events and mortality are varying.

The role of PAPP-A as an independent biomarker of ACS is debatable. One study has shown that PAPP-A might be able to contribute to the correct final diagnosis in certain subgroups of suspected ACS patients not treated with heparin (troponin-negative NSTE-ACS patients). However, other studies find the diagnostic accuracy of PAPP-A to be too low to conclude anything.

Since study results have not shown a clear sign of PAPP-A being the promising biological marker in coronary artery disease as initially hoped, the interest of the diagnostic companies for developing PAPP-A assays has declined. Thus, uniform results can be even harder to obtain. Some authors have developed their
own assays for optimizing the methods [19,41]; however, none of these assays have been able to break through in the standardization of an assay.

In general, it is poorly described which form of PAPP-A is measured by the assays used in the papers. Most simply do not state it. When stated, most measure total PAPP-A. Only three papers [65,79,80] measure both complex and total PAPP-A in different assays and are hereby able to calculate free PAPP-A by subtracting the two results. Interestingly, in one of these papers the prognostic performance of total PAPP-A was inferior to the performance of free PAPP-A [69]. They hypothesize that complex PAPP-A is variably found in all individuals without ACS and that the measured concentrations of circulating total PAPP-A in ACS are variably affected by interindividual variations in complex PAPP-A.

The consequences of the methodological differences in the assays and in many cases lack of descriptions of heparin treatment in relation to blood drawings are that the results presented should be looked upon with critical eyes and are not directly comparable. Measuring total PAPP-A instead of free PAPP-A overestimates the degree of elevation of the PAPP-A concentration, and heparin administration causes further elevation than what ACS itself causes. The relation with all-cause mortality is, however, still present.

**Conclusion & future perspective**

Studies show that patients with CHD have higher PAPP-A concentrations than controls have, and that there are varying results on PAPP-A as a predictor of future coronary events and mortality. However, methodological problems regarding the timing of heparin administration and different assays make direct comparisons of the studies difficult. Furthermore, heparin administration results in elevated PAPP-A concentrations in both ACS patients and healthy volunteers, and immunohistochemistry show conflicting results concerning the presence of PAPP-A in the vulnerable plaque. Thus, the original hypothesis of PAPP-A being released form vulnerable plaques is questioned.

As for now, absolute PAPP-A concentrations in plasma from different studies are difficult to compare. The main problem is the use of different assay technology and that the different assays measure different forms of PAPP-A (complex or total PAPP-A). Future research must aim at defining a (new) standard material for PAPP-A measurement in non-pregnant individuals making it possible to compare PAPP-A measured with different assays.

For further examination of the role of the vulnerable plaque in PAPP-A release, measuring PAPP-A

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**Executive summary**

**Historical aspects**
- Pregnancy-associated plasma protein-A (PAPP-A) was first discovered in pregnant women and found to be synthesized in the placenta.
- In 2001, elevated PAPP-A concentrations in acute coronary syndrome (ACS) patients were suggested to originate from the vulnerable plaque.

**Assay technology**
- Most assays are based on sandwich technology using different antibodies.
- Results from assays using different antibodies are not directly comparable.
- Only assays measuring free or total PAPP-A are useful in ACS, as PAPP-A here appears in the free form.

**Diagnostic PAPP-A**
- Patients with known or likely stable atherosclerotic disease have slightly elevated levels compared with controls, but the results are highly variable.
- In patients with ACS PAPP-A concentrations seem to be higher than in healthy controls and patients with stable coronary artery disease, but the circumstances regarding the blood drawings are generally poorly described.

**Prognostic PAPP-A**
- In patients with known or likely stable atherosclerotic disease elevated PAPP-A is related to mortality.
- Both in patients with non-ST-elevation ACS and ST-elevation ACS PAPP-A concentrations seem to be associated with all-cause mortality and to a lesser extent to nonfatal myocardial infarction.

**The role of heparin**
- Administration of heparin results in elevated PAPP-A concentrations in both ACS patients and healthy volunteers.
- Intravenous unfractionated heparin causes a rapid increase and decrease, whereas subcutaneous low-molecular-weight heparin elicits a lower but more sustained rise in PAPP-A concentration.

**Immunohistochemistry**
- Immunohistochemistry show conflicting results; two studies find PAPP-A in the vulnerable plaques but in different cell types, whereas two other do not find PAPP-A in the plaques at all.
concentrations in blood samples drawn during PCI in ACS patients treated with bivalirudin instead of heparin would be relevant. Furthermore, other hypotheses should be pursued; for example, could PAPP-A be bound to the endothelia of normal vessels and rapidly released into the circulation after treatment with UFH and analogs? The release may be due to competition for a common receptor on the endothelium.

References
Papers of special note have been highlighted as:
• of interest; •• of considerable interest
• One of the first descriptions of pregnancy-associated plasma protein-A (PAPP-A) in literature.
•• First paper describing PAPP-A in patients with ST-elevation myocardial infarction.
• Molecular distinction of PAPP-A in pregnancy and PAPP-A in myocardial infarction.
21 Lawrence JB, Oxvig C, Overgaard MT et al. The insulin-like growth factor (IGF)-dependent IGF binding protein-4 Financial & competing interests disclosure
The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.
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Review


80 *First demonstration of lower PAPP-A concentration in patients with acute coronary syndrome not treated with heparin than in patients treated with heparin.*


