Clinical Biochemistry 48 (2015) 322–328

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

PAPP-A and IGFBP-4 fragment levels in patients with ST-elevation myocardial infarction treated with heparin and PCI

Rikke Hjortebjerg [a,b,*], Søren Lindberg [c], Søren Hoffmann [c], Jan S. Jensen [c,d], Claus Oxvig [e], Mette Bjerre [a], Jan Frystyk [a,f]

[a] Medical Research Laboratory, Department of Clinical Medicine, Faculty of Health, Aarhus University, Aarhus, Denmark
[b] The Danish Diabetes Academy, Odense, Denmark
[c] Department of Cardiology P, Gentofte University Hospital, Copenhagen, Denmark
[d] Institute of Clinical Medicine, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark
[e] Department of Molecular Biology and Genetics, Faculty of Science and Technology, Aarhus University, Aarhus, Denmark
[f] Department of Endocrinology and Internal Medicine, Aarhus University Hospital, Aarhus, Denmark

ARTICLE INFO

Article history:
Received 22 August 2014
Received in revised form 4 November 2014
Accepted 27 November 2014
Available online 6 December 2014

Keywords:
Acute coronary syndrome (ACS)
Biomarker
Cardiovascular disease
Heparin
Insulin-like growth factor (IGF)
IGF binding protein-4 (IGFBP-4)
IGFBP-4 fragment
Pregnancy-associated plasma protein-A (PAPP-A)
ST-segment elevation myocardial infarction (STEMI)

ABSTRACT

Objectives: Circulating levels of pregnancy-associated plasma protein-A (PAPP-A) predict outcome in patients with acute coronary syndrome (ACS). Unfortunately, administration of heparin to patients with ACS increases circulating PAPP-A, probably by a detachment of PAPP-A from cell surfaces, inducing a considerable bias when using PAPP-A as a biomarker. It remains unknown whether PAPP-A-derived N- and C-terminal fragments of insulin-like growth factor binding protein-4 (NT-IGFBP-4/CT-IGFBP-4) are acutely affected by the increase in PAPP-A.

Methods: We prospectively included 78 patients with ST-segment elevation myocardial infarction (STEMI) treated with percutaneous coronary intervention (PCI). Prior to PCI, patients were injected with 10,000 IU of unfractionated heparin (UFH). Blood samples were collected immediately before PCI, but after UFH-injection, immediately after PCI and on day 1 and day 2.
Plasma IGFBP-4, CT-IGFBP-4 and NT-IGFBP-4 levels were determined by specific, novel immunoassays, and PAPP-A and IGF-I by commercial immunoassays.

Results: Plasma PAPP-A was strongly elevated upon STEMI, UFH-administration and PCI with mean concentrations (95%-confidence interval) pre-PCI, post-PCI, day 1, and day 2 of 13.0 (11.2;15.2), 14.8 (13.1;16.8), 1.03 (0.90;1.18), and 1.08 (0.92;1.28) μg/L, respectively ($p < 0.0001$). Pre-PCI concentrations of IGFBP-4, CT-IGFBP-4 and NT-IGFBP-4 were 154 (142;166), 53 (47;60) and 136 (122;150) μg/L, and levels were unaltered post-PCI. Concentrations increased on day 1 by 63 (43;87)%, 69 (36;110)%, and 47 (21;79)%, respectively ($p < 0.0001$), i.e. at a time point when PAPP-A levels had normalized.

Conclusion: Plasma IGFBP-4-fragment levels are not acutely altered in patients with STEMI treated with UFH and PCI. Thus, they possess potentials as prognostic markers in ACS patients.

Introduction

The metzincin metalloproteinase pregnancy-associated plasma protein-A (PAPP-A) has emerged as a candidate biomarker for acute coronary syndrome (ACS). Elevated circulating levels of PAPP-A are associated with ACS, reflect vulnerability of atherosclerotic plaques and predict clinical outcome in these patients [1,2]. Of notice, PAPP-A is ubiquitously present in eroded and ruptured plaques [1]. However, the clinical application of PAPP-A as a prognostic biomarker is hampered by technical and analytical difficulties.

In recent studies, intravenous administration of unfractionated heparin (UFH) and low molecular weight heparin (LMWH) to patients with ACS has been demonstrated to elicit a rapid increase in the circulating concentrations of PAPP-A [3–5]. These observations made the authors

Abbreviations: ACS, acute coronary syndrome; CI, confidence interval; CPS, counts per second; CT, carboxy terminal; ECG, electrocardiogram; GAG, glycosaminoglycan; IGF-I, insulin-like growth factor I; IGF-IR, insulin-like growth factor I receptor; IGFBP, insulin-like growth factor binding protein; kDa, kilo Dalton; LAD, left anterior descending; LMWH, low molecular weight heparin; MACE, major adverse cardiac events; MI, myocardial infarction; NSB, non-specific binding; NT, amino terminal; PAPP-A, pregnancy-associated plasma protein A; PBS, phosphate buffered saline; PCI, percutaneous coronary intervention; ProMBP, proform of eosinophil major basic protein; rh, recombinant human; SD, standard deviation; STEMI, ST-segment elevation myocardial infarction; TR-IFMA, time-resolved immunofluorometric assay; UFH, unfractionated heparin; VSMC, vascular smooth muscle cells.

* Corresponding author at: Medical Research Laboratory, Department of Clinical Medicine, Faculty of Health, Aarhus University, Nørrebrogade 44, Building 3b, DK-8000 Aarhus C, Denmark. Fax: +45 7846 2150.
E-mail address: rikke.hjortebjerg@clin.au.dk (R. Hjortebjerg).

http://dx.doi.org/10.1016/j.clinbiochem.2014.11.022
0009-9120/

suggest that heparin was able to detach PAPP-A from the vascular tissue and release it into the circulation, hereby increasing circulating PAPP-A concentrations [3,5,6]. This important interaction between heparin and PAPP-A was not recognized when the first prognostic studies of PAPP-A were conducted [1,7,8]. However, as heparin constitutes a mainstay in the treatment of ACS, previous results on PAPP-A need to be interpreted with caution. This knowledge also raises concerns regarding the reliability and consequently, the clinical value of PAPP-A measurements.

The active, dimeric form of PAPP-A consists of two identical PAPP-A subunits, whereas the inactive, heterotetrametric form is composed of two PAPP-A subunits covalently linked to two inhibitory subunits of the proform of eosinophil major basic protein (proMBP) [9,10]. The inactive PAPP-A/proMBP complex is the principal form present in the circulation during pregnancy [11]. By contrast, in ACS patients PAPP-A appears to circulate without being complexed with proMBP and therefore, most likely to be enzymatically active [10–14]. Importantly, PAPP-A assays generally lack the ability to differentiate between enzymatically active and inactive forms [15,16].

PAPP-A is expressed by numerous cell types, enhancing local insulin-like growth factor-I (IGF-I) signaling by proteolysis of primarily IGF-binding protein 4 (IGFBP-4) [17]. IGFBP-4 binds IGF-I with high affinity and prevents IGF-I-mediated activation of the cell-surface attached IGF-I receptor (IGF-IR) [18]. Proteolysis of IGFBP-4 leads to IGF-I release and IGF-IR activation, and thus, in the presence of PAPP-A, IGFBP-4 may serve as an IGF-I donor [19,20]. Indeed, accumulating evidence supports that a local increase in active PAPP-A in atherosclerotic plaques increases free IGF-I and hereby promotes plaque vulnerability.

In its mature form, IGFBP-4 weights approximately 24 kDa. Upon proteolytic cleavage by PAPP-A, which takes place at a single site in the central domain, IGFBP-4 is divided into highly specific IGFBP-4 N-terminal (NT-IGFBP-4) and C-terminal (CT-IGFBP-4) fragments with molecular masses of 18 and 14 kDa, respectively [21]. The PAPP-A mediated cleavage of IGFBP-4 is believed to occur primarily outside the circulation and accordingly, the circulating concentrations of the two IGFBP-4 fragments most likely reflect the tissue-localized activity of PAPP-A [10]. Consequently, we speculate that quantification of PAPP-A generated IGFBP-4 fragments in plasma reflects the in vivo PAPP-A activity, thus solving the analytical difficulties related to PAPP-A measurements [22,23]. Indeed, this notion gains support from a recent study by Postnikov et al., who demonstrated that the two IGFBP-4 fragments may serve as markers of major adverse cardiac events (MACE) in patients presenting with ischemia [22]. However, that study was made under the assumption that IGFBP-4 fragment levels are unaffected by the clinical condition and the heparin-mediated increase in circulating PAPP-A, but at present, no data on these effects are available. Accordingly, with the present study we aimed to evaluate levels of IGFBP-4-fragments in patients with ST-segment elevation myocardial infarction (STEMI) treated with heparin and percutaneous coronary intervention (PCI).

Materials and methods

Study populations and blood samples

We prospectively included 78 patients with STEMI treated with primary PCI at Gentofte University Hospital, Denmark, from February 2008 through March 2011 (Table 1). Inclusion criteria were significant ST-segment elevations (≥2 mV for men and ≥1.5 mV for women in lead V_2–V_3, and/or >1 mV in the other leads) in at least two contiguous leads of the electrocardiogram (ECG); significant increases in Troponin I (TnI) > 0.03 ng/L; less than 12 h from onset of symptoms to PCI; single vessel disease with 100% thrombotic occlusion of the left anterior descending (LAD) branch of the left coronary artery and a successful PCI resulting in TIMI 3 flow. Exclusion criteria were a history of myocardial infarction (MI) or heart failure and multi-vessel diseases.

Table 1
Baseline characteristics. Values are presented as mean ± SD* (parametric data) or median [25th and 75th percentile] ** (non-parametric data), whereas dichotomous variables are indicated as number (n) of total patients.

Characteristics	Patients ($n = 78$)
Age (years)*	59 ± 12
Males/females (n)	66/12
Body mass index (kg/m^2)*	26.9 ± 5.2
Diabetes mellitus (n)	5
Hypertension (n)	15
Hypercholesterolemia (n)	9
Currently smoking (n)	36
Symptom to balloon time (min)**	162 [120–275]
Creatinine (μmol/L)*	73 ± 19

Patients were defined as diabetic, hypertensive or hypercholesterolemic if treated with anti-diabetic, blood pressure-lowering or cholesterol-lowering medications, respectively.

Blood samples were collected four times during the admission: from the femoral sheath immediately before injection of contrast fluid and PCI procedure, but after UFH injection; immediately after PCI, and from the cubital vein on the first and second day following PCI. In some patients, only the first two ($n = 10$) or three blood samples were obtained ($n = 29$). Missing values were due to random effects. EDTA-plasma was obtained after centrifugation at 3500 *g* for 10 min within 30 min, and serum was obtained after cloth formation followed by centrifugation. Samples were stored in NuncCryo tubes (Nunc) at −80 °C until analysis.

Healthy control subjects were included in the study for comparisons. Control EDTA-plasma for IGFBP-4 measurements was collected from 100 randomly selected Danish registered blood donors at Aarhus University Hospital (50 women and 50 men, with 25 subjects younger than 50 years and 25 subjects older than 50 years for each gender group). Control serum for PAPP-A measurements was collected from 150 adults (75 women and 75 men from 20 to 70 years, with 15 women and 15 men in each age decade) and has recently been described [24]. IGF-I reference levels in healthy controls (age 56–60 years) have recently been described elsewhere [25]. All donors were healthy and received no medication.

The study was approved by the local scientific ethical committee and the terms of the Declaration of Helsinki were met. Informed consent was acquired from all participants.

Percutaneous coronary intervention (PCI)

The PCI procedure was performed according to contemporary interventional guidelines. Patients were pre-treated intravenously with 10,000 IU of UFH, 300 mg acetylsalicylic acid, and 600 mg clopidogrel. Glycoprotein IIb/IIIa inhibitors were used at the discretion of the operator. The transfemoral approach was used with 6- or 8-French sheaths, conventional devices and Iomeron contrast fluid (Bracco). Subsequent medical treatment included anti-ischemic, lipid-lowering and antithrombotic drugs according to current treatment guidelines.

Immunoassays for intact IGFBP-4, NT-IGFBP-4 and CT-IGFBP-4

EDTA-plasma levels of IGFBP-4, NT-IGFBP-4 and CT-IGFBP-4 were determined by newly established time-resolved immunofluorometric assays (TR-IFMA) based on monoclonal antibodies (IgG) and recombinant calibrators generously provided by HyTest Ltd. (Turku, Finland). The assays were performed essentially as recently described by Postnikov et al. [22], although with a few alterations.

For full-length IGFBP-4, monoclonal anti-human IGFBP-4 antibodies were used for coating (Cat# 4IGF4 IBP182) and detection (Cat# 4IGF4EU IBP144), abbreviated MAb IBP182 and MAb IBP144, respectively.