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Increased plasma cathepsin S and trombospondin-1 in patients with acute ST-segment elevation myocardial infarction

Rahel Befekadu[1], Kjeld Christiansen[2], Anders Larsson[3], Magnus Grenegård[4]

[1]Department of Laboratory Medicine, Section for Transfusion Medicine, Faculty of Medicine and Health Örebro University, Örebro, Sweden
[2]Department of Cardiology, Örebro University Hospital, Örebro, Sweden
[3]Department of Medical Sciences, Uppsala University, Uppsala, Sweden
[4]Cardiovascular Research Centre, School of Medical Sciences, Örebro University, Örebro, Sweden

Abstract

Background: *The role of cathepsins in the pathological progression of atherosclerotic lesions in ischemic heart disease have been defined in detail more than numerous times. This investigation examined the platelet-specific biomarker trombospondin-1 (TSP-1) and platelet function ex vivo, and compared this with cathepsin S (Cat-S; a biomarker unrelated to platelet activation but also associated this with increased mortality risk) in patients with ST-segment elevation myocardial infarction (STEMI).*

Methods: *The STEMI patients were divided into two groups depending on the degree of coronary vessel occlusion: those with closed (n = 90) and open culprit vessel (n = 40). Cat-S and TSP-1 were analyzed before, 1–3 days after and 3 months after percutanous coronary intervention (PCI).*

Results: *During acute STEMI, plasma TSP-1 was significantly elevated in patients with closed culprit lesions, but rapidly declined after PCI. In fact, TSP-1 after PCI was significantly lower inpatient samples compared to healthy individuals. In comparison, plasma Cat-S was significantly elevated both before and after PCI. In patients with closed culprit lesions, Cat-S was significantly higher compared to patients with open culprit lesions 3 months after PCI. Although troponin-I were higher ($p < 0.01$) in patients with closed culprit lesion, there was no correlation with Cat-S and TSP-1.*

Conclusions: *Cat-S but not TSP-1 may be a useful risk biomarker in relation to the severity of STEMI. However, the causality of Cat-S as a predictor for long-term mortality in STEMI remains to be ascertained in future studies.* (Cardiol J 2019; 26, 4: 385–393)

Key words: ST-segment elevation myocardial infarction, cathepsin S, percutaneous coronary intervention, platelets

Introduction

The role of cathepsins in the pathological progression of atherosclerotic lesions in ischemic heart disease have been described in detail more than a decade ago [1]. Cathepsin S (Cat-S) is one of the 11 family members, which are lysosomal proteases that participate in numerous physiological systems [1, 2]. Cat-S is stable at a neutral or slightly alkaline pH, thus holding most of its activity extracellularly [3–5]. Additionally, it has more specific roles such as MHC class II antigen presentation, where it is important in the degradation of the invariant chain [6–8].

Cathepsins have been implicated in various physiologic and pathophysiologic cellular processes, in which they act both as digestive and regulatory proteases [9]. The expression and

Address for correspondence: Dr. Rahel Befekadu, Department of Laboratory Medicine, Section for Transfusion Medicine, Örebro University hospital, 70185 Örebro, Sweden, 046-0762631428, e-mail: rshiferaw@hotmail.com

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activity of these proteins are changed during various inflammatory diseases, including rheumatoid arthritis, atherosclerosis, osteoporosis, abdominal aortic aneurysm and cancer [10]. Secretion of Cat-S is stimulated by pro-inflammatory interleukins such as IL-1β, and tumor necrosis factor as TNF-α [11, 12]. It was reported that normal cardiac tissues contained little or no Cat-S at all, but these proteins were richly expressed in cardiac myocytes, macrophages, intracoronary smooth muscle cells (SMCs) and endothelial cells, both in human and animals with heart failure and hypertension [12]. Numerous studies have recommended that the activity of Cat-S is amplified in the progression of atherosclerotic plaques towards plaque rupture [6, 13]. It has been reported that Cat-S has a powerful elastolytic and collagenolytic activity, and the lack of this enzyme activity apparently reduced the number of SMCs in the intima, and the disintegration of the elastic lamina atherosclerotic lesions [9]. This may explain that the interaction of Cat-S, released from SMC with extracellular matrix proteins, is involved in SMC migration and/or vascular remodeling a behavior that is likely to occur during the development of the atherosclerotic plaque [9].

The human Cat-S transgenic heart in Cat-S knockout model/mice, is characterized by a reduction in overload has been shown to exhibit a decrease in overload-induced hypertrophic responses, apoptosis and fibrosis [9]. This suggests that the inflammatory processes that prevail during cardiac remodeling locally increase the presence of the active form of these cathepsins. The ability of cardiac myocytes and macrophages to use cathepsins to degrade elastin and collagen support a function for these proteases in the cardiac wall in humans and animals [9]. The activation of platelet is fundamental in hemostasis and plays the most important role in initiating arterial thrombosis [14]. Platelet activation is also characterized by alpha-granule secretion leading to release of various proteins such as thrombospondin-1 (TSP-1). This glycoprotein exerts many biological functions and probably contributes to the pathogenesis of atherosclerosis [15].

Based on previous experimental and clinical studies, the aim of this study was to evaluate changes in Cat-S, TSP-1 and dysfunctional platelet responses *ex vivo* during ST-segment elevation myocardial infarction (STEMI) and to clarify if there were differences according to culprit vessel patency at angiography. The hypothesis was that complete occlusion of coronary blood flow (closed culprit lesion) may be associated with higher plasma levels of Cat-S and TSP-1, more severe myocardial tissue damage, and more pronounced dysfunctionality of platelet responses *ex vivo*.

Methods

Patient material

Blood samples from 130 patients were collected from the antecubital vein, into 3 mL sodium citrate vacutainer tubes, they were suffering from acute STEMI. Samples were collected and 1–3 days after and 3 months subsequent to percutaneous coronary intervention (PCI) at the Department of Cardiology, Örebro University Hospital. Patients were categorized into two groups, one with patent culprit vessel (n = 40), while the other group had a closed culprit vessel (n = 90) before PCI.

Control group

Serving as a control group, 3 mL sodium citrate vacutainer tubes were also collected from (n = 40) healthy individual blood/plasma donors with blood pressure 135/85 mmHg or less, aged between 18 and 65 years, with no known risk factors for coronary artery disease (CAD) or clinical symptoms of any other organic disease. As per the selection criteria in each group, subjects were recruited with their informed consent. Information regarding their demographic status, clinical history, and medication were noted down in detail. The study is approved by Regional Ethical Review Board in Uppsala, Dnr 2010/294 for the control subjects and Dnr 2010/277 for the patients.

Blood collection

Venous blood samples were collected and centrifuged at 2000 × g for 10 min prior to laboratory testing. The serum glucose, total cholesterol, low-density lipoprotein cholesterol (LDL-C), triglyceride, and high-density lipoprotein cholesterol (HDL-C) levels were measured (Randox enzymatic kits and a Roche-Hitachi modular system). The blood samples were divided into aliquots and stored at –80°C until the analysis was performed.

Baseline characteristics

Demographic data, cardiovascular risk factors, and medication use were retrieved from clinical records (Table 1). Hypertension was defined as systolic blood pressure > 140 mmHg, hypercholesterolemia was evaluated from clinical records, diabetes was defined as use of insulin or oral hypoglycemic agents, and smoking was defined by whether patients had smoked or not.

Table 1. Demographic and clinical features of the patients at admission.

	Subjects closed blood vessel	Subjects open blood vessel
Number of subjects	90	40
Age [years]	71 [46-96]	68 [43–93]
Sex M/F	68/22	25/15
BMI [kg/m^2]	27.3 [16.0–38.3]	27.3 [20.0–45.3]
Smoker	62%	60%
Previous MI	12%	20%
Diabetes mellitus	17%	20%
Hyperlipidemia	51%	40%
PCI [h]	0.11 [90]	0.15 [40]
PPT	224	228
WBC	13.5	10.1
Creatine kinase	82.8	83

Data are presented as mean values ± standard deviation. F — female; M — male; BMI — body mass index; MI — myocardial infarction; PCI — percutaneous coronary intervention; PPT — platelet particle concentration; WBC — white blood cell count

Plasma analyses

Troponin I was measured on an Architect 8000 (Abbott Laboratories, Abbott Park, IL, USA) with high sensitivity (hs)-troponin I reagents from the same manufacturer. The assay had a limit of detection of 2 ng/L and a 10% coefficient of variation (CV) at 5 ng/L. The plasma samples stored at –80°C, were used for the estimation of levels Cat-S and platelet-specific TSP-1 by the commercially available enzyme-linked immunosorbent assay (ELISA) kits with monoclonal antibodies against each, according to the manufacturer's instructions. The Cat-S (DY1183) and TSP-1 (DY3074) assays were performed with ELISA kits from R&D Systems (Minneapolis, MN, USA). The minimum detectable levels were 0.78 ng/mL for TSP-1 and 15.6 pg/mL for Cat-S. The CV for the ELISAs was approximately 6%. The assays were performed blinded without knowledge of the clinical diagnosis.

Platelet aggregation

Platelet function analysis was performed using the Multiplate analyzer, a whole blood impedance aggregometer (Roche/Dynabyte, Munich, Germany). Blood was collected into 4.5 mL tubes containing 25 μg/mL hirudin as an anticoagulant, according to the recommendations of the manufacturer. Analysis was performed within 3 h from sample collection. Platelet aggregation was initiated by using arachidonic acid (AA) ASPI test 0.5 mmol, thrombin receptor activating peptide (TRAP) test 32 μmol, and ADP test 6.5 μmol, using reagents supplied by the manufacturer instructions (Roche/ /Dynabyte, Munich, Germany).

Statistical analyses

Plasma Cat-S and TSP-1 were compared in the two categorized groups and with controls. Non-parametric ANOVA test (Kruskal-Wallis) followed by Dunn's multiple comparison test or Wilcoxon-Mann-Whitney test were used. Statistical significance was assumed when $p < 0.05$ was obtained. Correlations were analyzed using the Spearman test.

Results

Cat-S and TSP-1 in STEMI patients

In STEMI patients, analyses of plasma levels of Cat-S and TSP-1 were conducted at three timepoints; acute (prior to PCI), 1–3 days after PCI and 3 months after PCI. When compared to sample analysis from healthy individuals, plasma Cat-S was almost 10-fold higher during acute STEMI and 1–3 days after PCI (mean values ± standard deviation: 42807 ± 11285, 31988 ± 7748 vs. 5071 ± 1003 pg/L). Analyses 3 months after PCI revealed that plasma Cat-S was slightly higher in samples from STEMI patients compared to control samples (8937 ± 1706 vs. 5071 ± 1003 pg/L). Plasma TSP-1 was significantly higher during acute STEMI compared to control levels (29448 ± 26089 vs. 16428 ± 10285 ng/mL). On the other hand, plasma TSP-1 analyzed 1–3 days and 3 months after PCI was substantially lower compared to control (4912 ± 11551, 3123 ± ± 4441 vs. 16428 ± 10285 ng/mL). Cat-S did not correlate with TSP-1 prior to and after PCI ($r = –0.153$, $p = 0.11$ prior PCI; $r = 0.099$, $p = 0.33$ 1–3 days after PCI; $r = 0.104$, $p = 0.486$ 3 months after PCI).

STEMI patients with open and closed culprit vessels

For more detailed data analyses, patients were categorized into two groups, one with patent culprit vessel (n = 40) while the other group had closed culprit vessel (Table 1). Significant differences of baseline characteristics were found in hyperlipidemia, previous myocardial infarction and white blood cell count ($p < 0.05$). Plasma troponin I was analyzed as a biomarker of myocardial tissue injury. During the acute phase of STEMI (prior to PCI) there were no significant differences between the two patient groups ($p = 0.087$). However, after PCI intervention, troponin-I were significantly higher in patients with complete vessel occlusion (Fig. 1).

There were no significant differences in plasma Cat-S between the groups with respectively, open and closed culprit lesion in blood samples drawn before and shortly after PCI (Fig. 2). Analyses of samples obtained 3 months after PCI showed significantly lower Cat-S levels in both groups. However, plasma Cat-S was significantly higher ($p < 0.001$) in patient with closed culprit lesion (Fig. 2). Furthermore, in both patient groups (closed/open culprit lesions) plasma Cat-S 3 months after STEMI was significantly higher compared to samples from healthy individuals ($p < 0.001$).

Platelet-specific TSP-1 plasma levels are shown in (Fig. 3). Similar to Cat-S, patient plasma analyses were conducted at three time-points; acute, 1–3 days after PCI and 3 months after PCI. As shown in (Fig. 3), plasma TSP-1 was high during the acute phase of STEMI but decreased significantly ($p < 0.001$) in blood samples drawn 1–3 days after PCI. There were no significant differences in patient TSP-1 in samples obtained 1–3 days compared to 3 months after PCI. Furthermore, no significant differences in plasma TSP-1 between patients with open and closed culprit lesions were detected ($p = 0.860$ prior to PCI; $p = 0.396$ shortly after PCI; $p = 0.944$ 3 months after PCI). Notably, TSP-1 in healthy individuals were significantly higher compared to patient samples both 1–3 days and 3 months after PCI.

Platelet aggregation *ex vivo* was analyzed by measuring the increased impedance in whole blood samples. Analyses of blood samples drawn during the acute phase of STEMI revealed that the magnitude of ADP- and PAR-1 hexapeptide agonist SFLLRN-induced platelet aggregation were almost identical to that obtained from healthy volunteers (Fig. 4A, B). On the other hand, AA-induced platelet aggregation was significantly lower in the patient samples ($p < 0.001$; Fig. 4C). Platelet

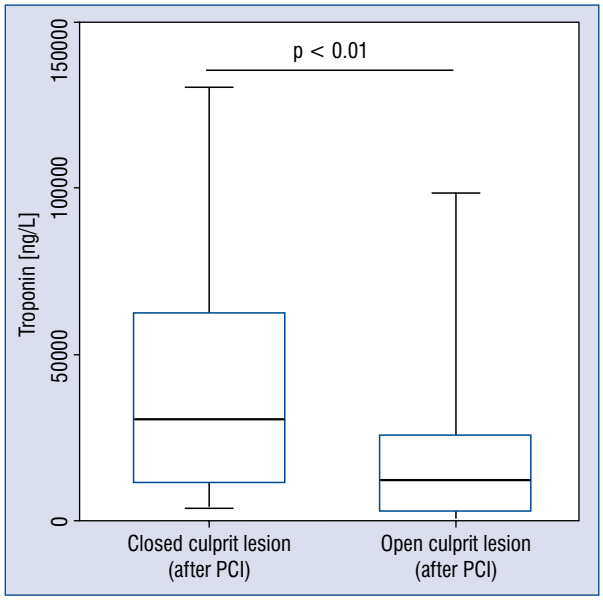

Figure 1. Plasma troponin-I during ST-segment elevation myocardial infarction. Troponin-I levels were analyzed in blood samples obtained before and shortly (1–3 days) after percutaneous coronary intervention (PCI) from patients with open and closed culprit lesion. Statistical analyses were conducted using the Mann-Whitney U test ($p < 0.01$) and data are shown as median and 5^{th}–95^{th} percentile.

aggregation responses measured 1–3 days after PCI were substantially reduced in blood samples stimulated by AA, ADP and SFLLRN. It should be emphasized that patients underwent prehospital medication with acetylsalicylic acid (ASA), and, moreover, patients received a bolus dose of 300 mg clopidogrel. This pharmacological intervention most likely has a major impact on the efficiency of platelet activators in *ex vivo* aggregation measurements. Despite this, no significant differences in platelet responsiveness between patients with open and closed culprit lesions were detected in any of the sampling times.

Discussion

ST-segment elevation myocardial infarction is most often the result of complete or partial occlusion of a major epicardial coronary vessel that must be diagnosed and treated promptly via coronary revascularization by PCI [16, 17]. It has been observed that patients suffering from non-STEMI but with occluded culprit lesion have higher degree of hypercholesterolemia, more complex coronary disease and left ventricle (LV)-dysfunction [18],

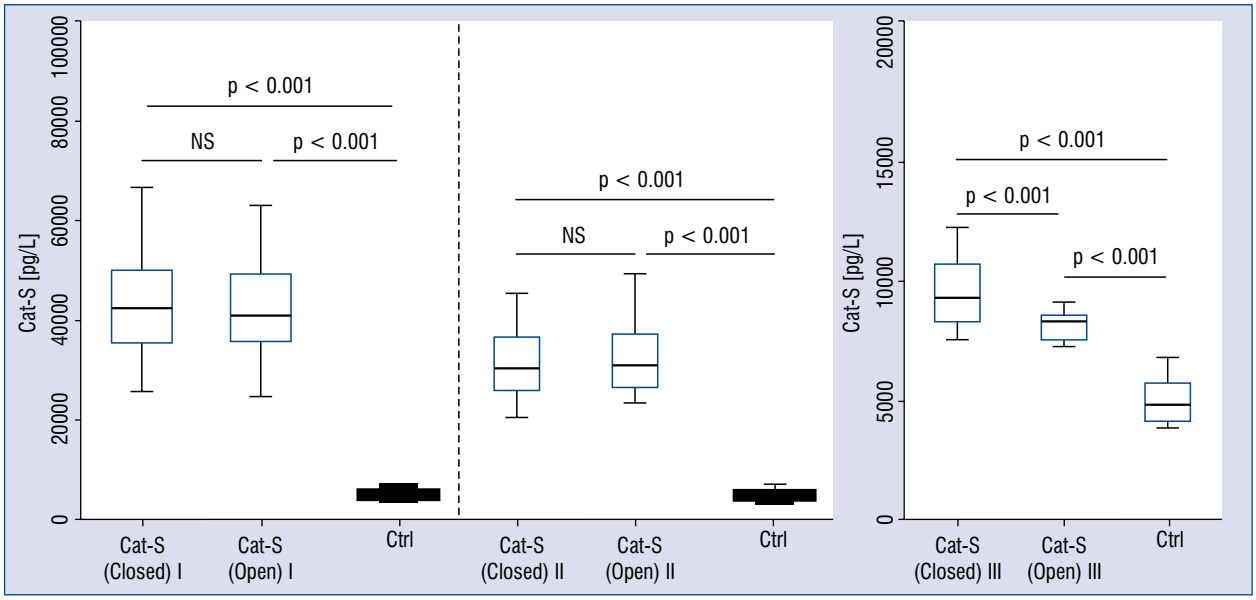

Figure 2. Plasma cathepsin S (Cat-S) in ST-segment elevation myocardial infarction patients. Cat-S levels were analyzed in blood samples obtained before (I), 1–3 days after (II) and 3 months after (III) percutaneous coronary intervention from patients with open/closed culprit lesion and healthy individuals (Ctrl). Statistical analyses were conducted by using Kruskal-Wallis followed by Dunn's multiple comparison test ($p < 0.001$) and data are shown as median and 5^{th}–95^{th} percentile; NS — non significant.

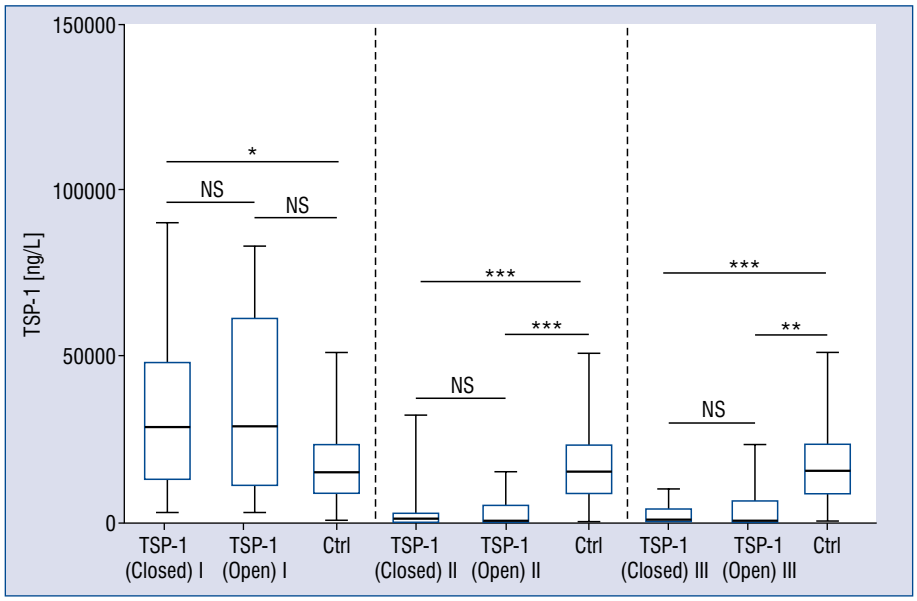

Figure 3. Plasma trombospondin-1 (TSP-1) in ST-segment elevation myocardial infarction patients. TSP-1 levels were analyzed in blood samples obtained before (I), 1–3 days after (II) and 3 months after (III) percutaneous coronary intervention from patients with open/closed culprit lesion and healthy individuals (Ctrl). Statistical analyses were conducted by using Kruskal-Wallis followed by the Dunn's multiple comparison test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$) and data are shown as median and 5^{th}–95^{th} percentile; NS — non significant.

and it is known that STEMI patients with occluded culprit lesion have a significant higher mortality rate than patients with open culprit lesion. Many mechanisms have been implicated in the formation of unstable atherosclerotic plaques leading to STEMI. For instance, it is believed that the activity

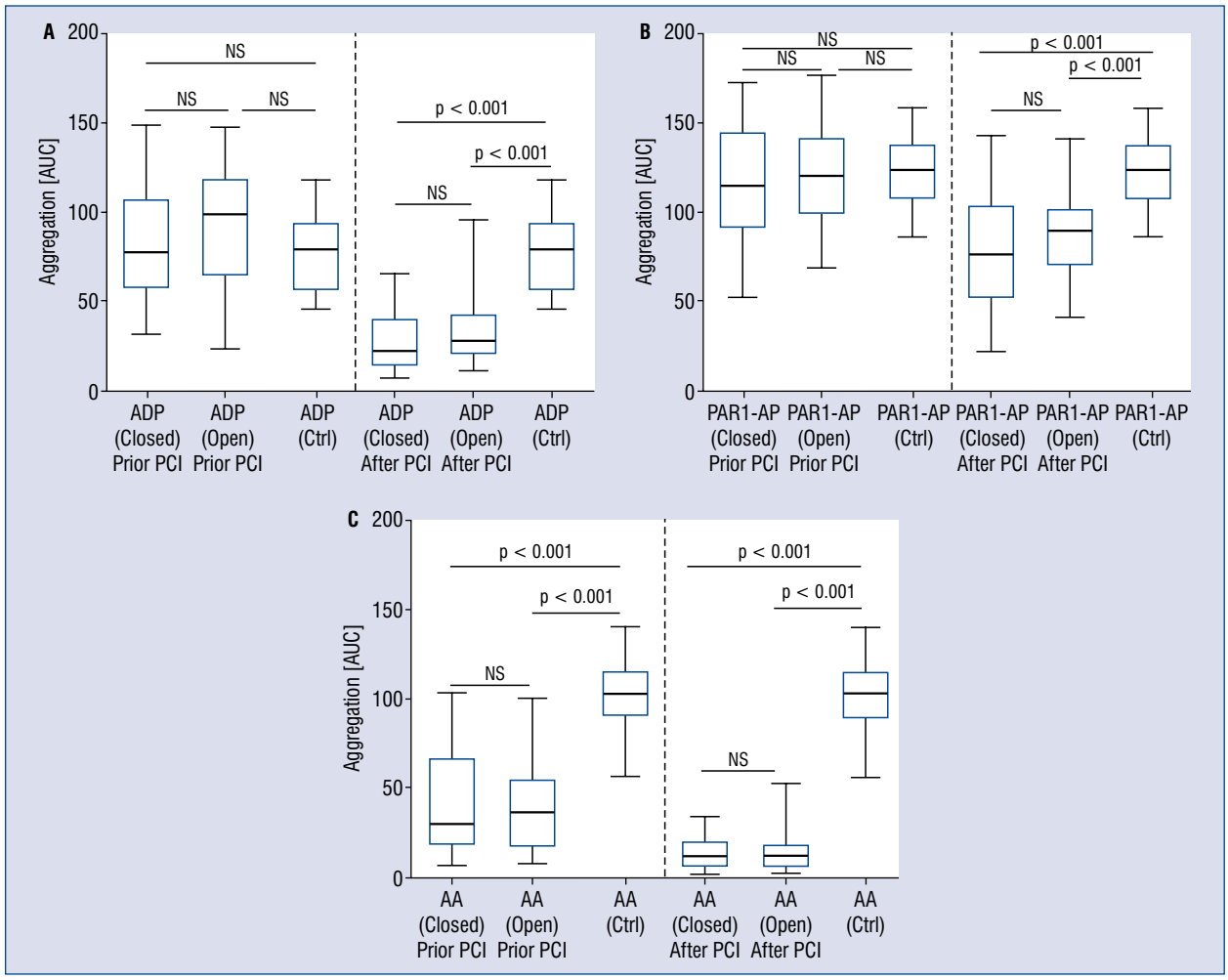

Figure 4. Platelet whole blood aggregation. Platelet aggregation responses in blood samples obtained before and 1–3 days after percutaneous coronary intervention (PCI) from patient with open and closed culprit lesion. Aggregation was induced by ADP (**A**), the PAR-1-AP SFLLRN (**B**), and AA (**C**) and is expressed as area under curve (AUC). Platelet aggregation in control samples (healthy volunteers [Ctrl]) was analyzed in parallel. Statistical analyses were conducted by using Kruskal-Wallis followed by the Dunn's multiple comparison test ($p < 0.001$) and data are shown as median and 5^{th}–95^{th} percentile; NS — non significant.

of the protease Cat-S is increased in the progress of atherosclerotic plaque with higher risk of rupture and uncontrolled platelet activation [19–22]. In this investigation, it was found that plasma Cat-S and platelet-specific TSP-1 was high during the acute phase of STEMI, but no significant differences were observed between patient groups with closed or open culprit lesion. However, in long time follow-up analyses, plasma Cat-S (but not TSP-1) was significantly higher in patient with more severe STEMI (i.e. patient with closed culprit lesion).

The involvement of Cat-S in atherosclerotic plaque rupture leading to platelet activation, thrombus formation and ultimately STEMI are not well studied. It is however believed that cathepsins may contribute to extracellular matrix damage which precedes atheroma rupture [6, 9]. In the present study, it was found that plasma Cat-S was high prior to PCI. This may indicate that analysis of Cat-S may be useful for evaluating patients with vulnerable plaques; patients with high risk of acute infarction. It is to be noted that Cat-S was only marginally reduced at days 1–3 post-PCI; a more significant reduction was observed 3 months post-PCI. This is in contrast to registered TSP-1 levels which returned to low levels shortly after PCI. It is possible that Cat-S remains higher as a result of ischemia reperfusion injury leading to e.g. endothelial cell activation. In accordance with our findings, Cat-S has been implicated in acute infarction and severe coronary vessel atherosclerosis in human atheroma lesions [23]. Cat-S detected in plasma probably

reflects ongoing inflammation associated with severe coronary atherosclerosis. It has also been proposed that Cat-S is released during ischemic necrosis following MI [24]. Furthermore, Cat-S has been linked to disease severity and is associated with increased mortality risk in different diseases [23, 25]. Interestingly, in the present long-term follow-up analysis, it was found that plasma Cat-S was significantly higher in patient with closed culprit lesion. This may be the result of a more extensive myocardial ischemia/reperfusion injury following complete occlusion of coronary vessel. In accordance with this, the present data showed that troponin-I levels were significantly higher in the group with closed culprit lesion. Taken together, it may be speculated that a rise in Cat-S may precede a thrombotic event whereas TSP-1 levels rise sharply following uncontrolled platelet activation. In accordance to this, it was recently shown that Cat-S levels were higher in stable as well as unstable CAD compared to healthy controls [26]. However, the significance of Cats-S and TSP-1 analyses in CADs that precede STEMI remains to be elucidated.

Trombospondin-1 is stored within platelet alpha-granules, from which it is quickly released during platelet activation, and may thus be a suitable biomarker indicative for uncontrolled platelet activation initiating artery thrombosis. TSP-1 acts as an extracellular matrix glycoprotein that influences cell adhesion, motility, and increases endothelial cell proliferation [27]. In this study it was observed that plasma TSP-1 rapidly declined far below basal, physiological levels (compared to healthy individuals). This took place during the first 72 h which indicates that TSP-1 may be useful as a marker of the most acute phase of MI, *i.e.* systemic biomarker in plasma indicative for misdirected platelet activation in coronary vessels. On the other hand, TSP-1 analysis is not useful for long-term follow-up analyses. The pharmacological intervention of the patients is probably the reason for low TSP-1 compared to the healthy control population. Interestingly, *ex vivo* analyses of aggregation revealed that platelets obtained from patients respond in a normal manner (as healthy individual) to ADP and the thrombin mimetic hexapeptide agonist SFLLRN during the acute phase of STEMI (the blood sample drawn prior to PCI). This implies that platelet functional tests even at acute and severe cardiovascular disease shed no light on platelet dysfunctionality following plaque rupture and arterial thrombus formation. A significant decrease in platelet aggregation was noticed in patient samples stimulated with AA (prior/after PCI), ADP (after PCI) and (to lesser extent) SFLLRN (after PCI). This is most likely caused by pharmacological interventions of the patients. In accordance, it has previously been shown that pre-hospital medication with 300 mg ASA caused a 50% reduction in platelet aggregation using AA stimulation whereas anti-platelet effect of clopidogrel was observed after 72 h [28]. The rapid effect of ASA is expected based on the known pharmacokinetics of ASA and the ISIS-2 study published three decades ago that showed a beneficial effect of platelet inhibition [29, 30]. Clopidogrel is metabolized by a two-step mechanism to acquire the active metabolite that inhibits platelet P2Y12 receptors. In this study, a loading dose of 300 mg clopidogrel was used. This may explain the modest effect on platelets stimulated by ADP. In comparison, other studies have shown that a higher loading dose (600 mg) induces a more rapid and significantly better effect of the drug [28]. The time from medication with clopidogrel to the arrival at the catherization laboratory was approximately 80 min. Furthermore, it is known that more than 20% of the population are not capable of a second metabolization due to a heritable defect in the enzyme [31]. Collectively, this may explain the relatively weak effect of the P2Y12 inhibitor in ex vivo aggregation analyses. Activation of platelet by the thrombin mimetic hexapeptide SFLLRN was marginally affected by ASA and clopidogrel treatments. This is probably explained by the efficiency of thrombin receptors in inducing intracellular signaling [32]. Specifically, this means that thrombin activation of platelets does not rely on secondary positive feedback loops such as thromboxane A2 and ADP. Overall, it was concluded that platelet aggregation *ex vivo* did not provide useful information in STEMI with closed or open culprit lesions.

In summary, the present results show significantly elevated levels of TSP-1 (indicative of platelet activation) and Cat-S during the acute stage of STEMI. No significant differences were observed between patients with closed culprit lesion as compared to patients with open culprit lesion during and after management of STEMI. However, from a long term perspective, high plasma Cat-S was associated with more severe STEMI characterized by closed culprit lesion and higher troponin-I. Long term analyses of TSP-1 and platelet aggregation measurement sheds no light on inappropriate platelet activation during STEMI. Analyses of platelet-specific granule constituents like TSP-1 may be a valuable diagnostic tool during the acute stage of the disease.

Conclusions

Cathepsin S and trombospondin-1 levels are high during acute STEMI and this may contribute to new knowledge related to foregoing plaque rupture. Elevated levels of Cat-S months after STEMI may reflect the severity of the heart disease and may be important for prognosis. Low levels of TSP-1 already 1–3 days after STEMI probably reflects the pharmacological interventions of the patients. Future studies with larger patient groups may reveal the causality of Cat-S as a biomarker in myocardial infarction.

Acknowledgements

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Conflict of interest: None declared

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