Turun yliopisto
University of Turku

PAPP-A AS A PROGNOSTIC MARKER IN SUSPECTED ACUTE CORONARY SYNDROMES

Juha Lund

TURUN YLIOPISTON JULKAISUJA - ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. D osa - tom. 1350 | Medica - Odontologica | Turku 2018

Turun yliopisto
University of Turku

PAPP-A AS A PROGNOSTIC MARKER IN SUSPECTED ACUTE CORONARY SYNDROMES

Juha Lund

TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. D osa - tom. 1350 | Medica - Odontologica | Turku 2018

University of Turku

Faculty of Medicine
Department of Cardiology and Cardiovascular Medicine
Doctoral Programme in Clinical Research
Heart Center, Turku University Hospital

Supervised by

Docent Liisa-Maria Voipio-Pulkki MD, PhD
Ministry of Social Affairs and Health
Helsinki, Finland

Professor Kim Pettersson P.hD
Department of Biochemistry and Biotechnology
University of Turku
Turku, Finland

Reviewed by

Docent Matti Niemelä MD, PhD
Department of Cardiology
Oulu University Hospital
Oulu, Finland

Docent Veli-Pekka Harjola MD, PhD
Department of Emergency Medicine
Helsinki University Hospital
Helsinki, Finland

Opponent

Docent Johan Lassus MD, PhD
Department of Cardiology
Jorvi Hospital
Espoo, Finland

The originality of this thesis has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service.

ISBN 978-951-29-7225-8 (PRINT)
ISBN 978-951-29-7226-5 (PDF)
ISSN 0355-9483 (Print)
ISSN 2343-3213 (Online)
Painosalama Oy – Turku, Finland 2018

To my Family

ABSTRACT

Juha Lund, MD

PAPP-A AS A PROGNOSTIC MARKER IN SUSPECTED ACUTE CORONARY SYNDROMES

University of Turku, Faculty of Medicine, Department of Cardiology and Cardiovascular Medicine, Doctoral Programme in Clinical Research, Heart Center, Turku University Hospital, Turku Finland

Annales Universitatis Turkuensis, Painosalama Oy, Turku Finland 2018

The major initial mechanism of acute coronary syndrome (ACS) is the rupture of the atherosclerotic coronary artery plaque. Clinical signs, electrocardiogram and cardiac troponins, as markers of myocardial ischemia or damage, give an accurate diagnosis, prognostic information and guidance for optimal treatment choices. The useful marker of unstable plaque or the early stage plaque rupture is still missing.

The primary purpose of this study was to assess the usefulness of pregnancy associated plasma protein A (PAPP-A) and its different forms abundantly expressed in unstable but not in stable atherosclerotic plaque as a prognostic marker in patients presenting with suspected ACS.

The basic study population comprised 541 consecutive patients who were admitted to the emergency department for symptoms consistent with ACS. Mortality data and data on other cardiac adverse events of nonfatal myocardial infarction, revascularization or hospitalization due to unstable angina, worsening heart failure or stroke, were collected during the 12 months follow up. The blood samples for PAPP-A and other measurements were obtained at admission, 12 h, 24 h and in patients representing with ST-elevation myocardial infarction (STEMI) also at 48 h. The PAPP-A analyses were performed by measuring the total fraction of PAPP-A (totalPAPP-A) in studies **I** (n=136) and **II** (n=62). After observing that the elevated PAPP-A concentration in ACS was almost entirely due to the free fraction of PAPP-A (freePAPP-A), a comparison of prognostic performance was done between freePAPP-A and totalPAPP-A measurements (study **III**, n=267).

The early elevation (< 24h) of circulating totalPAPP-A in patients who remained cardiac troponin I negative and highly elevated (>10 mIU/L) totalPAPP-A already at admission in patients with STEMI were predictive of higher risk of death or cardiac adverse events. In patients with STEMI the concentration of totalPAPP-A elevated early during the first hours of attack and normalized rapidly. The variability of totalPAPP-A kinetics at 48 hours reflects the success of reperfusion of the culprit artery. FreePAPP-A showed superiority as a prognostic marker compared to totalPAPP-A, giving independent and additive prognostic information when measured at the time of admission in patients hospitalized for non-ST-elevation ACS.

This study provides evidence of the prognostic performance of PAPP-A, a potential plaque instability marker, in patients representing with ACS. FreePAPP-A is the most prominent form in the circulation during ACS, and its prognostic performance is superior compared to totalPAPP-A.

Keywords: Prognosis, Acute Coronary Syndrome, Pregnancy Associated Plasma Protein A

TIIVISTELMÄ

LL Juha Lund

PAPP-A EPÄILLYN AKUUTIN SEPELVALTIMOTAUTIKOHTAUKSEN ENNUSTEMERKKIAINEENA

Turun yliopisto, Lääketieteellinen tiedekunta, Kardiologia ja kardiovaskulaarilääketiede, Turun kliininen tohtoriohjelma; Sydänkeskus, Turun yliopistollinen sairaala Turku, Suomi

Annales Universitatis Turkuensis, Painosalama Oy, Turku, Suomi 2018

Sepelvaltimoplakin repeäminen on tavallisin akuuttiin sepelvaltimotautikohtaukseen johtava mekanismi. Potilaan oireet, sydänfilmi ja sydänlihakselle spesifisen troponiinin lisääntynyt veripitoisuus ilmentävät sydänlihassolujen hapenpuutetta ja solutuhoa ja tarjoavat täsmällisen diagnoosin, ennustearvion sekä ohjaavat hoitovalintoja. Merkkiaine, jolla voitaisiin jo edeltävästi ennustaa epävakaan sepelvaltimoplakin repeämistä, ei toistaiseksi ole kliinisessä käytössä.

PAPP-A on proteiini, jonka on todettu ilmentyvän epävakaassa mutta ei niinkään vakaassa sepelvaltimoplakissa. Tämän tutkimuksen tavoitteena oli selvittää PAPP-A:n ja sen eri ilmenemismuotojen veripitoisuutta ja sen merkitystä arvioitaessa potilaan kuoleman ja uusintatapahtumien riskiä akuutin sepelvaltimotautikohtauksen eri alamuodoissa.

Tutkimusaineisto koostui 541 peräkkäisestä, epäillyn akuutin sepelvaltimotautikohtauksen takia ensiapuun hakeutuneesta potilaasta. PAPP-A:n veripitoisuus määritettiin tulovaiheessa, 12 ja 24 tunnin sekä ST-nousuinfarktipotilailla myös 48 tunnin kohdalla. Tiedot kuolleisuudesta, sairastetuista sydäninfarkteista, suoritetuista sydäntoimenpiteistä sekä sairaalahoidoista sydämen vajaatoiminnan, epävakaan rintakivun tai aivoinfarktin vuoksi kerättiin 12 kuukauden seuranta-aikana. Osatyössä **I** (n=136) määritettiin veren kokonais-PAPP-A:n pitoisuus potilailla, joilla ei todettu veren troponiinipitoisuuden lisääntymistä sekä osatyössä **II** (n=62) potilailla, joilla todettiin akuutti ST-nousuinfarkti. Havaittuamme, että akuutissa sepelvaltimotapahtumassa PAPP-A:n veripitoisuuden nousu johtui lähes täysin PAPP-A:n vapaan muodon lisääntymisestä, vertasimme osatyössä **III** (n=267) vapaan ja kokonais-PAPP-A:n ennustearvoa toisiinsa.

Totesimme, että varhainen (<24t) kokonais-PAPP-A:n pitoisuuden lisääntyminen troponiini negatiivisilla potilailla sekä jo tulovaiheessa huomattavasti (>10 mIU/L) lisääntynyt kokonais-PAPP-A:n pitoisuus ST-nousuinfarktipotilailla ennustivat korkeampaa kuoleman sekä uusinta sydäntapahtuman riskiä. Kokonais-PAPP-A:n veripitoisuus lisääntyi ST-nousuinfarktin yhteydessä ensimmäisten tuntien aikana ja väheni tämän jälkeen nopeasti ja pitoisuus 48 tunnin kohdalla korreloi infarktisuonen aukeamiseen. Tulovaiheessa määritetty vapaan PAPP-A:n veripitoisuus osoittautui kokonais-PAPP-A:ta paremmaksi merkkiaineeksi ennustamaan kuolemaa ja sydäntapahtumia potilailla, joilla todettiin akuutti sydäntapahtuma ilman ST-nousua.

Yhteenvetona voidaan todeta, että akuutin sepelvaltimotautikohtauksen yhteydessä todettava PAPP-A:n lisääntynyt veripitoisuus, potentiaalisena epästabiilin plakin merkkiaineena, liittyy korkeampaan kuolleisuuteen sekä uusinta sydäntapahtuman riskiin. Vapaa PAPP-A:n pitoisuus, selittäen valtaosan veripitoisuuden noususta akuutissa sepelvaltimotapahtumassa, osoittautui kokonais-PAPP-A:n määritystä paremmaksi ennustemerkkiaineeksi.

Avainsanat: ennuste, akuutti sepelvaltimotautikohtaus, PAPP-A

TABLE OF CONTENTS

ABBREVIATIONS

ACS	acute coronary syndrome
AMI	acute myocardial infarction
ApoE KO	apolipoprotein E–deficient knockout
AUC	area under the curve
BMI	body mass index
BNP	brain natriuretic peptide
CABG	coronary artery by-pass operation
CAD	coronary artery disease
CHF	congestive heart failure
CI	confidence interval
CK-MB	MB isoform of creatine kinase
CRP	C-reactive protein
CS	coronary sinus
cTn	cardiac troponin
cTnI	cardiac troponin I
cTnT	cardiac troponin T
CV	coefficient of variation
CVD	cardiovascular disease
ECG	electrocardiogram
ED	emergency department
ESC	European Society of Cardiology
freePAPP-A	free form of circulating PAPP-A
GAG	glycosaminoglycan
GRACE	Global Registry of Acute Coronary Events
HR	Hazard Ratio
hs-cTn	high sensitive cardiac troponin
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IGT	impaired glucose tolerance
IL	interleukin
IQR	interquartile range
IVUS	intravascular ultrasound
KO	knockout, inactivation of the gene coding a certain protein
LAD	left anterior descending coronary artery
LBBB	left bundle branch block
LDL	low-density lipoprotein
LMWH	low molecular weight heparin

proMBP	proform of eosinophil major basic protein
MI	myocardial infarction
mRNA	messenger ribonucleic acid
NSTEMI	myocardial infarction without ST-segment elevation in ECG
NSTE-ACS	acute coronary syndrome without ST-segment elevation in ECG
OD	odds ratio
PAPP-A	pregnancy associated plasma protein A
PCI	percutaneous coronary intervention
PCR	polymerase chain reaction
ROC	receiver operating characteristics
RR	risk ratio
SD	standard deviation
SMC	smooth muscle cell
STD	ST-segment deviation
STE-ACS	acute coronary syndrome with ST-segment elevation on ECG
STEMI	myocardial infarction with ST-segment elevation on ECG
totalPAPP-A	total form of circulating PAPP-A
TIMI	Thrombolysis in Myocardial Infarction
Tn	troponin
TnC	troponin C
TNF	tumor necrosis factor
UA	unstable angina pectoris
UFH	unfractionated heparin
URL	upper reference limit
VSMCs	vascular smooth muscle cell

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by Roman numerals (I-III):

I. Lund J, Qin QP, Ilva T, Pettersson K, Voipio-Pulkki LM, Porela P & Pulkki K (2003). Circulating pregnancy-associated plasma protein A (PAPP-A) predicts outcome in patients with acute coronary syndrome but no troponin I elevation. Circulation, 108(16), 1924-6.

II. Lund J, Qin QP, Ilva T, Nikus K, Eskola M, Porela P, Kokkala S, Pulkki K, Pettersson K & Voipio-Pulkki L-M (2006). Pregnancy associated plasma protein A (PAPP-A): A biomarker in reperfused STEMI. Ann Med, 38(3), 221-8.

III. Lund J, Wittfooth S, Qin QP, Ilva T, Porela P, Pulkki K, Pettersson K & Voipio-Pulkki LM (2010). Free vs Total Pregnancy-Associated Plasma Protein A (PAPP-A) as a Predictor of 1-Year Outcome in Patients Presenting with Non–ST-Elevation Acute Coronary Syndrome. Clin Chem, 56(7), 1158-65.

1 INTRODUCTION

The prognosis of acute coronary syndrome (ACS) has improved markedly during the last decades (McManus et al., 2011; Chung et al., 2015), but it is still the major cause of mortality and morbidity in the western world. Early, accurate diagnosis and prognostic evaluation play crucial roles in the triage and management of these patients.

The main ACS mechanism is the rupture or erosion of the underlying, vulnerable atherosclerotic plaque (Naghavi et al., [2003a;2003b]) that activates platelets and coagulation cascade (Toschi et al., 1997) and causes thrombus formation in the coronary artery (Libby et al., 2005). This compromises the blood flow and oxygen supply of the myocardium leading to varying levels of myocardial ischemia (Libby et al., 2005) and damage. This phenomenon causes also the typical clinical signs and symptoms in the acute phase of this syndrome.

The definition, final diagnosis and classification of ACS are based on clinical symptoms consistent with myocardial ischemia, electrocardiogram (ECG) and the determination of cardiac troponin I (cTnI) or T (cTnT) levels in blood samples supplemented, if necessary, by invasive or non-invasive imaging (Roffi et al., 2016). Using modern, highly sensitive cardiac troponin (hs-cTn) assays reveals even minimal myocardial damage early after the onset of symptoms (Ilva et al., 2005; Reichlin et al., 2009; Keller et al., 2009; Keller et al., 2011; Giannitsis et al., 2010; Haaf et al., 2012; Thygesen et al., 2012a; Rubini et al., 2013; Mueller et al., 2014), and these assays have also demonstrated good prognostic performance (Kavsak et al., 2009; Bonaca et al., 2010). Awareness is also increasing of the prognostic value of different ischemic changes in ECGs recorded at the acute phase of ACS (Savonitto et al., 1999; Kaul et al., 2001; Savonitto et al., 2005; Collinson et al., 2006; Jiménez-Candil et al., 2008; Yan et. al., 2010; Nikus et al., 2011).

The best treatment option for ACS would be prevention of a vulnerable plaque's rupture. However, the processes preceding this phenomenon are difficult or even impossible to foresee with methods currently available. When the clinical signs, cardiac troponin (cTn) and ECG in ACS, mirror the myocardial ischemia, useful markers have been missing of unstable plaque prone to rupture or of very early stage plaque rupture. Furthermore, even if the currently available tools give fairly good prognostic information, the outcome of patients with suspected ACS is variable in normal cTn levels and also in patients with cTn elevations (Wong et al., 2007). Additionally, the high sensitivity of modern cTn assays is partly achieved at the expense of lower specificity (Alcalai et al., 2007); thus, there is still room for improvement.

Pregnancy associated plasma protein A (PAPP-A) is a metzincin metalloproteinase (Kristensen et al., 1994) first detected in the serum of the women in late pregnancy by Lin and colleagues (1974). Bayes-Genis and colleagues (2001a) used immunohistochemical staining in the early 2000s to show the abundant expression of PAPP-A in both eroded and ruptured plaques but only minimal expression in stable plaques. This finding resulted in an awakening of interest in the potential role of PAPP-A as a marker of vulnerability or rupture of the atherosclerotic coronary artery plaque and as a prognostic marker inside the spectrum of ACS.

The primary aim of this study was to test the prognostic value of PAPP-A and its investigational novel forms when combined with traditional risk markers in patients admitted to the emergency department with symptoms and findings consistent with suspected ACS and, furthermore, to scrutinize the presumptive role of PAPP-A as a marker of plaque instability or rupture.

2 REVIEW OF LITERATURE

2.1 ACUTE CORONARY SYNDROMES

2.1.1 Pathogenesis

Acute coronary syndrome (ACS) is the manifestation of acute, severe narrowing or occlusion of coronary artery leading to variable degree of myocardial ischemia. Figure 1 illustrates the ruling phenomena of this cascade. Almost invariably the mechanism is the rupture or erosion of atherosclerotic plaque (Naghavi et al., [2003a; 2003b]) uncovering the thrombogenic collagen matrix and exposing blood to tissue factor, activating the coagulation cascade (Toschi et al., 1997) and platelets leading to thrombus formation inside the coronary artery (Libby et al., 2005). The coronary flow is rarely blocked by an embolus or spontaneous coronary dissection (Kamran et al., 2008). The plaque itself and the formed thrombus narrow or occlude the lumen of the coronary artery, reducing or stopping the nourishing coronary flow. The plaque debris and the fragments of the thrombus frequently embolize to the distal parts of the vessel, leading to reduced circulation in smaller arteries and microcirculation, though the site of plaque rupture may remain open (Topol et al., 2000; Heusch et al., 2009). Autopsy materials of individuals dying suddenly from an acute coronary event have shown evidence of distal microembolization in 54-79% of the cases (Falk et al., 1985; Frink et al., 1988; Schwartz et al., 2009). It is likely, therefore, that acute microvascular dysfunction driven by vasoactive agents released during the plaque rupture leads to dynamic vasospasm, playing a key pathogenetic role in the whole spectrum of ACS (De Caterina et al., 2011). The magnitude of myocardial damage that occurs will vary, depending on the level of impairment of coronary flow and myocardial perfusion and the size of the myocardium nourished by the blocked artery. Although the symptoms of acute coronary syndromes can manifest suddenly, the study of Falk and colleagues (1985) showed that an occluding thrombus per se is seldom formed abruptly but is very often preceded by recurrent thrombus formation and fragmentation that may last even for weeks before the occluding thrombosis will occur. The time of the plaque rupture itself is very difficult to define, though it is well known that vulnerable plaques with a lipid-rich core and thin, fibrous cap are prone to rupture.

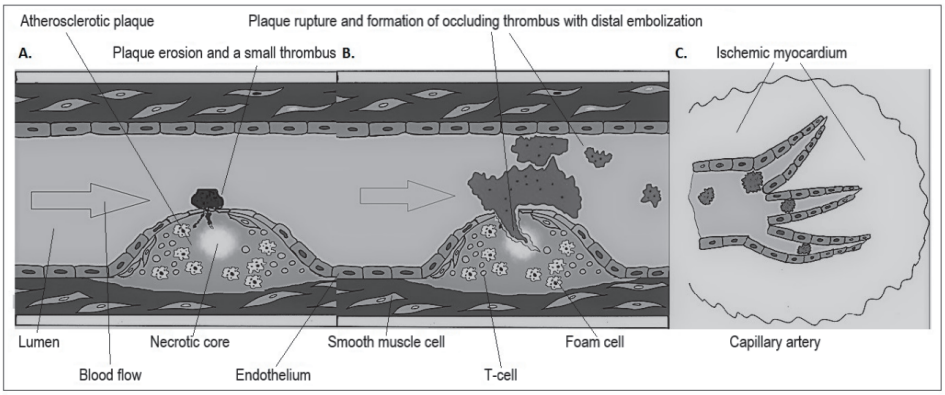

Figure 1. The ruling phenomena touching the rupture of atherosclerotic plaque causing myocardial ischemia. **A)** Early erosion of atherosclerotic plaque and small thrombus formation, **B)** rupture of atherosclerotic plaque activating coagulation cascade leading to formation of occluding thrombus and **C)** distal embolization of plaque material causing myocardial ischemia.

2.1.2 Diagnostics and classification

Chest pain or shortness of breath are typically the signs leading to the suspicion of ACS, while the intensity and type of symptoms can vary greatly inside the spectrum of the disease, ranging from lack of pain to sudden cardiac death. According to the current guidelines of the European Society of Cardiology (ESC), an ACS diagnosis is based on clinical signs, 12-leads electrocardiogram (ECG) and biochemical markers of myocardial damage, preferably the level of circulating cardiac troponin measured with a highly sensitive assay (hs-cTn) (Thygesen et al., 2012b). Strictly speaking, an ACS diagnosis can be used only when the symptoms and myocardial ischemia are driven by an occluded or stenotic coronary artery.

The current ACS classification is designed to serve simple, reliable and fast therapeutic decision making during the acute phase of the syndrome. The patients can be classified by ECG, as the main and the first step with acute coronary syndrome with ST-segment elevation (STE-ACS) or without ST-segment elevation (NSTE-ACS) on ECG (Table 1), followed by cardiac troponin I (cTnI) or T (cTnT) determination to ensure or exclude the myocardial necrosis. Figure 2 shows the overall ACS classification based on the ECG and the circulating level of cardiac troponin (cTn) (Roffi et al., 2016).

Table 1. The ECG classification of ACS

STE-ACS	In STE-ACS group the patients have acute chest pain with persistent (>20 minutes) ST-elevation or sometimes new left bundle branch block (LBBB) on ECG. The myocardial perfusion supplied by certain coronary artery is totally stagnant or very seriously disrupted leading to severe, usually transmural myocardial hypoxia. This causes almost without exception some degree of myocardial necrosis leading to cTn leakage and the diagnosis of ST-elevation myocardial infarction (STEMI) can be set.
NSTE-ACS	In NSTE-ACS the patients have symptoms suggesting acute coronary syndrome, but there is no persistent ST-elevation on ECG. Ischemic ECG changes without ST elevation can be present in both unstable angina (UA) and in myocardial infarction without ST-elevation on ECG (NSTEMI), but the ECG can be also normal especially in the asymptomatic phase. In NSTEMI detectable amount of cTn can be found in the circulation. In UA no sign of myocardial damage measured by cTn can be seen.

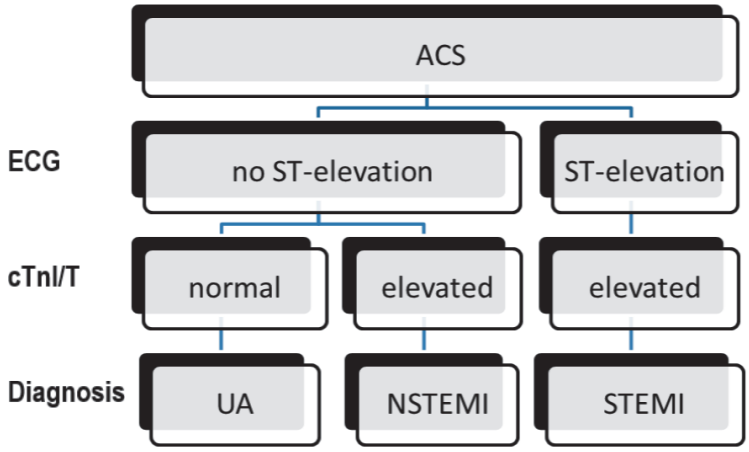

Figure 2. The classification of acute coronary syndrome. ACS=acute coronary syndrome, ECG=electrocardiogram, cTnI/T cardiac troponin I/T, NSTEMI=myocardial infarction without ST-elevation on ECG, STEMI=myocardial infarction with ST-segment elevation on ECG, UA=unstable angina pectoris.

The diagnosis of myocardial infarction (MI) can be confirmed when there is at least one clinical sign consistent with myocardial ischemia (ischemic symptoms, ischemic signs on ECG, recent myocardial damage in imaging investigations or intracoronary thrombus detected on angiography or autopsy) with cTn elevation over the 99th percentile of a healthy population in spontaneous situations and 5 times or 10 times of this upper reference limit (URL)(see section 2.1.4.2.1) after percutaneous coronary intervention (PCI) or coronary artery bypass grafting

(CABG), respectively (Thygesen et al., 2012b). According to the current guidelines, the myocardial infarctions are divided into five categories, depending on the clinical situation (Table 2).

Table 2. The clinical classification of MI based on the current guidelines (Thygesen et al., 2012b).

Type 1	Spontaneous MI.
Type 2	MI secondary to an ischemic imbalance.
Type 3	MI resulting in death when biomarker values are unavailable.
Type 4a	MI related to PCI.
Type 4b	MI related to stent thrombosis.
Type 5	MI related to CABG.

2.1.3 Overall prognosis of ACS

The patient population presenting with the signs and symptoms and also having the final diagnosis of ACS is very heterogeneous, and the exact overall prognosis is difficult to define. Overall, the outcome of ACS patients has improved during the last two decades surely due to increased education of the population, improvement of diagnostics and, especially, because invasive interventions have been established as a part of routine treatment of ASC patients. In the recently published registry data of over 500000 ACS patients in Sweden and the United Kingdom, the 30-day mortality rate was 9.3 – 12.8% during the year 2004 and 6.5 -7.6% in 2010 (Chung et al., 2015). Mortality was similar in patients with STEMI and NSTEMI (7.0 – 10% and 7.0 – 9.6%). The three-year total mortality rate of NSTEMI in the Swedish registry improved from 29.1% in the years 1996-1998 to 23.9% in the years 2005-2007 (Chung et al., 2015). A randomized FRISC II study assessing patients with unstable angina reported that the rates were 3.1% and 9.9% for death and 10.1% and 22.0% for myocardial infarction in one and five years of follow up, respectively (FRISC II investigators 1999, Lagerqvist et al., 2006).

2.1.4 Evaluation of prognosis in ACS

2.1.4.1 Clinical features and risk scores

Although the symptoms do not play a major role in the ACS classification, the clinical signs during ACS guide the treatment and the prognostic evaluation. Several risk scores have been developed to facilitate the prognostication among the wide spectrum of symptoms and signs. The Global Registry of Acute Coronary Events (GRACE) and the Thrombolysis in Myocardial Infarction (TIMI) risk

scores are the most widely used, but neither has gained real routine status in everyday clinical practice, and the impact of risk score implementation on patient outcomes has not been adequately investigated. Table 3 shows the risk factors and scoring of the GRACE risk factors. GRACE seems to provide better prognostic accuracy than the TIMI risk score both at the time of admission and at six months after the index event (de Araujo et al., 2005; Aragam et al., 2009), and the use of the GRACE risk score and GRACE2 risk calculation is recommended in the recently published ESC guidelines for the management of the ACS in patients presenting without persistent ST-segment elevation on ECG (Roffi et.al., 2016).

Table 3. The GRACE risk score. Adapted from de Araujo et al. 2005 and Aragam et al. 2009.

		Score
Age (≤30 to ≥90 yrs)		0 to 100
Heart rate (≤50 to ≥200 beats/minutes)		0 to 46
Systolic blood pressure (≤80 to ≥200 mmHg		58 to 0
Killip class I-IV		0 - 59
Serum creatinine level 0 to ≥354 µmol/l		0 to 28
ST-segment deviation on ECG No/Yes		0/14
Cardiac arrest at hospital admission No/Yes		0/39
Elevated serum cardiac marker No/Yes		0/14
	In hospital death	6-month death after discharge
Score < 108	<1%	<3%
Score 109 – 140	1-3%	3–8%
Score > 140	>3%	>8%

2.1.4.2 Biochemical markers

2.1.4.2.1 Troponins

The research on Troponins started in the early 1960s. Ebashi (Ebashi et al., 1963) published the first report about the protein complex regulating the calcium-dependent relaxation of the skeletal muscle. The protein complex was first called ´´native tropomyosin´´ until Ebashi and Kodama (Ebashi et al., 1965) found the native tropomyosin was formed of two proteins, tropomyosin and another protein they called troponin (Tn). In 1971 Greaser and Gergely succeeded in separating the Tn complex into one inactive and three active parts that they first time named TnI, TnT and troponin C (TnC) (Greaser et al., 1971). A few years later, the expression of different isoforms of TnI was identified in slow and fast skeletal muscle and in myocardium (Syska et al., 1974; Wilkinson et al., 1975; Grand et al., 1976; Grand et al., 1977).

The observation of the different molecular sizes and structure of the N-terminal site of the TnI isoforms between skeletal and cardiac muscles (Wilkinson et al., 1978) allowed the development of cardiac-specific cTnI assay (Cummins et al., 1987). The

isoforms of cardiac cTnT are also expressed in fetal skeletal muscle but are not found in the normal skeletal muscle of adults (Anderson et al., 1991). TnC, in turn, is also expressed after the postnatal period in the slow skeletal muscle, compromising its use as a specific clinical cardiac marker (Parmacek et al., 1989).

Cummins et al. (1987) published the first reported use of the plasma concentration of cTnI to detect myocardial necrosis and diagnosis in 32 acute MI patients. A few years later, Katus et al. proved the efficiency of circulating cTnT to detect myocardial necrosis in 50 patients with transmural MI (Katus et al., 1989) and also its superior efficiency in diagnosis compared to the MB isoform of creatine kinase (CK-MB) in 388 suspected ACS and 101 muscle damage patients (Katus et al., 1991).

Hamm et al. (1992) published the first study on the prognostic significance of cTnT. This study reported that the circulating cTnT was detectable (> 0.20 ug/L) in 33 of 84 (39%) patients with UA and ischemic chest pain at rest, while CK-MB was elevated in only three of those patients. The mortality during the hospitalization was 15.2% and 1.9% in patients with elevated and normal cTnT, respectively. Several studies during the 1990s showed the myocardial damage, as detected by elevation of cTn, as a sign of unfavorable prognosis (Ravkilde et al., 1993; Antman et al., 1996; Stubbs et al., [1996a; 1996b]; Lindahl et al., 1996; Galvani et al., 1997; Polanczyk et al., 1998; Ottani et al., 2000). The determination of cTn was accepted as a diagnostic criteria of MI for the first time in 2000 (Alpert et al., 2000). Immediately after this, Heidenreich and colleagues (2001) published a large meta-analysis of 26 studies with 11963 patients in which the prognostic performance of cTnI or cTnT was tested in NSTE-ACS. In cTnT studies 99 of 1635 (6.1%) cTnT positive vs. 53 of 3524 (1,5%) cTnT negative patients died during the mean follow-up time of 28 weeks; the corresponding number for cTnI studies with a mean follow up of 10 weeks was 108 of 1981 (5,5%) and 77 of 4422 (1.74%) respectively.

The first generation assays were still quite insensitive, and the cut-off points were high, although the prognostic performance of cTn was unequivocally demonstrated. The observations that even a minor cTn elevation increases the risk of adverse events in patients with acute coronary syndrome (Morrow et al., 2001; Pham et al., 2004; Kontos et al., 2004) led to changing the recommendation to use the diagnostic cut-off limit for MI corresponding to the 99% URL of healthy subjects, when using assays with coefficient of variation (CV) <10 % (see Table 4) at URL (Hamm et al., 2011). Recently, the modern, highly sensitive assays have shown superiority by detecting even smaller myocardial damage more quickly after the onset of chest pain and by also giving more reliable prognostic information (Keller et al., 2009; Venge et al., 2009). Today cTn plays a key role in the current recommendations for the diagnostics, classification and prognostic evaluation of ACS and in identifying the patients who benefit, for example, from antiplatelet, antithrombotic and early invasive treatment. A rapid rule-out protocol (0 h and 3 h) is recommended using hs-cTn

tests, and even a 0 h and 1 h rule-out can be used if a hs-cTn test with a validated 0 h/1 h algorithm is available (Roffi et al.,2016). Additional testing after 3–6 h is indicated if the first two troponin measurements are inconclusive and the clinical condition is still suggestive of ACS (Roffi et al., 2016).

2.1.4.2.2 C-reactive protein (CRP)

Inflammation plays an important role in atherosclerosis (Ross, 1993) and its consequences of plaque formation and rupture (Moreno et al., 1994; van der Wal et al., [1994a; 1994b; 1994c]; Kovanen et al., 1995) underlying the process of acute coronary syndrome. C-reactive protein (CRP) is an acute phase protein representing a highly sensitive marker of inflammation produced in hepatocytes when stimulated by interleukin (IL)-1,-6 and tumor necrosis factor-α (TNFα) (Volanakis et al., 2001). Evidence also exists of CRP expression in coronary smooth muscle cells induced by inflammatory cytokines (Calabro et al., 2003). CRP can itself also affect the inflammation reaction and, possibly, the atherotrombotic effects by inducing endothelial cells to express vascular-cell adhesion molecules (Pasceri et al., 2000) and monocytes to produce tissue factor (Cermak et al., 1992).

Elevated CRP has been associated with increased risk of cardiovascular events in the general population (Ridker et al., 1997; Koenig et al., 1999; Ford et al., [2000a; 2000b]; Ridger et al., 2000; Strandberg et al., 2000) and with recurrent instability in patients with stable coronary disease (Haverkate et al., 1997). Several studies have confirmed the correlation between elevated CRP and the risk of repeated cardiovascular events in unstable coronary disease (Haverkate et al., 1997, Toss et al., 1997; Rebuzzi et al., 1998; Ferreiros et al., 1999; Heeschen et al., 2000). The meta-analysis of 20 studies with 17422 ACS patients also found that the CRP levels of 3.1–10.0 mg/l and >10.0 mg/l after ACS were associated with 1.40-fold and 2.18-fold higher risks of adverse outcomes as compared with the low levels (CRP ≤3.0 mg/l) (He et al., 2010). However, as the prospective analysis of 52 studies with 246669 participants without a history of cardiovascular disease shows, the use of CRP as a prognostic marker in ACS contains a number of uncertainties, such as poor specificity and the lack of decision-making levels. In this cohort, the assessment of CRP level in individuals at intermediate risk for a cardiovascular event would prevent one additional event over a period of 10 years for every 400 to 500 screened (Kaptoge et al., 2012). In conclusion the current guidelines do not recommend the use of CRP as prognostic marker in ACS (Roffi et al., 2016).

2.1.4.3 ECG

The ECG, an immediately accessible, simple and noninvasive tool, is nowadays the cornerstone of ACS diagnostics, classification and prognostication.

The ECG can be inconclusive in patients with acute chest pain (due to, for example, LBBB or pacemaker), but the first line discrimination between ST-elevation and non-ST-elevation can easily be done in the majority of cases. In STEMI the ECG gives immediate and reliable information on location, size and even prognosis of the threatening myocardial infarction (Eskola et al., [2007a; 2007b; 2007c]; Eskola et al., [2009a; 2009b]; Tierala et al., 2009). However, in NSTE-ACS a wide variation exists in the ischemic ECG changes in the different ACS populations. For example, in the PRAIS UK registry (Collinson et al., 2006), SYNERGY ECG substudy (Yan et al., 2010) and Paragon B cTnT substudy (Kaul et al., 2003), 19%, 28,6% and 60 % of the patients had ST-segment deviation upon their admission ECG, respectively. Around one fifth of the patients have a normal ECG (Antman et al., 1999; Collinson et al., 2006) with the rest having some degree of T-wave changes or Q-waves. However, an ECG can be normal even in very severe coronary artery disease (CAD) in asymptomatic phase (Atie et al., 1991), and the ECG should be registered repeatedly in ACS according to the patients' symptoms.

ECG changes also provide information on the severity and prognosis of the underlying CAD in NSTE-ACS. Prominent (Savonitto et al., 2005) and/or widespread ST depressions (Holmvang et al., 2003) increase the likelihood of 3-vessel or left main disease and, when associated with inverted T-waves in V4-V5, are signs of poor prognosis (Nikus et al., 2004). Kaul et al.'s (2001) study found the ST depression >0.1 mV was associated with an 11% rate of death and MI at one year, while ST depression >0.2 mV carried about a six-fold increase in mortality. In the case of regional ischemia, the ECG changes (ST depression, T-wave changes) are usually evident in less than six leads (Nikus et al., 2010). However, the localization of culprit stenosis in NSTE-ACS is not necessarily unambiguous except in the case of ST depression in leads V1-V5 with positive T-waves, indicating the culprit localization in the left anterior descending coronary artery (LAD). The patients with isolated T-inversion without ST depression have a better prognosis than the patients with ST elevation or depression or both during ACS (Savonitto et al., 1999).

Table 4. Clarification of quality criterions of the diagnostic tests.

	Clarification	Calculation
Variance		The average of the squared from the mean
Standard deviation (SD)	Amount of variation of data values	The square root of the variance
Coefficient of variation (CV)	Extent of variability in relation to the mean of the measurements	Ratio of the standard deviation to the mean
Imprecision	Measurement deviation from true value	

2.2 PREGNANCY ASSOCIATED PLASMA PROTEIN A (PAPP-A)

The PAPP-A protein was first detected by Lin and coworkers in 1974 from the serum of the woman in late pregnancy (Lin et al., [1974a; 1974b]). A few years later, the level of circulating PAPP-A was noticed to increase when gestation and at the highest level just before the delivery (Smith et al., 1979; Folkersen et al., 1981). In the early 1990s, Wald and colleagues (1992) demonstrated the correlation between low levels of PAPP-A in maternal circulation, especially during the first trimester, and 21 trisomy (Down's syndrome) pregnancies. The concentration of PAPP-A in blood has widely been used as one of the components in multimarker screening protocols for Down's syndrome and possible other abnormities or complications during the pregnancy as a result of these findings.

2.2.1 The molecular structure of PAPP-A

PAPP-A has a similar zinc binding site as found in other metalloproteinases belonging to the metzincin superfamily (Kristensen et al., 1994). As a monomer, the polypeptide contains 1547 amino acid residues (Kristensen et al., 1994). When PAPP-A was found, the protein sequence did not conform to any of the existing four families of metzincins, and PAPP-A was declared as the founding member of a new, fifth subfamily of metzincins named as Pappalysins (Boldt et al., 2001). It was first believed that PAPP-A consists of two disulfide-bridged subunits, but it was later realized that during pregnancy and in healthy populations, PAPP-A is manifested in blood predominantly as a 500-kDa 2:2 heterotetramer covalent complex (Figure 3) of two PAPP-A and two proform of eosinophil major basic protein (proMBP) subunits (complexed PAPP-A) (Oxvig et al., 1993; Qin et al., 2005). ProMBP is a heterogeneously glycosylated proteoglycan with moderately variable molecular mass of 30-100 kDa (Oxvig et al., 1993; Oxvig et al., 1994) and is produced by the maturing eosinophils (Popken-Harris et al., 1998). ProMBP regulates the activity of PAPP-A (Figure 3), being proteolytically active when presenting its free, noncomplexed form and inactive when complexed with proMBP (Overgaard et al., 2000).

Complexed PAPP-A is variably found in circulation in all individuals, while the level of free form (not complexed with proMBP) PAPP-A (freePAPP-A) is near to zero in healthy subjects (Wittfooth et al., 2006). The circulating fraction of free-PAPP-A is <1% also during the second and third trimester of pregnancy (Overgaard et al., 2000) while a variable fraction of active freePAPP-A exist during the early pregnancy (Gyrup et al., 2007).

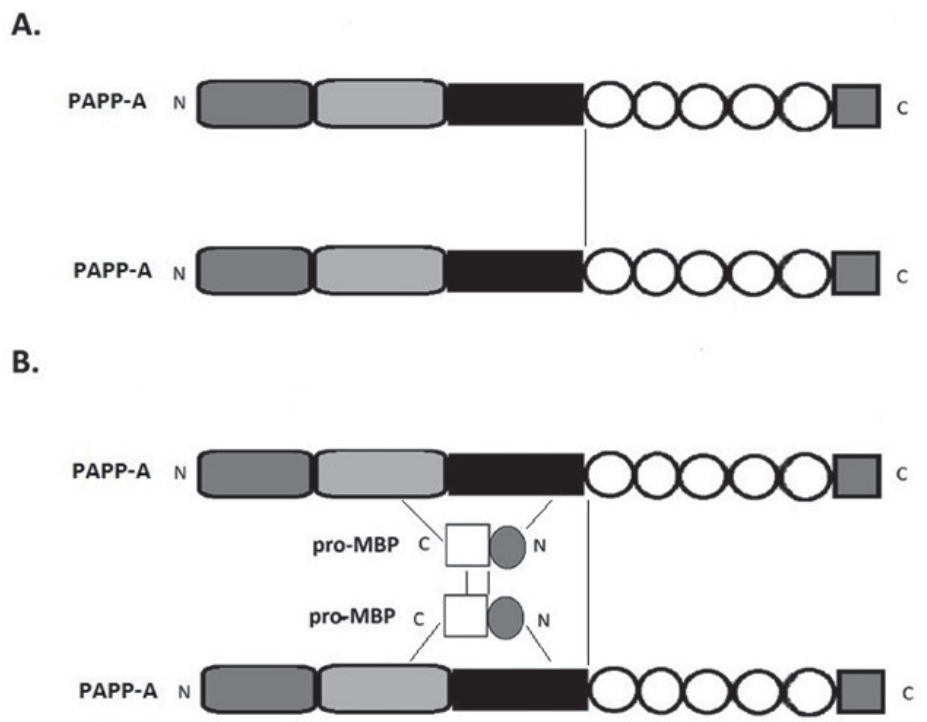

Figure 3. The rough structure of PAPP-A and pro-MBP molecules and the regulation of PAPP-A activity by pro-MBP. **A)** Proteolytically active noncomplexed free form PAPP-A. **B)** Proteolytically inactive PAPP-A complexed with pro-MBP. Adapted from Boldt et al., 2007 and Glerup et al., 2005.

2.2.2 The expression of PAPP-A

The expression of both PAPP-A in the syncytiotrophoblast and proMBP in extravillous trophoblasts is strongest during late pregnancy (Bonno et al., 1994). By using a sensitive, semi-quantitative, reverse transcription polymerase chain reaction (PCR) method, Overgaard and colleagues (1999) could demonstrate the expression of both PAPP-A and proMBP in a wide range of tissues. PAPP-A and proMBP messenger ribonucleic acid (mRNA) were synthesized in all other tested, postmenopausal reproductive tissues (ovary, tuba uterina, endometrium, and myometrium), in addition to the placenta, and the non-reproductive tissues of non-pregnant individuals (kidney, colon, prostate, prostate carcinoma, bone marrow cells, breast, and breast carcinoma) were also tested. PAPP-A is additionally expressed by human fibroblasts, osteoblasts and osteoprogenitor cells (Lawrence et al., 1999). Chen and colleagues (2003) demonstrated staining for PAPP-A in uninjured skin in the epidermis, sweat and sebaceous gland epithelial cells, hair follicles and blood vessels. The expression of PAPP-A was augmented by injury in the dermal granulation tissue, small blood vessels and on collagen near the injury.

The basal PAPP-A expression in endothelial cells of human coronary arteries is low (Conover et al., 2008), but the stimulation by pro-inflammatory cytokines, especially TNF-α and interleukin (IL)-1ß, increase the expression (Conover et al., 2006; Conover et al., 2008). Interestingly, resveratrol, a polyphenol thought to explain the salutary effect of grapes and red wine, inhibits the expression of PAPP-A and its proteolytic activity (Conover et al 2006). Bayes-Genis and colleagues 2001 demonstrated the expression of PAPP-A in human and porcine vascular smooth muscle cells (VSMCs) in vitro and that PAPP-A was markedly up-regulated in vivo in (porcine) neointimal hyperplasia after coronary angioplasty (Bayes-Genis et al., 2001).

2.2.3 The main physiologic function of PAPP-A

The active, non-complexed form of PAPP-A acts as a protease cleaving locally insulin-like, growth factor binding proteins (IGFBP) -2, -4 and -5 (Lawrence et al., 1999; Laursen et al., 2001; Gerard et al., 2004) and is the predominant protease of IGFBP-4 (Overgaard et al. 2000). The cleavage of IGFBPs by PAPP-A releases insulin growth factors (IGF) I and II from their binding proteins (Jones et al., 1995; Laursen et al., 2002). IGFs are small (7.5 kDa) polypeptide hormones with structural similarity with pro-insulin and are mostly (ca. 80%) synthesized in the liver and in periphery by connective tissue type cells to a lesser extent (Clemmons et al., 2007). In an extra-cellular environment, the IGFs are mainly (ca. 99%) bound to IGF-binding proteins, but cleavage of IGFBP enables bioactive IGFs to bind predominantly to cell-surface receptors, mediating the most actions of IGFs (Hsieh et al., 2003). IGFs are acting in various cell functions like cell proliferation and differentiation. The effects of IGF-1 resemble the effects of insulin, and IGF-1 generally promotes insulin signaling in the cell (Blundell et al., 1980).

2.2.4 PAPP-A and the process of atherosclerosis

Bayes-Genis and colleagues (2001a) collected coronary artery plaques in the early 2000s from eight patients who had died suddenly of cardiac causes and showed by immunohistochemical staining an abundant expression of PAPP-A, especially in the shoulder regions of both eroded and ruptured plaques, but only a minimal expression in stable plaques. Sangiorgi and colleagues (2006) later observed similar findings in carotid plaques collected by surgical endarterectomy. They demonstrated a strong correlation between a decrease in plaque cap thickness and an increase in inflammatory infiltrate and the expression of PAPP-A in the plaque. Brügger-Andersen and colleagues (2010) collected the plaque debris and thrombus material by aspiration thrombectomy during a PCI procedure from 13 acute

STEMI patients. They demonstrated PAPP-A expression in the plaques' extracellular matrix but not in the thrombus itself. Bayes-Genis and colleagues' study showed the abundant expression of PAPP-A in the cytoplasm of medial and neointimal cells of porcine arteries at 7, 14, and 28 (highest labeling) days after angioplasty (Bayes-Genis et al., 2001b). Smith and colleagues (2001) showed similar results by balloon dilatation of the femoral artery in rats, as did Resch and colleagues (2006) by ligation of the carotid artery in mice. On the contrary, Rossen and colleagues (2007) could not show any PAPP-A expression in the atherosclerotic plaque materials collected during PCI of acute STEMI, while the circulating PAPP-A level was elevated.

The mechanism of PAPP-A in atherosclerosis and its acute manifestations is still not fully understood. The research trying to explain the mechanism is partly contradictory and there have been confounding factors in the studies. However, the conceivable role of PAPP-A in the atherosclerotic processes is possibly, at least in part, mediated by IGFBPs and/or biologically active IGFs (Bayes-Genis et al., 2000; Bayes-Genis et al., 2001b; Bayes-Genis et al., 2003). PAPP-A (Bayes-Genis et al., 2001a; Sangiorgi et al., 2006) and IGF-1 (Grant et al., 1994; Grant et al., 1996) are abundantly expressed in human atherosclerotic lesions. However, whether the excessive local PAPP-A expression is the primary pathological process causing the atherosclerotic progress or secondary to tissue healing is not well understood. Liu and coworkers (2008) demonstrated a significant increase of circulating IGF-1 and PAPP-A after the PCI. Grant and colleagues' (1996) study did not detect IGF-I, its receptor and IGFBPs in VSMCs of normal coronary arteries, while the expression of IGF-I, IGF-I receptor, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4 and IGFBP-5 in de novo and restenotic atherosclerotic plaques was noticed. IGFs induce not only the growth and migration of VSMCs but also their extracellular matrix synthesis in the atherosclerotic plaque (Delafontaine et al., 2004). IGF-I has an ability to promote macrophage activation, chemotaxis, low-density lipoprotein (LDL) cholesterol uptake by macrophages and the release of pro-inflammatory cytokines by these cells (Bayes-Genis et al., 2000); in turn, a high expression of PAPP-A by monocyte–macrophage cells was shown by Sangiorgi and colleagues (2006) in complicated and vulnerable carotid plaques but only to a lesser extent in stable plaques. However, a later study of Conover and colleagues did not detect any expression of PAPP-A in macrophages or in human peripheral monocytes (Conover et al., 2007).

Interestingly, the recently published study by Bale and colleagues (2014) showed a significant inhibition of both atherosclerotic plaque progression and development of advanced plaque with necrotic cores by decreasing the PAPP-A gene expression in mice. Similar results were already shown earlier in Harrington and colleagues' (2007) study, in which they demonstrated the resistance of atherosclerotic lesion

progression, but not full inhibition, in apolipoprotein E–deficient knockout (ApoE KO) mice with genetically deleted PAPP-A compared to a control group of ApoE KO mice with normal PAPP-A expression predisposed to the same high-fat diet. In the same work, the expression of PAPP-A mRNA, IGF1 and IGFBP-4 mRNA in the control group was significantly higher (20-, 20- and 8-fold respectively) in the formed atherosclerotic lesions compared to the areas without atherosclerotic lesions (Harrington et al., 2007). Conover and colleagues (2010) performed a study with a reversed design with ApoE KO mice expressing the human PAPP-A transgene at relatively high levels in arterial smooth muscle cells (SMC). They noticed a 3,5 - fold increase in atherosclerotic lesion areas in mice aortas with high expression of human PAPP-A compared to the control ApoE KO mice while, as in earlier studies, no difference in the lesion number was noticed. Interestingly, the PAPP-A KO mice seems to live significantly longer (even 35%) compared to the wild-type controls (Conover et al., 2010). These observations strongly support the pivotal role of PAPP-A, IGFs and IGFBPs axis in the process of atherosclerotic plaque formation.

2.2.5 The anticipated mechanism of circulating PAPP-A release in atherosclerotic manifestations

There is only indirect evidence of the release of PAPP-A into the circulation from the atherosclerotic plaque or from the atherosclerotic vascular bed. Although partly controversial, the currently available data supports the plaque hypothesis, while other theories also explain the PAPP-A release.

2.2.5.1 Circulating PAPP-A and the character of atherosclerotic plaque

In 2001 Bayes-Genis and coworkers showed an abundant expression of PAPP-A in ruptured and eroded, but not any or only minimal expression in stable coronary artery plaques (Bayes-Genis et al., 2001a) and after this observation the levels of circulating PAPP-A and the atherosclerotic plaque character have been clarified in several studies. In the work of Sangiorgi and colleagues (2006) the PAPP-A level, determined the day before the planned endarterectomy, was significantly higher in patients with vulnerable and ruptured plaques with thrombus versus in patients with stable carotid plaques collected by surgical endarterectomy. Also, Heider and colleagues (2010) reported significantly higher PAPP-A level in patients with unstable vs. stable carotid artery plaques. Recently Wu and colleagues (2013) detected with intravascular ultrasound (IVUS) larger plaque burden, more thrombus and more complex plaques in patients with higher (taken before any heparin treatment) compared to patients with lower PAPP-A levels. Similarly, PAPP-A levels were significantly higher in patients with any fulfilled criteria of unstable or complex lesions showed by IVUS compared to patients with plaques lacking those

signs. In the work of Zhao and colleagues (2013) the PAPP-A level was positively correlated with plaque necrotic core area and negatively correlated with plaque fibrotic area evaluated with IVUS. In the same study, the PAPP-A level was significantly higher in patients with no-reflow phenomenon during the PCI. Recently, similar results have been shown by Daidoji and colleagues (2015). Interestingly, Mei and colleagues (2011) noticed the higher restenosis rate after PCI done in acute phase in ACS patients with high PAPP-A compared to patients with low PAPP-A levels measured at the time of admission due to ACS.

To summarize, the available data shows the positive correlation between the atherosclerotic plaque complexity and the level of circulating PAPP-A. This supports, albeit indirectly, the hypothesis of PAPP-A release due to plaque rupture and the over-expression of PAPP-A in unstable or complex atherosclerotic vascular bed.

2.2.5.2 The elevation of circulating PAPP-A due to tissue ischemia or damage

While there is a strong suspicion of the plaque rupture as a mechanism behind the release of PAPP-A into the circulation, there are also doubts that myocardial ischemia itself can explain the phenomenon. In a recent work by Steffensen and coworkers (2015), the myocardial ischemia was induced by ligation of pigs' LAD for 45 minutes; the blood samples were collected from the aorta, coronary sinus (CS) and left femoral vein at baseline and every 10-15 minutes (ad 85 minutes) after the induction of myocardial ischemia. The PAPP-A level started to elevate 40 minutes after the induction of ischemia and, interestingly, the levels were comparable in the aorta, femoral vein and in CS at every time point. The same work demonstrated higher PAPP-A levels in the femoral vein compared to the aorta or CS after the ligation of femoral artery. They conclude that PAPP-A can be released in situations of myocardial ischemia in the absence of atherosclerosis, and part of the PAPP-A elevation could be due to hypoperfusion of non-cardiac striated muscle. This observation is in line with a previous finding of localization of PAPP-A in injured skin (Chen et al., 2003), most likely due to part of the healing process.

2.2.5.3 The effect of heparin administration on the level of circulating PAPP-A

As recently shown, the concomitant heparin treatment may significantly affect the concentration of circulating PAPP-A. Terkelsen and colleagues (2009) first noticed a trend towards higher PAPP-A levels in 84 STEMI patients treated with intravenous unfractionated heparin (UFH) before primary PCI, compared to 14 historical control patients with acute STEMI not treated with heparin. The animal experiment, published in the same paper, showed the higher PAPP-A levels in mice receiving concomitant heparin, and the reinjection of heparin caused a re-

elevation of PAPP-A. At the same time, Hájek and coworkers (2010) showed parallel results in patients heparinized for elective coronary angiogram with or without PCI and in patients with PCI for acute STEMI. In their study, intravenous heparin caused a dose-dependent, precipitous and high PAPP-A elevation followed by a quite rapid decrease during the next one-to-two hours and reaching the normal level in 12 hrs. No PAPP-A elevation was noticed during or after the elective coronary angiogram done without any heparinization and without any angiographic signs of atherosclerotic coronary disease. Tertti and colleagues (2009) demonstrated the rapid, intense increase and decrease of the levels of circulating total- and freePAPP-A after the intravenous dispensing of low molecular weight heparin (LMWH) or UFH during coronary angiography or hemodialysis. In vitro, adding heparins to whole blood samples of the same dialysis patients did not cause elevation of PAPP-A. Later, Wittfooth and colleagues (2011) demonstrated the elevation of freePAPP-A after intravenous injection of UFH and LMWH. The elevation of freePAPP-A was lower and slower after the subcutaneous bolus of LMWH. The extracted PAPP-A from atherosclerotic plaques was in free form, and the extraction was significantly enhanced by LMWH in the same work. The circulating PAPP-A level also increases after the subcutaneous injection of LMWH and normalizes quite slowly during the 36-48 hrs after the last subcutaneous injection (Wang et al., 2011, Wang et al., 2013).

Speculation exists about the mechanism of how heparin increases the concentration of circulating PAPP-A. Laursen and colleagues (2002) demonstrated reversible binding of PAPP-A to the cell surface of several cell types mediated by the glycosaminoglycan binding site, and heparin and heparan sulfate competed for PAPP-A surface binding. PAPP-A is also known to bind heparin (Sinosich et al., 1981; Davey et al., 1983).

2.2.6 Measurement and the levels of circulating PAPP-A

2.2.6.1 Assays for circulating PAPP-A

Existing commercial PAPP-A assays are based on the measurement of the total form of PAPP-A (totalPAPP-A) by detecting, with specific antibodies for the PAPP-A subunit, both the complexed PAPP-A/proMBP and the non-complexed free form of PAPP-A (Figure 4).

Complexed PAPP-A is variably found in all individuals (Wittfooth et al., 2006), and the normal reference level of circulating PAPP-A varies between the used assays; until now, there is no general approval for any cut-off point or normal range

for PAPP-A assays. Table 5 illustrates the detection methods and the levels of totalPAPP-A in a normal population of a certain assay.

Table 5. The detection methods and the levels of totalPAPP-A in normal population by various PAPP-A assays.

Author	Assay	TotalPAPP-A mIU/L in normal population			Detection method
		Median (25^{th}, 75^{th})	Median [range]	Mean ±SD	
Mueller et al. 2006	DSL	0.64 (0.40, 1.04)			ELISA (enzyme linked immuno-sorbent assay)
Coskun et al. 2007	DSL	3.9 (3.1, 5.2)			
Aso et al. 2004	DSL	4.54 (4.00, 6.01)			
Lauzurica et al. 2005	DSL		0.96 [0.16 to 3.08]		
Joaquin et al. 2008	DSL			0.92 ±0.56	
Khosravi et al. 2002	DSL		1.09 [0 to 10.4] (men) 0.03 [0 to 3.60] (women)		
Wittfooth et al. 2006		2.4 (2.0, 3.1)	2,4 [1.0 to 5.9]		Time-resolved immunofluorometric assay
Qin et al. 2002	Qin		3.01 [1.51 to 7.59]		
Stulc et al. 2003	BRAHMS Kryptor			6.5 ±2.5	TRACE (Time-resolved amplified cryptate emission assay)
Kalousova et al. 2003	BRAHMS Kryptor	8.0 (7.0, 10.0)			
Beaudeux et al. 2003	BRAHMS Kryptor			8.0 ±2.8	
Hodkova et al. 2006	BRAHMS Kryptor			11.4 ±1.9	
Liu et al. 2008	Bayes-Genis			8.4 ±2.0	Biotin tyramide amplified enzyme immuno assay
Bayes-Genis et al. 2001a	Bayes-Genis		7.4 [3.8 to 10.4]		

Interestingly, freePAPP-A predominantly represents the fraction that changes dynamically in ACS (Qin et al., 2005; Wittfooth et al., 2006). This observation raised the idea that the measurement of freePAPP-A could be a more specific and sensitive biomarker in ACS than totalPAPP-A. Our group developed and evaluated the first, new point-of-care assay for the determination of the ACS-related non-complexed PAPP-A in 2006 (Wittfooth et al., 2006). This assay measures freePAPP-A by using two separate assays measuring total and complexed PAPP-A forms, and the concentration of freePAPP-A is calculated from the difference of the results given by these two assays (Figure 4).

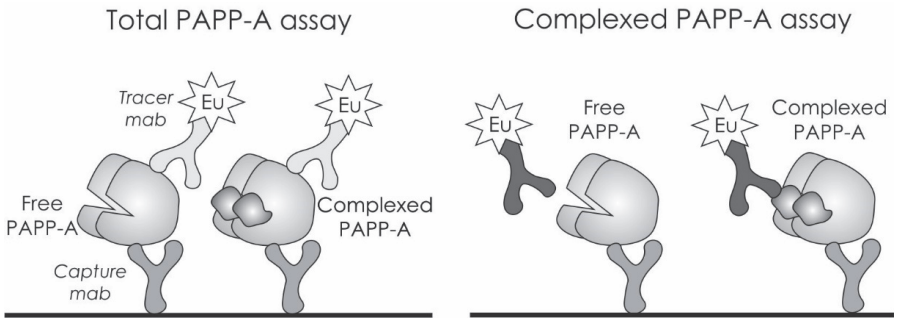

Figure 4. The basics of detection of total and complexed PAPP-A. PAPP-A subunits bind to surface capture antibody regardless of complexation with pro-MBP or not. In total PAPP-A assay the labeled antibody binds to PAPP-A molecule. In complexed PAPP-A assay the labeled antibody binds to pro-MBP molecule thus detecting only the PAPP-A/pro-MBP complex. Reproduced with the permission of S. Wittfooth.

2.2.6.2 The level of circulating PAPP-A related to gender, age, diabetes, hypercholesterolemia and smoking

The concentration of totalPAPP-A seems to be significantly higher in men compared with non-pregnant women in an apparently healthy population, as shown by Wittfooth and colleagues (2006) (2.8 vs. 2.1 mIU/L, p<0.0001), Coskun and colleagues (2013) (6.85 vs. 3.40 ng/mL, p <0.011) and also by Pellitero and colleagues (2007) (1.04 vs. 0.52 mIU/L, p=0.025). A weak positive correlation might exist between the PAPP-A level and age (Cosin-Sales et al., 2005; Coskun et al., 2013).

According to a few case control studies with diabetic patients, the PAPP-A level is higher in patients with type 2 diabetes than in non-diabetic controls. Studies by Heidari with coworkers (2015) and Aso with coworkers (2004) showed that the serum concentrations of PAPP-A were significantly higher in diabetic patients vs. control subjects (mean ± standard deviation (SD) [0.61 ±0.24 mIU/L vs. 0.35 mIU/L ±0.08, p <0.001] and median [interquartile range (IQR)] 7.82 [6.31 to 9.93] mIU/L vs. 4.54 [4.00 to 6.01] mIU/L, p<0.0001, respectively). Astrup and colleagues's (2007) study reported that the PAPP-A was higher in type 1 diabetes patients with diabetic nephropathy vs. patients without albuminuria (median [range] 3.6 [0.4 to 51.1] mIU/l vs. 2.1 [0.4 to 46.6] mIU/ l, p<0.0001). Conversely, Pellitero and colleagues's (2007) study showed that the PAPP-A level was lower in type 2 diabetic patients than in normal controls (median [IQR] 0.73 [0.48 to 1.33] mIU/L vs. 0.45 [0.19 to 0.82] mIU/L, p <0.0001).

The level of circulating PAPP-A in patients with hypercholesterolemia has been studied in some trials. Stulc and coworkers's (2003) small, controlled study

demonstrated that the PAPP-A concentration was significantly higher in hypercholesterolemic (total cholesterol >7 mmol/l) patients without any other significant diseases vs. controls (mean ±SD 8.02 ±1.86 mU/L vs. 6.50 ±2.54 mU/L, p=0.018), while atorvastatin treatment had no effect on PAPP-A levels. However, Miedema and coworkers (2008) studied 59 patients with ACS or stable CAD and noticed a significant decrease of the circulating PAPP-A level at one month with high dose (80 mg per day) atorvastatin treatment but not with low dose (10mg per day) treatment.

The level of circulating PAPP-A seems to be lower in smokers vs. non-smokers (Coskun et al., 2013; Szumska et al., 2013), which has been established also during pregnancy (Zhang et al., 2011; Chełchowska et al., 2012).

2.2.6.3 The level of circulating PAPP-A in CAD

2.2.6.3.1 The level of circulating PAPP-A in stable coronary artery disease

While few trials showing divergent results exist, the greater part of the studies done on stable CAD show no correlation of circulating levels of PAPP-A or PAPP-A/proMBP ratio and the extent or complexity of underlying CAD. In Cosin-Sales and colleagues' study (2005), the PAPP-A concentration, determined just before the diagnostic angiogram in 643 patients with stable angina pectoris, was found as an independent predictor for the presence and extent of CAD in patients with chronic stable CAD. The concentration (mean ±SD) of PAPP-A was significantly higher in patients with multivessel disease (6.45 ±2.58 mIU/L) vs. in patients with single vessel disease (5.49 ±1.54 mIU/L, p <0.001) or without CAD (4.62 ±1.17 mIU/L, p <0.001). The PAPP-A concentration >4.5 mIU/L was found to predict the presence of significant (>50%) stenosis with a sensitivity of 45% and a specificity of 84%. The same researchers had previously observed a significant relationship between PAPP-A/proMBP ratio, PAPP-A levels, and the presence of complex coronary stenosis and PAPP-A/proMBP ratio was an independent predictor of plaque complexity in patients with stable angina pectoris (Cosin-Sales et al., 2004). Bayes-Genis and coworkers's (2001a) study reported that the (median [range]) levels of PAPP-A were comparable between the patients with stable CAD and age-matched controls without any clinical or angiographic evidence of CAD (8.4 [4.4 to 22.5] mIU/L and 7.4 [3.8 to 10.4] mIU/L, p=0.07). Liu and coworkers' (2008) small study showed similar results, wherein the corresponding (mean ±SD) PAPP-A levels in stable CAD and control group were comparable (8.5 ±3.1 mIU/L and 8.4 ±2.0 mIU/L). Schulz and coworkers (2011) measured PAPP-A two-to-three days prior to a diagnostic coronary angiogram and without any heparin treatment

from 228 outpatients suffering from stable angina pectoris type symptoms. No correlation was noticed between the (median [25th, 75th percentiles]) PAPP-A levels and the presence of CAD (2.12 [1.38, 2.64] mIU/L in patients with and 1.65 [1.18, 2.61] mIU/L without CAD, p=NS) or the complexity of atherosclerotic lesions in the angiography. The available evidence logically reflects the character of the stable coronary disease with atherosclerotic plaques compromising the blood flow but not prone to rupture.

2.2.6.3.2 The level of circulating PAPP-A in ACS

The circulating PAPP-A level has been widely investigated inside the whole spectrum of ACS manifestations. In summary, the PAPP-A level elevates in patients with ACS, reflecting the mechanism of acute plaque rupture. Bayes-Genis and coworkers (2001a) were the first to notice significantly higher (median [range]) PAPP-A levels taken at the time of a coronary angiogram in patients with UA (14.9 [6.3 to 63.4] mIU/L) or acute myocardial infarction (AMI) (20.6 [9.2 to 46.6] mIU/L) compared to patients with stable angina pectoris or to a control group of patients without evidence of CAD (7.4 [3.8 to 10.4] mIU/L), p<0.001. The PAPP-A level of 10.0 mIU/L had the best composite of sensitivity and specificity for the identification of ACS, being 89.2% and 81.3%, respectively. Only one of 13 of the control subjects without any evidence of atherosclerosis had PAPP-A >10.0 mIU/L. Similarly, Khosravi with colleagues (2002) reported with serial blood sampling in 71 cardiac patients and in 47 healthy controls (women and men), collected together with markers of myocardial damage, significantly higher (median [range]) PAPP-A levels in patients with elevated CK-MB or cTnT (4.47 [0.31 to 16.5] mIU/L or 3.89 [0.01 to 97.0] mIU/L) compared to patients with CK-MB in the normal range (0.29 [0 to 4.9] mIU/L, p<0.001) or healthy controls of men (1.09 [0 to10.4] mIU/L (p<0.001)) and women (0.03 [0 to 3.60] mIU/L (p<0.001)). Miedena and colleagues' (2008) study, performed with 86 patients suffering from ACS (n=35) or stable angina pectoris (n=51), reported that the level of (median [25th, 75th percentiles]) PAPP-A taken before diagnostic coronary angiography was significantly higher in patients with ACS than in patients with stable angina pectoris (2.05 [1.02, 4.45] mIU/L vs. 0.75 [0.42, 1.47] mIU/L, p=0.001). Liu and coworkers (2008) determined PAPP-A before the coronary angiogram in patients with UA and cTn and CK-MB in the normal range, in patients with acute STEMI (n=12), in patients with stable angina pectoris and in 16 normal controls. They noticed the significantly higher (median ±SD) PAPP-A levels in patients with UA (15.2 ±10.5 mIU/L) and STEMI (16.9 ±10.3 mIU/L) compared to a control group (8.4 ±2.0 mIU/L, p<0.01) or to patients with stable angina pectoris (8.5 ±3.1 mIU/L, p<0.01). Rossen and colleagues' (2007) study observed the lowest PAPP-A levels (0 to 4.8 mIU/L) in healthy controls (n=103), while the concentrations

were significantly higher (0 to 18.9 mIU/L) in NSTEMI (n=20) and the highest (4.5 to 49.8 mIU/L) in patients with STEMI (n=14).

Iversen and colleaques (2009) conducted the first study with a bigger number of NSTE-ACS patients (n=573) clarifying the release patterns and the levels of circulating PAPP-A in different types of NSTE-ACS. This study showed that the PAPP-A level stayed constantly elevated at all time points (determined at every six-eight hrs) except at admission during the first 36 hours in patients with high risk ACS (defined as cases with elevated CK-MB and cTnT and/or ischemia on ECG) and also in low risk ACS with normal EGC and no elevation in cardiac markers. No significant difference existed in (mean (95% confidence interval (CI)) peak PAPP-A levels between the groups being 14.0 (11.9–16.0) mIU/L for patients with high-risk and 12.3 (11.0–13.6) mIU/L for those at low risk (p=0.20). However, PAPP-A was significantly higher in these groups compared to a reference population of 1448 patients admitted with non-cardiac reason, of which PAPP-A was under the detection limit (<4.0 mIU/L) in 80.8 %; the mean cPAPP-A was 6.3 (6.1–6.5) mIU/L in the rest (Iversen et al., 2008a).

Some studies with ACS patients have determined the level of circulating PAPP-A at the time of admission that reduced the possible effects of confounding factors, such as, in particular, the effect of heparin and invasive treatments. McCann and colleagues' (2008) study revealed that the (median [25th, 75th percentiles]) admission PAPP-A was slightly higher in patients with AMI as the final diagnosis vs. in patients without the evidence of AMI (6.7 [2.6, 12.4] mIU/L vs. 5.0 [1.7, 11.0] mIU/L, p=0.044). However, PAPP-A did not demonstrate significant, independent predictive value for an MI diagnosis. Elesber and colleagues (2007) included patients with an intermediate or high likelihood of a coronary event at presentation and determined PAPP-A at the time of admission. The (median [25th, 75th percentiles]) PAPP-A concentration was significantly higher in patients with ACS as a final diagnosis vs. in patients with chest pain from non-cardiac reasons (2.0 [1.2, 4.9] mIU/L vs. 1.2 [0.7, 1.6] mIU/L, p=0.001). Heeschen and colleagues (2005) measured PAPP-A at admission time and before any possible heparin treatment in a heterogeneous group of 644 patients representing with acute chest pain consistent with suspected ACS. This study showed that (median [range]) PAPP-A was significantly higher in patients with chest pain of ischemic origin vs. in patients with stable angina pectoris or no evidence of coronary artery disease confirmed by coronary angiography (4.9 [0.1 to 362.5] mIU/L vs. 1.9 [0.1 to 113.9] mIU/L, p=0.007) or 1.4 [0.1 to 54.1] mIU/L; p< 0.001, respectively).

Hajek and coworkers' (2012) study, with a limited number (n=67) of heparin-naïve acute chest pain patients, reported that the (median) admission PAPP-A level was significantly higher in patients with a final ACS diagnosis vs. in the patients classified into the non-ACS group (8.6 vs. 7.3 mIU/l, p=0.006).

2.2.6.4 Circulating PAPP-A as a prognostic marker in CAD

The use of the level of circulating PAPP-A as a prognostic marker in stable coronary artery disease and during the acute manifestations of CAD has been broadly investigated.

2.2.6.4.1 Circulating PAPP-A as a prognostic marker in stable CAD

Evidence exists that supports the prognostic value of the concentration of circulating PAPP-A in the stable phase of atherosclerotic coronary disease. The decision limit has been quite comparable in these studies, varying between 2.7 mIU/L to 4.8 mIU/L. Elesber and colleagues (2006) measured PAPP-A just before the diagnostic coronary angiogram showing at least one coronary artery diameter stenosis of >50% in 103 patients suffering the symptoms consistent with stable angina pectoris. During the median follow-up time of 4.9 yrs, an elevated (>4.8 mIU/L) PAPP-A concentration was significantly associated with the combined endpoint of future death (adjusted hazard ratio (HR) 5.2, 95% CI 1.27–22.0, p=0.023), death and ACS (adjusted HR 3.56, 95% CI 1.27–10.0, p=0.015) but not with death and revascularization. The mortality rate was 9 % and 29 % in patients with PAPP-A < 4.8 mIU/L and > 4.8 mIU/L, respectively, while the parallel ACS rates were 2 % and 16 %. Consuegra-Sanchez and colleagues' (2008) study followed up 663 patients with stable angina pectoris for a median time of 8.8 years. An increased PAPP-A concentration (>4.8 mIU/L) taken at the time of a diagnostic coronary angiogram was an independent predictor of all-cause mortality (HR 1.95, 95% CI 1.14–3.36, p=0.016) during the follow-up time. The PAPP-A was determined at the time of study entry in a multicenter CLARICOR trial comparing clarithromycin to placebo in 4242 stable CAD patients with a history of hospitalization due to angina pectoris, myocardial infarction or revascularization. During the median follow-up time of 2.8 years, the PAPP-A level >4.0 mIU/L was significantly related to the composite outcome of MI and death (HR 1.99, 95% CI 1.62–2.45, p<0.0005), all-cause mortality (HR 2.42, 95% CI 1.92–3.06, p <0.0005) and MI (HR 1.40, 95% CI 1.01–1.94, p=0.046) (Iversen et al., 2011). Schulz and colleagues' (2011) study showed similar results, wherein the PAPP-A level > 2.7 mUI/L, determined from outpatients (without any heparin treatment) two-to-three days prior to the scheduled coronary angiography indicated by the symptoms of stable angina pectoris, was predictive for all-cause death (HR 4.73, 95% CI 1.46–15.31, p=0.01), all-cause death or nonfatal MI (HR 4.01, 95% CI 1.58–10.13, p=0.003) and all-cause death, nonfatal MI or hospitalization (HR 1.96, 95% CI 1.03–3.70, p=0.04). The prognostic power of PAPP-A did not change substantially after correction for values of high-sensitive cTnI.

2.2.6.4.2 Circulating PAPP-A as a prognostic marker in suspected ACS

The suspicion of ACS is a complex continuum of clinical pictures from non-cardiac reasons that mimic the symptoms of cardiac origin, such as NSTEMI, STEMI and, in the worst case, death. Thus, the prognosis in patients presenting with acute coronary syndrome is also highly variable. As section (2.2.6.3.2) previously showed, there is more interindividual and between-studies variation in measured PAPP-A levels. The risk of influence of confounding factors is higher in this ACS group, and no clear cut-off points of PAPP-A are currently available for clinical decision making. The follow-up times also vary between the studies.

However, while there are few studies with divergent results showing no correlation of elevated levels of circulating PAPP-A and worse prognosis, the majority of the trials show that the patients with elevated PAPP-A have higher mortality, a higher risk of recurrent MI and, in some studies, higher risk on revascularization. Table 6 shows the summary of the prognostic PAPP-A studies

In 2004 Laterza with coworkers (2004) determined the concentration of PAPP-A at the time of admission from 346 patients hospitalized due to suspected ACS. PAPP-A >0.22 mUI/L was calculated by receiver operating characteristics curve (ROC) to be the best cut-off point (area under the curve (AUC) 0.56 with sensitivity and specificity [95% CI] of 66.7% [48.2–82.0] and 51.1% [45.4–56.8], respectively) to forecast the combined adverse event of death, MI and revascularization during the 30 days follow up. Elevated PAPP-A (>0.22 mIU/L) was found as a predictor of adverse events (risk ratio (RR) 4.7, 95% CI 2.2-9.8), although not as specific as cTnT. Heeschen and colleagues (2005) clarified the additive and independent prognostic information given by PAPP-A levels compared to already established biomarkers in 626 concomitant patients with acute chest pain suggestive of ACS. Elevated PAPP-A >12.6 mIU/L, taken at the time of admission and before the start of any anticoagulant treatment, was associated with higher risk of death or myocardial infarction within 30 days in patients with low (<0.1ug/l) cTnT levels (odds ratio (OR) 2.55, 95% CI 1.22 - 5.36) and also in patients with high (>0.1ug/l) TnT levels (OR 4.38, 95% CI 1.79 - 10.75). The event rates of the whole study group in univariate analysis were 16.9% vs. 7.9% (OR 2.38, 95% CI 1.4 - 4.05, p=0.001) in patients with elevated PAPP-A vs. patients with PAPP-A <12.6 mIU/L, respectively. Using a multimarker approach (cTnT, hs-CRP, sCD40-ligand, interleukin-10), PAPP-A emerged as a powerful, independent predictor of cardiovascular events during the 30-day follow up (OR 3.11, 95% CI 1.74 - 5.56, p<0.001).

In Kavsak and colleagues' (2009) study, 320 patients were admitted with symptoms suggestive of cardiac ischemia. They observed that the subjects with baseline PAPP-A concentrations in the highest tertile (>1.62 mIU/L) compared to the lowest tertile (<0.92 mIU/L) were at higher risk for death during the two-year follow

up, even after adjusting for age, sex, and baseline cTnI (RR 2.15, 95% CI 1.00–4.63, p=0.050). The statistical significance was even higher in a group of patients with hs-cTnI elevation (p=0.02), while the prognostic significance was not noticed in patients without hs-cTnI elevation (n=66). The proportion of patients having heparin treatment during the index hospital stay was only 22% in this study, and no difference existed in the baseline PAPP-A level between the patients with and without heparin treatment, while the peak PAPP-A level was higher in the heparin-treated group, indicating that the PAPP-A taken at the baseline was not significantly influenced by heparin treatment. Iversen and colleagues (2010) measured PAPP-A at the time of admission and, thereafter, every six-to-eight hrs in 415 patients with suspected ACS, but no evidence of ischemia on ECG and normal (<0.03 ug/l) cTnT occurred during the hospitalization. The risk of death or non-fatal MI (combined endpoint) was 15% in the highest (>12.4 mIU/L) compared to 3% in the lowest (<4.0 mIU/L) PAPP-A quartile (highest measured) (relative risk 3.7, p=0.01) after three months of follow up. The corresponding numbers after one year were 24% and 10% (RR 2.4, p=0.01), respectively. In the post hoc analyses performed only with admission PAPP-A sample, the patients with admission PAPP-A >4.0 mIU/L had higher risk for the combined endpoint (p=0.001). PAPP-A >4.0 mIU/L was also significantly predictive for the combined endpoint (HR 1.85, 95% CI 1.17 – 2.93. p=0.01) in the multivariate analyses.

Schaub and colleagues' (2011) later study measured the concentration of PAPP-A at the time of admission and before any heparin treatment in 398 patients presenting with the symptoms suggestive of myocardial infarction. The PAPP-A level was significantly higher in patients with AMI than in patients with another diagnosis (median [IQR] 4.6 [4.0 to 9.3] vs 4.0 [4.0 to 5.6] mIU/L, p <0.001). However, the trend towards the higher risk of mortality was only in the patients with PAPP-A in the highest tertile (>4.9 mIU/L) (RR 1.29, 95% CI 0.68 – 2.46). Mei and coworkers showed, in the same year, the predictive prognostic value of PAPP-A, measured before any anticoagulant treatment, in 129 ACS (42 NSTEMI and 87 UA) patients with only single coronary stenosis treated successfully by PCI during the index hospitalization. They found PAPP-A >11.33 mIU/L as an independent predictor of a combined adverse event of cardiac death, nonfatal MI, revascularization and rehospitalization for angina pectoris during the two-year follow-up (adjusted RR 4.1, 95% CI 1.0 - 16.2, p=0.037). The event rate was only 6.2% in patients with PAPP-A <11.33 mIU/L, while it increased to 25.0% and 36.8% in patients with PAPP-A 11.33 - 24.71 mIU/L (p=0.035) and > 24.71 mIU/L (p=0.002), respectively (Mei et al., 2011). The elevated PAPP-A was found to be an independent risk factor for all-cause mortality (RR 1.78, 95% CI 1.33 - 2.40), combination of all-cause mortality and non-fatal MI (RR 1.75, 95% CI 1.36 - 2.26), and combined cardiovascular events (RR 1.58, 95% CI 1.19 - 2.11) in the recent meta-analysis,

including a total of 12,830 participants and 1813 cases. Interestingly, the prognostic value of PAPP-A was not influenced by the assay methods, CAD type or follow-up duration (Li et al., 2013). von Haehling and coworkers' (2013) study measured the PAPP-A at the time of admission before any heparin treatment in 2568 patients hospitalized for chest pain of cardiac origin and confirmed by coronary angiogram during the first 24 hrs after admission. Of those patients, 1229 had ACS (including UA, STEMI and NSTEMI patients) with cTnI elevation (>0.04 ng/ml); the rest remained cTnI negative. Elevated PAPP-A (>34.6 mIU/L) was independently associated with an increased risk of combined endpoint of stent thrombosis, myocardial infarction, ischemic stroke or cardiovascular death in the whole study group and also in the patients with (RR 1.91, 95% CI 1.48 - 2.46) and without (RR 2.15, 95% CI 1.81 - 2.56) cTnI elevation during the 90- day follow up. Bonaca and coworkers (2012) determined PAPP-A at baseline in 3782 NSTEMI patients randomized to MERLIN-TIMI 36 study. Patients with elevated PAPP-A (>6.0 mIU/L) had higher rates of cardiovascular death or MI at 30 days (7.4% vs. 3.7%, RR 2.01, 95% CI 1.43 - 2.82, p= 0.001) and at one year (14.9% vs. 9.7%, RR 1.63, 95% CI 1.29 to 2.05, p 0.001). Zengin and coworkers' (2015) recent analysis found that PAPP-A was determined in 927 patients presenting with cardiac chest pain between the admission and coronary angiogram and, if possible, before heparin treatment. However, the blood sample from the 393 patients was collected after the start of recommended heparin treatment. Out of the whole study group, the elevated PAPP-A (>11.4 mIU/L) was predictive for cardiac mortality in the long-term (median 5 years) follow up in the patients with ACS or stable angina.

Some studies also show no clear evidence of the prognostic value of the level of circulating PAPP-A in patients with ACS. Brügger-Andersen and colleagues' (2008) study of 298 patients with MI were included and PAPP-A, determined late during the index hospitalization (4-5 days after the admission), was not found to be related to the primary endpoint of MI during the median follow-up time of 45 months. McCann and colleagues (2009) enrolled 555 acute chest-pain patients, of whom 53% had an AMI diagnosis (19% STEMI, 34% NSTEMI), 26% UA, and the rest had non-ischemic chest pain during the index hospitalization. The follow-up time for composite endpoint of death or MI was one year. PAPP-A taken at the time of admission before the start of any thrombolysis or anticoagulant treatment was not a predictor of adverse events in this study. Sanchis and colleagues (2008) enrolled 422 patients presenting to the emergency department with chest pain without ST-segment deviation on ECG or troponin elevation during the hospital stay. Using ROC analysis, the association during the median 60 weeks of follow up between PAPP-A and death or myocardial infarction was borderline significant (AUC 0.62, p=0.07) and significant with combined adverse event of death or MI or revascularization (AUC 0.58, p=0.04). However, PAPP-A was not statistically significantly associated with adverse events in the multivariate analysis.

Table 6. Summary of the prognostic studies of circulating PAPP-A in patients with suspected ACS

Study and patient profile	n	Highest detected/ admission PAPP-A	Heparin effect	PAPP-A cut-off mIU/L	FU- time	Study endpoint	All patients			cTn positive patients			cTn negative patients		
							PAPP-A mIU/L	Event Rate	RR (95% CI)/p	PAPP-A mIU/L	Event rate	RR (95% CI)/p	PAPP-A mIU/L	Event Rate	RR (95% CI)/p
Laterza et al., 2004															
Suspected ACS patients	346	Admission	Probable	0.22	30 days	MI+revas-cularization + death	≤ 0.22 > 0.22	NA NA	4.7 (2.2-9.8)						
Heeschen et al., 2005															
ACS patients with ECG changes	626	Admission	Yes	12.6	6 months	MI + death	≤12.6 >12.6	7.9% 17.4%	2.64 (1.55-4.50) / p=0.001	≤12.6 >12.6	13.2% 22.8%	NA/p<0.01	≤12.6 >12.6	5.4% 13.5%	NA/p<0.01
Suspect ACS patients	644	Admission	No	12.6	30 days	MI + death	≤12.6 >12.6	7.9% 16.9%	2.38 (1.40-4.05) p=0.001	≤12.6 >12.6	**(57%)[b]** **(23%)[b]**	4.38 (1.79-10.75)	≤12.6 >12.6	**(5%)[b]** **(10%)[b]**	2.55 (1.22-5.36)
Brügger-Andersen et al., 2008									4th vs. 1st Quartile						
Patients with MI	298	4-6 days after index MI	Highly probable	0.72	45 months	MI	<0.31 0.31-0.49 0.49-0.72 >0.72	28.0% 25.7% 20.6% 35.1%	1.58 (0.96−2.62) / p=0.073						
Sanchis et al., 2008															
Chest pain without ECG changes and without cTn elevation.	422	<24 hours af-ter admission	Probable		60 weeks	MI + death + revascu-larization								NA	AUC 0.58 p=0.04
						MI + death								NA	AUC 0.62 p=0.07
Kavsak et al., 2009									3rd vs. 1st Tertile			3rd vs. 1st Tertile			
NSTEMI and UA patients	320	Admission	Limited	1.62	2 years	Death	<0.92 0.92-1.62 >1.62	**(8%)[b]** **(14%)[b]** **(23%)[b]**	2.96 (1.38–6.35) p= 0.005	<0.92 0.92-1.62 >1.62	**(15%)[b]** **(15%)[b]** **(20%)[b]**	2.15 (1.00–4.63)/ p=0.050			p=NS

Table 6. continued

Study and patient profile	n	Highest detected/ admission PAPP-A	Heparin effect	PAPP-A cut-off mIU/L	FU- time	Study endpoint	All patients			cTn positive patients			cTn negative patients		
							PAPP-A mIU/L	Event Rate	RR (95% CI)/p	- PAPP-A mIU/L	Event rate	RR (95% CI)/p	PAPP-A mIU/L	Event Rate	RR (95% CI)/p
McCann et al., 2009							ng/ml								
STEMI, NSTEMI, UA and non-cardiac chest pain patients	550	Admission	No		1 year	MI + death	<12. 4 >12.4		1.1 (0.6-2.2)/ p=0.67						
Iversen et al., 2010															4th vs. 1st Quartile
Low risk ACS patients	415	Highest	Obvious		1 year	MI + death							< 4.0 4.0 –7.7 7.8-12.4 >12.4	10.0% **(13%)[b]** **(20%)[b]** 24.0%	 2.4 (NA) / p=0.01
					1 year	Death							< 4.0 4.0 –7.7 7.8-12.4 >12.4	6.0% **(7%)[b]** **(10%)[b]** 14.0%	 2.4 (NA)/ p=0.05
															Multivariate analysis
Post hoc analysis with the same population without heparin effect	NA	Admission	No		NA	MI+death							<4.0 ≥4.0	NA NA	 1.85(1.17–2.93)/ p=0.01
						Death							<4.0 ≥4.0	NA NA	 2.09(1.16-3.77)/ p=0.01
Schaub et al., 2011															
NSTEMI, UA and non-cardiac chest pain patients	398	Admission	No		2 years	Death	≤4.0 4.1-4.9 >4.9	7.6% 7.1% 12.0%	 p=0.59						
Mei et al., 2011									vs. 1st Tertile						
NSTEMI and UA patients	129	Admission	No	11.3	2 years		<11.3 11.3-24.7 >24.7	6.2% 25.0% 36.8%	 NA/p=0.035 NA/p=0.002						

Table 6. continued

Study and patient profile	n	Highest detected/ admission PAPP-A	Heparin effect	PAPP-A cut-off mIU/L	FU- time	Study endpoint	All patients			cTn positive patients			cTn negative patients		
							PAPP-A mIU/L	Event Rate	RR (95% CI)/p	PAPP-A mIU/L	Event rate	RR (95% CI)/p	PAPP-A mIU/L	Event Rate	RR (95% CI)/p
Bonaca et al., 2012															
NSTEMI ACS patients	3782	Admission	Probable, was taken account	6.0	30 days	MI + death	≤6.0 >6.0	3.7% 7.4%	2.01(1.43- 2.82)/ p<0.001						
					1 year		≤6.0 >6.0	9.7% 14.9%	1.63 (1.29- 2.05)/ p< 0.001	≤6.0 >6.0	12.7% 17.4%	NA/p=0.0047	≤6.0 >6.0	5.5% 8.0%	NA/p=0.15
Li et al., 2013									Elevated PAPP-A and risk						
ACS patients	12830		Probable			MI+death		NA	1.80 (1.26-2.57)						
						Death		NA	1.62 (0.93-2.81)						
von Haehling et al., 2013									Adjusted RR						
Whole study group of patients with cardiac chest pain	2568	Admission	No	34.6	90 days	MI + cardiac death + stent thrombosis + stroke	<34.6 ≥34.6	**(4%)[b]** **(22%)[b]**	5.3 (4.02-6.98)/NA						2.15(1.81–2.56)/ p=NA
TnI positive ACS patients	1229									<34.6 ≥34.6	NA NA	1.91(1.48–2.46)/ p=NA			
Zengin et al., 2015									4th vs. 1st Quartile						
Whole study group of patients with cardiac chest pain	927	Between admission and coronary angiogram	Probable	11.4	7 years	CV mortality	<7.2 7.2-9.3 9.3-11.7 >11.7	**(4%)[b]** **(4%)[b]** **(6%)[b]** **(12%)[b]**	2.64 (1.26–5.54)						
ACS patients	393		Highly probable				<7.2 7.2-9.3 9.3-11.7 >11.7	**(4%)[b]** **(3%)[b]** **(11%)[b]** **(13%)[b]**	NA/p=0.01				≤11.4 >11.4	NA NA	NA/p=0.01

[b]Estimated percentages from Kaplan Meier curves
Abbreviations not appearing outside this table in the thesis: NA=not available

3 AIMS OF THE STUDY

The primary purpose of this study was to test the usefulness of circulating PAPP-A and its investigational novel forms as prognostic markers in patients presenting with suspected ACS.

The specific aims were

I. to investigate the prognostic value of totalPAPP-A in patients with symptoms of ACS without elevation in troponin I,

II. to evaluate 48-hour release pattern of totalPAPP-A and its association with dynamic ECG changes, the patency of infarct related artery and 12 month outcome in patients with acute STEMI, and

III. to estimate the prognostic value of free form of PAPP-A vs. totalPAPP-A concentrations in predicting death and nonfatal myocardial infarction in patients with NSTE-ACS.

4 MATERIALS AND METHODS

4.1 MAIN STUDY POPULATION AND DESIGN

The basic population of this study comprised 541 consecutive patients who were admitted to the Turku University Hospital Emergency Department for symptoms consistent with acute coronary syndrome and who gave their written, informed consent to participate (Table 7). The recruitment of study patients started in May 2000 and was completed in June 2001. Of these 541 patients, 366 were hospitalized during the index visit (during the same visit the patient was enrolled in the study) for at least 12 hours, and the rest 175 were discharged from the emergency department (ED). All patients were treated according to routine clinical protocols of Turku University Hospital. The mortality data were obtained from Statistics Finland during the 12-month follow up, and the data of other endpoints were collected by mail, telephone interviews and from the hospital records, which were retrospectively reviewed for classification. Random discrepancies were settled by mutual consensus. The study was conducted in accordance with the Declaration of Helsinki as revised in 1996 and approved by the Ethics Committee of Turku University Hospital.

Table 7. Baseline demographics and follow-up data of the main study population (n=541)

	n=541
Age *	67.0 (29-94) ±12.0
Diabetes (%)	91 (16.8)
Gender, male (%)	320 (59.1)
Hypertension (%)	238 (44.0)
Hyperlipidemia (%)	300 (55.5)
Killip class ≥2 (%)	165 (30.5)
Statin treatment (%)	141 (26.1)
Current smoker (%)	104 (19.2)
Former smoker (%)	160 (29.6)
Coronary artery disease (%)	213 (39.4)
Previous MI (%)	148 (27.4)
Previous PCI (%)	58 (10.7)
Previous CABG (%)	65 (12.1)
UA or non-cardiac chest pain at index hospitalization (%)†	344 (63.6)
MI as a diagnosis at index hospitalization (%)†	197 (36.4)
STEMI	62 (11.5)
NSTEMI	135 (24.9)
Follow-up data (12 months)	
Revascularization (PCI or CABG) (%)	97 (17.9)
Stroke (%)	7 (1.3)
Myocardial infarction (%)	52 (9.6)
Cardiac mortality (%)	45 (8.3)
Total mortality (%)	62 (11.5)

*Mean (range)±SD
†The diagnosis set by treating clinician according to guidelines.

4.2 PATIENTS AND DESIGN IN SUB-STUDIES

All 366 patients hospitalized for at least 12 hours and the 64 discharged from the ED were screened in the final analysis of the substudies. Of the 541, 111 (the patients discharged from ED with ID between 1-240 and 441-541) patients were not included in the analyses of this study.

4.2.1 Troponin negative subgroup (study I)

Two hundred consecutive (patients with ID 241-440 of the basic population) patients were enrolled in this study template. Serum samples for PAPP-A and cTnI determinations were collected immediately at admission, 6–12 and 24 hours. CRP was measured at admission. One patient was excluded from the analysis because of incomplete follow-up data. During the first 24 hrs of hospital stay, 136/199 (69 men and 67 women; mean ±SD age, 66 ±16 years) remained cTnI-negative for up to 24 hours. These 136 patients were followed up for six months for the primary endpoint as a combination of cardiovascular mortality, first episode of nonfatal MI or revascularization (PCI or CABG) and secondary endpoint as a combination of

non-cardiovascular death, hospitalization for UA, worsening heart failure (congestive heart failure) or stroke.

4.2.2 STEMI subgroup (study II)

This analysis included all 62 patients in the main study population who met the STEMI criteria of acute typical chest pain >20 minutes and new >1.0 mV and/or >2.0 mV ST-elevation in two consecutive limb and/or precordial leads, respectively. An additional 14 patients were selected to a subgroup with frequent early sampling to clarify the release pattern of circulating PAPP-A and cTnI. These samples were collected immediately at admission and at the time points of 6–12, 24 and 48 hours and in 14 patients selected for frequent sampling group also at 1, 2 and 4 hours. CRP was analyzed at admission. The patency of the infarct-related coronary artery was estimated by clinically driven coronary angiography performed for half of the patients (n=31), 19 of whom during the first 7 days after the index event. The angiography data were analyzed offline by two cardiologists to identify the culprit lesion, infarct-related vessel patency (Thrombolysis In Myocardial Infarction (TIMI) flow >2) and the overall extent of CAD. Special attention was drawn in this study to a late PAPP-A elevation and its possible correlations with prognosis, early reperfusion or vessel patency. The patients were followed up for 12 months for the study endpoint as a combination of cardiovascular mortality and the first episode of non-fatal MI after enrollment.

4.2.3 Comparison of free and totalPAPP-A (study III)

This study's population comprised all patients in the basic study population who were hospitalized for at least 12 hours (n=366) during the index visit. Individuals with acute ST elevation on electrocardiogram (ECG) (n=62) or missing blood samples (n=9) or inadequate follow up data (n=6) were excluded. LMWH was given to 136 patients during the hospitalization. Of these patients, 22 received LMWH before the admission blood sampling and were excluded. Thus, 267 (136 men and 131 women, median [25^{th}, 75^{th} percentile] age 70 [60, 78] years) patients were eligible for this study's final analysis. The blood samples were collected at admission for PAPP-A and CRP and at admission, 6-12 hours and 24 hours for cTnI analysis. The prognostic value of freePAPP-A compared to totalPAPP-A concentrations to forecast death and nonfatal MI was estimated during the 12-month follow up.

4.3 METHODS

4.3.1 Biochemical measurements

4.3.1.1 Circulating PAPP-A

PAPP-A levels were determined post hoc in study **I** and **II** as totalPAPP-A, i.e., whether in free form or in complex with the proMBP, by an investigational point-of-care, time-resolved immunofluorometric assay (Qin et al., 2002). The lower limit of detection was 0.5 mIU/L and the functional sensitivity (imprecision <20%) 1.5 mIU/L. The between-assay imprecision at the lowest standard (2.5 mIU/L) was 13.7%. The PAPP-A samples were studied in study **III** by investigational point-of-care, time-resolved immunofluorometric assays for totalPAPP-A and PAPP-A/proMBP complex (Wittfooth et al., 2006). In the totalPAPP-A assay, the capture antibody and the detection antibody both specifically bind to the PAPP-A subunit of freePAPP-A and complexed PAPP-A molecules. In the PAPP-A/proMBP assay, the same antibody is used for capture as in the totalPAPP-A assay; however, the detection antibody specifically binds to the proMBP subunit of PAPP-A/proMBP complex, enabling the exclusive detection of this complexed PAPP-A form. The analytical detection limits (zero calibrator + 3SD) and functional detection limits (imprecision < 20%) were 0.18 mIU/L and 0.27 mIU/L for the totalPAPP-A assay and 0.23 mIU/L and 0.70 mIU/L for the PAPP-A/proMBP assay, respectively. The total coefficient of variation (CV) for the totalPAPP-A assay was 7.2% at 4.9 mIU/L and 9.7% at 3.1 mIU/L for PAPP-A/proMBP assay. The concentration of freePAPP-A was calculated from the difference of the results given by these two assays.

4.3.1.2 Troponin

cTnI was determined in study **I** using the Innotrac AIO (Innotrac Diagnostics Corp) with analytical sensitivity 0.05 µg/L and the cutoff value at 10% imprecision (CV) of 0.22 µg/L, the level used retrospectively to define cTnI negativity in the trial. Bayer Immuno I assay (Bayer Diagnostics, Tarrytown, NY, USA), MDC 0.1 µg/L, cutoff value for AMI at 10% CV 0.3 µg/L (Morrow et al., 2000) was used in study **II** to measure the troponin I levels. For the purposes of study **III**, cTnI was analyzed using the Innotrac Aio! second-generation assay (Innotrac Diagnostics Corp.), which has been classified as a level 1 contemporary, clinically useable cTn assay (Eriksson 2005). The minimum detectable concentration of the assay is 0.012 µg/L, and the cutoff value for myocardial infarction was defined as the concentration with a 10% CV is 0.06 µg/L. The 99th percentile reference concentration was determined

to be 0.025 µg/L. Thus, a cTnI concentration >0.03 µg/L was deemed to be increased in this study.

4.3.1.3 C-reactive protein

CRP was determined **(I, II, III)** by an ultrasensitive Aio! assay with an analytical detection limit of 0.003 mg/L and a functional detection limit of 0.1 mg/L. 2.0 mg/L was used as a cutoff concentration in multivariate analysis.

The clinicians had no access to the investigational AIO cTnI, CRP, or cPAPP-A information, but the results of cTnI measured by Bayer Immuno I were **(II)** made available for the treating physicians.

4.3.2 ECG

ECG was recorded at admission **(II, III)** and also 120 ± 30 minutes after the start of trombolysis treatment in patients with acute STEMI **(II)**. The patients with LBBB or non-diagnostic ECG were identified by manual coding. ST-segment elevation >0.1 mV (except V1-3 ≥0.2 mV) at the J-point in two continuous leads was classified as ST elevation. Ischemic ST-segment depression ≥0.05 mV at the J + 80 ms point in at least one lead was coded as having ST depression. If the previous criteria were unfulfilled, then the T-wave was measured. T-inversion was coded if it was present in ≥2 contiguous leads. If none of these criteria was fulfilled, the ECG was coded as having no ischemic changes. Early reperfusion was determined as >75% ST-resolution during the first 150 minutes after the initiation of reperfusion therapy, obtained in a single lead with the highest ST elevation on primary ECG.

4.3.3 Statistical analysis

Categorical variables between the groups were compared with the Chi-square test **(II, III)** and 2-tailed Fisher exact test **(I)**. Continuous variables were compared with the use of Wilcoxon's rank-sum test **(I, II, III)**. Survival curves were estimated using the Kaplan-Meier method, and differences between the curves were tested with the log rank test **(I, II, III)**. Correlations were tested using Spearman's correlation test **(I, II, III)**. Uni-**(I)** and multivariate **(I, II)** associations were analyzed using Cox proportional-hazards modeling to evaluate the independent contributions of the variables to the risk of cardiovascular events during the follow up. Statistical analyses were performed using SAS statistical software (versions 6.12 **(I)**, 8.1 **(II)** and 9.2 **(III)**; SAS Institute, Cary, NC, USA). *P* values <0.05 were considered significant.

5 RESULTS

5.1 CIRCULATING PAPP-A AND PATIENT CHARACTERISTICS IN SUB-STUDIES

5.1.1 The levels of circulating PAPP-A

The median [25th, 75th percentiles] admission totalPAPP-A was 2.3 [1.6, 3.0] mIU/L in study **I**. The highest detected totalPAPP-A was 2.35 [1.6, 2.9] mIU/L in discharged and 3.3 [2.1, 6.5] mIU/L in hospitalized patients ($p<0.001$), respectively. The median [25th, 75th percentiles] admission and the highest detected totalPAPP-A in STEMI frequent sampling group was 8.0 [3.7, 12.2] mIU/L and 11.6 [4.7, 18,8] mIU/L, respectively **(II)**. Median freePAPP-A was 1.43 [1.13, 1.95] mIU/L (1.49 [1.12, 1.97] mIU/L in men and 1.43 [1.13, 1.89] mIU/L in women, p=NS) in study **III**'s admission samples. The corresponding values for totalPAPP-A were 2.41 [1.82, 3.26] mIU/L (2.53 [1.90, 3.39] mIU/L in men and 2.32 [1.80, 3.18] mIU/L in women, p=NS).

5.1.2 Definition of the prognostic cut-off points of circulating PAPP-A

TotalPAPP-A value 2.9 mIU/L (the highest level of any time point) was found to be the best predictive cut-off value for the combined endpoint (RR, 3.7, 95% CI 1.6-8.9, P=0.0028) in study **I**. Patients were then divided into four groups according to the highest detected totalPAPP-A levels: <2.0, 2.0 to 2.8, 2.9 to 4.4, and ≥4.5 mIU/L **(I)**. The patients were divided in tertiles based on admission totalPAPP-A value in study **II**: <3.0, 3.0 – 10.0 and >10.0 mIU/L and divided in study **III** according to tertiles of admission freePAPP-A (<1.27, 1.27–1.74, >1.74 mIU/L) and admission totalPAPP-A (<1.98, 1.98–2.99, >2.99 mIU/L).

5.1.3 Characteristics of patient with different circulating PAPP-A levels

Tables 8 **(I)**, 9 **(II)** and 10 **(III)** show the patient characteristics between the groups with different PAPP-A levels in the substudies. Briefly, the groups were well balanced in all substudies for most background variables. However, diabetes **(I, II, III)** and previous MI **(I, II)** were more frequent in patients with higher PAPP-A levels, and there was also a trend of patients with higher PAPP-A being older. Admission cTnI and CRP were significantly higher in the third tertile compared with the lowest one in study **III**; the same trend for admission cTnI can be seen in study **II**.

Table 8. Patient characteristics between the groups with different totalPAPP-A level in troponin negative sub-study (Study **I**).

	totalPAPP-A		
	<2.9 mIU/L	**≥2.9 mIU/L**	
	n=75	**n=61**	**p**
Age*	64±13	69±13	ns
Diabetes (%)	6 (8.0)	13 (21.7)	0.027
Gender, male (%)	35 (46.7)	34 (56.7)	ns
Hypertension (%)	30 (40.0)	34 (56.7)	ns
Previous MI (%)	15 (20.0)	22 (36.7)	0.035
Current smoker (%)	21 (28.0)	11 (18.3)	ns
Aspirin (%)	31 (41.3)	25 (41.6)	ns
Statins (%)	18 (24.0)	20 (33.3)	ns
Warfarin (%)	5 (6.7)	11 (18.3)	ns
Killip class ≥2 (%)	7 (9.3)	20 (33.3)	ns

*Mean ±SD, ns = non significant.

Table 9. Patient characteristics between the the groups with different totalPAPP-A level in STEMI sub-study (Study **II**).

	Admission totalPAPP-A mIU/L			
	<3.0	**3.0 – 10.0**	**>10.0**	
	n=20	**n=20**	**n=22**	**p***
Age †	62.5 (34 to 84)	66.0 (44 to 82)	71.5 (51 to 83)	**0.045**
BMI †	26.9 (17.3 to 34.9)	27.8 (21.6 to 32.7)	26.9 (21.2 to 34.6)	**ns**
Male gender (%)	10 (50.0)	17 (85.0)	13 (59.0)	**ns**
ST-elevation on ECG				
Anterior (%)	5 (25.0)	5 (25.0)	11 (50.0)	**0.046**
Inferior (%)	8 (40.0)	12 (60.0)	8 (36.0)	**ns**
Posterolateral (%)	7 (35)	3 (15)	3 (13.6)	**ns**
Thrombolysis (%)	14 (70.0)	15 (75.0)	22 (100)	**0.007**
Delay (min) from symptom onset to reperfusion therapy‡	180 [120, 400]	120 [60, 360]	160 [90, 270]	**ns**
Early reperfusion on ECG (%)	17 (85.0)	12 (60.0)	14 (63.6)	**ns**
Revascularization (PCI or CABG) (%)§	7 (35.0)	10 (50.0)	10 (45.0)	**ns**
Current smoking (%)	9 (45.0)	5 (25.0)	6 (27.3)	**ns**
Hypertension (%)	7 (35.0)	7 (35.0)	7 (31.8)	**ns**
Previous MI (%)	1 (5.0)	3 (15.0)	6 (27.3)	**0.076**
Diabetes mellitus (%)	1 (5.0)	1 (5.0)	5 (22.7)	**0.035**
Killip class ≥2 (%) #	8 (40.0)	9 (45.0)	13 (59.1)	**ns**
Family history of CVD (%)	5 (25.0)	11 (55.0)	9 (40.9)	**ns**
AdmcTnI ng/ml ‡	0 [0, 0.3]	0.2 [0, 0.7]	0.25 [0, 2.0]	**ns**
48 hrs cTnI ng/ml ‡	7.4 [3.6, 16.4]	11.1 [2.9, 17.3]	5.9 [2.0, 13.2]	**ns**
MaxcTnI ng/ml ‡	33.9 [11.9, 153.8]	34,2 [11.0, 89.8]	27.9 [13,2,109.4]	**ns**
AdmCRP mg/l ‡	2.3 [0.75, 4.35]	2.1 [1.0, 5.1]	2.7 [1.6, 5.7]	**ns**

*P-value for the difference between the groups with admPAPP-A > and <10mIU/L.

†Median (range).

‡Median [25th, 75th].

§ during 1 year follow up.

#Killip class during index hospitalization

Abbreviations not appearing outside this table in the thesis: AdmcTnI=admission cardiac troponin I, MaxcTnI=maximal cardiac troponin I, AdmCRP= admission C-reactive protein, ns = non significant.

Table 10. Patient characteristics between the the groups with different PAPP-A level in the study of comparison of free and totalPAPP-A (Study **III**).

	freePAPP-A (mIU/L)					totalPAPP-A (mIU/L)				
	<1.27 n=89	**1.27–1.74 n=89**	**>1.74 n=89**	**p***	**p†**	**<1.98 n=89**	**1.98–2.99 n=89**	**>2.99 n=89**	**p***	**p†**
Age (years) ‡	64 [57, 74]	70 [62, 78]	73 [64, 79]	**ns**	**ns**	67 [58, 76]	68 [59, 78]	73 [66, 78]	**ns**	**ns**
Male gender (%)	46 (51.7)	43 (48.3)	47 (52.8)	**ns**	**ns**	37 (41.6)	49 (55.1)	50 (56.2)	**ns**	**ns**
Diabetes (%)	12 (13.5)	19 (21.3)	27 (30.3)	**ns**	**0.012**	13 (14.6)	19 (21.3)	26 (29.2)	**ns**	**0.018**
Smoking (%)	23 (23.6)	21 (25.8)	23 (23.6)	**ns**	**ns**	22 (24.7)	23 (25.8)	22 (24.7)	**ns**	**ns**
Previous MI (%)	24 (27.0)	35 (39.3)	32 (36.0)	**ns**	**ns**	32 (36.0)	26 (29.2)	33 (37.1)	**ns**	**ns**
Previous reperfusion (%)	17 (19.1)	22 (24.7)	12 (13.5)	**ns**	**ns**	18 (20.2)	17 (19.1)	16 (18.0)	**ns**	**ns**
Hospitalization time (days) ‡	4 [2, 6]	5 [3, 7]	5 [2, 7]	**ns**	**ns**	4 [2, 6]	5 [2, 7]	5 [2, 7]	**ns**	**ns**

* 1st versus 2nd tertile
† 1st versus 3rd tertile.
‡ Median [25th, 75th percentile]

5.2 THE PROGNOSTIC PERFORMANCE OF CIRCULATING PAPP-A

5.2.1 In troponin negative patients (I, III)

The prognostic performance of totalPAPP-A (admission and highest detected) and freePAPP-A (admission) investigated in cTnI- negative patients in study **I** (n=136) and in study **III** (n=146), respectively. Figure 5 shows how the cumulative risk of endpoint was only 8% if the highest detected totalPAPP-A was below 2.9 mIU/L, but the risk increased to 25.0% if totalPAPP-A was 2.9 to 4.4 mIU/L (p=0.035) and to 37.9% if the level was >4.5 mIU/L (p=0.0012) **(I)**. Using only the admission totalPAPP-A value, 12 of 40 (30.0%) with totalPAPP-A ≥2.9 mIU/L versus 14 of 96 (14.6%) with totalPAPP-A <2.9 mIU/L patients experienced a combined primary endpoint during the 6-month follow up (RR 2.3, 95% CI 1.1 to 5.0, p=0.03) **(I)**. A similar trend was also noticed in patients (n=64) who were discharged directly from the emergency room **(I)**. Adjusting for age, congestive heart failure (CHF) (during index event), CRP, current smoking, diabetes (dietary or drug therapy), gender, hypertension and previous MI, the totalPAPP-A ≥2.9 mIU/L was an independent predictor of a combined primary adverse event during the 6-month follow up (adjusted RR 4.6, 95% CI 1.8 to 11.8, p=0.002) **(I)**. The patients with normal cTnI but elevated (>1.74 mIU/L) freePAPP-A at admission in study **III** had significantly higher risk for adverse events vs. the patients with low (<1.27 mIU/L) freePAPP-A (8 of 45 (17.8%) vs. 3 of 55 (5.5%), p=0.042) Figure 6.

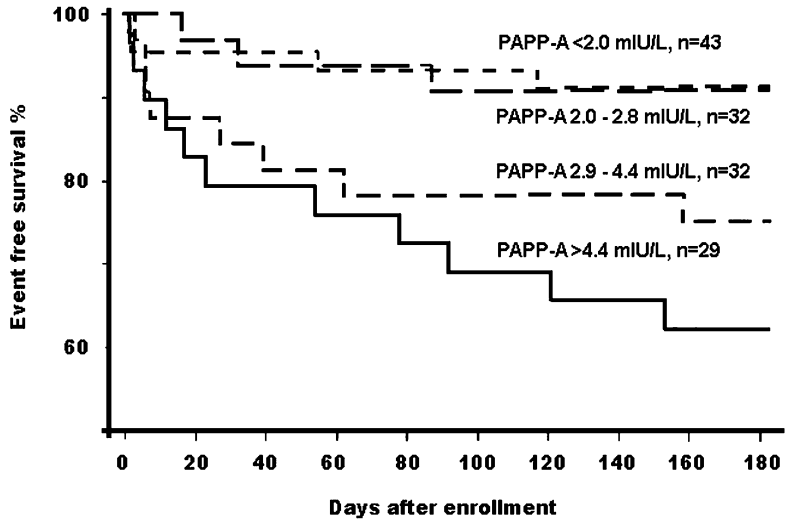

Figure 5. Kaplan-Meier curves of event free survival for cardiovascular death, myocardial infarction and revascularization according to totalPAPP-A level **(I)**.

5.2.2 In NSTEMI patients (III)

Study **III** included 121 patients with elevated cTnI during their hospitalization. As Figure 6 shows, the highest risk was observed in patients with increased cTnI and admission freePAPP-A >1.74 mIU/L, as 19 of 44 (43.2%) met the endpoint. Although, there was a trend of higher risk for an adverse event in patients in the highest freePAPP-A tertile compared to the patients in the lowest tertile, the difference was not statistically significant (p=0.084).

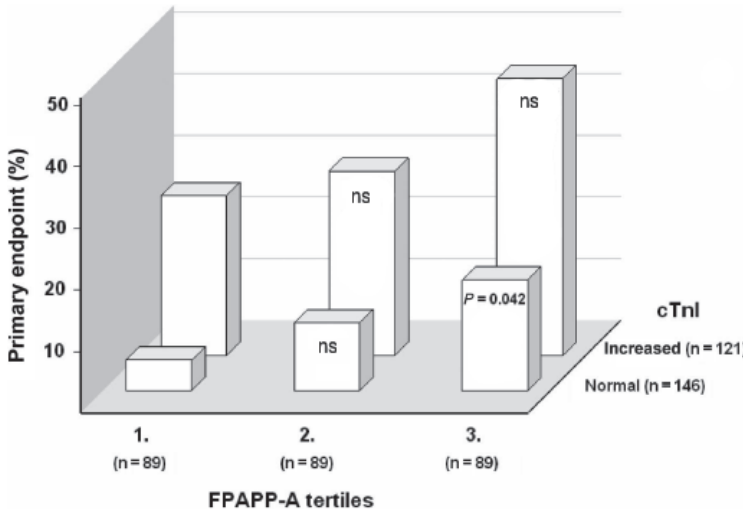

Figure 6. Cumulative frequency (%) of endpoints according to pooled maximal cTnI and free-PAPP- A status. P values are between the first and second and first and third freePAPP-A tertiles. ns=non-significant.

5.2.3 In STEMI patients (II)

Study **II** invested the prognostic value of totalPAPP-A determined at early and late phases in patients with STEMI. At the end of follow up, 17/62 (25.7%) patients with STEMI had met a primary endpoint. Cardiovascular causes accounted for 100% of the 11 deaths (total mortality 16.6%). Six patients (9.1%) suffered non-fatal MI. The cumulative risk of a primary endpoint was 15% and 20.0% if admission totalPAPP-A was <3.0 mIU/L and 3.0–10.0 mIU/L, respectively, but it increased to 45% when the admission totalPAPP-A was >10.0 mIU/L (p=0.049), as shown by Figure 7. The presence or absence of a late totalPAPP-A elevation was not statistically significantly associated with outcome as a single variable. However, 7/13 (53.8%) of those patients who showed late totalPAPP-A elevation after having failed reperfusion by ECG criteria met the primary endpoint in 12 months (versus 20.4% out of the other 49 patients, p=0.016).

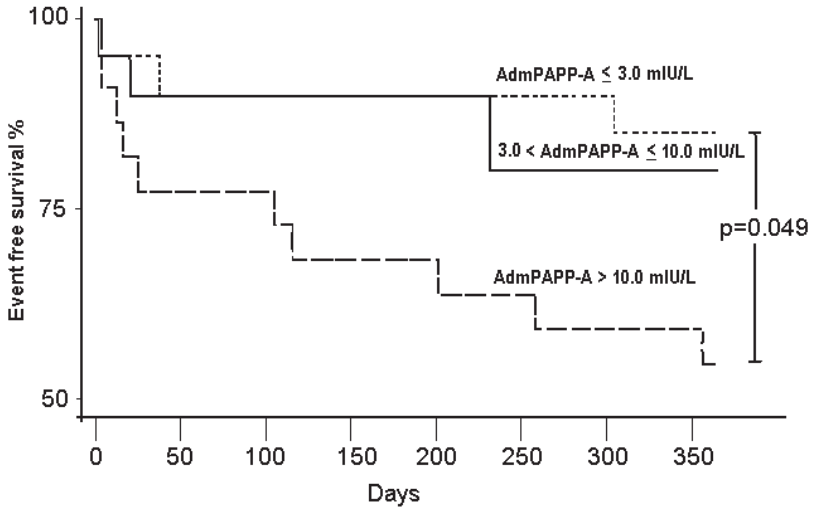

Figure 7. Kaplan-Meier analysis of the 12-month outcome according to admission totalPAPP-A tertiles **(II)**.

5.2.4 Free vs. totalPAPP-A (III)

Study **III** compared the prognostic performance of freePAPP-A and totalPAPP-A determined at the time of admission and before any heparin treatment. During the 12 month follow up, 57 patients (21.3%) met an endpoint (22 deaths and 35 non-fatal MIs). Total mortality was 32 of 267 (12.0%), as 10 patients who had MI as an endpoint died during the entire follow up. An endpoint was met by 12 (13.5%), 18 (20.2%), and 27 (30.3%) (p=0.02) subjects and 17 (19.1%), 17 (19.1%), and 23 (25.8%) (p=0.54) subjects in freePAPP-A and totalPAPP-A tertiles, respectively. The Kaplan–Meier survival curves for the various freePAPP-A tertiles diverged

early, but this was not the case for totalPAPP-A (Figure 8). Adjusting for age / 10 year, gender, diabetes (dietary or drug treated), previous AMI and ischemic ECG findings, freePAPP-A >1,74 mIU/L (RR 2.0, 95% CI 1.0 - 4.2, p=0.048), elevated admission cTnI (RR 1.9, 95% CI 1.1 - 3.5, p=0.024) and CRP >2.0 mg/L (RR 2.4, 95% CI 1.2 - 4.7, p<0.01) were independent, early-phase predictors of adverse outcome. Replacing admission cTnI with maximal cTnI gave comparable results. However, in the same settings, totalPAPP-A did not reach independency as a predictor of an endpoint (RR 1.1-1.2, p=0.49-0.88).

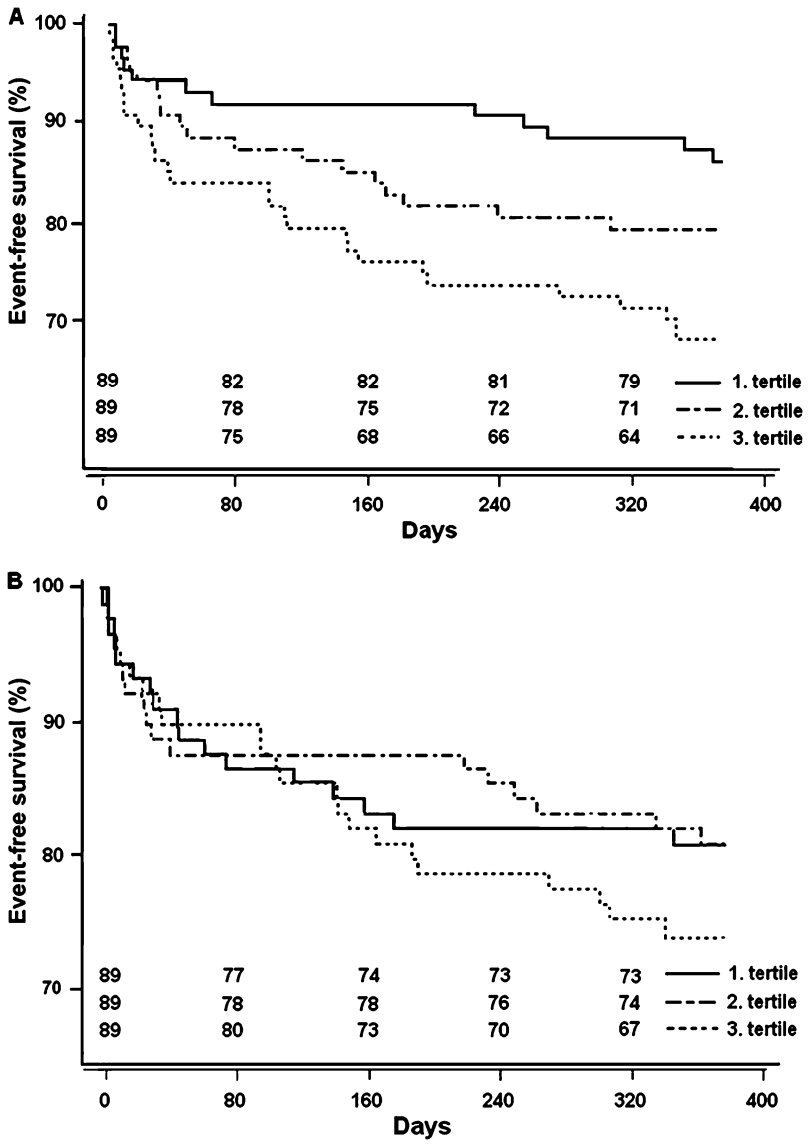

Figure 8. Kaplan–Meier curves of event-free survival for myocardial infarction and total mortality according to freePAPP-A (A) and totalPAPP-A (B) tertiles **(III)**.

5.3 THE RELEASE PATTERN OF CIRCULATING PAPP-A IN STEMI (II)

The early release pattern of circulating PAPP-A was investigated in detail in 14 STEMI patients treated with thrombolysis **(II)**. Figure 9 shows the trends of totalPAPP-A and cTnI levels as median [25th and 75th percentiles] values in all 14 patients with frequent sampling. The highest PAPP-A value was reached at one hour; thereafter, the values started to decline, and the lowest level was measured at 48 hours after admission. The peak TnI value was measured at 12 hrs. The lower PAPP-A levels at one hour was noticed in patients with a longer (>285 min) delay between the onset of symptoms to thrombolysis (Figure 10).

5.4 THE LATE ELEVATION OF CIRCULATING PAPP-A IN STEMI (II)

Using >9.5 mIU/L (75th percentile at 48 hours) as the cut-off for late totalPAPP-A elevation, 20/62 (32.3%) patients were identified as having a second peak. Delayed totalPAPP-A elevations were associated with less successful, early reperfusion by ECG criteria (35% versus 88%, P<0.001). All patients in the coronary angiography subgroup (n=31) who demonstrated early ECG reperfusion (n=20) were found to have a patent infarct-related coronary artery by angiography. A late totalPAPP-A elevation was found in only two of them (10.0%). In contrast, the late totalPAPP-A elevation occurred in eight (72.7%) patients with only late or no reperfusion (P<0.01 versus early reperfusion).

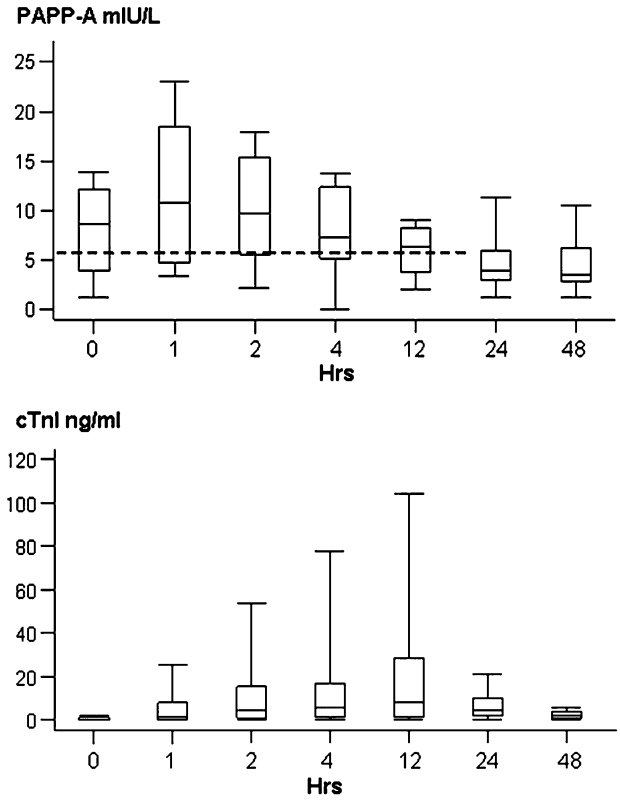

Figure 9. The averaged (median 10th, 25th, 50th, 75th, 90th percentile) circulating levels of totalPAPP-A and cTnI in the 14 patients with frequent early blood sampling (box plots). X-axis: Time after admission (hours). The bolded dotted line represents the upper 97.5th PAPP-A percentile in healthy normal males (Qin et al., 2002).

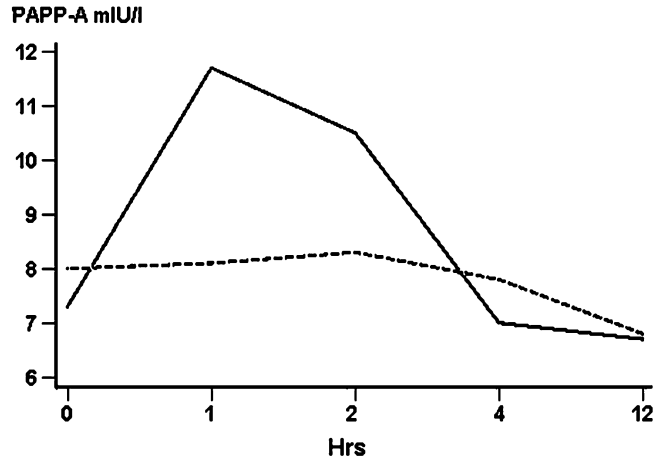

Figure 10. The release patterns of totalPAPP-A into the circulation during the first 12 hours in the frequent sampling group according to delay from symptom onset to thrombolysis. Solid line: short delay (<285 min), dotted line: long delay (>285 min). X-axis: time after admission (hours).

5.5 CORRELATIONS OF CIRCULATING PAPP-A WITH OTHER CARDIAC MARKERS

The correlations were tested in substudies between cPAPP-A and CRP and TnI. Briefly, there was no correlation between totalPAPP-A and CRP **(I, II)** or cTnI **(II)** in study **I** or study **II**. Furthermore, only very weak correlations were found between freePAPP-A and admission cTnI (r=0.15, p= 0.012), maximal cTnI (r =0.12, p=0.043) or CRP (r=0.17, P= 0.005) in study **III**.

6 DISCUSSION

Early, accurate diagnosis and prognostic evaluation play crucial roles in triage and management of the patients with ACS. The mechanism of ACS is almost invariably the rupture or erosion of atherosclerotic plaque that activates the coagulation cascade (Toschi et al., 1997). The currently used diagnostic and prognostic markers, mainly highly sensitive troponins, have an excellent prognostic performance but are mostly mirroring the myocardial damage. As shown already by Falk and colleagues (1985), the occluding thrombus per se is seldom formed abruptly but is very often preceded by recurrent thrombus formation and fragmentation that may last even for weeks, opening variable time windows for potential preventive interventions before the occluding thrombosis will occur. However, despite intensive research efforts, an ideal marker to forecast the plaque rupture does not exist. This study was motivated by the presumptive role of PAPP-A as an early marker of plaque instability or rupture, a concept evoked for the first time by Bayes-Genis and colleagues (2001a), reflecting also the prognosis of these patients.

6.1 STUDY POPULATION

The study population consists of 541consecutive patients admitted to the ED of Turku University Hospital between May 2000 and June 2001 with symptoms consistent of ACS. The data collection during the follow-up period was comprehensively managed by mail and telephone interviews and review of hospital records. Only one patient in study **I** and 6 patients in study **III** were dropped out due to incomplete follow up data. The (mean ±SD) age was 67 $\pm$12 years and 59.1 % were men. The proportion of diabetics was 16.8 %, and 19.2 % were current and 29.6 % former smokers. The smoking habits correspond well with the general Finnish population. The proportion of patients with previously known diabetes at baseline was quite low. However, the real prevalence of diabetes was probably higher, because the diagnosis of diabetes or impaired glucose tolerance (IGT) made during or after the index hospitalization was not included in this analysis. It is well known that even two-thirds of the patients with acute myocardial infarction without previously known diabetes will have type 2 diabetes (25 %) or IGT (41%) (Bartnik et al., 2004). At baseline, 26.1 % had statin medication and hypercholesterolemia was diagnosed in 55.5 % of the patients before the index hospitalization. The proportion of patients with previously known coronary artery disease was 39.4 % and 27.4 % had history of myocardial infarction. Total mortality during the 12 months follow up was 9.6 % and 72.6% were cardiac deaths in line with the recently published registry data from Sweden and UK (Chung et al., 2015). Thus,

this cohort can be considered as representative of non-selected ACS patients in ED.

Revascularization procedures were quite infrequent (17.9 %) even during the follow-up period. The main studies showing the superiority of early invasive treatment in NSTE-ACS patients with elevated cTn (FRISC II Investigtors 1999, Morrow et al., 2001) were published just before or during the patient enrollment of this study and the treatment strategy had not yet changed to favor more invasive diagnostics and treatment as coronary angiogram, PCI and CABG. This might be also the strength of this study cohort without conceivable bias caused by routine, not symptom driven invasive treatment especially during the follow-up period. On the other hand, this study population does not fully represent the current practice, which is the weakness of this material.

6.2 NORMAL VARIATION, DECISION LIMITS AND POTENTIAL SOURCES OF ERROR OF CIRCULATING PAPP-A DETERMINATION

Excluding study **III,** our own totalPAPP-A assays are the ones that so far have been used in published clinical reports on circulating PAPP-A as a cardiac or ACS marker. Although the level of totalPAPP-A correlates with the prognosis in this and previous studies, both the wide interindividual and interassay variations without established cut-off values make the comparison of the different studies with different decision limits complicated. Complexed PAPP-A is found in variable concentrations in circulation in all individuals without ACS (Qin et al., 2005; Wittfooth et al., 2006). The studies conducted with apparently healthy populations show substantial variation in totalPAPP-A levels between different populations and assays, as Table 5 illustrated. Serteser and colleagues (2012) evaluated the biological variation of circulating PAPP-A levels by weekly standardized measurements for five weeks in 24 healthy, non-pregnant subjects and noticed 12.6% within-subject and 14.0% between-subject variations. These observations express a wide variation in totalPAPP-A levels in healthy individuals and in ACS mainly due to interindividual variations in circulating concentrations of complexed PAPP-A. The significance of interindividual and inter-assay variation is emphasized especially in minor changes in PAPP-A levels, while conversely being diminutive in greater changes. This partly explains the superiority of freePAPP-A compared to totalPAPP-A assay as a prognostic marker, as study **III** has shown. The future development challenges are to minimize the inter-assay variations and standardize the PAPP-A assays.

As recently shown, heparin treatment may significantly increase the circulating PAPP-A concentration (Terkelsen et al., 2009; Tertti et al., 2009; Hájek et al.,

2011; Wang et al., 2011; Wittfooth et al., 2011; Wang et al., 2013). This phenomenon should be taken into account when assessing the results of the PAPP-A studies in which the possible heparin effect has not been taken into consideration, as is the case in the greater part of clinical trials, including two of our own **(I**, **II)**. The confounding effect of heparin treatment on the results is difficult to estimate. The heparin treatment may strengthen the results and others dilute the "real" PAPP-A elevation in some studies. Nonetheless, the results of the studies performed in patients with stable CAD and also in ACS mainly advocate the observation of circulating PAPP-A as a prognostic marker in acute manifestations of atherosclerotic heart disease. Furthermore, there are studies in which the possible heparin effect has been taken into account or the PAPP-A level has been determined before heparin treatment, supporting the prognostic value of PAPP-A. von Haehling and coworkers' (2013) study determined the presence of circulating PAPP-A in 2568 acute chest pain patients before any heparin treatment; 45 % of the patients had ACS and the rest had stable disease. A coronary angiogram confirmed the CAD. Patients with ACS had slightly higher PAPP-A compared to patients with stable disease. The PAPP-A level was significantly higher in patients who did vs. those who did not experience the primary endpoint of stent thrombosis, myocardial (re)infarction, ischemic stroke or cardiovascular-related death during 90 days of follow up; the unadjusted risk of an adverse event above the optimal cut off was 5.30 (4.02–6.98). The PAPP-A also remained a highly significant prognostic factor in multivariate analysis. Mjelva and coworkers' (2013) study evaluated the prognostic value of PAPP-A, determined before any heparin treatment, in a long-term (84 months) follow up. The mortality was significantly higher in patients with PAPP-A >4.8 mIU/L compared to the patients with low (< 4.8 mIU/L) PAPP-A (42.4 % vs. 32.4%, $p< 0.05$). Similarly, the rate of combined endpoint of death or myocardial infarction was higher in patients with high PAPP-A. The prognostic power of circulating PAPP-A was analyzed in the post hoc analysis of the study of Iversen and coworkers (2010) including low risk ACS patients with normal cTn and without new ischemic ECG changes. Only the patients with admission PAPP-A sample collected before heparin treatment were included in the analysis. The patients with admission PAPP-A >4.0 mIU/L had higher risk for the combined endpoint of death or non-fatal MI ($p=0.001$), and PAPP-A >4.0 mIU/L was also a significant predictor of the combined endpoint in multivariate analyses. Similarly, in the studies of Heeschen and coworkers (2005) and Kavsak and coworkers (2009), PAPP-A was a significant predictor of adverse events. Interestingly, in the two studies on stroke or transient ischemic attack, the circulating PAPP-A determined before any heparin treatment was predictive for recurrent events (Wang et al., 2014; Wang et al., 2016).

The effect of heparin may also partly explain the discrepancy in the prognostic performance of totalPAPP-A between our previous studies **I** and **III**, although the

patient material and PAPP-A sampling protocols were not identical. As we previously reported, the maximal totalPAPP-A within 24 h predicts adverse cardiac events in six months in ACS patients without cTnI elevation (study **I**). Patients received heparin medication within the first 24 h after admission in this work, which may have had a substantial effect on the results, i.e., the inclusion of higher-risk patients on heparin treatment led to higher observed increases of PAPP-A in these patients due to heparin effects. Only admission freePAPP-A and totalPAPP-A were analyzed in study **III** to avoid the heparin effect, and patients receiving heparin or low molecular weight heparin treatment before the first blood sampling were excluded. The freePAPP-A gave independent and additive prognostic information, even with these prerequisites.

6.3 CIRCULATING PAPP-A IN TROPONIN NEGATIVE PATIENTS (I, III)

Studies **I** and study **III (**partly) evaluated the prognostic performance of circulating PAPP-A in patients without elevation of troponin. The proportion of patients representing with ACS and remaining troponin negative varies according to the study population, troponin assays and the URL used for the assay. For example, in a meta-analysis of 4422 suspected ACS patients, the percentage of troponin negative patients was 67 % in clinical trials and 77 % in cohort studies using the first-generation troponin I assay (Heidenreich et al., 2001). Morrow and colleagues' (2001) study with 1821 UA or NSTEMI patients found that 734 (40.3%) remained troponin I negative when using the first generation TnI assay with a cut-off point of 0.1 ng/mL.

Study **I** was the first to show the predictive power of circulating PAPP-A determined as totalPAPP-A in patients with suspected ACS and negative cTnI. A single PAPP-A level, determined at the time of admission, also showed a significant predictive value. Similar results were shown, for example, in Heeschen and coworkers' (2005) study, in which the level of circulating PAPP-A >7.0 mIU/L, taken at the time of admission and before the start of any anticoagulant treatment, was associated with a higher risk of death or myocardial infarction within 30 days compared to PAPP-A ≤7.0 mIU/L in patients with low (<0.1 µg/L) admission TnT levels (OR 2.55, 95% CI 1.22-5.36).

Study **I** measured the cTnI using the first generation Innotrac AIO assay with analytical sensitivity 0.05 µg/L. The cut-off value at 10% imprecision (CV) was 0.22 µg/L, which was used to define cTnI negativity. According to ESC/ACC guidelines published already in 2000 (Alpert et al., 2000), the 99th percentile with CV < 10% should be used as the URL for troponin assays. However, none of the assays available at that time met these criteria; therefore, the lowest concentration with

imprecision of 10% was used as a cut-off point in the studies published at the early 2000s. The currently used hs-cTnI and hs-cTnT assays fulfill the strict guideline criteria. The current guidelines also recommend using these high sensitivity troponin assays in clinical practice (Roffi et al., 2016).

It is a matter of debate whether or not the results would be the same using hs-cTn to indicate the troponin negativity. Our own group (Eriksson et al., 2005) studied the concordance between the first (innotrac AIO with cut off limit 0.22 µg/) and the second generation cTnI (detection limit 0.012 µg/L, with cut-off value at 10 % imprecision 0.06 µg/L); 14.3 %, 10.2 % and 8.3 % of patients classified as cTn negative using the first generation assay had elevated (>0.06 µg/L) cTnI when measured with the second generation assay at the time points of admission, 6-12 h and at 24 h, respectively. This indicates about 10% crossover from the cTn negative to the cTn positive group, expressing a not very significant diluting effect, especially for the results of study **I**. When using the second generation cTnI, 142 (54%) patients remained cTnI negative in study **III**. The prognostic value of free-PAPP-A for death and non-fatal MI was good (study **III**) in this cTnI negative group. Iversen and colleagues (2010) published similar results in patients with suspected ACS but no evidence of ischemia on ECG and normal (<0.03 µg/L) cTnT determined by second generation assay. The prognosis for death or non-fatal MI was also good (3% event rate) in the lowest PAPP-A quartile compared to significantly worse prognosis (event rate 15 %) in the highest quartile at three months in this study (Iversen et al., 2010).

These results taken together support the view that the level of circulating PAPP-A at admission, especially determined as freePAPP-A, may carry additional prognostic information in suspected ACS patients with low or normal troponin levels in situations in which the expected change in PAPP-A concentration is also small, even when measured with high sensitivity cTn assays,

6.4 Release kinetics and prognostic performance of circulating PAPP-A in STEMI (II)

The main mechanism of ST-elevation MI is the acute atherosclerotic plaque rupture that activates platelets and a coagulation cascade, leading to intracoronary thrombus formation and blockage of the nourishing blood flow to the myocardium. The time of symptoms onset is possible to determine in most of the cases, in contrast with NSTEMI. Our study **(II)** was the first to clarify the release pattern and the prognostic performance of circulating PAPP-A in the acute phase of STEMI. The main finding was the early and high elevation of PAPP-A during the first

hours, followed by a quite rapid decrease near to normal level, particularly in patients with clinical and ECG signs of early reperfusion, which fits the hypothesis of PAPP-A release due to plaque rupture. The similar release pattern was repeated by Iversen and coworkers (2008b) and Schoos and coworkers (2009). However, Terkelsen and colleagues (2009) noticed a trend towards higher circulating concentration of PAPP-A in heparinized STEMI patients compared to historical controls without heparin treatment and a clear re-elevation of PAPP-A with reinjection of heparin in mice already heparinized. Hájek and colleagues (2011) confirmed the effect of heparin on the release pattern of PAPP-A in STEMI by showing a comparable release pattern of PAPP-A to Study **II**. However, the observed heparin effect does not exclude the possible plaque derived PAPP-A release. In the work of Terkelsen and coworkers, the median circulating PAPP-A level of STEMI patients without heparin treatment was 5.5 mIU/L, and one quarter of patients had PAPP-A concentration over 24.0 mIU/l, while the normal reference limit was <10 mIU/L. To conclude, the natural release patterns of cPAPP-A in STEMI patients remains obscure.

The prognosis of STEMI patients with high PAPP-A already at the time of admission was very poor, and the event-free survival rate for cardiovascular death or non-fatal MI was significantly lower compared to patients with low PAPP-A **(II)**. Interestingly, the absence of late PAPP-A elevation was strongly associated with early reperfusion and patency of the infarct-related artery, while the patients with a biphasic release pattern were characterized by late or no reperfusion in a majority of the patients **(II)**. The difference cannot be explained by heparin treatment, because the recommendation was to continue LMWH treatment for several days after STEMI in all patients at the time of patient enrollment. One explanation is that the longer myocardial ischemia and larger myocardial damage increases PAPP-A concentration. A similar correlation with late or persistent PAPP-A elevation and the concomitant clinical complications was later noticed in a smaller study by Hájek and coworkers (2011). Although the precise mechanisms of PAPP-A elevation in STEMI remain unclear, the elevated level of circulating PAPP-A also seems to carry prognostic information in this patient group.

6.5 THE PROGNOSTIC PERFORMANCE OF FREE FORM OF PAPP-A (III)

As shown by our group, the freePAPP-A predominantly represents the circulating PAPP-A fraction changing dynamically in ACS (Qin et al., 2005). Furthermore, the concentration of freePAPP-A is negligible in an apparently healthy population (Wittfooth et al., 2006) and <1% of totalPAPP-A concentration is in circulation in the late phase of pregnancy (Overgaard et al., 2000). It would be expected that freePAPP-A could be a more precise marker for ACS compared to totalPAPP-A

when also taking the smaller interindividual variation into account (Wittfooth et al., 2006). Contrary to previous studies, study **III** used freePAPP-A as a prognostic marker in ACS, and the prognostic power was compared to totalPAPP-A in heparin-naïve patients. FreePAPP-A, measured at the time of admission before any heparin treatment, turned out to be an independent predictor of the combined endpoint of death or myocardial infarction during the one-year follow up. These results also confirmed the hypothesis of the superiority of freePAPP-A compared to totalPAPP-A as a prognostic marker in ACS. Theoretically, the main advantage of using freePAPP-A would be achieved in low PAPP-A levels supported by the finding of the correlation of totalPAPP-A and freePAPP-A in higher levels but not the lower ones.

No commercially available assays exist for freePAPP-A to our knowledge, and study **III** is the first - and up to this point, the only - to use freePAPP-A as a prognostic marker in the settings of ACS. While freePAPP-A seems to be a superior marker compared to total PAPP-A, the fact that two separate assays are needed is a clear methodological limitation; thus, an obvious need exists for a direct freePAPP-A assay, which is in the development phase in our group's laboratory.

7 CONCLUSIONS

The main conclusions based on the current study's results are summarized as follows:

1. The level of circulating totalPAPP-A, notably when determined at an early phase, i.e., at admission, has prognostic value for cardiac adverse events especially in patients representing with suspected ACS with minimal or no troponin I elevation and in patients with STEMI.

2. A circulating free form of PAPP-A holds promise to be superior as a prognostic marker for death or myocardial infarction compared to totalPAPP-A, giving independent and additive prognostic information when measured at the time of admission and before any heparin treatment in patients hospitalized for NSTE-ACS.

3. The heparin treatment increases the level of circulating PAPP-A and should be taken into account when assessing the utility of PAPP-A as a prognostic marker in ACS. With the currently available technology, the samples should be collected before any heparin treatment.

Although the level of circulating PAPP-A has prognostic value in patients representing with suspected ACS, the uncertainties (like interaction with concomitant heparin treatment) linked to available PAPP-A assays, the lack of clear decision cut points and the wide interindividual variation in the concentrations limit the use of PAPP-A in clinical practice so far. Nevertheless, PAPP-A may still have potential as a marker of plaque activity, and our results are not in conflict with this theory. However, an obvious need exists for a direct freePAPP-A assay to eliminate the interindividual and the interassay variation; further research is ongoing by our group to reach this target. At present, there are no data on potential pharmacological or interventional measures to improve the prognosis of patients with elevated circulating PAPP-A, which remains to be clarified in further studies.

ACKNOWLEDGEMENTS

The present study was carried out between 2000 – 2018 at the Heart Center, Department of Medicine and Department of Clinical Chemistry of University of Turku in collaboration with the Department of Biochemistry and Biotechnology, University of Turku.

I want to express my warmest thanks to:

Docent Liisa-Maria Voipio-Pulkki, MD, for her role as a teacher of scientific thinking, and who is the great supervisor and promoter of this work. Liisa-Maria's scientific comprehension, skills in scientific work and medical writing are admirable. Without Liisa-Maria's extensive efficiency, strict but always constructive criticism, friendly and forbearing support, especially in troublesome times during these years, the work would never have been completed.

Professor Kim Pettersson, PhD, my second supervisor, for excellent expertise in the field of biomarkers and laboratory science, enabling the great collaboration between the laboratory and clinical scientists, and for encouraging me to keep moving and finish this long-lasting project.

Professor Juhani Airaksinen, MD, the Head of the Heart Center, for familiarizing me with the world of cardiology, for teaching and pushing me towards new challenges of invasive cardiology and, especially, cardiac electrophysiology. I also wish to thank him for patiently motivating me to finish this project and for valuable help in revising the final manuscript.

Professor Auli Toivanen, MD, and Professor Jorma Viikari, MD, the former Heads of the Department of Medicine, for encouraging me and for providing the opportunity to work in their department.

Docent Matti Niemelä, MD, from the Department of Cardiology, Oulu University Hospital, and Docent Veli-Pekka Harjola, MD, from the Department of Emergency Medicine, Helsinki University Hospital, as the official examiners of this thesis for their expeditious review, great suggestions and constructive criticism to improve the final manuscript.

All co-authors of the original communications added in this thesis: Saara Wittfooth, PhD, for fruitful collaboration, for tremendous work in the development process of PAPP-A assays and also for revising the final manuscript - without Saara's hard and unyielding input, the finishing of this thesis would not be possible; Dr. Tuomo Ilva, MD, for friendship and excellent collaboration and teamwork in the planning period, patient recruitment and data collection of this project; Do-

cent Pekka Porela, MD, for friendship, analyzing the ECG data, constructive criticism and great ideas, especially when finishing the original publications; Professor Kari Pulkki, MD, for excellent expertise in laboratory science and enabling the collaboration with the laboratory service of Turku University Hospital; Professor Kjell Nikus, MD, and Docent Markku Eskola, MD, for analyzing the angiographic and ECG data and for valuable criticism and advice; and Dr. Qiu-Ping Qin, PhD, "the father of PAPP-A" and one of the promoters of this study for innovative and teaching collaboration, especially during the early phase of this project.

Professor Emeritus Kerttu Irjala, former Head of the Department of Clinical Chemistry and Mrs. Tuula Laukkanen for permitting the collaboration and taking care of the logistics of laboratory service; Mrs. Taina Mertamo for excellent work with preparing the database; and Hans Helenius, M.Sc., for valuable advice when preparing the statistical analyses.

The current and former colleagues and workmates in the Heart Center and in the Department of Medicine for collaboration and support. Special thanks to Dr. Samuli Jaakkola, MD, for advice with the thesis book template.

Carolyn Abbott for revising and editing the English of this thesis.

All my good friends and their families, but especially Mr. Jukka Laakso, for friendship and support lasting already for decades. The weekly badminton match and confabulations have been an excellent counterbalance and reminded me about the life outside of medicine; Docent Marko Neva, MD, for friendship and for motivating me to finish this work. The yearly sailing weekends and skiing tours with Marko and his family, enjoying incredible food and excellent wines, have especially kept my spirit high.

My parents Sirkka and Leif Lund for persistent encouragement, love and support during my life and during this project, my sister Outi and my brother Vesa and their families for friendship, support and for being there if needed and my parents-in-law Ralf and Paula Karlström for support and, when needed, irreplaceable assistance with the children.

Finally, my warmest thanks to my dear wife Mervi and our lovely daughters Ida and Nelli for love, patience and support and for helping me to remember the life outside the hospital and medicine during these years.

The study was financially supported by EVO grants from the Turku University Hospital, the Helsinki University Hospital and the Finnish Funding Agency for Technology and innovation (TEKES), which is gratefully acknowledged.

REFERENCES

Alcalai R, Planer D, Culhaoglu A, Osman A, Pollak A & Lotan C (2007). Acute coronary syndrome vs nonspecific troponin elevation: clinical predictors and survival analysis. *Arch Intern Med, 167*(3), 276-281.

Alpert JS, Thygesen K, Antman E & Bassand J P (2000). Myocardial infarction redefined - a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *J Am Coll Cardiol, 36*(3), 959-969.

Anderson PA, Malouf NN, Oakeley, AE, Pagani ED & Allen PD (1991). Troponin T isoform expression in humans. A comparison among normal and failing adult heart, fetal heart, and adult and fetal skeletal muscle. *Circ Res, 69*(5), 1226-1233.

Antman EM, McCabe CH, Gurfinkel EP, Turpie AG, Bernink, PJ, Salein D, et al. (1999). Enoxaparin prevents death and cardiac ischemic events in unstable angina/non-Q-wave myocardial infarction. Results of the thrombolysis in myocardial infarction (TIMI) 11B trial. *Circulation, 100*(15), 1593-1601.

Antman EM, Tanasijevic MJ, Thompson B, Schactman M, McCabe CH, Cannon, C P, et al. (1996). Cardiac-specific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. *N Engl J Med, 335*(18), 1342-1349.

Aragam KG, Tamhane UU, Kline-Rogers E, Li J, Fox KA, Goodman SG, et al. (2009). Does simplicity compromise accuracy in ACS risk prediction? A retrospective analysis of the TIMI and GRACE risk scores. *PLoS One, 4*(11), e7947.

Aso Y, Okumura K, Wakabayashi S, Takebayashi K, Taki S & Inukai T (2004). Elevated pregnancy-associated plasma protein-a in sera from type 2 diabetic patients with hypercholesterolemia: associations with carotid atherosclerosis and toe-brachial index. *J Clin Endocrinol Metab, 89*(11), 5713-5717.

Astrup AS, Tarnow L, Christiansen M, Hansen PR, Parving HH & Rossing P (2007). Pregnancy-associated plasma protein A in a large cohort of Type 1 diabetic patients with and without diabetic nephropathy-a prospective follow--up study. *Diabet Med, 24*(12), 1381-1385.

Atie J, Brugada P, Brugada J, Smeets JL, Cruz FE, Roukens MP, et al. (1991). Clinical presentation and prognosis of left main coronary artery disease in the 1980s. *Eur Heart J, 12*(4), 495-502.

Bale LK, Chakraborty S & Conover CA (2014). Inducible reduction in pregnancy-associated plasma protein-A gene expression inhibits established atherosclerotic plaque progression in mice. *Endocrinology, 155*(4), 1184-1187.

Bartnik M, Malmberg K, Hamsten A, Efendic S, Norhammar A, Silveira A, et al. (2004). Abnormal glucose tolerance--a common risk factor in patients with acute myocardial infarction in comparison with population-based controls. *J Intern Med, 256*(4), 288-297.

Bayes-Genis A, Conover CA, Overgaard MT, Bailey KR, Christiansen M, Holmes D R, et al. (2001a). Pregnancy-associated plasma protein A as a marker of acute coronary syndromes. *N Engl J Med, 345*(14), 1022-1029.

Bayes-Genis A, Conover CA & Schwartz RS (2000). The insulin-like growth factor axis: A review of atherosclerosis and restenosis. *Circ Res, 86*(2), 125-130.

Bayes-Genis A, Schwartz RS, Bale LK & Conover CA (2003). Effects of insulin-like growth factor-I on cultured human coronary artery smooth muscle cells. *Growth Horm IGF Res, 13*(5), 246-253.

Bayes-Genis A, Schwartz RS, Lewis DA, Overgaard MT, Christiansen M, Oxvig, C, et al. (2001b). Insulin-like growth factor binding protein-4 protease produced by smooth muscle cells increases in the coronary artery after angioplasty. *Arterioscler Thromb Vasc Biol, 21*(3), 335-341.

Beaudeux JL, Burc L, Imbert-Bismut F, Giral P, Bernard M, Bruckert E, et al. (2003). Serum plasma pregnancy-associated protein A: a potential marker of echogenic carotid atherosclerotic plaques in asymptomatic hyperlipide-mic subjects at high cardiovascular risk. *Arterioscler Thromb Vasc Biol*, 23(1): e7-10.

Blundell TL & Humbel RE (1980). Hormone families: pancreatic hormones and homologous growth factors. *Nature, 287*(5785), 781-787.

Boldt HB, Overgaard MT, Laursen LS, Weyer K, Sottrup-Jensen L & Oxvig C (2001). Mutational analysis of the proteolytic domain of pregnancy-associated plasma protein-A (PAPP-A): classification as a metzincin. *Bio chem J, 358*(Pt 2), 359-367.

Boldt HB & Conover CA (2007). Pregnancy-associated plasma protein-A (PAPP-): a local regulator of IGF bioavailability through cleavage of IGFBPs. *Growth Horm IGF Res*, 17(1), 10-8. Review

Bonaca M, Scirica B, Sabatine M, Dalby A, Spinar J, Murphy SA, et al. (2010). Prospective evaluation of the prognostic implications of improved assay performance with a sensitive assay for cardiac troponin I. *J Am Coll Cardiol, 55*(19), 2118-2124.

Bonaca MP, Scirica BM, Sabatine MS, Jarolim P, Murphy SA, Chamberlin JS, et al. (2012). Prospective evaluation of pregnancy-associated plasma protein-a and outcomes in patients with acute coronary syndromes. *J Am Coll Cardiol, 60*(4), 332-338.

Bonno M, Oxvig C, Kephart GM, Wagner JM, Kristensen T, Sottrup-Jensen L, et al. (1994). Localization of pregnancy-associated plasma protein-A and colocalization of pregnancy-associated plasma protein-A messenger ribonucleic acid and eosinophil granule major basic protein messenger ribonucleic acid in placenta. *Lab Invest, 71*(4), 560-566.

Brügger-Andersen T, Aarsetøy H, Grundt, H, Staines H & Nilsen DW (2008). The long-term prognostic value of multiple biomarkers following a myocardial infarction. *Thromb Res, 123*(1), 60-66.

Brügger-Andersen T, Bostad L, Sandnes DA, Larsen AI, Bonarjee VV, Barvik S, et al. (2010). The activity of pregnancy-associated plasma protein A (PAPP-A) as expressed by immunohistochemistry in atherothrombotic pla-ques obtained by aspiration thrombec-tomy in patients presenting with a ST-elevation myocardial infarction: a brief communication. *Thromb J, 8*(1), 1.

Brügger-Andersen T, Hetland Ø, Pönitz V, Grundt H & Nilsen DW (2007). The effect of primary percutaneous coronary intervention as compared to tenecteplase on myeloperoxidase, pregnancy-associated plasma protein A, soluble fibrin and D-dimer in acute myocardial infarction. *Thromb Res, 119*(4), 415-421.

Calabró P, Willerson JT & Yeh ET (2003). Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells. *Circulation, 108*(16), 1930-1932.

Cermak J, Key NS, Bach RR, Balla J, Jacob H S & Vercellotti GM (1993). C-reactive protein induces human peripheral blood monocytes to synthesize tissue factor. *Blood, 82*(2), 513-520.

Chelchowska M, Maciejewski T, Gajewska J, Ambroszkiewicz J, Laskowska-Klita T & Leibschang J (2012). The pregnancy-associated plasma protein A and insulin-like growth factor system in response to cigarette smoking. *J Matern Fetal Neonatal Med, 25*(11), 2377-2380.

Chen BK, Leiferman KM, Pittelkow MR., Overgaard MT, Oxvig C & Conover CA (2003). Localization and regulation of pregnancy-associated plasma protein a expression in healing human skin. *J Clin Endocrinol Metab, 88*(9), 4465-4471.

Chung SC, Sundström J, Gale CP, James S, Deanfield J, Wallentin L, et al. (2015b). Comparison of hospital variation in acute myocardial infarction care and outcome between Sweden and United Kingdom: population based cohort study using nationwide clinical registries. *BMJ, 351*, h3913.

Clemmons DR (2007). Modifying IGF1 activity: an approach to treat endocrine disorders, atherosclerosis and cancer. *Nat Rev Drug Discov, 6*(10), 821-833.

Collinson J, Bakhai A, Taneja A, Wang D & Flather MD (2006). Admission ECG predicts long-term outcome in acute coronary syndromes without ST elevation. *QJM, 99*(9), 601-607.

Conover CA, Bale LK, Harrington SC, Resch ZT, Overgaard MT & Oxvig C (2006). Cytokine stimulation of pregnancy-associated plasma protein A expression in human coronary artery smooth muscle cells: inhibition by resveratrol. *Am J Physiol Cell Physiol, 290*(1), C183-188.

Conover CA, Bale LK, Mader JR, Mason MA, Keenan KP & Marler RJ (2010). Longevity and age-related pathology of mice deficient in pregnancy-associated plasma protein-A. *J Gerontol A Biol Sci Med Sci, 65*(6), 590-599.

Conover CA, Harrington SC & Bale LK (2008). Differential regulation of pregnancy associated plasma protein-A in human coronary artery endothelial cells and smooth muscle cells. *Growth Horm IGF Res, 18*(3), 213-220.

Conover CA, Harrington SC, Bale LK & Oxvig C (2007). Surface association of pregnancy-associated plasma protein-A accounts for its colocalization with activated macrophages. *Am J Physiol Heart Circ Physiol, 292*(2), H994-H1000.

Conover CA, Mason MA, Bale LK, Harrington SC, Nyegaard M, Oxvig C, et al. (2010). Transgenic overexpression of pregnancy-associated plasma protein-A in murine arterial smooth muscle accelerates atherosclerotic lesion development. *Am J Physiol Heart Circ Physiol, 299*(2), H284-291.

Consuegra-Sanchez L, Petrovic I, Cosin-Sales J, Holt DW, Christiansen M & Kaski JC (2008). Prognostic value of circulating pregnancy-associated plasma protein-A (PAPP-A) and proform of eosinophil major basic protein (pro-MBP) levels in patients with chronic stable angina pectoris. *Clin Chim Acta, 391*(1-2), 18-23.

Cosin-Sales J, Christiansen M, Kaminski P, Oxvig C, Overgaard MT, Cole D, et al. (2004). Pregnancy-associated plasma protein A and its endogenous inhibitor, the proform of eosinophil major basic protein (proMBP), are related to complex stenosis morphology in patients with stable angina pectoris. *Circulation, 109*(14), 1724-1728.

Cosin-Sales J, Kaski JC, Christiansen M, Kaminski P, Oxvig C, Overgaard MT, et al. (2005a). Relationship among pregnancy associated plasma protein-A levels, clinical characteristics, and coronary artery disease extent in patients with chronic stable angina pectoris. *Eur Heart J, 26*(20), 2093-2098.

Coskun A, Bicik Z, Duran S, Alcelik A, Soypacaci Z, Yavuz O, et al. (2007). Pregnancy-associated plasma protein A in dialysis patients. *Clin Chem Lab Med, 45*(1), 63-66.

Coskun A, Serteser, M, Duran S, Inal TC, Erdogan BE, Ozpinar A, et al. (2013). Reference interval of pregnancy-associated plasma protein-a in healthy men and non-pregnant women. *J Cardiol, 61*(2), 128-131.

Cummins B, Auckland ML & Cummins P (1987). Cardiac-specific troponin-I radioimmunoassay in the diagnosis of acute myocardial infarction. *Am Heart J, 113*(6), 1333-1344.

Cummins B & Cummins P (1987). Cardiac specific troponin-I release in canine experimental myocardial infarction: development of a sensitive enzyme-linked immunoassay. *J Mol Cell Cardiol, 19*(10), 999-1010.

Daidoji H, Takahashi H, Otaki Y, Tamura H, Arimoto T, Shishido T, et al. (2015). A combination of plaque components analyzed by integrated backscatter intravascular ultrasound and serum pregnancy-associated plasma protein a levels predict the no-reflow phenomenon during percutaneous coronary intervention. *Catheter Cardiovasc Interv, 85*(1), 43-50.

Davey MW, Teisner B, Sinosich M & Grudzinskas JG (1983). Interaction between heparin and human pregnancy-associated plasma protein A (PAPP-A): a simple purification procedure. *Anal Biochem, 131*(1), 18-24.

de Araújo Gonçalves P, Ferreira J, Aguiar C & Seabra-Gomes R (2005). TIMI, PURSUIT, and GRACE risk scores: sustained prognostic value and interaction with revascularization in NSTE-ACS. *Eur Heart J, 26*(9), 865-872.

De Caterina AR, Galiuto L, Fedele E & Crea F (2011). Microvascular dysfunction in the spectrum of coronary instability. *Am J Cardiol, 108*(10), 1513-1516.

Delafontaine P, Song YH & Li Y (2004). Expression, regulation, and function of IGF-1, IGF-1R, and IGF-1 binding proteins in blood vessels. *Arterioscler Thromb Vasc Biol, 24*(3), 435-444.

Ebashi S (1963). Third component participating in the superprecipitation of 'natural actomyosin'. *Nature, (200)*, 1010.

Ebashi S & Kodama A (1965). A new protein factor promoting aggregation of tropomyosin. *J Biochem, 58*(1), 107-108.

Elesber AA, Conover CA, Denktas AE, Lennon RJ, Holmes DR, Overgaard MT, et al. (2006). Prognostic value of circulating pregnancy-associated plasma protein levels in patients with chronic stable angina. *Eur Heart J, 27*(14), 1678-1684.

Elesber AA, Lerman A, Denktas AE, Resch Z T, Jared Bunch T, Schwartz RS, et al. (2007). Pregnancy associated plasma protein-A and risk stratification of patients presenting with chest pain in the emergency department. *Int J Cardiol, 117*(3), 365-369.

Eriksson S, Ilva T, Becker C, Lund J, Porela P, Pulkki K, et al. (2005). Comparison of cardiac troponin I immunoassays variably affected by circulating autoantibodies. *Clin Chem, 51*(5), 848-855.

Eskola MJ, Holmvang L, Nikus KC, Sclarovsky S, Tilsted HH, Huhtala H, et al. (2007a). The electrocardiographic window of opportunity to treat vs. the different evolving stages of ST-elevation myocardial infarction: correlation with therapeutic approach, coronary anatomy, and outcome in the DANAMI-2 trial. *Eur Heart J, 28*(24), 2985-2991.

Eskola MJ, Kosonen P, Sclarovsky S, Vikman S & Nikus KC (2007b). The ECG pattern of isolated right ventricular infarction during percutaneous coronary intervention. *Ann Noninvasive Electrocardiol, 12*(1), 83-87.

Eskola MJ, Nikus KC, Holmvang L, Sclarovsky S, Tilsted HH, Huhtala H, et al. (2009a). Value of the 12-lead electrocardiogram to define the level of obstruction in acute anterior wall myocardial infarction: correlation to coronary angiography and clinical outcome in the DANAMI-2 trial. *Int J Cardiol, 131*(3), 378-383.

Eskola MJ, Nikus KC & Sclarovsky S (2009b). Persistent precordial "hyperacute" T waves signify proximal left anterior descending artery occlusion. *Heart, 95*(23), 1951-1952; author reply 1952-1953.

Eskola MJ, Nikus KC, Voipio-Pulkki LM, Huhtala H, Lund J, Ilva T, et al. (2007c). Detection of proximal coronary occlusion in acute coronary syndrome: a feasibility study using computerized electrocardiographic analysis. *Ann Noninvasive Electrocardiol, 12*(4), 301-305.

Falk E (1985). Unstable angina with fatal outcome: dynamic coronary thrombosis leading to infarction and/or sudden death. Autopsy evidence of recurrent mural thrombosis with peripheral embolization culminating in total vascular occlusion. *Circulation, 71*(4), 699-708.

Ferreirós ER, Boissonnet CP, Pizarro R, Merletti, PF, Corrado G, Cagide A, et al. (1999). Independent prognostic value of elevated C-reactive protein in unstable angina. *Circulation, 100*(19), 1958-1963.

Folkersen J, Grudzinskas JG, Hindersson P, Teisner B & Westergaard JG (1981). Pregnancy-associated plasma protein A: circulating levels during normal pregnancy. *Am J Obstet Gynecol, 139*(8), 910-914.

Ford ES & Giles WH (2000a). Serum C-reactive protein and fibrinogen concentrations and self-reported angina pectoris and myocardial infarction: findings from National Health and Nutrition Examination Survey III. *J Clin Epidemiol, 53*(1), 95-102.

Ford ES & Giles WH (2000b). Serum C-reactive protein and self-reported stroke: findings from the Third National Health and Nutrition Examination Survey. *Arterioscler Thromb Vasc Biol, 20*(4), 1052-1056.

Frink RJ, Rooney PA, Trowbridge JO & Rose J P (1988). Coronary thrombosis and platelet/fibrin microemboli in death associated with acute myocardial infarction. *Br Heart J, 59*(2), 196-200.

Galvani M, Ottani F, Ferrini D, Ladenson JH, Destro A, Baccos D, et al. (1997). Prognostic influence of elevated values of cardiac troponin I in patients with unstable angina. *Circulation, 95*(8), 2053-2059.

Giannitsis E, Becker M, Kurz K, Hess G, Zdunek D & Katus HA (2010). High-sensitivity cardiac troponin T for early prediction of evolving non-ST-segment elevation myocardial infarction in patients with suspected acute coronary syndrome and negative troponin results on admission. *Clin Chem, 56*(4), 642-650.

Glerup S, Boldt HB, Overgaard MT, Sottrup-Jensen L, Giudice LC & Oxvig C (2005). Proteinase inhibition by proform of eosinophil major basic protein (pro-MBP) is a multistep process of intra- and intermolecular disulfide rearrangements. J Biol Chem, 280(11), 9823-32.

Grand RJ & Wilkinson JM (1976). The amino acid sequence of rabbit cardiac troponin I. *Biochem J, 159*(3), 633-641.

Grand RJ & Wilkinson JM (1977). The amino acid sequence of rabbit slow-muscle troponin I. *Biochem J, 167*(1), 183-192.

Grant MB, Wargovich TJ, Ellis EA, Caballero S, Mansour M & Pepine CJ (1994). Localization of insulin-like growth factor I and inhibition of coronary smooth muscle cell growth by somatostatin analogues in human coronary smooth muscle cells. A potential treatment for restenosis? *Circulation, 89*(4), 1511-1517.

Grant MB, Wargovich TJ, Ellis EA, Tarnuzzer R, Caballero S, Estes K, et al. (1996). Expression of IGF-I, IGF-I receptor and IGF binding proteins-1, -2, -3, -4 and -5 in human atherectomy specimens. *Regul Pept, 67*(3), 137-144.

Greaser ML & Gergely J (1971). Reconstitution of troponin activity from three protein components. *J Biol Chem, 246*(13), 4226-4233.

Gyrup C & Oxvig C (2007). Quantitative analysis of insulin-like growth factor-modulated proteolysis of insulin-like growth factor binding protein-4 and -5 by pregnancy-associated plasma protein-A. *Biochemistry, 46*(7), 1972-1980.

Gérard N, Delpuech T, Oxvig C, Overgaard M T & Monget P (2004). Proteolytic degradation of IGF-binding protein (IGFBP)-2 in equine ovarian follicles: involvement of pregnancy-associated plasma protein-A (PAPP-A) and association with dominant but not subordinated follicles. *J Endocrinol, 182*(3), 457-466.

Haaf P, Drexler B, Reichlin T, Twerenbold R, Reiter M, Meissner J, et al. (2012). High-sensitivity cardiac troponin in the distinction of acute myocardial infarction from acute cardiac noncoronary artery disease. *Circulation, 126*(1), 31-40.

Hamm CW, Bassand JP, Agewall S, Bax J, Boersma E, Bueno H, et al (2011). ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J, 32*(23), 2999-3054.

Hamm CW, Ravkilde J, Gerhardt W, Jørgensen P, Peheim E, Ljungdahl L, et al. (1992). The prognostic value of serum troponin T in unstable angina. *N Engl J Med, 327*(3), 146-150.

Harrington SC, Simari RD & Conover CA (2007). Genetic deletion of pregnancy-associated plasma protein-A is associated with resistance to atherosclerotic lesion development in apolipoprotein E-deficient mice challenged with a high-fat diet. *Circ Res, 100*(12), 1696-1702.

Haverkate F, Thompson SG, Pyke SD, Gallimore JR & Pepys MB (1997). Production of C-reactive protein and risk of coronary events in stable and unstable angina. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. *Lancet, 349*(9050), 462-466.

He LP, Tang XY, Ling WH, Chen WQ & Chen YM (2010). Early C-reactive protein in the prediction of long-term outcomes after acute coronary syndromes: a meta-analysis of longitudinal studies. *Heart, 96*(5), 339-346.

Heeschen C, Dimmeler S, Hamm CW, Fichtlscherer S, Simoons ML, Zeiher AM, et al. (2005). Pregnancy-associated plasma protein-A levels in patients with acute coronary syndromes: comparison with markers of systemic inflammation, platelet activation, and myocardial necrosis. *J Am Coll Cardiol, 45*(2), 229-237.

Heeschen C, Hamm CW, Bruemmer J & Simoons ML (2000). Predictive value of C-reactive protein and troponin T in patients with unstable angina: a comparative analysis. CAPTURE Investigators. Chimeric c7E3 AntiPlatelet Therapy in Unstable angina REfractory to standard treatment trial. *J Am Coll Cardiol, 35*(6), 1535-1542.

Heidari B, Fotouhi A, Sharifi F, Mohammad K, Pajouhi M, Paydary K, et al. (2015). Elevated Serum Levels of Pregnancy-Associated Plasma Protein-A in Type 2 Diabetics Compared to Healthy Controls: Associations with Subclinical Atherosclerosis Parameters. *Acta Med Iran, 53*(7), 395-402.

Heidenreich PA, Alloggiamento T, Melsop K, McDonald KM, Go AS & Hlatky MA (2001). The prognostic value of troponin in patients with non-ST elevation acute coronary syndromes: a meta-analysis. *J Am Coll Cardiol, 38*(2), 478-485.

Heider P, Pfäffle N, Pelisek J, Wildgruber M, Poppert H, Rudelius M, et al. (2010). Is serum pregnancy-associated plasma protein A really a potential marker of atherosclerotic carotid plaque stability? *Eur J Vasc Endovasc Surg, 39*(6), 668-675.

Heusch G, Kleinbongard P, Böse D, Levkau B, Haude M, Schulz R, et al. (2009). Coronary microembolization: from bedside to bench and back to bedside. *Circulation, 120*(18), 1822-1836.

Hodkova M, Dusilova-Sulkova S, Kalousova M, Soukupova J, Zima T, Mikova D, et al. (2006). Influence of oral vitamin E therapy on micro-inflammation and cardiovascular disease markers in chro-nic hemodialysis patients. *Ren Fail,* 28(5):395-9.

Holmvang L, Clemmensen P, Lindahl B, Lagerqvist B, Venge P, Wagner G, et al. (2003). Quantitative analysis of the admission electrocardiogram identifies patients with unstable coronary artery disease who benefit the most from early invasive treatment. *J Am Coll Cardiol, 41*(6), 905-915.

Hsieh T, Gordon RE, Clemmons DR, Busby WH & Duan C (2003). Regulation of vascular smooth muscle cell responses to insulin-like growth factor (IGF)-I by local IGF-binding proteins. *J Biol Chem, 278*(44), 42886-42892.

Hájek P, Macek M, Lashkevich A, Klučková H, Hladíková M, Hansvenclová E, et al. (2011). Influence of concomitant heparin administration on pregnancy-associated plasma protein-A levels in acute coronary syndrome with ST segment elevation. *Arch Med Sci, 7*(6), 977-983.

Hájek P, Macek M, Pešková M, Hladíková M, Hansvenclová E, Malý M, et al. (2012). High positive predictive value of PAPP-A for acute coronary syndrome diagnosis in heparin-naïve patients. *J Thromb Thrombolysis, 34*(1), 99-105.

Ilva T, Eriksson S, Lund J, Porela P, Mustonen H, Pettersson K., et al. (2005). Improved early risk stratification and diagnosis of myocardial infarction, using a novel troponin I assay concept. *Eur J Clin Invest, 35*(2), 112-116.

FRISC investigators (1999). Invasive compared with non-invasive treatment in unstable coronary-artery disease: FRISC II prospective randomised multicentre study. FRagmin and Fast Revascularisation during InStability in Coronary artery disease Investigators. (1999). *Lancet, 354*(9180), 708-715.

Iversen KK, Dalsgaard M, Teisner AS, Schoos M, Teisner B, Nielsen H, et al. (2010). Pregnancy-associated plasma protein-A, a marker for outcome in patients suspected for acute coronary syndrome. *Clin Biochem, 43*(10-11), 851-857.

Iversen KK, Teisner AS, Teisner B, Kliem A, Bay M, Kirk V, et al. (2008a). Pregnancy-associated plasma protein A in non-cardiac conditions. *Clin Biochem, 41*(7-8), 548-553.

Iversen KK, Teisner AS, Teisner B, Kliem A, Thanning P, Grande, P, et al. (2008b). Pregnancy associated plasma protein A, a novel, quick, and sensitive marker in ST-elevation myocardial infarction. *Am J Cardiol, 101*(10), 1389-1394.

Iversen KK, Teisner AS, Teisner B, Kliem A, Thanning P, Nielsen H, et al. (2009). Pregnancy associated plasma protein A, a potential marker for vulnerable plaque in patients with non-ST-segment elevation acute coronary syndrome. *Clin Biochem, 42*(9), 828-834.

Iversen KK, Teisner B, Winkel P, Gluud C, Kjøller E, Kolmos HJ, et al. (2011). Pregnancy associated plasma protein-A as a marker for myocardial infarction and death in patients with stable coronary artery disease: a prognostic study within the CLARICOR Trial. *Atherosclerosis, 214*(1), 203-208.

Jiménez-Candil J, Cruz González I, Martín F, Pabón P, León V, Hernández J, et al. (2008). Relationship between QRS duration and prognosis in non-ST-segment elevation acute coronary syndrome. *Int J Cardiol, 126*(2), 196-203.

Joaquin C, Aguilera E, Granada ML, Pastor MC, Salinas I, Alonso N, et al. (2008). Effects of GH treatment in GH-deficient adults on adiponectin, leptin and pregnancy-associated plasma protein-A. *Eur J Endocrinol, 158*(4), 483-490.

Jones JI & Clemmons DR (1995). Insulin-like growth factors and their binding proteins: biological actions. *Endocr Rev, 16*(1), 3-34.

Kalousová M, Sulková S, Fialová L, Soukupová J, Malbohan IM, Spacek P, et al (2003). Glycoxidation and inflammation in chronic haemodialysis patients. *Nephrol Dial Transplant,* 18(12), 2577-81.

Kamran M, Guptan A & Bogal M (2008). Spontaneous coronary artery dissection: case series and review. *J Invasive Cardiol, 20*(10), 553-559.

Kaptoge S, Di Angelantonio E, Pennells L, Wood AM, White IR, Gao P, et al. (2012). C-reactive protein, fibrinogen, and cardiovascular disease prediction. *N Engl J Med, 367*(14), 1310-1320.

Katus HA, Remppis A, Looser S, Hallermeier, K, Scheffold T & Kübler W (1989). Enzyme linked immuno assay of cardiac troponin T for the detection of acute myocardial infarction in patients. *J Mol Cell Cardiol, 21*(12), 1349-1353.

Katus HA, Remppis A, Neumann FJ, Scheffold T, Diederich KW, Vinar G, et al. (1991). Diagnostic efficiency of troponin T measurements in acute myocardial infarction. *Circulation, 83*(3), 902-912.

Kaul P, Fu Y, Chang WC, Harrington RA, Wagner GS, Goodman SG, et al. (2001). Prognostic value of ST segment depression in acute coronary syndromes: insights from PARAGON-A applied to GUSTO-IIb. PARAGON-A and GUSTO IIb Investigators. Platelet IIb/IIIa Antagonism for the Reduction of Acute Global Organization Network. *J Am Coll Cardiol, 38*(1), 64-71.

Kaul P, Newby LK, Fu Y, Hasselblad V, Mahaffey KW, Christenson RH, et al. (2003). Troponin T and quantitative ST-segment depression offer complementary prognostic information in the risk stratification of acute coronary syndrome patients. *J Am Coll Cardiol, 41*(3), 371-380.

Kavsak PA, Wang X, Henderson M, Ko DT, MacRae AR & Jaffe AS (2009). PAPP-A as a marker of increased long-term risk in patients with chest pain. *Clin Biochem, 42*(10-11), 1012-1018.

Kavsak PA, Wang X, Ko DT, MacRae AR & Jaffe AS (2009). Short- and long-term risk stratification using a next-generation, high-sensitivity research cardiac troponin I (hs-cTnI) assay in an emergency department chest pain population. *Clin Chem, 55*(10), 1809-1815.

Keller T, Zeller T, Ojeda F, Tzikas S, Lillpopp L, Sinning C, et al. (2011). Serial changes in highly sensitive troponin I assay and early diagnosis of myocardial infarction. *JAMA, 306*(24), 2684-2693.

Keller T, Zeller T, Peetz D, Tzikas S, Roth A, Czyz E, et al. (2009). Sensitive troponin I assay in early diagnosis of acute myocardial infarction. *N Engl J Med, 361*(9), 868-877.

Khosravi J, Diamandi A, Krishna RG, Bodani U, Mistry J & Khaja N (2002). Pregnancy associated plasma protein-A: ultrasensitive immunoassay and determination in coronary heart disease. *Clin Biochem, 35*(7), 531-538.

Koenig W, Sund M, Fröhlich M, Fischer HG, Löwel H, Döring A, et al. (1999). C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. *Circulation, 99*(2), 237-242.

Kontos MC, Shah R, Fritz LM, Anderson FP, Tatum JL, Ornato JP, et al. (2004). Implication of different cardiac troponin I levels for clinical outcomes and prognosis of acute chest pain patients. *J Am Coll Cardiol, 43*(6), 958-965.

Kovanen PT, Kaartinen M & Paavonen T (1995). Infiltrates of activated mast cells at the site of coronary atheromatous erosion or rupture in myocardial infarction. *Circulation, 92*(5), 1084-1088.

Kristensen T, Oxvig C, Sand O, Møller NP & Sottrup-Jensen L (1994). Amino acid sequence of human pregnancy-associated plasma protein-A derived from cloned cDNA. *Biochemistry, 33*(6), 1592-1598.

Lagerqvist B, Husted S, Kontny F, Ståhle E, Swahn E, Wallentin L, et al. (2006). 5-year outcomes in the FRISC-II randomised trial of an invasive versus a non-invasive strategy in non-ST-elevation acute coronary syndrome: a follow-up study. *Lancet, 368*(9540), 998-1004.

Laterza OF, Cameron SJ, Chappell D, Sokoll LJ, & Green GB (2004). Evaluation of pregnancy-associated plasma protein A as a prognostic indicator in acute coronary syndrome patients. *Clin Chim Acta, 348*(1-2), 163-169.

Laursen LS, Overgaard MT, Søe R, Boldt HB, Sottrup-Jensen L, Giudice LC, et al. (2001). Pregnancy-associated plasma protein-A (PAPP-A) cleaves insulin-like growth factor binding protein (IGFBP)-5 independent of IGF: implications for the mechanism of IGFBP-4 proteolysis by PAPP-A. *FEBS Lett, 504*(1-2), 36-40.

Laursen LS, Overgaard MT, Weyer K, Boldt HB, Ebbesen P, Christiansen M, et al. (2002). Cell surface targeting of pregnancy-associated plasma protein A proteolytic activity. Reversible adhesion is mediated by two neighboring short consensus repeats. *J Biol Chem, 277*(49), 47225-47234.

Lauzurica R, Pastor C, Bayés B, Hernández JM & Romero R (2005). Pretransplant pregnancy-associated plasma protein-a as a predictor of chronic allograft nephropathy and posttransplant cardiovascular events. *Transplantation, 80*(10), 1441-1446.

Lawrence JB, Oxvig C, Overgaard MT, Sottrup-Jensen L, Gleich GJ, Hays LG, et al. (1999). The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. *Proc Natl Acad Sci U S A, 96*(6), 3149-3153.

Li Y, Zhou C, Zhou X, Song L & Hui R (2013). PAPP-A in cardiac and non-cardiac conditions. *Clin Chim Acta, 417*, 67-72.

Libby P & Theroux P (2005a). Pathophysiology of coronary artery disease. *Circulation, 111*(25), 3481-3488.

Lin TM, Galbert SP, Kiefer D, Spellacy WN & Gall S (1974a). Characterization of four human pregnancy-associated plasma proteins. *Am J Obstet Gynecol, 118*(2), 223-236.

Lin TM, Halbert SP & Spellacy WN (1974b). Measurement of pregnancy-associated plasma proteins during human gestation. *J Clin Invest, 54*(3), 576-582.

Lindahl B, Venge P & Wallentin L (1996). Relation between troponin T and the risk of subsequent cardiac events in unstable coronary artery disease. The FRISC study group. *Circulation, 93*(9), 1651-1657.

Liu ZY, Zhang JY, Sun TW, Zhang YJ, Zhang L & Wang LX (2008). Levels of pregnancy-associated plasma protein A in patients with coronary artery disease. *Clin Invest Med, 31*(2), E85-89.

McCann CJ, Glover BM, Menown IB, Moore MJ, McEneny J, Owens CG, et al. (2008). Novel biomarkers in early diagnosis of acute myocardial infarction compared with cardiac troponin T. *Eur Heart J, 29*(23), 2843-2850.

McCann CJ, Glover BM, Menown IB, Moore MJ, McEneny J, Owens CG, et al. (2009). Prognostic value of a multimarker approach for patients presenting to hospital with acute chest pain. *Am J Cardiol, 103*(1), 22-28.

McManus DD, Gore J, Yarzebski J, Spencer F, Lessard D & Golbberg RJ (2011). Recent trends in the incidence, treatment, and outcomes of patients with STEMI and NSTEMI. *Am J Med*, 124(1),40–47.

Mei WY, Du ZM, Zhao Q, Hu CH, Li Y, Luo CF, et al. (2011). Pregnancy-associated plasma protein predicts outcomes of percutaneous coronary intervention in patients with non-ST-elevation acute coronary syndrome. *Heart Lung, 40*(3), e78-83.

Miedema MD, Conover CA, MacDonald H, Harrington SC, Oberg D, Wilson D, et al. (2008). Pregnancy-associated plasma protein-A elevation in patients with acute coronary syndrome and subsequent atorvastatin therapy. *Am J Cardiol, 101*(1), 35-39.

Mjelva OR, Brügger-Andersen T, Pönitz V, Grundt H, Kontny F, Staines H, et al. (2013). Long-term prognostic utility of PAPP-A and calprotectin in suspected acute coronary syndrome. *Scand Cardiovasc J, 47*(2), 88-97.

Moreno PR, Falk E, Palacios IF, Newell JB, Fuster V & Fallon JT (1994). Macrophage infiltration in acute coronary syndromes. Implications for plaque rupture. *Circulation, 90*(2), 775-778.

Morrow DA, Rifai N, Tanasijevic MJ, Wy benga DR, de Lemos JA, Antman EM (2000). Clinical efficacy of three assay for cardiac troponin I for risk stratification in acute coronary syndromes: a Thrombolysis In Myocardial Infarction (TIMI) 11B Substudy. *Clin Chem*, 46(4), 453-60.

Morrow DA, Cannon CP, Rifai N, Frey MJ, Vicari R, Lakkis N, et al. (2001). Ability of minor elevations of troponins I and T to predict benefit from an early invasive strategy in patients with unstable angina and non-ST elevation myocardial infarction: results from a randomized trial. *JAMA, 286*(19), 2405-2412.

Mueller C (2014). Biomarkers and acute coronary syndromes: an update. *Eur Heart J, 35*(9), 552-556.

Mueller T, Dieplinger B, Poelz W & Haltmayer M (2006). Increased pregnancy-associated plasma protein-A as a marker for peripheral atherosclerosis: results from the Linz Peripheral Arterial Disease Study. *Clin Chem, 52*(6), 1096-1103.

Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, et al. (2003a). From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part I. *Circulation, 108*(14), 1664-1672.

Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, et al. (2003b). From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part II. *Circulation, 108*(15), 1772-1778.

Nikus KC, Pahlm O, Wagner, G, Birnbaum Y, Cinca J, Clemmensen P, et al. (2010). Electrocardiographic classification of acute coronary syndromes: a review by a committee of the International Society for Holter and Non-Invasive Electrocardiology. *J Electrocardiol, 43*(2), 91-103.

Nikus KC, Eskola MJ, Virtanen VK, Vikman S, Niemelä KO, Huhtala H, et al. (2004). ST-depression with negative T waves in leads V4-V5--a marker of severe coronary artery disease in non-ST elevation acute coronary syndrome: a prospective study of Angina at rest, with troponin, clinical, electrocardiographic, and angiographic correlation. *Ann Noninvasive Electrocardiol, 9*(3), 207-214.

Nikus KC, Sclarovsky S, Huhtala H, Niemelä K, Karhunen P & Eskola MJ (2012). Electrocardiographic presentation of global ischemia in acute coronary syndrome predicts poor outcome. *Ann Med*, 44(5), 494-502.

Ottani F, Galvani M, Nicolini FA, Ferrini D, Pozzati A, Di Pasquale G, et al. (2000). Elevated cardiac troponin levels predict the risk of adverse outcome in patients with acute coronary syndromes. *Am Heart J, 140*(6), 917-927.

Overgaard MT, Haaning J, Boldt HB, Olsen IM, Laursen LS, Christiansen M, et al. (2000). Expression of recombinant human pregnancy-associated plasma protein-A and identification of the proform of eosinophil major basic protein as its physiological inhibitor. *J Biol Chem, 275*(40), 31128-31133.

Overgaard MT, Oxvig C, Christiansen M, Lawrence JB, Conover CA, Gleich GJ, et al. (1999). Messenger ribonucleic acid levels of pregnancy-associated plasma protein-A and the proform of eosinophil major basic protein: expression in human reproductive and nonreproductive tissues. *Biol Reprod, 61*(4), 1083-1089.

Oxvig C, Haaning J, Højrup P & Sottrup-Jensen L (1994). Location and nature of carbohydrate groups in proform of human major basic protein isolated from pregnancy serum. *Biochem Mol Biol Int, 33*(2), 329-336.

Oxvig C, Sand O, Kristensen T, Gleich GJ & Sottrup-Jensen L (1993). Circulating human pregnancy-associated plasma protein-A is disulfide-bridged to the proform of eosinophil major basic protein. *J Biol Chem, 268*(17), 12243-12246.

Parmacek MS & Leiden JM (1989). Structure and expression of the murine slow/cardiac troponin C gene. *J Biol Chem, 264*(22), 13217-13225.

Pasceri V, Willerson JT & Yeh ET (2000). Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation, 102*(18), 2165-2168.

Pellitero S, Reverter JL, Pizarro E, Pastor MC, Granada ML, Tàssies D, et al. (2007). Pregnancy-associated plasma protein-a levels are related to glycemic control but not to lipid profile or hemostatic parameters in type 2 diabetes. *Diabetes Care, 30*(12), 3083-3085.

Pham MX, Whooley MA, Evans GT, Liu, C, Emadi H, Tong W, et al. (2004). Prognostic value of low-level cardiac troponin-I elevations in patients without definite acute coronary syndromes. *Am Heart J, 148*(5), 776-782.

Polanczyk CA, Lee TH, Cook EF, Walls, R, Wybenga D, Printy-Klein G, et al. (1998). Cardiac troponin I as a predictor of major cardiac events in emergency department patients with acute chest pain. *J Am Coll Cardiol, 32*(1), 8-14.

Popken-Harris P, Checkel J, Loegering, D, Madden B, Springett M, Kephart, G, et al. (1998). Regulation and processing of a precursor form of eosinophil granule major basic protein (ProMBP) in differentiating eosinophils. *Blood, 92*(2), 623-631.

Qin QP, Christiansen M & Pettersson K. (2002) Point-of-care time-resolved immunofluorometric assay for human pregnancy-associated plasma protein A: use in first-trimester screening for Down syndrome. *Clin Chem, 48*(3), 473-483.

Qin QP, Kokkala S, Lund J, Tamm, N, Voipio-Pulkki LM & Pettersson K (2005). Molecular distinction of circulating pregnancy-associated plasma protein A in myocardial infarction and pregnancy. *Clin Chem, 51*(1), 75-83.

Qin QP, Laitinen P, Majamaa-Voltti K, Eriksson S, Kumpula EK & Pettersson, K (2002). Release patterns of pregnancy associated plasma protein A (PAPP-A) in patients with acute coronary syndromes. *Scand Cardiovasc J, 36*(6), 358-361.

Ravkilde J, Hørder M, Gerhardt W, Ljungdahl L, Pettersson T, Tryding N, et al. (1993). Diagnostic performance and prognostic value of serum troponin T in suspected acute myocardial infarction. *Scand J Clin Lab Invest, 53*(7), 677-685.

Rebuzzi AG, Quaranta G, Liuzzo G, Caligiuri G, Lanza GA, Gallimore JR, et al. (1998). Incremental prognostic value of serum levels of troponin T and C-reactive protein on admission in patients with unstable angina pectoris. *Am J Cardiol, 82*(6), 715-719.

Reichlin T, Hochholzer W, Bassetti S, Steuer S, Stelzig C, Hartwiger S, et al. (2009). Early diagnosis of myocardial infarction with sensitive cardiac troponin assays. *N Engl J Med, 361*(9), 858-867.

Resch ZT, Simari RD & Conover CA (2006). Targeted disruption of the pregnancy-associated plasma protein-A gene is associated with diminished smooth muscle cell response to insulin-like growth factor-I and resistance to neointimal hyperplasia after vascular injury. *Endocrinology, 147*(12), 5634-5640.

Ridker PM, Cushman M, Stampfer MJ, Tracy RP & Hennekens CH (1997). Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med, 336*(14), 973-979.

Ridker PM, Hennekens CH, Buring JE & Rifai N (2000). C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med, 342*(12), 836-843.

Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, et al. (2016). 2015 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: Task Force for the Management of Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation of the European Society of Cardiology (ESC). *Eur Heart J, 37*(3), 267-315.

Ross R (1993). The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature, 362*(6423), 801-809.

Rossen M, Iversen K, Teisner A, Teisner B, Kliem A & Grudzinskas G (2007). Optimisation of sandwich ELISA based on monoclonal antibodies for the specific measurement of pregnancy-associated plasma protein (PAPP-A) in acute coronary syndrome. *Clin Biochem, 40*(7), 478-484.

Rubini Giménez M, Hoeller R, Reichlin T, Zellweger C, Twerenbold R, Reiter M, et al. (2013). Rapid rule out of acute myocardial infarction using undetectable levels of high-sensitivity cardiac troponin. *Int J Cardiol, 168*(4), 3896-3901.

Sanchis J, Bosch X, Bodí V, Bellera, N, Núñez J, Benito B, et al (2008). Combination of clinical risk profile, early exercise testing and circulating biomarkers for evaluation of patients with acute chest pain without ST-segment deviation or troponin elevation. *Heart, 94*(3), 311-315.

Sangiorgi G, Mauriello A, Bonanno E, Oxvig C, Conover, CA, Christiansen M, et al. (2006). Pregnancy-associated plasma protein-a is markedly expressed by monocyte-macrophage cells in vulnerable and ruptured carotid atherosclerotic plaques: a link between inflammation and cerebrovascular events. *J Am Coll Cardiol, 47*(11), 2201-2211.

Savonitto S, Ardissino D, Granger CB, Morando G, Prando MD, Mafrici A, et al. (1999). Prognostic value of the admission electrocardiogram in acute coronary syndromes. *JAMA, 281*(8), 707-713.

Savonitto S, Cohen MG, Politi A, Hudson MP, Kong DF, Huang Y, et al. (2005). Extent of ST-segment depression and cardiac events in non-ST-segment elevation acute coronary syndromes. *Eur Heart J, 26*(20), 2106-2113.

Schaub N, Reichlin T, Meune C, Twerenbold R, Haaf P, Hochholzer W, et al. (2011). Markers of Plaque Instability in the Early Diagnosis and Risk Stratification of Acute Myocardial Infarction. *Clin Chem*, 58(1), 246-256.

Schoos M, Iversen K, Teisner A, Teisner, B, Thaning P, Kliem A, et al. (2009). Release patterns of pregnancy-associated plasma protein A in patients with acute coronary syndromes assessed by an optimized monoclonal antibody assay. *Scand J Clin Lab Invest, 69*(1), 121-127.

Schulz O, Reinicke M, Krämer J, Berghöfer G, Bensch R, Schimke I, et al. (2011). Pregnancy-associated plasma protein A values in patients with stable cardiovascular disease: use of a new monoclonal antibody-based assay. *Clin Chim Acta, 412*(11-12), 880-886.

Schwartz RS, Burke A, Farb A, Kaye D, Lesser JR, Henry TD, et al. (2009). Microemboli and microvascular obstruction in acute coronary thrombosis and sudden coronary death: relation to epicardial plaque histopathology. *J Am Coll Cardiol, 54*(23), 2167-2173.

Serteser M, Coskun A, Unsal I & Inal TC (2012). Biological variation in pregnancy-associated plasma protein-A in healthy men and non-pregnant healthy women. *Clin Chem Lab Med, 50*(12), 2239-2241.

Sinosich MJ, Teisner B, Davey M & Grudzinskas JG (1981). Pregnancy associated plasma protein-A: interaction with heparin in crossed affinity immunoelectrophoresis. *Aust N Z J Med, 11*(4), 429-433.

Smith EP, Kamyar A, Niu W, Wang J, Cercek B, Chernausek SD, et al. (2001). IGF-binding protein-4 expression and IGF-binding protein-4 protease activity are regulated coordinately in smooth muscle during postnatal development and after vascular injury. *Endocrinology, 142*(10), 4420-4427.

Smith R, Bischof P, Hughes G & Klopper A (1979). Studies on pregnancy-associated plasma protein A in the third trimester of pregnancy. *Br J Obstet Gynaecol, 86*(11), 882-887.

Steffensen LB, Poulsen CB, Shim J, Bek M, Jacobsen K, Conover CA, et al. (2015). Myocardial and Peripheral Ischemia Causes an Increase in Circulating Pregnancy-Associated Plasma Protein-A in Non-atherosclerotic, Non-heparinized Pigs. *J Cardiovasc Transl Res, 8*(9), 528-535.

Strandberg TE & Tilvis RS (2000). C-reactive protein, cardiovascular risk factors, and mortality in a prospective study in the elderly. *Arterioscler Thromb Vasc Biol, 20*(4), 1057-1060.

Stubbs P, Collinson P, Moseley D, Greenwood T & Noble M (1996a). Prognostic significance of admission troponin T concentrations in patients with myocardial infarction. *Circulation, 94*(6), 1291-1297.

Stubbs P, Collinson P, Moseley D, Greenwood T & Noble M (1996b). Prospective study of the role of cardiac troponin T in patients admitted with unstable angina. *BMJ, 313*(7052), 262-264.

Stulc T, Malbohan I, Malík J, Fialová L, Soukupová J & Ceska R (2003). Increased levels of pregnancy-associated plasma protein-A in patients with hypercholesterolemia: the effect of atorvastatin treatment. *Am Heart J, 146*(6), E21.

Syska H, Perry SV & Trayer IP (1974). A new method of preparation of troponin I (inhibitory protein) using affinity chromatography. Evidence for three different forms of troponin I in striated muscle. *FEBS Lett, 40*(2), 253-257.

Szumska M, Krystyna T, Wielkoszyński T, Długaszek M, Krywult A, Czubilińska J, et al. (2013). Evaluation of the influence of exposure to tobacco smoke on the concentration of the pregnancy-associated plasma protein A in the population of healthy men and non-pregnant women. *Przegl Lek, 70*(10), 813-817.

Terkelsen CJ, Oxvig C, Nørgaard BL, Glerup S, Poulsen TS, Lassen JF, et al. (2009). Temporal course of pregnancy-associated plasma protein-A in angioplasty-treated ST-elevation myocardial infarction patients and potential significance of concomitant heparin administration. *Am J Cardiol, 103*(1), 29-35.

Tertti R, Wittfooth S, Porela P, Airaksinen KE, Metsärinne K & Pettersson K (2009). Intravenous administration of low molecular weight and unfractionated heparin elicits a rapid increase in serum pregnancy-associated plasma protein A. *Clin Chem, 55*(6), 1214-1217.

Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, et al. (2012a). How to use high-sensitivity cardiac troponins in acute cardiac care. *Eur Heart J, 33*(18), 2252-2257.

Thygesen K, Alpert JS, Jaffe AS, Maarten L, Simoons ML, Bernard R, Chaitman BR, White HD, et al. (2012b). Third universal definition of myocardial infarction. European Heart Journal, 33(20), 2551–2567.

Tierala I, Nikus KC, Sclarovsky S, Syvänne M, Eskola M & Group HS (2009). Predicting the culprit artery in acute ST-elevation myocardial infarction and introducing a new algorithm to predict infarct-related artery in inferior ST-elevation myocardial infarction: correlation with coronary anatomy in the HAAMU Trial. *J Electrocardiol, 42*(2), 120-127.

Topol EJ & Yadav JS (2000). Recognition of the importance of embolization in atherosclerotic vascular disease. *Circulation, 101*(5), 570-580.

Toschi V, Gallo R, Lettino M, Fallon JT, Gertz SD, Fernández-Ortiz A, et al. (1997). Tissue factor modulates the thrombogenicity of human atherosclerotic plaques. *Circulation, 95*(3), 594-599.

Toss H, Lindahl B, Siegbahn A & Wallentin L (1997). Prognostic influence of increased fibrinogen and C-reactive protein levels in unstable coronary artery disease. FRISC Study Group. Fragmin during Instability in Coronary Artery Disease. *Circulation, 96*(12), 4204-4210.

van der Wal AC, Becker AE & Das PK (1994a). The expression of adhesion molecules on endothelial cells in atherosclerosis has a topographic relationship with the underlying inflammatory process. *Histopathology, 24*(2), 200-201.

van der Wal AC, Becker AE, van der Loos CM & Das PK (1994b). Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques is characterized by an inflammatory process irrespective of the dominant plaque morphology. *Circulation, 89*(1), 36-44.

van der Wal AC, Becker AE, van der Loos CM, Tigges AJ & Das PK (1994c). Fibrous and lipid-rich atherosclerotic plaques are part of interchangeable morphologies related to inflammation: a concept. *Coron Artery Dis, 5*(6), 463-469.

Venge P, Johnston N, Lindahl B & James S (2009). Normal plasma levels of cardiac troponin I measured by the high-sensitivity cardiac troponin I access prototype assay and the impact on the diagnosis of myocardial ischemia. *J Am Coll Cardiol, 54*(13), 1165-1172.

Volanakis JE (2001). Human C-reactive protein: expression, structure, and function. *Mol Immunol, 38*(2-3), 189-197.

von Haehling S, Doehner W, Jankowska EA, Ponikowski P, Stellos K, Puntmann VO, et al. (2013). Value of serum pregnancy-associated plasma protein A for predicting cardiovascular events among patients presenting with cardiac chest pain. *CMAJ, 185*(7), E295-303.

Wald N, Stone R, Cuckle HS, Grudzinskas JG, Barkai G, Brambati B, et al. (1992). First trimester concentrations of pregnancy associated plasma protein A and placental protein 14 in Down's syndrome. *BMJ, 305*(6844), 28.

Wang G, Zhang A, Han X, Zhang J, Zhang G & Sun L (2013). Effect of routine heparins treatment in acute coronary syndrome on serum pregnancy-associated plasma protein a concentration. *Ann Clin Lab Sci, 43*(3), 274-277.

Wang L, Jiang J, Du L, Zhang X & Wang C (2014). The prognostic value of serum pregnancy-associated plasma protein A, S100 and high sensitivity C-reactive protein in acute ischemic stroke patients without heparin administration. *Clin Biochem, 47*(16-17), 187-191.

Wang S, Jiang J, Qu C, Wang C & Sun Z (2017). Predictive value of serum pregnancy-associated plasma protein A for patients with ischemic cerebrovascular disease. *J Clin Lab Anal*, 31(5), e22091.

Wang S, Wang L, Zhang X, Wang C, Wu W, Dong Z, et al. (2011). Profile of serum pregnancy-associated plasma protein A after sustained subcutaneous low molecular weight heparin administration in patients with cerebrovascular diseases. *Clin Chem, 57*(3), 526-527.

Wilkinson JM & Grand RJ (1975). The amino acid sequence of troponin I from rabbit skeletal muscle. *Biochem J, 149*(2), 493-496.

Wilkinson JM & Grand RJ (1978). Comparison of amino acid sequence of troponin I from different striated muscles. *Nature, 271*(5640), 31-35.

Wittfooth S, Qin, QP, Lund J, Tierala, I, Pulkki K, Takalo H, et al. (2006). Immunofluorometric point-of-care assays for the detection of acute coronary syndrome-related noncomplexed pregnancy-associated plasma protein A. *Clin Chem, 52*(9), 1794-1801.

Wittfooth S, Tertti R, Lepäntalo M, Porela P, Qin QP, Tynjälä J, et al. (2011). Studies on the effects of heparin products on pregnancy-associated plasma protein A. *Clin Chim Acta, 412*(3-4), 376-381.

Wong P, Murray S, Ramsewak A, Robinson A, van Heyningen C & Rodrigues E (2007). Raised cardiac troponin T levels in patients without acute coronary syndrome. *Postgrad Med J, 83*(977), 200-205.

Wu XF, Liu XH, Dong JZ, Wang LY, Yang Y, Nie SP, et al. (2013). Pregnancy-associated plasma protein-A as a marker of culprit lesion instability in unstable angina patients: an intravascular ultrasound study. *Cardiology, 126*(4), 244-251.

Yan RT, Yan AT, Mahaffey KW, White HD, Pieper K, Sun JL, et al. (2010). Prognostic utility of quantifying evolutionary ST-segment depression on early follow-up electrocardiogram in patients with non-ST-segment elevation acute coronary syndromes. *Eur Heart J, 31*(8), 958-966.

Zengin E, Sinning C, Zeller T, Rupprecht HJ, Schnabel RB, Lackner KJ, et al. (2015). The utility of pregnancy-associated plasma protein A for determination of prognosis in a cohort of patients with coronary artery disease. *Biomark Med, 9*(8), 731-741.

Zhang J, Lambert-Messerlian G, Palomaki GE & Canick JA (2011). Impact of smoking on maternal serum markers and prenatal screening in the first and second trimesters. *Prenat Diagn, 31*(6), 583-588.

Zhao XY, Wang XF, Li L, Zhang JY, Du YY & Yao HM (2013). Plaque characteristics and serum pregnancy-associated plasma protein A levels predict the no-reflow phenomenon after percutaneous coronary intervention. *J Int Med Res, 41*(2), 307-316.

Annales Universitatis Turkuensis

Turun yliopisto
University of Turku

Painosalama Oy, Turku, Finland 2018

ISBN 978-951-29-7225-8 (PRINT)
ISBN 978-951-29-7226-5 (PDF)
ISSN 0355-9483 (PRINT) | ISSN 2343-3213 (PDF)