Clinical Biochemistry 43 (2010) 851–857

Contents lists available at ScienceDirect

Clinical Biochemistry

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

Pregnancy-associated plasma protein-a, a marker for outcome in patients suspected for acute coronary syndrome☆

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ARTICLE INFO

Article history:
Received 23 January 2010
Received in revised form 29 March 2010
Accepted 30 March 2010
Available online 11 April 2010

Keywords:
Pregnancy-associated plasma protein A
Acute coronary syndrome
Prognosis

ABSTRACT

Objectives: To examine if pregnancy-associated plasma protein-A (PAPP-A) in patients with chest pain, could identify patients at risk for death or myocardial infarction.

Design and methods: Patients admitted with chest pain and both normal ECG and normal biomarkers were evaluated by serial measurement of PAPP-A. Main outcome measures were mortality and non-fatal myocardial infarction.

Results: Median age of patients included (415) was 67 years and 43% were women. The risk of death or non-fatal myocardial infarction after 3 months was 15% in the highest quartile of circulating PAPP-A compared with 3% in the lowest quartile (relative risk 3.7, $p<0.01$). Corresponding numbers after 1 year were 24% and 10% (relative risk 2.4, $p=0.01$).

Conclusion: In patients admitted with chest pain and both normal ECG and normal biomarkers PAPP-A seems to be valuable for predicting patients at high risk of death or non-fatal myocardial infarction.

Introduction

Management of patients presenting with clinical signs of acute coronary syndrome (ACS) without elevation of cardiac biomarkers or electrocardiographic signs of ischemia can be a clinical challenge. In general, the prognosis is considered to be relatively benign, but recent guidelines have paid increasing attention to the fact that some of these patients may have unstable plaques and therefore be at high risk of death or myocardial infarction. A marker of coronary plaque instability could potentially identify a subset of high-risk patients admitted with chest pain but without elevation of biomarkers or electrocardiographic changes.

Pregnancy-associated plasma protein A (PAPP-A) is a new and promising marker of unstable plaques in the coronary arteries. Since 2001, when Bayes-Genis and coworkers managed to stain PAPP-A from unstable, but not stable plaques [1], increasing evidence for the use of PAPP-A as a marker of unstable plaques has come to light [2–11]. Two studies have examined the role of PAPP-A in troponin-negative patients and found conflicting results [12,13]. The present study aimed to investigate the role of PAPP-A in predicting mortality and non-fatal myocardial infarction in patients presenting with signs of ACS but without elevation of biomarkers or ECG changes.

Method

Patients

Consecutive patients admitted to Amager University Hospital, Copenhagen, Denmark, between 3 January 2005 and 14 February 2006 with chest pain and suspicion of non-ST-segment elevation acute coronary syndrome (NSTE-ACS) were included in the study.

High-risk NSTE-ACS was diagnosed if patients presented with symptoms of ACS and had newly developed ischemic changes on the ECG (ST-depression or negative T-waves) and/or elevated troponin-T. The diagnostic criteria's are based on recommendations in recent guidelines [14]. ST-segment depression was defined as depression of the ST-segment compared with the isoelectric line of more than 1 mm in two contiguous leads. Negative T-waves were defined as negative

Abbreviations: ACS, acute coronary syndrome; PAPP-A, pregnancy-associated plasma protein A; NSTE-ACS, non-ST-segment elevation acute coronary syndrome; CKMB, creatine kinase isoenzyme MB; CI, confidence interval.

☆ There was no external funding for this study.

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doi:10.1016/j.clinbiochem.2010.03.018

deflections of the T-wave of more than 1 mm in two contiguous leads excluding aVR and V1. ECG's were analyzed by one of the investigators (KI) prior to analyses of PAPP-A. Information regarding other clinical variables was obtained from patient records.

All patients were treated wilt enoxaparin 1 mg/kg bid subcutaneously until high-risk NSTE-ACS was excluded (after 12-16 h).

Blood samples

Following admission, blood samples for analysis of PAPP-A, creatine kinase isoenzyme MB (CKMB) and troponin-T were drawn every 6–8 h, until levels of biomarkers of myocardial necrosis were found to be falling consistently (typically, from three sequential samples). The first sample was drawn prior to treatment with enoxaparin.

Samples for analysis of CKMB (Roche Diagnostics GmbH, Germany) and troponin-T (Roche Diagnostics GmbH) were analyzed in the local laboratory. Serum samples for PAPP-A measurements were centrifuged for 10 min at 3000 rpm and sera were stored at −20°C until analysis. Samples were stored for 12–18 months, since the stability of PAPP-A has previously been shown to be very high [15]. Sera were thawed only once. The diagnostic cut-offs of cardiac troponin-T were based on the lowest value with a coefficient variation <10%, as recommended in the current guidelines [14] (>0.03 μg/L for troponin-T). For patients with renal failure elevation of troponin-T was only considered diagnostic if there was a rise and fall in troponin-T concentration of more than 50%.

Timing of samples and other study details has been further described elsewhere [16].

Assay technique

The assay used to measure circulating PAPP-A was a sandwich ELISA technique based on two monoclonal antibodies reacting with distinct epitopes on the PAPP-A molecule developed on our own laboratory. Candidate monoclonal antibodies were identified by immunohistochemistry, Western blot and the absence of positive signals (ELISA) with normal, non-pregnant serum as antigen source. The ELISA technology was standardized against the original PAPP-A radioimmunoassay and the WHO reference preparation (WHO 78/610). Results different from those obtained by the original radioimmunoassay led to ELISA modifications with respect to dilution buffer and enzymatic digestion of the monoclonal antibodies. In order to avoid influence from human anti-animal IgG antibodies the dilution buffer contained 1% (v/v) bovine serum, and the indicator antibody was applied as biotinylated $F(ab')_2$ in order to avoid interference from rheumatory factors [17,18]. The development and characteristics of the assay have been described in detail elsewhere [18]. The detection threshold was 4.0 mIU/L. The intra-assay coefficient variations at 71.7 mIU/L and 10.4 mIU/L ($n=24$) were 2.0% and 5.7%, respectively, and the corresponding inter-assay coefficient variations ($n=14$) were 6.4% and 8.7%.

Follow-up

Information about death came from the Danish Central Civil Register, which records the vital status of all inhabitants. Information about admissions with myocardial infarction (ICD code I21.0–I21.9, 20.9) came from the Danish National Hospital Register, a database of all somatic hospital admissions.

Ethics

The study complied with the Helsinki Declaration II and was approved by the local ethics committee. Following oral and written provision of information, all patients gave written informed consent to their participation in the study.

Statistics

For the statistical analyses the highest measured PAPP-A value for each patients were used. Quartiles were constructed so identical values of PAPP-A were included in the lower quartile. Associations between categories of variables were measured by the χ^2 test and means of continuous data were compared using Student's independent samples *t*-test. Categorical data are presented as frequencies (percentages), and continuous variables are summarized as means (± 95% confidence interval [CI]). One-way ANOVA was used to compare the means of continuous clinical variables with respect to quartiles of circulating PAPP-A and to compare mean PAPP-A values in sample one, two and three. Kaplan–Meier plots were used to illustrate survival curves and the log-rank test was used for initial comparison. Univariate and multivariate comparisons were performed using a Cox proportional hazard model (fitted by backward elimination) after checking assumptions of proportionality. All variables in the univariate analyses were included in the multivariate model. Continuous variables are summarized as means and 95% CI, while categorical data are summarized as frequencies and percentages. All confidence intervals were constructed to have a coverage of 95%. Statistical calculations were performed with SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Serial PAPP-A samples were drawn from 538 consecutively admitted patients with NSTE-ACS. High-risk NSTE-ACS was confirmed in 123 patients (23%) and was accordingly excluded from further analyses. Of the remaining 415 patients (77%) one was lost to follow-up.

The median age was 67 years (range 20–92 years) and 179 patients (43%) were women. Three or more samples for circulating PAPP-A were drawn from 261 (63%) of the patients. Two samples were drawn from 123 patients (30%) and 31 patients (7%) had only one sample drawn. The number of samples with detectable PAPP-A was lower in the first sample 71 (20.1%) compared to the second 236 (60.6%) and third sample 212 (62.4%), $p<0.001$. Among samples with detectable PAPP-A there was no difference in mean PAPP-A between the first sample (10.7 mIU/L), second sample (10.3 mIU/L) and third sample (11.0 mIU/L), $p=0.83$ (see Fig. 1).

Analyses of the effect of time from onset of pain, divided in quartiles, on PAPP-A levels in the baseline sample was performed. They show that there were no difference in the percentage of samples with detectable PAPP-A ($p=0.42$) or the mean PAPP-A between samples ($p=0.29$), in the first quartile (0.5–3.7 h) 17/67 (25%) detectable, mean 19.5 mIU/L,

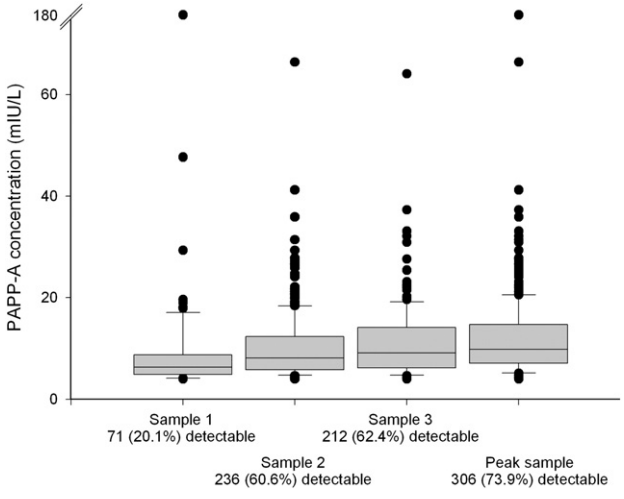

Fig. 1. Boxplot of PAPP-A levels in the first, second, third and peak sample. Boxes present 25–75 percentile, error bars present 10–90 percentile, dots present outliers.