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RESEARCH ARTICLE

Thrombin activity throughout the acute phase of acute ST-elevation myocardial infarction and the relation to outcome

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Abstract

Background: Thrombin and plasmin play a central role in ongoing thrombosis and platelet activation in patients with acute ST-elevation myocardial infarction (STEMI). Data of thrombin and plasmin activity in the early course of STEMI and the relation to outcome are scarce.

Methods: We included 68 consecutive patients (53 male, 59 ± 11.4 years) with STEMI who underwent acute catheter-based reperfusion therapy within the first 12 h after onset of symptoms. Blood samples were taken at admission and after 4, 8, 12 and 24 h. Thrombin activity and generation was measured by changes in the thrombin/antithrombin-III complex (TAT) and prothrombin fragment (F1.2); plasmin was measured by changes in the plasmin-α_2/antiplasmin complex (PAP). A follow-up with respect to the combined primary endpoint consisting of death, acute myocardial infarction or urgent need for revascularization up to 6 weeks post-discharge was carried out.

Results: TAT values showed no significant change over time in patients with and without the primary endpoint but there was a borderline difference between these groups at 4 h after admission (event group 9.0 vs no event group 4.7 µg l^{-1}, $p = 0.057$). F1.2 values were different between groups only after 24 h (event group 1.5 vs no event group 0.9 nmol l^{-1}, $p = 0.028$) and did not differ in serial sampling of 24 h. PAP values were higher in patients with events after 4 and 8 h and declined over time in the group without events ($p < 0.001$). Odds ratios (OR) with respect to the primary endpoint were highest for TAT >4.8 µg l^{-1} at 0 h and TAT >8.4 µg l^{-1} at 4 h (OR 7.1, 95% confidence interval (CI) 1.5–34, $p = 0.015$ and OR 5.5, 95% CI 1.5–20.0, $p = 0.01$, respectively). The predictive value of plasmin concentrations were equally high after 4 h (PAP >962 µg l^{-1}; OR 6.8, 95% CI 1.8–26.2, $p = 0.005$) and 8 h (PAP >495 µg l^{-1}, OR 6.7, 95% CI 1.4–32.9, $p = 0.024$). Values for F1.2 were only predictive after 24 h (F1.2 >0.85 nmol l^{-1}, OR 13, 95% CI 1.4–117.8, $p = 0.023$).

Conclusions: Markers of thrombin and plasmin activity in acute STEMI are related to outcome. The marker for thrombin generation F1.2 becomes a significant predictor of outcome at 24 h after admission, reflecting the potentially adverse effects of ongoing thrombin generation. This underlines the potential for direct thrombin inhibition and individualization of treatment by thrombin markers in STEMI.

Keywords: *STEMI; reperfusion therapy; thrombin; major cardiac events*

Introduction

Thrombolytic therapy and acute catheter-based intervention are currently the standard treatments for patients with ST-elevation myocardial infarction although direct percutaneous coronary intervention (PCI) is preferred if performed within 90 min after first medical contact (Antman et al. 2004, Faxon & Heger 1999, Vermeer et al. 1999, Zijlstra et al. 1999). Since the angiographic data from the GUSTO trial (GUSTO Angiographic Investigators

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1993) revealed a re-occlusion rate in thrombolysed patients of 4.9–6.4%, depending on the thrombolytic regimen, in the era of acute PCI, diminished epicardial flow despite successful reopening of the vessel due to microvascular obstruction is perhaps one mechanism that underlies adverse clinical events such as re-infarction or death. Thrombin seems to play a pivotal role in the pathophysiology of these events (Agnelli 1996). Thrombin and, in high concentrations, plasmin are strong platelet activators and platelets are highly important in the pathophysiology of acute coronary syndromes (Davies et al. 1986). In contrast, high concentrations of plasmin may cleave the thrombin receptor on platelets and, therefore, limit thrombin-induced platelet activation (Bode 2006, Kimura et al. 1996).

Earlier angiographic studies have highlighted the prediction of re-occlusion after fibrinolysis by thrombin concentration. Investigators found that the elevated levels of the thrombin/antithrombin-III complex (TAT) 3 h after thrombolysis were predictive for re-occlusion (Gulba et al. 1988, 1991). In contrast, Nordt et al. found lower TAT values in patients with re-occlusion after thrombolysis, and that in 31 patients platelet aggregation was the best predictor of early and late re-occlusion (Nordt et al. 1998).

Data for hemostatic markers especially thrombin and plasmin in patients who underwent catheter-based reperfusion therapy which is now the strategy of choice in ST-elevation myocardial infarction (STEMI) (Antman et al. 2004) are very limited (Christersson et al. 2007, van der Putten et al. 2006). Therefore, we investigated the early course of thrombin and plasmin markers in acute myocardial infarction (AMI) and we tested the hypothesis that markers of thrombin generation and activity and plasmin activity could predict the outcome of patients with AMI who underwent catheter-based reperfusion therapy.

Methods

Patients

We investigated 68 consecutive patients with acute STEMI (53 male, 59 ± 11.4 years). The patients were mostly admitted directly by the emergency physician from the mobile intensive care unit (ICU). According to our standard treatment strategies direct PCI was the recommended therapy. Acute PCI was performed on the basis of catheter laboratory availability. Thus, the maximum delay from diagnosis to the start of angiography did not exceed 30 min. Exclusion criteria were severe concomitant diseases and lack of informed consent.

Prothrombin fragments (F1.2) and the TAT levels were measured on admission (median 240 min after symptom onset) and after 4, 8, 12 and 24 h as markers (Moser et al. 1999) of thrombin generation and activity, respectively. Plasmin-α_2/antiplasmin complex (PAP) was determined for the assessment of plasmin concentrations at the same time points (Goto et al. 1994). Troponin I was measured from the admission sample (Dimension®, Dade Behring (now Siemens), Schwalbach, Germany). All patients received unfractionated heparin intravenously with a target activated partial thromboplastin time (aPTT) of 65–85 s and aspirin 500 mg i.v. on admission. Baseline samples were taken before any heparin was given in 19 (27.9%) patients. GPIIB/IIIA inhibitors were not used at the time of the investigations and all patients received bare metal stents. Ticlopidine (250 mg twice daily; 500mg loading dose) was prescribed for 4 weeks after stent implantation. Blood samples were taken by repetitive puncture of the cubital vein.

Follow-up

A 6-week follow-up was completed for all patients with regard to the combined primary endpoint of death, re-infarction and the urgent need for revascularization (catheter-based or surgical intervention or thrombolysis). The follow-up was performed in three steps: (1) at the time of transfer from the ICU; (2) at the time of discharge from the hospital; and (3) 6 weeks after discharge.

Analytical methods

At each time point of blood sampling (see above) 5 ml for citrated plasma for the hemostatic markers was obtained and stored immediately in an ice bag, centrifuged and frozen at –80°C within 2 h. Analysis of markers were performed batchwise within 1 year after samples were obtained.

Concentrations of F1.2, TAT and plasmin-α_2/antiplasmin complex (PAP) were measured by enzyme immunoassays (EIA; Dade Behring). The range of measurement for F1.2 was 0.04–10 nmol l^{-1}, for PAP 50–5000 μg l^{-1} and for TAT 2–60 μg l^{-1}. The intra-assay coefficient of variation (CV) was 5–7.5% for F1.2, 4–9% for PAP and 4–6% for TAT. The interassay CV was 6–13% for F1.2, 5–10% for PAP and 6–9% for TAT.

Statistics

Quantitative data in the tables are listed as median and 20%/80% percentiles (quintiles), except the age of patients, which is shown as mean and standard deviation (SD), or event rates with 95% confidence limits (CI). Differences between quantitative variables were calculated by the non-parametric Wilcoxon's test for independent samples. Differences between qualitative

Table 1. Characteristics of the 68 patients by the combined primary endpoint of death, re-infarction and urgent unplanned revascularization after 6 weeks.

	All patients (n=68; 53 male, 59±11.4 years)	
	No events (n=54)	Events (n=14)
Age (years)	59.6	56.6
	SD 11.9	SD 9.1
Body mass index (kg m^{-2})	25.7	26.5
	22.6/29.6	24.7/31.5
Admission troponin I (μg l^{-1})	2.1	3.2
	0.6/10.9	0.0/19.8
aPTT (s)	160	91
	32/250	28/250
Platelets (nl^{-1})	236	246
	185/269	168/275
Time to treatment (min)	240	270
	150/426	120/480
Anterior AMI (%)	62 (p=0.006)	21
Complete reperfusion (%)	100 (p<0.001)	71
Risk factors (%)		
Male sex	76	86
Arterial hypertension	46	62
Diabetes mellitus	22	23
Previous AMI	17 (p=0.135)	36
Family history of CAD	48 (p=0.092)	75
Hyperlipoproteinemia	43 (p=0.046)	75
Smoking	60	62

Values are means and standard deviation (SD) or medians and quintiles for continuous variables; p-values (<0.2) are listed versus the corresponding group with events. aPTT, activated partial thromboplastin time; AMI, acute myocardial infarction; CAD, coronary artery disease.

variables were calculated by the χ^2 test. An α-error of 5% was used. All analyses were performed according to the intention-to-treat principle. The odds ratios (ORs) for the primary endpoint were calculated by logistic regression analysis with gender and age as control variables. The cut-offs were optimized with the help of receiver operating characteristics (ROC) analyses. All calculations were performed using SPSS® v13.01 statistical software.

Results

Characteristics of patients

The patients in this single-centre study were included from 10 July 1995 to 12 January 1997. Table 1 shows the patient characteristics by primary endpoint after 6 weeks. The reperfusion rate was 94%. One stroke occurred post-discharge. In the group with events the rate of successful reperfusion was significantly lower and fewer patients presented with anterior AMI (Table 1). There was no significant correlation between initial aPTT levels and the studied markers at admission.

Time course of thrombin and plasmin markers

Table 2 shows the values of TAT, F1.2 and PAP in patients with and without events. Figure 1 shows the serial TAT values in patients with events and without events. TAT values showed no significant change over time in both groups but there was a borderline difference between groups at 4 h after admission (Table 2; 9.0 vs 4.7 μg l^{-1}, p=0.057). F1.2 values were different between groups only after 24 h (Table 2) and did not differ in serial sampling

Table 2. Time courses of plasma concentrations for thrombin/antithrombin-III complex (TAT), prothrombin fragment (F1.2) and plasmin-α_2/antiplasmin complex (PAP) in 68 acute percutaneous coronary intervention (PCI) patients with and without events in the follow-up.

	Time (h)				
	0	4	8	12	24
TAT (μg l^{-1})					
Events*	6.6	9.0	7.4	8.6	8.2
	3.8/15.2	3.0/25.0	5.1/52.5	6.3/17.6	4.6/53.6
No events	4.3 (p=0.149)	4.7 (p=0.057)	5.4 (0.116)	7.2	5.2 (p=0.139)
	2.0/14.5	3.0/9.2	2.9/14.1	3.4/21.8	2·9/12·5
F1.2 (nmol l^{-1})					
Events	0.8	1.0	0.8	0.7	1.5
	0.6/1.8	0.5/2.2	0.6/2.5	0.7/2.2	0.9/2.3
No events**	1.0	0.8	0.9	1.0	0.9 (p=0.028)
	0.5/1.3	0.5/1.4	0.7/1.4	0.8/1.8	0·7/1·7
PAP (μg l^{-1})					
Events	574	1132	816	645	367
	297/2005	303/30151	432/10254	255/3612	211/1469
No events***	501	490 (p=0.073)	447 (p=0.027)	473	370
	268/1138	316/962	317/727	243/709	187/501

*p=0.179; **p=0.194; ***p<0.001 denote values of Friedman test of the corresponding line of values. Values are medians and quintiles; p values <0.2 (Wilcoxon's test) vs corresponding group with events in brackets; events denote the cumulative primary endpoint consisting of death, acute myocardial infarction and need for urgent revascularization within 6 weeks of discharge.

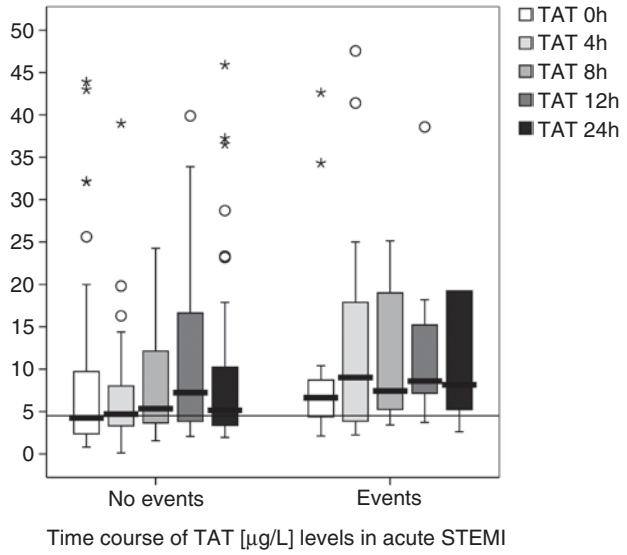

Figure 1. Serial thrombin/antithrombin-III complex (TAT) values in patients who underwent direct percutaneous coronary intervention (PCI) for ST-elevation myocardial infarction (STEMI).

of 24 h. PAP values were higher in patients with events after 4 and 8 h and declined significantly over time in the group without events (Table 2).

Follow-up

The cumulative event rate (primary endpoint) at 6 weeks was 14/68 (21%). Most of the events occurred during the ICU period and none during the remainder of the hospital stay. Table 3 shows the event rates (primary endpoint and components) in detail over the three steps of follow-up. Urgent revascularization (UR) during the ICU stay included six catheter-based interventions including PCI of high-grade symptomatic stenoses not primarily treated, four patients with additional fibrinolytic treatment and one surgical intervention (coronary artery bypass grafting). Planned secondary PCIs are also listed in Table 3 and occurred only after ICU discharge.

The prediction of the primary endpoint was optimized by TAT values >4.8 µg l^{-1} at 0 h and TAT >8.4 µg l^{-1} at 4 h (OR 7.1, 95% CI 1.5–34, $p = 0.015$ and OR 5.5, 95% CI 1.5–20.0, $p = 0.01$, respectively). The predictive value of plasmin concentrations were equally high after 4 h (PAP >962 µg l^{-1}; OR 6.8, 95% CI 1.8–26.2, $p = 0.005$) and 8 h (PAP >495 µg l^{-1}, OR 6.7, 95% CI 1.4–32.9, $p = 0.024$). Values for F1.2 were only predictive after 24 h (F1.2 >0.85 nmol l^{-1}, OR 13, 95% CI 1.4–117.8, $p = 0.023$) (Table 4).

Figure 2 shows the event rates by quintiles of TAT at 4 h. The risk seems to be represented by a J-shaped curve. An additional logistic regression analysis that included TAT (two lowest risk quintiles vs higher values) resulted in a significant prediction of the combined primary endpoint (OR 6.96, $p = 0.0204$).

Table 3. Events in the 68 patients during the 6-week follow-up.

Events	ICU	ICU – discharge	Discharge – 6 weeks
Primary endpoint	18 (9–29)	0 (0–6)*	3 (0–11)
Death	4 (1–12)	0 (0–6)*	0 (0–6)*
AMI	2 (0–8)	0 (0–6)*	3 (0–11)
UR	16 (8–27)	0 (0–6)*	0 (0–6)*
PCI	0 (0–52)*	6 (2–15)	12 (5–23)

Values are % and 95% confidence limits. The primary endpoint consisted of acute myocardial infarction (AMI), death or need for urgent revascularization (UR). ICU, intensive care unit; PCI, planned (secondary) percutaneous transluminal coronary intervention; *one-sided 97.5% confidence interval.

Table 4. Prediction of outcome by elevation of different hemostatic markers at different time points during the early course of ST-elevation myocardial infarction (STEMI).

Marker	Odds ratio	*p*-Value	Cut-off
TAT 0 h	7.069	0.015	4.8
TAT 4 h	5.508	0.010	8.4
TAT 24 h	6.274	0.037	5.2
PAP 0 h	4.803	0.036	1239
PAP 4 h	6.824	0.005	962
PAP 8 h	6.712	0.024	495
PAP 12 h	4.716	0.031	749
F 1.2 24 h	12.966	0.023	0.85

TAT, thrombin/antithrombin-III complex (µg l^{-1}); F1.2, prothrombin fragment 1.2 (nmol l^{-1}); PAP, plasmin-α_2/antiplasmin complex (µg l^{-1}); logistic regression analysis was controlled for gender and age.

Discussion

We describe activation of coagulation and fibrinolysis in patients with STEMI and acute catheter-based reperfusion therapy. The results show a strong significant prediction of outcome by TAT and PAP values. Although data were obtained some years ago, the now worldwide preferred strategy of direct PCI was already established in our centre and therefore the data reveal important new insights, especially on thrombin activity and generation during the acute early phase of STEMI.

Antithrombin therapy with heparin is still the current standard of concomitant AMI therapy. Patients with high persistent thrombin activity were shown to be prone to an adverse outcome, which might be due to persistent microvascular occlusion. Earlier angiographic studies showed that TAT predicts re-occlusion in thrombolysed patients with a critical value of 6 µg l^{-1} around 3 h after the start of thrombolysis (Gulba et al. 1988, 1991), a value which is similar to our cut-off (8.4) in PCI patients.

Thrombin is a potent platelet activator, and many studies have emphasized the role of antiplatelet

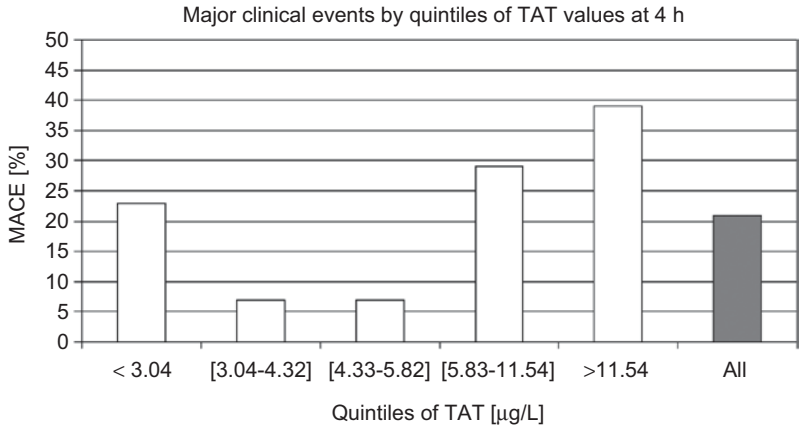

Figure 2. Major clinical event (MACE) rate until 6 weeks post-discharge by quintiles of thrombin/antithrombin-III complex (TAT) values 4 h after admission.

therapy in acute coronary syndromes (Brener et al. 1998). In addition, the use of direct thrombin inhibition in acute ischemic syndromes has shown promising results (Organisation to Assess Strategies for Ischemic Syndromes (OASIS-2) Investigators 1999, Stone et al. 2006) and has been being investigated in STEMI (HORIZONS-AMI-study) (Stone et al. 2008) showing significant reduction of mortality by bivalirudin.

Our data are in line with more recent data which indicate that patients with early decrease of coagulation activity after myocardial infarction have a lower risk for recurrent ischemic events (Christersson et al. 2007). This is especially reflected by the significantly lower values of F1.2 in patients without events 24 h after admission (Table 2).

The occurrence of major clinical events in patients with higher coagulation activation can possibly be explained by pathogenic factors, such as deteriorated perfusion of microvessels as a result of the persistent hemostatic activation.

Finally, Figure 2 demonstrates a J-shaped relationship, which means that patients with both very low and very high TAT values have an increased risk for adverse events.

Thrombin is a strong activator of platelets (Bode 2006). Nevertheless, increases of thrombin markers TAT and F1.2 do not correlate with platelet activation in health (Mockel et al. 1999, 2001) and disease (Moser et al. 1999). It remains unclear whether the thrombin markers do not accurately reflect the amount of thrombin that acts on platelets or whether other concomitant hemostatic changes, such as an increase of plasmin, mask these effects. In higher concentrations, plasmin exerts platelet activation and seems to inhibit the thrombin-mediated platelet activation by cleaving the thrombin receptor (Kimura et al. 1996). In our study plasmin elevations, as measured by PAP concentrations, had a similar predictive value for clinical events as the TAT values. This confirms the hypothesis that persistent and pronounced hemostatic activation, which usually involves coagulation and fibrinolysis, is an important cause of an adverse outcome after reperfusion therapy in STEMI. Recent studies have also demonstrated that persistent occlusion of the infarct-related artery in STEMI is associated with markers related to higher thrombin and plasmin production (Huisse et al. 2007).

In our study, all patients received intravenous heparin. No significant differences occurred between aPTT values in the different groups (Table 1) although the median seems lower in the event group. The broad range of aPTT values was also due to the fact that some patients were admitted already pretreated with heparin and others had the initial blood sample prior to every acute medical treatment. Additionally, no acute severe bleeding complications were observed.

The correlation of high PAP values and adverse events has to be interpreted as fibrinolysis activation in response to the increased coagulation activity (Table 2). Prothrombin fragments are not similarly predictive when compared with TAT and PAP. As F1.2 reflects thrombin generation, which needs time to evolve compared with persistent thrombin activity as reflected by TAT, it is consistent that values increase in the event group of acute PCI patients after 24 h (Table 2) and were predictive for outcome only at that time (Table 4).

Our data show that plasmin and thrombin are pathophysiologically relevant factors in STEMI and as markers are potentially useful tools for risk stratification after reperfusion therapy. The biochemical markers may additionally have the advantage of being suitable for monitoring intensified antithrombin or antiplatelet therapy during the early course of STEMI. This is in line with other studies in different populations with coronary artery disease, which have been summarized in a recent review (van der Putten et al. 2006). A study is ongoing to determine if additional treatment options such as direct thrombin inhibition (Schwienhorst 2006) could

be beneficial in patients with increased hemostatic markers.

In conclusion, markers of thrombin and plasmin activity in acute STEMI are related to outcome. The marker for thrombin generation, F1.2, becomes a significant predictor of outcome at 24 h after admission, reflecting the potentially adverse effects on ongoing thrombin generation. This underlines the potential for direct thrombin inhibition and individualization of treatment by thrombin markers in STEMI.

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