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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 ☆

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Abstract

Background: Pregnancy-associated plasma protein-A (PAPP-A) concentrations predict outcome in patients with acute coronary syndromes. PAPP-A levels and PAPP-A/pro-MBP ratio are increased in chronic stable angina (CSA) patients with complex coronary artery stenoses. Little is known however, about the long-term prognostic value of PAPP-A and pro-MBP in "real-life" CSA patients. We sought to assess whether PAPP-A, the proform of eosinophil major basic protein (pro-MBP) and PAPP-A/pro-MBP levels predict long-term all-cause mortality in patients with CSA.
Methods: We recruited 663 consecutive patients (169 women [25.5%]; mean age 62.9±9.7 years) undergoing routine diagnostic coronary angiography. Samples for PAPP-A and pro-MBP were taken at study entry. Patients were followed for a median of 8.8 years (interquartile range 3 – 10.6 years).
Results: 106 patients (16%) died during follow-up. On a Cox proportional hazards model, increased PAPP-A concentration (>4.8 mIU/L) was an independent predictor of the occurrence of all-cause mortality (HR 1.953, 95% CI 1.135–3.36, $p=.016$). Neither pro-MBP nor PAPP-A/pro-MBP ratio were markers of all-cause mortality ($p=.45$ and .54, respectively).
Conclusions: High PAPP-A levels (>4.8 mIU/L) showed an association with all-cause mortality during long-term follow-up in patients with CSA.

Keywords: PAPP-A; pro-MBP; Long-term mortality; Chronic stable angina

1. Introduction

Circulating levels of pregnancy-associated plasma protein-A (PAPP-A), a zinc-binding metalloproteinase, are increased in patients with acute coronary syndromes (ACS), and represent a marker of adverse cardiovascular events in these patients. [1–3].

We have previously reported that circulating PAPP-A is increased in chronic stable angina (CSA) patients with extensive coronary artery disease (CAD) [4] and patients with angiographically complex coronary stenoses [5]. Recently, a study in selected patients showed that high PAPP-A levels correlated with the occurrence of death and ACS in patients with CSA [6]. The proform of eosinophil major basic protein (pro-MBP) is the endogenous inhibitor of the proteolytic activity of PAPP-A [7]. Low pro-MBP levels and/or an increased PAPP-A/pro-MBP ratio may be a marker of cardiovascular risk. Cosin et al demonstrated that a high PAPP-A/proMBP ratio is associated with the presence of vulnerable coronary stenoses in CSA patients [5]. In the present study we sought to assess whether

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PAPP-A levels and PAPP-A/pro-MBP ratio are markers of all-cause mortality during long term follow up in CSA patients.

2. Methods

2.1. Patients

This was a prospective study in 663 consecutive CSA patients undergoing routine diagnostic coronary angiography in our institution. CSA was defined as typical chest pain during exercise, relieved by rest and/or sublingual nitrates, with symptoms unchanged for at least 6 months before study entry. We did not include patients with any of the following: <12 weeks ACS, life threatening arrhythmias, acute or chronic liver disease, renal failure, and chronic inflammatory and/or immunological conditions.

2.2. Angiographic analyses

As described previously [4,5], during coronary angiography (Judkins technique) images of the coronary tree were obtained in routine standardized projections (Philips Integris 3000 system, Philips, Holland). In every coronary angiogram, we assessed the number of major coronary arteries showing ≥75% lumen diameter reduction ("vessel score") and quantified the extent of angiographic coronary atherosclerosis using the Sullivan scoring system [8]. The Sullivan "extension score" refers to the proportion of the coronary tree showing angiographically detectable atheroma. The observed proportion of atheroma in each vessel is multiplied by a factor that varies according to the artery involved: left main stem, 5; left anterior descending coronary artery, 20; main diagonal branch, 10; first septal perforator, 5; left circumflex artery, 20; obtuse marginal and posterolateral vessels, 10; right coronary artery, 20; and main posterior descending branch, 10. When the major lateral wall branch was a large obtuse marginal or intermediate, this was given a factor of 20, and left circumflex artery, a factor of 10. When a vessel was occluded and the distal bed was not fully visualized by collateral flow, the proportion of vessel not visualized was given the mean extent score of the remaining vessels. The scores for each vessel or branch were added to give a total score out of 100, representing the percentage of the coronary lumenal surface involved by atheroma. Interobserver agreement (calculated as intraclass correlation coefficient) regarding extension score in the present study was 97%.

2.3. Study end-point

The primary study endpoint was all-cause mortality. Follow-up data were available in 94% of patients. Follow-up information on mortality was obtained by a periodic review of the patients' clinical notes, postal questionnaires sent to the patients' general practitioners, and telephone contact with patients and/or their families. We limited our analysis to all cause mortality as this information could be obtained accurately during the long term follow up.

The study protocol, which is in accordance with the current revision of the Helsinki Declaration, was approved by the Local Research Ethics Committee, and all patients gave written informed consent before study entry.

2.4. Biochemical measurements

Fasting blood samples were obtained from every patient at the time of coronary angiography. Blood was drawn and centrifuged immediately and the serum was then aliquoted and stored at −80°C. PAPP-A levels were determined using a biotin–tyramide-amplified enzyme immunoassay with a limit of detection of 0.03 mIU/L. Intra-assay and inter-assay coefficients of variation were 10 and 15%, respectively. PAPP-A polyclonal antibodies were used for capture and a combination of monoclonal antibodies were used for detection. The assay detects and quantifies total PAPP-A (*free* PAPP-A plus the PAPP-A/proMBP complex) and was calibrated against the World Health Organization's International Reference Standard 78/610.

C-reactive protein (CRP) measurements were performed on the COBAS Integra (Roche Diagnostics Limited, Lewes, East Sussex, UK) using the CRP-Latex assay in both the high sensitivity application (analytical range 0.2–12 mg/L) and the normal application (analytical range 2–160 mg/L). Analytical precision of the high-sensitivity CRP-latex assay was 7.6% at a level of 1.02 mg/L, 3.3% at 1.79 mg/L, and 1.3% at a level of 4.36 mg/L. Samples outside the analytical range of the high sensitivity CRP-Latex assay were analysed by the CRP-Latex in the normal application. Analytical precision of the normal CRP-latex assay was 2.4% at a level of 29.5 mg/L and 1.3% at a level of 113 mg/L. Left ventricular ejection fraction (LVEF) was assessed in 563 patients (79%).

Total pro-MBP levels were assessed in 385 patients with an immunoassay developed at the Statens Serum Institute, Copenhagen. Within the calibrator range used, the interassay variation coefficient was <5% [9].

2.5. Statistical analysis

In this study involving 663 patients, the statistical power was 95% to detect a difference of 12 percent units (4th quartile vs 1st quartile) assuming an expected event rate of 8% in subjects in the first PAPP-A quartile -based on previous pilot work from our group- and a type I error of 0.05. Differences in variables between groups were assessed using the Chi-Square test or Fisher's exact test for categorical variables and Student *t* test or the Mann–Whitney test for continuous variables, as appropriate. The Spearman two way test was used to assess the relation between two quantitative variables with non-normal distribution. Long-term mortality was analyzed by the Kaplan–Meier method, and differences were compared with log-rank (Cox–Mantel) test. We tested the proportional hazard assumption with the Schöenfeld residuals test and visual plots, and appeared valid for all analysis. Variables included in the multivariable analysis (covariates) were those that showed a significant association both with the study endpoint and higher values of PAPP-A (Group 2=PAPP-A >4.6 mIU/L) on univariate analysis ($p<.05$), variables that showed a trend ($p<.10$) towards an association, and variables considered to be of clinical relevance (gender, type 2 diabetes mellitus, revascularization at index hospitalization and CRP). In patients in whom ejection fraction was not measured, the value was imputed with the sample median to include all subjects in the model. The backward stepwise likelihood ratio was used to derive the final model for which significance levels of .1 and .05 were chosen to exclude and include terms, respectively. The hazard ratio (HR) and their 95% confidence interval (CI) were calculated with a proportional Cox multivariable regression model. χ^2 statistic analysis was performed to assess the importance of each covariate in the final multivariable model. We used a multivariate fractional polynomial approach to test the probability of a threshold effect. Logarithmic transformation was performed to normalize the distribution of CRP. The Harrell's C-statistic (equivalent to the area under the Receiver Operating Characteristic curve) was calculated for the model. Tests were considered to be statistically significant if the null hypothesis could be rejected with ≥95% confidence. Statistical analyses were performed with SPSS 13.0 (SPSS Inc., Chicago, IL).

3. Results

Six-hundred and sixty-three CSA patients (mean age 62.9± 9.7 years, 494 (74.5%) male) were recruited. Patients were followed for a median of 8.8 years (interquartile range 3–10.6 years). Table 1 shows demographic, clinical, angiographic and biochemical data of all patients included in the study. PAPP-A levels were *a priori* stratified in quartiles (1st quartile ≤ 4.6 mIU/L; 2nd quartile 4.6–5.6 mIU/L; 3rd quartile 5.6–6.8 mIU/L; 4th quartile ≥6.8 mIU/L). Analysis of PAPP-A quartiles in relation to survival with Kaplan–Meier curves (Fig. 1) showed little, if any, difference in the cumulative mortality rate between patients in quartiles 2, 3 and 4 suggesting the presence of a threshold effect between patients in the 1st quartile and those in the remaining quartiles (log rank=9.39, $p=.002$). Consequently, patients were subdivided into two groups according to the 25th percentile, i.e. Group 1 comprised patients in the 1st PAPP-A quartile, and Group 2, patients