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Low protein Z plasma level is a risk factor for acute myocardial infarction in coronary atherosclerosis disease patients

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Abstract

Objectives—To examine plasma protein Z (PZ) levels in acute myocardial infarction (AMI) and chronic coronary atherosclerosis disease (CCAD) patients without history of AMI and explore its potential clinical significance.

Methods—Plasma PZ concentrations were measured in 90 AMI patients (Group A), 87 CCAD patients without AMI history who remained free of major clinical events at least one year (Group B), and 88 clinically healthy controls (Group C).

Results—PZ was found to be significantly lower (P<0.001) in A (1508.5 ± 486.2 ng/mL) compared with B (1823.0 ± 607.8 ng/mL) and C (2001.7 ± 733.0 ng/mL) groups and in A+B compared with C Group (A+B 1663.1±570.0, P<0.001). No statistically significant difference was reached between B and C groups (P=0.081). PZ level was significantly correlated with concentration of creatine kinase MB, high sensitive-cardiac troponin T, high sensitive C reactive protein, D-dimer and coagulation factor II and may be a useful predictor for AMI (OR: 1.38, 95% CI: 1.13-1.77, P=0.03). Subgroup analysis showed PZ concentration below the lowest tertile (< 1398 ng/mL) had a significantly increased risk for AMI and CCAD (OR: 3.39; 95% CI: 1.12-10.31; P=0.03 and OR: 7.39; 95% CI: 2.62-20.79; P<0.001 respectively).

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Authors' contributions: B. Liu, J. Luo, L. Dai, J. Zhao, H. Li and D. Wang enrolled the patients and collected clinical data and samples. B. Liu, Y. Li and Q. Jie performed the experiments. Y. Wei and X. Huang designed the study. B. Liu, Y. Li, Y. Wei and X. Huang analyzed the data and wrote the manuscript.

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Conclusions—PZ deficiency is found in AMI patients and could potentially reflect the myocardium injury, local coagulation activation and inflammation response during the acute phase of coronary atherosclerosis disease.

Keywords

Blood coagulation; Coronary atherosclerosis; Myocardial infarction; Plasma Protein Z; Inflammation

Introduction

Protein Z (PZ) is a plasma vitamin K dependent glycoprotein sharing similar structure with other vitamin K dependent coagulant factors like factors VII, IX, X, protein C and protein S, but with a protease domain lacking enzymatic activity due to replacement of the Ser and His residues in the typical catalytic triad of a serine protease active site (1, 2). In the coagulation process, PZ acts as a cofactor for the anticoagulant protein, protein Z-dependent protease inhibitor (ZPI), by accelerating the rate at which ZPI inhibits FXa by 3 orders of magnitude in the presence of phospholipid and calcium(3, 4). The importance of the PZ/ZPI anticoagulant system is evidenced by the findings that PZ or ZPI deficient mice show a significantly increased thrombosis after vascular injuries, and a severe thrombosis phenotype when combined with FV Leiden(5).

Plasma PZ and ZPI levels vary over a broad range. There are many factors that could affect PZ and ZPI levels. Stable warfarin therapy was found to reduce the PZ level significantly(6). ZPI/PZ gene mutations and polymorphisms could also influence the PZ and ZPI plasma levels(7, 8).

Because PZ and ZPI deficiency has been linked with a procoagulant state, many studies have tried to correlate reduced PZ and ZPI levels with the development of arterial vascular and venous thromboembolic diseases. However, the results were not always consistent and many researchers have reported conflicting results: some reported low ZPI and PZ levels were correlated with venous thrombosis, whereas others reported no such correlation could be established, as reviewed by Bafunno et.al. (9). There are conflicting reports about PZ levels in ischemic stroke. Kobelt and Mcquillan et.al. reported that increased plasma PZ level was related with ischemic stroke (10, 11), whereas Vasse et.al. found low level PZ was related (12), or no association could be detected as reported by Lopaciuk et.al. (13).

PZ was also found involved in the development of arterial atherosclerotic diseases. There are three reports showing low levels of PZ are correlated with acute coronary syndrome (ACS) including acute myocardial infarction (AMI) and unstable angina (UA) (14-16), while two other reports showing no significant association could be detected (17, 18). This study has measured PZ levels in AMI and chronic coronary atherosclerosis disease (CCAD) patients without AMI history, with the aim to further explore its clinical significance and evaluate its role in the development of atherosclerotic diseases.

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Materials and methods

Study population

The study population was constituted by three groups: From October 2014 to July 2015, 90 consecutive AMI patients who had undergone primary percutaneous coronary intervention (PCI) at the Catheterization Laboratory of Department of Cardiology, Shanghai Tenth People's Hospital, were enrolled into AMI group (Group A). A total of 87 patients for follow-up examinations in our department who were clinically diagnosed as CCAD but without a history of AMI and who remained free of a major clinical event for at least one year were enrolled as CCAD group (Group B). The clinical symptoms of CCAD patients were well controlled by oral medications and were free from hospitalization treatment for at least one year. Population in healthy control group were 88 clinically healthy subjects for routine physical examination in outpatient department (Group C). All subjects in these groups were comparable for age and gender, respectively. None of the subjects had liver or renal dysfunction and patients under warfarin therapy were excluded.

Patient demographics, including risk factors for coronary atherosclerosis disease (CAD) (age, gender, hypertension, smoking status, diabetes mellitus and lipid disorders), drug therapy, left ventricular ejection fraction (LVEF), high sensitive cardiac troponin T (hs-cTnT), creatine kinase MB (CK-MB), high sensitive C reactive protein (hs-CRP) and coagulant parameters including activated partial thromboplastin time (APTT), prothrombin time (PT), PT ratio (the ratio of a patient's prothrombin time to a normal sample), D-dimer and coagulation factor II (FII, prothrombin) were also documented. Patients were treated according to standard clinical protocols included aspirin, clopidogrel, intravenous nitrates, statins, β-blockers, angiotensin-converting enzyme (ACE) inhibitors/angiotensin II receptor blockers (ARB) where appropriate. Early invasive management, including emergency coronary angiography (CAG) within 4 h of admission followed by PCI, was routinely performed on AMI patients. Patients with 1) autoimmune, malignant or infectious diseases or diseases of the connective tissue within the previous month; 2) liver and renal dysfunction; 3) warfarin therapy; 4) anemia (hemoglobin < 90 g/L); and 5) major operations or trauma within the previous 3 months were excluded. AMI was diagnosed according to criteria established by the American College of Cardiology and the European Society of Cardiology (19): typical elevated and gradual fall cTnT concentration above the 99th percentile of the upper reference limit (hs-cTnT > 0.014 ng/mL), with an acute onset of typical ischemic angina, or surface ECG showing at least one of the following: ST-segment elevation (≥ 0.2 mV in men or ≥ 0.15 mV in women in leads V2-V3 and/or ≥ 0.1 mV in other leads), ST-segment depression ≥ 0.05 mV or T-wave inversion ≥ 0.1 mV in at least two contiguous leads. CCAD referred to patients with a diagnosis of CAD but in a stable phase including stable angina, normal coronary angina syndrome, asymptomatic myocardial ischemia, ischemic cardiomyopathy. The patients were diagnosed as CCAD based on previous hospitalization and out-patient documents such as symptoms, medications and biophysical investigations. This study complies with World Medical Association's Declaration of Helsinki and was approved by the Ethics Committee of Shanghai tenth people's hospital. All patients recruited in the current study gave written informed consent.

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CAG

CAG was performed using a standard Judkins technique or through a radial approach. Luminal diameter narrowing > 50% in a major epicardial coronary artery was defined as clinical significance. Multi-vessel disease was diagnosed as the presence of lesions in ≥ 3 coronary vessels. The presence of occlusion in the main and secondary branch of a vessel was defined as single-vessel disease. Coronary flow over the culprit lesion was graded according to the Thrombolysis in Myocardial Infarction Trial (TIMI) criteria (20).

Blood sample

Blood samples were collected from the antecubital vein into evacuated plastic tubes containing 0.109 mol/L sodium citrate at admission to the Catheterization Laboratory for emergency reperfusion therapy (Group A) or in the morning after at least 4 hours of fat fasting and before 10 a.m. (Group B and C and Group A patients after PCI procedure). All blood samples were centrifuged at 3000× g for 10 min at 4°C, and then the plasma samples were obtained and frozen at -80°C before processing.

Protein Z assay

Quantitative measurement of PZ in the plasma was performed using an Enzyme-linked Immunosorbent Assay (ELISA) Kit (USCN Life Science Inc., China) and followed the manufacturer's instructions. Samples from the same patient at different time intervals (pre-operation or post-operation) were assessed in the same plate at the same time and using an internal control sample assessed in duplicate to validate our results. The experiment was repeated at least three times.

Definition of risk factors

Hypertension was diagnosed when systolic blood pressure/diastolic blood pressure was ≥ 140/90 mmHg or if patients were treated with hypotensive drugs. Diabetes mellitus was diagnosed based on a fasting plasma glucose ≥ 7.0 mmol/L, or random plasma glucose ≥ 11.1 mmol/L. Lipid disorders were defined as the presence of total cholesterol ≥ 5.7 mmol/L, or LDL ≥ 3.6 mmol/L, or HDL < 1.04 mmol/L, or being actively treated with anti-hyperlipidemic agents. Tobacco use was defined by using ≥ 1 pack (20 cigarettes) per day at least 1 year.

Statistical analysis

Continuous variables are expressed as the mean ± standard deviation, and categorical variables as a percentage. Distributions of samples were tested by Shapiro-Wilk test. Differences between groups were determined using t-Student test for independent samples with normal distribution and Mann-Whitney test for nonparametric samples. Pearson correlation analysis was done for identifying the correlation between parameters. We also divided PZ levels into tertiles according to the distribution of plasma PZ concentration of all the enrolled participants (first tertile: <1398 ng/mL; second tertile: 1398-1926 ng/mL; third tertile: > 1926 ng/mL) in order to test if decreasing PZ was a risk factor. The highest tertile PZ distribution was used as the reference and Group C as the reference patients. The risks for incidence of AMI or CCAD were assessed in a logistic regression analysis. In the

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univariate logistic regression model, only tertiles of PZ served as the independent variable and AMI or CCAD as the dependent variable. For the multivariate logistic regression model, other variables were entered into the model in a step-wise procedure. Data were analyzed using the software program SPSS 16.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$, which is two-sided, was considered significant.

Results

Baseline characteristics of study subjects and plasma PZ level

Among the 396 patients inquired initially, 32 were not eligible and 61 not interested in the study. During the period of data collection, 14 patients were no longer interested and 24 patients with missing data. The mean age of the final enrolled 265 patients was 65.3 ± 12.0 years, 62.3% were men. The demographic characteristics and risk factors for CAD of different groups, PZ levels and other parameters are listed in Table 1. The CAD risk factors such as age and gender were not significantly different among the three groups. Hypertension, lipid disorders, diabetes mellitus, tobacco use were not significantly different between Groups A and B. However, they were significantly higher than Group C. Plasma PZ level was found to be significantly lower in Group A (1508.5 ± 486.2 ng/mL) than in Group B and Group C (Group B: 1823.0 ± 607.8 ng/mL ; Group C: 2001.7 ± 733.0 ng/mL) (both $P < 0.001$). The PZ level in Group B was also relatively lower than that in Group C but did not reach statistical significance ($P = 0.08$). In addition, combining Group A and Group B together, PZ level was also found to be significantly lower compared with Group C (Group A+B: 1663.1 ± 570.0 ng/mL, $P < 0.001$, Figure 1). Among the AMI patients (Group A), there existed no significant difference in plasma PZ level between pre- and post-PCI (1508.5 ± 486.2 vs. 1505.4 ± 522.5 ng/mL; $P = 0.21$; Figure 1).

Comparison of PZ level in patients with or without risk factors of CAD

We also investigated the relationship between plasma PZ level and several risk factors of CAD, such as gender, hypertension, diabetes mellitus, lipid disorders, and smoking habit. Among all enrolled in the study population, 105 were with hypertension, 62 were with diabetes mellitus, 48 were with lipid disorders, and 108 patients had a smoking habit for at least one year. According to the 2013 American College of Cardiology Foundation/ American Heart Association Guideline for the management of patients with ST-elevation myocardial infarction (STEMI), old age (≥ 65 years) is a major risk factor of CAD (21). Thus, we also reclassified the research subjects into two groups, ≥ 65 years (n=119) and < 65 years (n = 146). We analyzed the differences in PZ level between these groups with or without above-mentioned risk factors. However, whether the subjects possessed any of these risk factors or not, no significant difference in plasma PZ concentration was detected (all $P > 0.05$).

The relationship between plasma PZ level and CAG results as well as myocardial indicators

Of the 90 AMI patients, 68 showed right coronary artery dominance, 18 had a balanced coronary system and 4 showed left coronary artery dominance. Approximately half of the patients were diagnosed as 3-vessel disease and 57 vessels were graded into the 70-90%

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group among all coronary lesions. The pre procedural TIMI flow of most patients was classified into grade 0, and the post procedural TIMI flow of most AMI patients was evaluated as grade 3. Levels of hs-cTnT (Group A: 0.79 ± 0.67 ng/mL; Group B: 0.007 ± 0.002 ng/mL; Group C: 0.007 ± 0.002 ng/mL, P < 0.001, Table 1) and CK-MB (Group A: 94.0 ± 69.7 U/L; Group B: 13.5 ± 5.2 U/L; Group C: 13.8 ± 4.4 U/L, P < 0.001, Table 1) were apparently higher in Group A compared with that of Groups B and C. We assessed the correlation between the level of PZ and the degree of coronary stenosis, as well as the number of coronary lesions. However, the results indicated no statistical significance. Further, we conducted correlation analysis between PZ and other AMI-related parameters including hs-cTnT, CK-MB, LVEF, degree of coronary stenosis and number of coronary lesions, respectively. Plasma PZ concentration was found to be significantly inversely correlated with hs-cTnT (r = -0.21; P = 0.001) and CK-MB (r = -0.13; P = 0.03) (Table 2).

The relationship between plasma PZ, coagulation factor II level and hs-CRP

Hs-CRP value <1, 1-3, and >3mg/L indicate lower, average or higher relative cardiovascular risk (22). We detected a significantly higher hs-CRP level in Group A than in Group B and C (Group A: 6.3 ± 2.0 mg/L; Group B: 2.9 ± 1.6 mg/L; Group C: 2.7 ± 1.6 mg/L; P < 0.001, Table 1). The hs-CRP of group A patients was significantly correlated with plasma PZ level (r = -0.25, P = 0.02, Table 3). More than 60% patients and subjects (including Group A, B and C) were with a hs-CRP value > 3 mg/L, and hs-CRP also significantly correlated with PZ level in these patients (r =-0.18; P = 0.03). No significant correlation was detected between hs-CRP and PZ level in Group B, Group C patients and in patients and control subjects with a hs-CRP value 1-3 mg/L as well as < 1 mg/L. PZ level in patients with a hs-CRP value > 3 mg/L was significantly lower than in patients with a hs-CRP value 1-3 mg/L and with a hs-CRP< 1mg/L (1710.4 ± 628.1 mg/L vs. 1874.7 ± 666.7 mg/L; P = 0.02). On the other hand, no correlation was found between coagulation factor II with hs-CRP level in group A, B and C subjects (r=0.025, P=0.81; r=0.186,P=0.08; r=-0.115,P=0.28 for group A,B and C, respectively).

The relationship between PZ level and coagulation parameters

The concentration of D-dimer was significantly higher in Group A than in Group B and Group C (Group A: 343.5 ± 130.0 ng/mL; Group B: 140.1 ± 71.0 ng/mL; Group C: 136.5 ± 73.1 ng/mL; P < 0.001, Table 1). The coagulation factor II was significantly lower in Group A compared with Group B and Group C (Group A: 104.3 ± 30.0 μg/mL; Group B: 134.9 ± 29.4 μg/mL; Group C: 139.5 ± 41.0 μg/mL; P < 0.001,Table 1). Among the three groups, PT decreased from Group A, B to C patients, although the difference did not reach statistical significance (P = 0.17, Table 1). The PT ratio (> 1.2) was relatively higher in Group A compared with Group C (P = 0.02, Table 1). The proportion of patients with abnormal PT ratio was relatively higher in group A than in Group B and C, although no statistical significance was reached. Further correlation analysis showed that plasma PZ level was significantly correlated with D-dimer (r = -0.29,-0.43,-0.56 for Group A,B,C, respectively; P < 0.001) and with coagulation factor II (r = 0.51, 0.48, 0.60 for Group A,B and C, respectively; P < 0.001) (Table 4). No significant correlation was detected between PZ and PT, PT ratio or APTT. The ratio of PZ/FII was not significantly different in all groups (P=0.16, Table 1)

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Plasma PZ level was independently associated with AMI

We performed a step-wise logistic regression model analysis including age, gender, hypertension, diabetes mellitus, lipid disorders, tobacco use, LVEF, hs-CRP, hs-cTnT, CK-MB, PZ, PT, APTT, D-dimer, and coagulation factor II. Diabetes (OR: 2.67; 95% CI: 1.27-5.63; P = 0.01), hs-cTnT (OR: 0.36; 95% CI: 0.21-0.60; P < 0.001), CK-MB (OR: 0.50; 95% CI: 0.34-0.72; P < 0.001), hs-CRP (OR: 0.11; 95% CI: 0.03-0.36; P < 0.001), PZ (OR: 1.38; 95% CI: 1.13-1.77; P = 0.03), D-dimer (OR: 0.83; 95%CI: 0.79-0.89; P < 0.001) and coagulation factor II (OR: 0.79; 95%CI: 0.68-0.90; P =0.01) were significantly related to the incidence of AMI (Table 5). We furthermore detected the crude and adjusted risks of different tertiles of PZ levels for AMI or CCAD in the studied population, and the results showed that the PZ level in the first tertile had a significantly increased risk for AMI and CCAD (OR: 3.39; 95% CI: 1.12-10.31; P=0.031 and OR: 7.39; 95% CI: 2.62-20.79; P<0.001 respectively, Table 6).

Discussion

The present study has investigated the role of PZ in CAD patients with or without AMI. Our data showing that the plasma PZ level is relatively lower in CAD patients and low PZ may be a risk factor for AMI in CAD patients without AMI history, reinforces the role of PZ in the acute phase of CAD. In addition, we found the PZ level was inversely correlated with CK-MB, cTnT, and CRP to a statistically significant extent, suggesting PZ may play a role in the myocardium injury and inflammatory state of AMI. Moreover, PZ was also significantly correlated with coagulant parameters such as D-dimer and coagulation factor II, which indicated PZ may be also involved in the coagulation activation during occurrence of AMI. Interestingly, the data showed a progressive decrease of the PZ level from the healthy group to the CCAD group and then to the AMI group, the most severe type of CAD, although the difference between the CCAD and healthy groups was not statistically significant. Tertile analysis further showed that in all studied populations, PZ level in first tertile was associated with both AMI and CCAD. The results showed no difference of PZ levels before and after patients underwent the PCI procedure.

Three previous studies also have shown that lower PZ was found in ACS patients (contain both AMI and UA) (14-16). For stable CAD, Sofi et al observed the PZ level was significantly decreased compared with both healthy subjects and ACS patients that include AMI and UA patients. Our data showed AMI patients have a lower PZ level than Group B patients (similar to Sofi's stable CAD group, but without AMI history), but the PZ level of Group B was not significantly lower than the healthy subjects (Group C). The reason for such a discrepancy appears multifactorial. One reason is that the subjects we enrolled in our Group B (CCAD patients) are the stable CAD patients without a history of AMI, whereas in Sofi' s stable CAD group the patients with a history of AMI were ~55%. In addition, we only included the AMI patients in our AMI group, whereas Sofi's ACS group contained both AMI (56%) and UA (44%) patients (15). Therefore, patients we enrolled in our Group A (AMI patients) may represent a more developed stage of atherosclerosis compared with Sofi's ACS group, while our Group B patients may represent a comparatively earlier stage of coronary atherosclerosis development compared with Sofi's stable CAD group. There is a trend of lower PZ level in Group B compared with healthy subjects in our data, though not

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significant, probably due to the limited number of subjects recruited by our study. Combining Group B (CCAD group) and Group A (AMI group) together (all CAD patients), PZ level was still found to be significantly lower compared with the healthy control group (Group C).

The reason for PZ deficiency in AMI patients seems to be multiple and needs to be interpreted cautiously. The pathogenesis of AMI involves both local blood coagulation activation and local inflammation response. In both cases PZ may be involved. A previous study suggested that ZPI function could be consumed after coagulation activation(23), although no studies have shown how the antigen level changed, or how its cofactor, PZ, changes in function and antigen levels after coagulation is induced. PZ function could be lost through its degradation by many proteases such as thrombin that are activated during blood coagulation(24). So PZ deficiency in AMI patients could simply be due to the consumption caused by local coagulation activation around a clotted vessel. The significantly higher concentration of D-Dimer in AMI patients suggests such activation. The comparably lower concentration of coagulation factor II, higher PT ratio, longer PT and abnormal PT ratio (though the latter two parameters were not statistically significant different compared with healthy subjects, Table 1) in AMI patients suggests similar coagulation factor II consumption, consistent with the previous report that lower coagulation factor II and higher D-dimer concentration were observed in AMI patients (25).

On the other hand, PZ levels in AMI patients also inversely correlate with hs-CRP significantly, whereas no such negative correlation was detected for coagulation factor II and hs-CRP, suggesting PZ may also be involved in the inflammation contribution to AMI pathogenesis. In this regard, firstly, an increasing number of studies support the notion that reduced PZ levels may constitute an independent risk factor for the development of atherosclerosis. A recent animal study from Butschkau et al. (28) showed PZ contributes to a reduced neointima formation after vascular injury, and PZ knock-out mice showed a significantly increased area and thickness of the neointima and subsequently increased luminal stenosis in carotid arteries after injury compared to wild type mice, suggesting PZ could mitigate the development of atherosclerosis. PZ is biosynthesized by vascular endothelial cells (26)and Greten et al. (27) reported that PZ antigen was detected in atherosclerotic vascular lesions. Secondly, PZ down-regulation during the acute phase of disease has also been reported by some previous investigators. Vasse et al. (29)only found weak down-regulation of PZ biosynthesis by inflammatory cytokines in cultured HepG2 cells. Undar et al. (30)showed that in patients with hematologic malignancies, the PZ plasma level was significantly inversely correlated with IL-6, a strong proinflammatory cytokine. More recently, Krawiec et al. (31)confirmed that in active rheumatoid arthritis (RA) patients that the PZ level was inversely correlated with CRP, IL-6, fibrinogen, etc. Consistent with these findings, Butschkau et al. (32)showed in a murine model of the generalized Shwartzman reaction that ZPI deficiency enhanced the thrombotic response to vascular injury, whereas PZ deficiency increased the inflammatory response, as evidenced by PZ deficient mice developing the highest concentrations if IL-6 and IL-10, and showing greater leukocytic tissue infiltration than their wild-type littermates. Together, these studies suggest that PZ plays an anti-inflammatory role in inflammation.

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Our study observed PZ deficiency in AMI patients and this was correlated with biomarkers reflecting both coagulation activation and inflammation in AMI patients. In both cases, it could exacerbate pathogenesis of AMI. It is interesting to note that Sofi et al reported much poorer prognosis for ACS patients with lower PZ level (16), consistent with PZ anti-coagulation and anti-inflammation function. However, it is unclear which mechanism could be the dominant path for PZ down regulation in AMI patients. What also remains unclear is the exact timing by which low PZ levels are associated with AMI. Whether low PZ level occurs before AMI as a result of coagulation activation or increased inflammation response in these patients, or after AMI as a result from coagulation activation or due to a negative acute phase protein responding to an acute coronary event, or maybe both situations still requires further investigations.

There are also some studies that suggest no difference in PZ level was detected between CAD patients and healthy subjects. In the PRIME study, Morange et al. (18)found that PZ levels do not seem to have an effect on the risk of coronary events in the following 5 years after measurement. Similarly, in the ARIC study, Rafaai et al. (17) reported neither PZ nor ZPI levels were significantly associated with the development of CAD or stroke in the overall groups. However, in a subgroup analysis, they reported there was a relationship between lower PZ levels and the development of CAD in females and smokers and a trend for the development of stroke in blacks, consistent with the report of Fedi's (14), who found a 9.5 fold increased risk of ACS in smokers with low PZ levels (< 5th percentile). It should be noted that PRIME and ARIC are prospective studies. When measurements were made for ZPI or PZ, the subjects were not confirmed for either stable or acute phase CAD, which probably are the reasons why the conclusions are different.

As more studies consistently reported that lower PZ was detected in CAD patients, it would be expected that the results would be similar from ischemic stroke clinical studies. However, there are discrepancies about PZ level changing in ischemic stroke studies. One potential reason could be that PZ levels were measured at different time points of patient admission in these studies, as McQuilian et al measured PZ level within 7 days of ischemic stroke and found it was increased(11), while Vasse M measured PZ level 3 months after thrombotic events and found it was decreased(12). It should also be noted that it is harder to distinguish ischemic stroke from hemorrhagic stroke at the very earlier stage of patient admission, which may further confound the studies that tried to measure PZ level at the acute phase of ischemic stroke.

Our data has not analyzed the genotype of AMI patients. However, FV Leiden, which could increase the thrombosis phenotype of PZ deficiency and which is comparably common in European population, is very rare in the Chinese population (33). So it is should not be considered as a significant factor for patients with AMI and low PZ levels.

Limitations of our studies include: i) we did not perform a case-controlled clinical study to analyze the prognostic value of PZ in AMI, ii) due to the comprehensive laboratory protocol, we included only a limited number of patients, so some results are not conclusive, and iii) we only described statistical associations, not the underlying mechanism of various parameter interaction.

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Conclusions

This study found PZ deficiency exists in AMI patients, being correlated with the inflammation and pathological coagulation changes of AMI patient, suggesting PZ may be involved in the pathogenesis of AMI. Further studies are still required to elucidate the exact mechanism of PZ modulation on inflammation and coagulation status in AMI.

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Highlights

- Protein Z is a plasma protein involved in regulating blood coagulation and inflammation.
- We studied plasma Protein Z levels in coronary atherosclerosis disease patients with or without acute myocardial infarction (AMI).
- Low Protein Z was found to be associated significantly with AMI and could be involved in AMI pathogenesis and be a useful clinical parameter.

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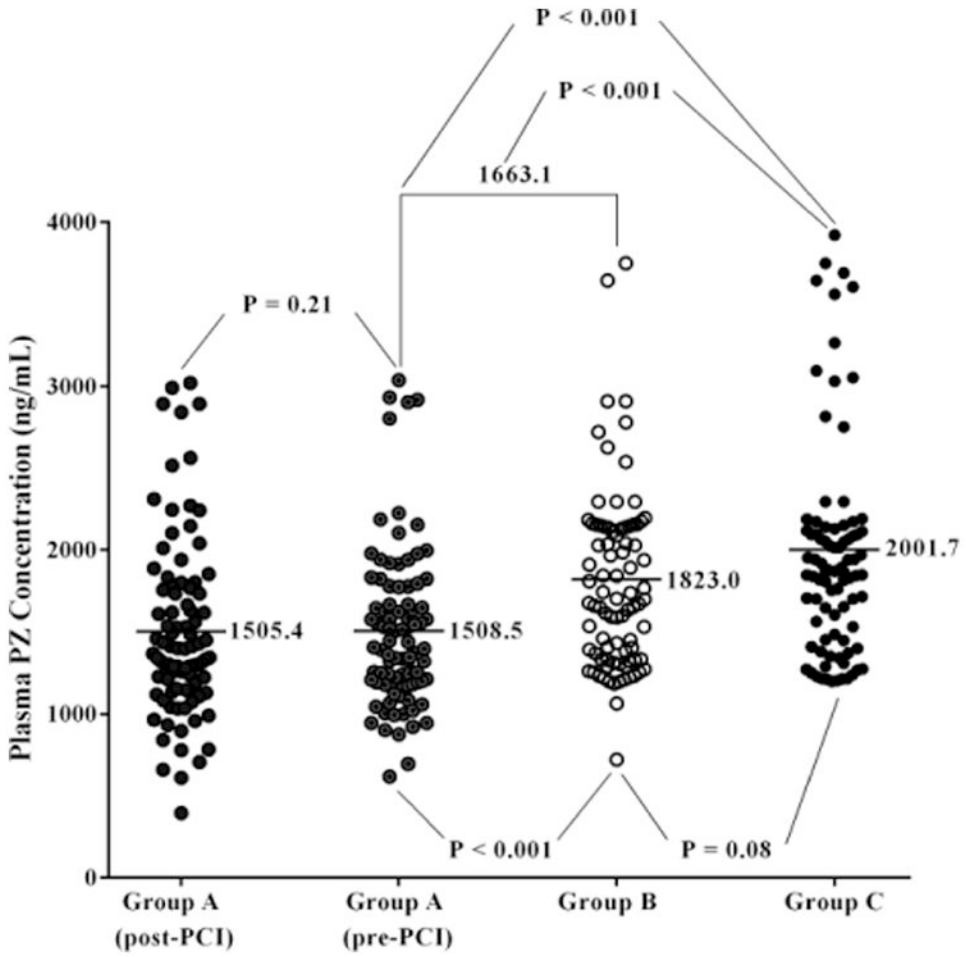

Figure 1. Comparison of plasma PZ level of the enrolled study population

Group A, AMI patients; Group B, CCAD patients without AMI history and free of major clinical events; Group C, healthy control group. Post-PCI was same with Group A patients but measured again after PCI procedure. Plasma PZ level was lower in Group A than that in the Group B and Group C, and lower in A+B compared with Group C. However, no significant difference was detected between Group B and Group C and between Post-PCI and Pre-PCI Group A. CCAD, chronic coronary atherosclerosis disease; AMI, acute myocardial infarction; PCI, percutaneous transluminal coronary intervention.

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Table 1
Characteristics of the enrolled patients in different groups

Variables	Group A (n=90)	Group B (n=87)	Group C (n=88)	P value
Age (years, mean ± SD)	67.9 ± 9.7	66.1 ± 11.4	66.3 ± 11.0	0.76
Gender (Male/Female)	61/29	52/35	53/35	0.41
Hypertension (n, %)	36 (40.0%)	39 (44.8%)	30 (34.1%)	0.35
Lipid disorders (n, %)	#*20 (22.2%)	#Δ19 (21.8%)	*Δ9 (10.2%)	# 0.98 * 0.04 Δ 0.04
Diabetes mellitus (n, %)	#*32 (35.6%)	#Δ24 (27.6%)	*Δ6 (6.8%)	# 0.26 * < 0.001 Δ < 0.001
Tobacco use (n, %)	#*39 (43.3%)	#Δ48 (55.2%)	*Δ25 (28.4%)	# 0.13 * 0.04 Δ < 0.001
LVEF (%, mean ± SD)	63.3 ± 9.5	63.7 ± 10.4	63.8 ± 9.8	0.92
hs-cTnT (ng/mL, mean ± SD)	#*0.79 ± 0.67	#Δ0.007 ± 0.002	*Δ0.007 ± 0.002	# < 0.001 * < 0.001 Δ 0.99
CK-MB (U/L, mean ± SD)	#*94.0 ± 69.7	#Δ13.5 ± 5.2	*Δ13.8 ± 4.4	# < 0.001 * < 0.001 Δ 0.96
hs-CRP (mg/L, mean ± SD)	#*6.3 ± 2.0	#Δ2.9 ± 1.6	*Δ2.7 ± 1.6	# < 0.001 * < 0.001 Δ 0.38
PT (s, mean ± SD)	14.0 ± 5.3	13.4 ± 4.4	12.7 ± 3.9	0.17
PT ratio (mean ± SD)	#*1.08 ± 0.45	#Δ1.02 ± 0.38	*Δ0.94 ± 0.32	# 0.30 * 0.02 Δ 0.19
Abnormal PT ratio (n, %)	20 (22.2%)	18 (20.7%)	12 (13.6%)	0.29
APTT (s, mean ± SD)	35.7 ± 12.2	35.2 ± 12.2	37.6 ± 12.9	0.39
PZ (ng/mL; mean ± SD)	#*1508.5 ± 486.2	#Δ1823.0 ± 607.8	*Δ2001.7 ± 733.0	# < 0.001 * < 0.001 Δ 0.08
D-dimer (ng/mL, mean ± SD)	#*343.5 ± 130.0	#Δ140.1 ± 71.0	*Δ136.5 ± 73.1	# < 0.001 * < 0.001 Δ 0.81
Coagulant factor II (μg/mL, mean ± SD)	#*104.3 ± 30.0	#Δ134.9 ± 29.4	*Δ139.5 ± 41.0	# < 0.001 * < 0.001 Δ 0.37
PZ/coagulant Factor II ($\times 10^{-3}$, mean ± SD)	15.6 ± 7.2	13.8 ± 3.9	14.9 ± 4.8	0.16

Group A, AMI patients; Group B, CCAD patients without AMI history and free of major clinical events; Group C, healthy control group. PT ratio value > 1.2 is regarded as an abnormal PT ratio. hs-cTnT, high sensitive cardiac troponin T; CK-MB, creatine kinase MB; hs-CRP, high sensitive C reaction protein; LVEF, left ventricular eject fraction.

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Table 2

The correlation analysis between PZ and AMI-related parameters

	hs-cTnT (ng/m)	CK-MB (U/L)	LVEF (%)	Degree of coronary stenosis (%)	Number of coronary lesions
r	-0.21	-0.13	-0.03	0.05	0.08
P value	0.001	0.03	0.63	0.67	0.46

hs-cTnT, high sensitive cardiac troponin T; CK-MB, creatine kinase MB; LVEF, left ventricular eject fraction.

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Table 3

The correlation analysis between PZ and hs-CRP levels

		hs-CRP (mg/L)			hs-CRP		
		< 1mg/L (n=27)	**1-3 mg/L (n=78)**	**> 3mg/L (n=160)**	**Group A (n=90)**	**Group B (n=87)**	**Group C (n=88)**
PZ	**r**	-0.10	-0.09	-0.18	-0.25	-0.14	-0.04
	P value	0.64	0.41	0.03	0.02	0.17	0.72

Group A, AMI patients; Group B, CCAD patients without AMI history and free of major clinical events; Group C, healthy control group; hs-CRP, high sensitive C reaction protein

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Table 4

The correlation analysis between PZ and coagulant parameters

	Coagulant factor II				D-dimer			
	Total (n=265)	**Group A (n=90)**	**Group B (n=87)**	**Group C (n=88)**	**Total (n=265)**	**Group A (n=90)**	**Group B (n=87)**	**Group C (n=88)**
r	0.59	0.51	0.48	0.60	-0.46	-0.29	-0.43	-0.56
P	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.01	< 0.001	< 0.001

Group A, AMI patients; Group B, CCAD patients without AMI history and free of major clinical events; Group C, health healthy control group

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Table 5

Multivariate logistic regression for identification of independent predictors of AMI

Variables	OR	95% CI	P value
Age	1.10	0.97-1.23	0.13
Gender	1.30	0.67-2.53	0.45
Hypertension	1.26	0.64-2.50	0.50
Diabetes Mellitus	2.67	1.27-5.63	0.01
Lipid Disorders	2.08	0.86-5.02	0.10
Tobacco use	1.11	0.57-2.14	0.77
LVEF	1.11	0.80-1.41	0.53
hs-cTnT	0.36	0.21-0.60	< 0.001
CK-MB	0.50	0.34-0.72	< 0.001
hs-CRP	0.11	0.03-0.36	< 0.001
PZ	1.38	1.13-1.77	0.03
PT	0.96	0.77-0.21	0.76
APTT	1.01	0.98-1.04	0.60
Factor II	0.79	0.68-0.90	0.01
D-dimer	0.83	0.79-0.89	< 0.001

AMI, acute myocardial infarction; LVEF, left ventricular eject fraction; hs-cTnT, high sensitive cardiac troponin T; CK-MB, creatine kinase MB; hs-CRP, high sensitive C reaction protein; OR, odds ratio; CI, confidence interval.

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Table 6

Relative risks for AMI according to the tertiles of PZ levels

	Univariate			Multivariate[#]		
	OR	**95% CI**	**P value**	**OR**	**95% CI**	**P value**
Group A						
First tertile (< 1398 ng/ml)	4.41	2.06-9.44	< 0.001	3.39	1.12-10.31	0.031
Second tertile (1398-1926 ng/ml)	2.08	0.94-4.64	0.073	2.46	0.84-7.21	0.102
Third tertile (> 1926 ng/ml)		Reference			Reference	
Group B						
First tertile (< 1398 ng/ml)	3.64	1.74-7.60	0.001	7.39	2.62-20.79	0.001
Second tertile (1398-1926 ng/ml)	1.40	0.63-3.13	0.407	1.82	0.68-4.92	0.236
Third tertile (> 1926 ng/ml)		Reference			Reference	

[#]Adjusted for age, gender, hypertension, lipid disorders, diabetes mellitus, and tobacco use, LVEF, hs-CRP, PT, D-dimer, coagulation Factor II. AMI, acute myocardial infarction; OR, odds ratio; CI, confidence interval. Group A, AMI patients; Group B, CCAD patients without AMI history and free of major clinical events.