Plasma levels of thrombin and activated protein C in patients with acute myocardial Infarction: An observational study

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A R T I C L E   I N F O

A B S T R A C T

Introduction: Activation of the plasmatic coagulation system is a major contributor to acute myocardial infarction (AMI). Markers of plasmatic coagulation and thrombin activation are correlated with clinical, laboratory and outcome parameters. In this study, we sought to evaluate if the catalytically active coagulation factors thrombin and activated protein C (APC) can be measured in patients with AMI and whether there are associations with laboratory or clinical parameters.
Methods: Thrombin and APC was quantified using oligonucleotide-enzyme-capture assays (OECAs) in 132 patients presenting with AMI immediately before and 24 h after percutaneous coronary intervention (PCI).
Results: APC was measured above the lower limit of quantification (LLOQ) in 43 (32.6%) patients before PCI (day 0) and in 55 (41.7%) patients on the following day (day 1). Thrombin was measured in 62 (47.0%) patients on day 0 and 60 (45.5%) on day 1. Both APC and thrombin were correlated with markers of thrombin generation including F1 + 2 and TAT. Additionally, APC values correlated with CK and CK-MB while thrombin correlated with CK and troponin I after PCI. APC levels above a cutoff of 0.141 ng/ml after PCI, but not thrombin, predicted 30 day major adverse cerebrovascular events.
Conclusion: Both thrombin and APC were elevated above the LLOQ in a subset of patients with AMI before and after PCI and correlated with surrogate markers of myocardial injury. Our results indicate that enzymatically active APC and thrombin are present in the circulation of patients with AMI.

1. Introduction

Cardiovascular diseases (CVD) are a major burden on global health and the absolute number of deaths due to CVD have been increasing steadily throughout the last three decades [1]. Coronary artery disease is a major contributor to CVD mortality, often through acute myocardial infarction (AMI) and its complications.

In the majority of patients with AMI, the disruption of plaque architecture, either through plaque rupture or erosion [2], contributes to the activation of the coagulation system, the formation of intravascular thrombi and the subsequent occlusion of the affected coronary artery [3,4].

This exemplifies why the inhibition of the coagulation system, besides the immediate reopening of occluded coronary arteries, is main target of pharmacological therapy [5,6]. Therapeutic options include platelet inhibition through aspirin, P2Y12 receptor antagonists and GPIIb/IIIa inhibitors, as well as inhibition of the plasmatic coagulation cascade through heparin (incl. low-molecular weight heparins), direct factor Xa and thrombin inhibition [6].

The serine protease thrombin is the central enzyme of the plasmatic coagulation cascade and modulates the conversion of fibrinogen into fibrin [7]. In addition, thrombin is also involved in the thrombomodulin- and endothelial protein C receptor-facilitated activation of the zymogen protein C (PC) into activated protein C (APC). APC plays a multi-faceted

role as an anticoagulant by inhibiting activated coagulation cofactors V and VIII and through its cytoprotective properties [8].

Previous studies have indicated that surrogate markers of thrombin generation (e.g. thrombin-antithrombin complexes [TAT], prothrombin fragment F1 + 2 [F1 + 2]), the endogenous thrombin generation potential as well as APC-protein C inhibitor complex (APC-PCI) are elevated and associated with tissue damage and outcome after AMI [3,9–12]. In addition, prolonged increase of these markers beyond the initial presentation and intervention (either PCI or thrombolysis) are associated with worse outcome [13,14].

It is however unclear whether catalytically active thrombin or APC can be detected in the systemic circulation of patients with AMI and what the implications may be. The purpose of this study was therefore to characterize thrombin and APC in patients with AMI and to compare patients with elevated values vs those without to assess whether elevated values may indicate potential myocardial tissue damage and outcome.

2. Materials and methods

This was a prospective, observational cohort study that recruited patients who underwent percutaneous coronary intervention (PCI) from 05/2015 to 02/2019 at a German tertiary referral hospital. We included patients admitted with AMI (both ST-elevation myocardial infarction [STEMI] and Non-ST-elevation myocardial infarction [NSTEMI]) planned for immediate or early PCI (within 24 h). Patients with unstable angina were excluded.

The initial diagnosis and indication for PCI were provided by the cardiologist on call and confirmed by a senior attending cardiologist according to the 3rd Universal Definition of Myocardial Infarction [15]. All patients included in the study were treated based on the current Guidelines of the European Society of Cardiology [5,16,17]. The study was carried out according to the principles of the Declaration of Helsinki and was approved by the medical ethics commission II of the Faculty of Medicine Mannheim, University of Heidelberg, Germany (IRB approval number 2015–526 N-MA). Informed consent was obtained from all participating patients or their legal representatives.

2.1. Collection of blood samples and follow-up

Blood samples for APC, thrombin and the measurement of coagulation parameters were obtained directly before PCI (day 0 or pre-PCI) and on the day following PCI (day 1 or post-PCI). All other laboratory parameters (i.e. high-sensitivity troponin I) as well as echocardiography was performed at the discretion of the treating physician. Left ventricular ejection fraction (LVEF) was measured using the biplane Simpson's method and was collected according to 4 categories: I) LVEF $\geq$ 50%, II) LVEF 49–40%, III) LVEF 39–30%, and IV) LVEF < 30%.

For plasma thrombin and APC measurements, blood collection tubes were prepared to prevent inactivation of thrombin and APC ex vivo as previously described [18,19]. All other coagulation parameters were measured in plasma prepared from citrated blood. Blood tubes were carefully inverted after blood draw and were immediately centrifuged at 2,500 × g for 15 min. Plasma was collected and stored at −80 °C until analyzed.

2.2. Measurement of established plasmatic coagulation markers

Prothrombin activation fragment F1 + 2 (F1 + 2) and thrombin-antithrombin complexes (TAT) were determined using the Enzygnost TAT micro and F1 + 2 (monoclonal) enzyme-linked immunosorbent assay (ELISA) kits (Siemens Healthineers, Erlangen, Germany). Plasmin-alpha2-antiplasmin complexes (PAP) were measured using the TECH-NOZYM® PAP Complex ELISA Kit (Technoclone, Vienna, Austria).

Plasma samples from healthy blood donors were used to determine the following reference ranges (95% confidence intervals): INR: 0.85–1.27, aPTT: 27–35 sec., TAT: 0–3.9 ng/ml, F1 + 2: 0–0.34 nmol/l, PAP: 150 – 440 ng/ml.

2.3. Measurement of plasma thrombin and APC levels by oligonucleotide-based enzyme capture assays (OECAs)

Plasma thrombin- and APC levels were measured using the oligonucleotide-based enzyme capture assays (OECA) Oligobind® Thrombin and Oligobind® APC (Loxo, Dossenheim, Germany) as previously described [18,19]. The limit of detection (LOD) and the lower limit of quantification (LLOQ) were determined by measuring hexaplicates of the respective plasma-based calibrator dilutions and the LOD and LLOQ were calculated as the concentrations that correspond to change in fluorescence of 3 (LOD) or 9 times (LLOQ) the standard deviation (SD) of the blanks. Determination was at least repeated 3 times on different days and calculated mean values (±SD) defined as the LOD and LLOQ of the assays.

Thrombin and APC levels were previously measured below the respective lower limit of quantification (LLOQ) of these assays (Thrombin: 0.039 ng/ml, APC: 0.116 ng/ml) in the plasma of 20 healthy blood donors (10 male, 10 female, age range 21–53 years) [18,19].

2.4. Outcome and follow-up

Follow-up for stroke (including hemorrhagic and ischemic stroke as well as transient ischemic attack), all-cause mortality, myocardial infarction and any unplanned revascularization procedures after the index event (both PCI and coronary artery bypass grafting (CABG)) was conducted 30 days after PCI by chart-review and structured questionnaire. Analysis was performed for major adverse cardiovascular and cerebrovascular events (MACCE) as a composite of stroke, all-cause mortality, myocardial infarction and revascularization procedures, as well as for each of the outcomes individually.

2.5. Statistical analysis

Statistical analysis was performed using SAS Version 9.4 (SAS, Cary, NC, USA) and GraphPad Prism Version 9.2.0 (GraphPad, La Jolla, California, USA). Data is presented as mean ± standard deviation (SD) for normally distributed continuous variables, median ± interquartile range (IQR) for non-normally distributed continuous variables and as frequency (%) for categorical variables. The distribution of values was assessed by the Shapiro-Wilk test.

The Student's t-test and the Mann–Whitney U test were used to compare continuous variables with normal and non-normal distributions, respectively. To assess differences for biomarker measurements between day 0 and day 1, paired analysis using the Wilcoxon matched-pairs signed rank test was used. Qualitative parameters were analyzed using a 2 × 2 contingency table and chi-squared test or Fisher's exact test as appropriate. Correlations between continuous variables were assessed by calculating Pearson's r or Spearman's r, respectively. For assessing the correlation between two binary variables, the Phi coefficient was calculated. Diagnostic performance was assessed using receiver operating curves (ROC) and optimal cutoffs were identified by Youden's index. To identify predictors for patient outcome, we used univariable logistic regression analysis. Kaplan-Meier curves were created and differences in MACCE-free survival between two groups were assessed using the log-rank test. In case of missing data, patients were excluded and a complete-case analysis was performed. As this was an exploratory study and no data are available that report differences in APC or thrombin in patients with myocardial infarction, no power analysis was conducted. A two-tailed p-value of < 0.05 was considered statistically significant for all tests.

3. Results

3.1. Patient characteristics

We included 132 patients with AMI, of which 86 (65.2%) had a STEMI while 46 (34.8%) were diagnosed with NSTEMI. Median age of the cohort was 59 years (IQR 52–73) and 105 (79.5%) patients were male. Patients enrolled in the study had a pronounced cardiovascular risk profile with 106 patients (80.3%) suffering from hypertension, 77 (58.3%) from dyslipidemia, 33 (25.0%) from diabetes while 70 (53.0%) were active smokers and 31 (23.5%) had a family history of cardiovascular disease. 33 patients (25.0%) were previously diagnosed with coronary artery disease. Nine patients (6.8%) were previously treated with CABG while 32 (24.2%) had previously received PCI (Table 1). Of all patients included, 8 (6.1%) underwent CPR before PCI. 72 patients (54.5%) were diagnosed with multivessel disease (MVD). Median number of implanted stents was 2 (IQR 1–3) and median stent length was 46 mm (IQR 28–68 mm).

123 patients (93.2%) had echocardiography performed after PCI, and left ventricular function was ≥ 50% in 70 patients (56.9%), between 40 and 49% in 30 patients (24.4%), between 30 and 39% in 17 patients (13.8%) and < 30% in 6 patients (4.9%) (Table 1). 25 patients (18.9%) suffered a MACCE during 30 day follow up. Of these, 7 patients (5.3%) succumbed while 4 patients (3.0%) suffered a myocardial infarction, 16 patients (12.1%) required additional cardiovascular revascularization and 2 patients (1.5%) suffered a stroke (Table 2).

3.2. APC and thrombin in patients with ACS

Plasma APC levels above the LLOQ were detected in 75 patients (56.8%) in one of the two measurements and in 23 patients (17.4%) in both measurements. Regarding thrombin plasma levels, 72 patients (54.5%) showed elevations above the LLOQ in one of the two measurements and 25 patients (18.9%) in both measurements (Fig. 1A).

APC plasma levels above the LLOQ were measured in 43 (32.6%) patients at the start of PCI (day 0) and in 55 (41.7%) patients on the following day (day 1). Thrombin was quantifiable in the plasma of 62 (47.0%) patients on day 0 and in 60 patients (45.5%) on day 1 (Fig. 1B). When analyzing plasma levels above the LLOQ as categorial variable, there was a statistically significant association between APC and thrombin on day 0 (Phi coefficient = 0.3499, P-value < 0.0001) and day 1 (Phi coefficient = 0.2777, P-value = 0.0014). There was however neither a statistically significant correlation for APC (Phi coefficient = 0.1667, P-value = 0.0555) nor for thrombin (Phi coefficient = -0.0970, P-value = 0.2651) when comparing day 0 and 1.

In patients with APC plasma levels above the LLOQ, median concentrations were 0.50 ng/ml (IQR 0.18–0.95) on day 0 and 0.20 ng/ml on day 1 (IQR 0.15–0.51). Median thrombin plasma levels in patients with values above the LLOQ were 0.15 ng/ml (IQR 0.06–0.60) on day 0 and 0.12 ng/ml (IQR 0.07–0.79) on day 1. APC and thrombin did not show statistically significant changes when comparing values between both days (P-value > 0.05) (Fig. 1C).

In summary, APC and thrombin values could be quantified in>50% of our cohort at least once and in>15% twice.

Table 1
Baseline characteristics.

	All (n = 132)	APC day 1 (post-PCI)			Thrombin day 1 (post-PCI)		
		Below LLOQ (n = 77)	Above LLOQ (n = 55)	P-value	Below LLOQ (n = 72)	Above LLOQ (n = 60)	P-value
Demographics							
Age [years], median (IQR)	59 (52–73)	59 (53–73)	59 (52–72)	0.9632	61 (54–74)	58 (51–69)	0.2013
Sex [male], n (%)	105 (79.5)	65 (84.4)	40 (72.7)	0.1007	56 (77.8)	49 (81.7)	0.5813
Cardiovascular risk profile, n (%)							
Hypertension	106 (80.3)	66 (85.7)	40 (72.7)	0.0644	61 (84.7)	45 (75.0)	0.1620
Dyslipidemia	77 (58.3)	45 (58.4)	32 (58.2)	0.9762	45 (62.5)	32 (53.3)	0.2875
Diabetes	33 (25.0)	23 (29.9)	10 (18.2)	0.1263	19 (26.4)	14 (23.3)	0.6864
Active smoker	70 (53.0)	42 (54.6)	28 (50.9)	0.6798	40 (55.6)	30 (50.0)	0.5242
Family history cardiovascular disease	31 (23.5)	17 (22.1)	14 (25.5)	0.6519	14 (19.4)	17 (28.3)	0.2303
Coronary artery disease	33 (25.0)	23 (29.9)	10 (18.2)	0.1263	21 (29.2)	12 (20.0)	0.2259
Previous PCI	**32 (24.2)**	**24 (31.2)**	**8 (14.6)**	**0.0280**	19 (26.4)	13 (21.7)	0.5284
Previous CABG	9 (6.8)	7 (9.1)	2 (3.6)	0.3037	6 (8.3)	3 (5.0)	0.5096
Clinical characteristics							
STEMI, n (%)	86 (65.2)	47 (61.0)	39 (70.9)	0.2407	**41 (56.9)**	**45 (75.0)**	**0.0302**
Pre-PCI DOAC, n (%)	5 (3.8)	5 (6.5)	0 (0.0)	0.0540	4 (5.6)	1 (1.7)	0.3760
Pre-PCI VKA, n (%)	3 (2.3)	3 (3.9)	0 (0.0)	0.2653	2 (2.8)	1 (1.7)	1.0000
CPR before PCI, n (%)	8 (6.1)	2 (2.6)	6 (10.9)	0.0666	3 (4.2)	5 (8.3)	0.4675
Multivessel disease, n (%)	72 (54.5)	43 (55.8)	29 (52.7)	0.7229	41 (56.9)	31 (51.7)	0.5443
Number of vessels involved, median (IQR)	2 (1–2)	2 (1–3)	2 (1–2)	0.5581	2 (1–2)	2 (1–2)	0.7075
Number of stents implanted, median (IQR)	**2 (1–3)**	**2 (1–3)**	**3 (1–4)**	**0.0149**	2 (1–3)	2 (1–3)	0.3395
Stent length [mm], median (IQR)	**46 (28–68)**	**35 (24–62)**	**58 (40–84)**	**0.0015**	40 (24–66)	52 (28–76)	0.2222
Left ventricular ejection fraction (post PCI), n (%)*							
	All (n = 123)	Below LLOQ (n = 72)	Above LLOQ (n = 51)	P-value	Below LLOQ (n = 67)	Above LLOQ (n = 56)	P-value
≥50%	70 (56.9)	48 (66.7)	22 (43.1)	0.0328	43 (64.2)	27 (48.2)	0.2426
40–49%	30 (24.4)	16 (22.2)	14 (27.5)		15 (22.4)	15 (26.8)	
30–39%	17 (13.8)	6 (8.3)	11 (21.6)		6 (9.0)	11 (19.6)	
<30%	6 (4.9)	2 (2.8)	4 (7.8)		3 (4.5)	3 (5.4)	

PCI, percutaneous coronary intervention; LLOQ, lower limit of quantification; IQR, inter quartile range; CABG, coronary artery bypass grafting; STEMI, ST-elevation myocardial infarction; DOAC, direct oral anticoagulants; c CPR, cardio pulmonary resuscitation.

Table 2
Outcome after PCI in relation to APC and thrombin levels respectively.

	All	APC day 1 (post-PCI)			Thrombin day 1 (post-PCI)		
		Below OC (n = 87)	Above OC (n = 45)	P-value	Below OC (n = 88)	Above OC (n = 44)	P-value
Outcome							
30-day MACCE, n (%)	**25 (18.9)**	**12 (13.8)**	**13 (28.9)**	**0.0359**	13 (14.8)	12 (27.3)	0.0840
Mortality	**7 (5.3)**	**2 (2.3)**	**5 (11.1)**	**0.0322**	2 (2.3)	**5 (11.4)**	**0.0409**
Myocardial infarction	4 (3.0)	2 (2.3)	2 (4.4)	0.4955	3 (3.4)	1 (2.3)	1.0000
Revascularization	**16 (12.1)**	**7 (8.1)**	**9 (20.0)**	**0.0461**	10 (11.4)	6 (13.6)	0.7061
Stroke	2 (1.5)	2 (2.3)	0 (0.0)	0.3054	2 (2.3)	0 (0.0)	0.5522

OC, optimal cutoff; APC, activated protein C; PCI, percutaneous coronary intervention; LLOQ, lower limit of quantification; MACCE, major adverse cardiovascular or cerebrovascular events.
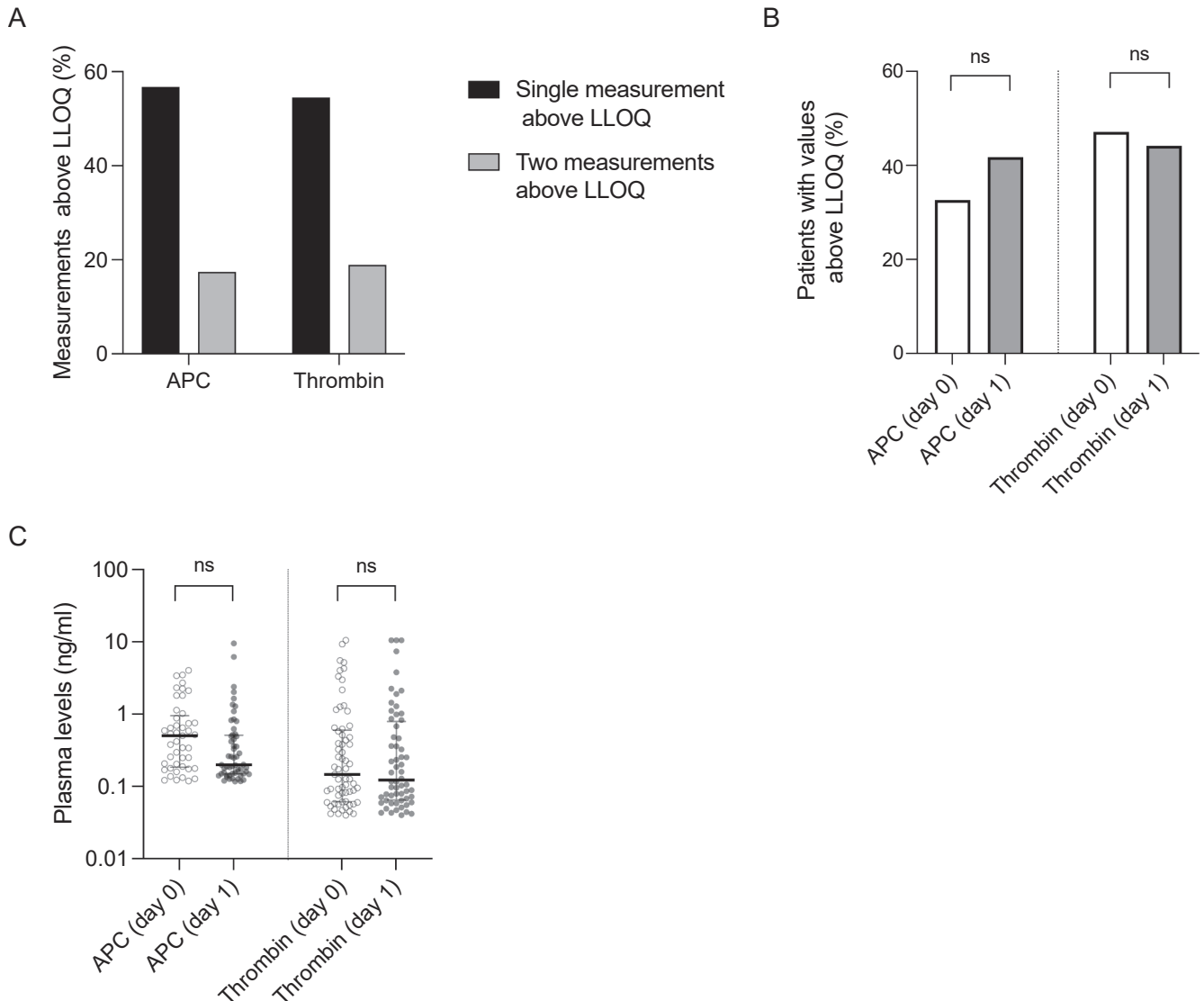

Fig. 1. a, Percentage of APC and thrombin measurements above the lower limit of quantification on one or on both measurement time points on a per patient basis. **b**, Percentage of APC and thrombin measurements above the lower limit of quantification on day 0 and on day 1. **c**, APC and thrombin values above the lower limit of quantification. Bars are medians, whiskers depict interquartile ranges.

3.3. Correlation of APC and thrombin levels with established coagulation markers

APC plasma levels showed statistically significant correlations with thrombin levels both on day 0 (Spearman's r = 0.4510, P-value < 0.0001) and day 1 (Spearman's r = 0.3456, P-value = 0.0001). In addition, APC showed statistically significant correlations with F1 + 2 fragments (Spearman's r = 0.3314, P-value = 0.0002 on day 0 and r = 0.3160, P-value = 0.0004 on day 1) and TAT (Spearman's r = 0.5122 on day 0 and r = 0.5199 on day 1, both P-values < 0.0001), the fibrinolysis indicator PAP (Spearman's r = 0.3266, P-value = 0.0002 on day 0 and r = 0.1849, P-value = 0.0432 on day 1) and INR on day 0 (Spearman's r = -0.2501, P-value = 0.0070) (**Suppl. Table 1**).

Statistically significant associations were also apparent for thrombin

and F1 + 2 fragments (Spearman's r = 0.3196, P-value = 0.0003 on day 0 and r = 0.3212, P-value = 0.0003 on day 1) and TAT (Spearman's r = 0.4873 on day 0 and r = 0.3803 on day 1, both P-values < 0.0001) (**Suppl. Table 1**). There were no statistically significant correlations with aPTT and platelet counts for both APC and thrombin (P-values > 0.05).

In summary, APC and thrombin moderately correlated with markers of thrombin activation while APC also correlated with INR and the fibrinolysis indicator PAP.

3.4. Correlation of APC and thrombin with clinical parameters

APC on day 1 exhibited statistically significant correlations with CK (Spearman's r = 0.2814, P-value = 0.0021), CK-MB (Spearman's r = 0.2373, P-value = 0.0199) but not troponin (P-value > 0.05). In contrast, thrombin on day 1 showed statistically significant correlations with CK (Spearman's r = 0.2087, P-value = 0.0239) and troponin (Spearman's r = 0.2047, P-value = 0.0297). There were however no statistically significant correlations between APC or thrombin and the delta between troponin on day 0 and 1 as indicator of myocardial infarction extent.

In summary, both APC and thrombin on day 1 were weakly associated with some surrogate markers of myocardial infarction (**Suppl. Table 1**).

3.5. Factors associated with increased APC and thrombin plasma levels

To assess which factors were associated with elevation of APC and

thrombin plasma levels above the respective LLOQ, we used univariate logistic regression analysis. To this extent, analysis revealed that demographic parameters did not predict APC or thrombin plasma levels above the LLOQ (**Suppl. Table 2**). For APC plasma levels, procedural characteristics including number of stents implanted (OR 1.293, 95% CI 1.014–1.649, P-value = 0.0383) and total stent length (OR 1.017, 95% CI 1.005–1.029, P-value = 0.0043) were predictors. For thrombin, STEMI was identified as predictor (OR 2.268, 95% CI 1.074–4.790, P-value = 0.0318), however this was not the case for door to needle time (OR 0.990, 95% CI 0.964–1.016, P-value > 0.05).

The usage of the GP IIB/IIIA inhibitor abciximab, which was applied in patients with high intracoronary thrombus load, was also a statistically significant predictor for both APC (OR 3.538, 95% CI 1.389–9.014, P-value = 0.0081) and thrombin (OR 4.714, 95% CI 1.731–12.836, P-value = 0.0024) plasma levels above the LLOQ (**Suppl. Table 2**).

3.6. Plasma APC and thrombin levels above the LLOQ are associated with markers of myocardial injury

To assess if quantifiable APC and thrombin plasma levels could identify patients with impaired outcome, we first stratified our cohort into individuals with and without APC and thrombin plasma levels above the LLOQ on day 1, respectively (**Suppl. Table 3**).

In patients with quantifiable APC levels on day 1, CK levels were higher compared to those without (1120 vs 399 IU/L, P-value = 0.0096). For thrombin, patients with quantifiable thrombin levels on day 1 exhibited higher CK (766 vs 381 U/L, P-value = 0.0110), CK-MB (101 vs 59 U/L, P-value = 0.0273) and troponin (23.6 vs 12.8 ng/L, P-
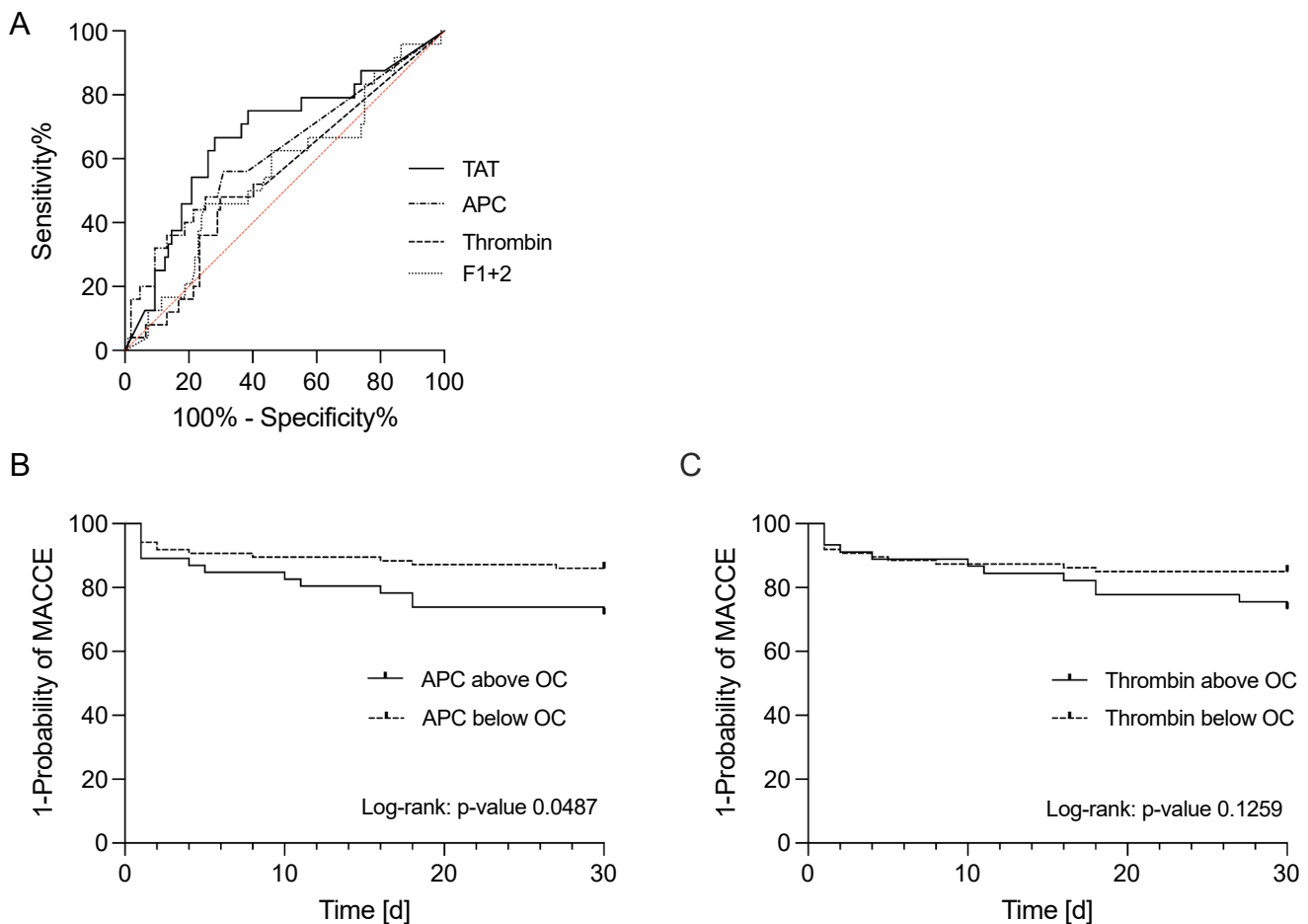

Fig. 2. a, Receiver operating characteristic curves for TAT, APC, thrombin and F1 + 2 for the endpoint MACCE. **b,** Kaplan Meier curves for patients with APC values above and below the optimal cutoff (OC). **c,** Kaplan Meier curves for patients with thrombin values above and below the optimal cutoff (OC).

value = 0.0328) values (**Suppl. Table 3**). When comparing LVEF function, LVEF between 49 and 40% (27.5 vs 22.2%), between 39 and 30% (21.6 vs 8.3%) and below 30% (7.8 vs 2.8%) was more prevalent in patients with APC values above the LLOQ (P-value = 0.0328) (Table 1).

3.7. APC but not thrombin levels are associated with outcome

Next, we used ROC analysis and Youden's index to assess whether thrombin, APC, F1 + 2 or TAT could predict outcome and to determine corresponding optimal cut-offs (OC; Fig. 2A, **Suppl. Table 4**). Highest AUCs were derived for TAT (AUC 0.6736, OC = 7.56 ng/ml) followed by APC (0.6247, OC = 0.141 ng/ml), F1 + 2 (0.5569, OC = 0.39 ng/ml) and thrombin (0.5425, OC = 0.070 ng/ml) when measured on day 1.

Based on these findings, we assessed whether the 30-day outcome was different between patients with and without APC and thrombin above the respective OC. There were statistically significant differences in MACCE rates when comparing patients with APC above versus below the OC (28.9 vs 13.8%, P-value = 0.0359), but not when comparing thrombin values above and below the OC (27.3 vs 14.8%, P-value = 0.0840) (Table 2). Regarding individual MACCE components, revascularization procedures were more frequent in patients with APC above the OC (20.0 vs 8.1%, P-value = 0.0461) while mortality was significantly increased in patients with both APC (11.1 vs 2.3%, P-value = 0.0322) and thrombin (11.4 vs 2.3%, P-value = 0.0409) above the OC.

Kaplan-Meier curves revealed differences in 30-day MACCE for patients with TAT (log-rank test p-value = 0.0004), APC (log-rank test p-value = 0.0487) and F1 + 2 (log-rank test p-value = 0.0188), but not for thrombin (log-rank test p-value = 0.1259) (Fig. 2B and C, Suppl. Fig. 1A and B).

4. Discussion

In this study we report that I) APC and thrombin can be quantified in patients with ACS before and after undergoing PCI in a subset of patients, that II) both APC and thrombin are correlated with surrogate markers of thrombin activation, that III) thrombin (and to a lesser extent APC) is associated with markers of myocardial injury and IV) when calculating optimal cutoffs, patients with APC values above the corresponding cutoff also had a worse 30-day outcome in an unadjusted analysis. Our data did not demonstrate an additional clinical value of thrombin or APC over more established markers such as TAT or F1 + F2.

Both cellular and plasmatic coagulation and their interplay have been identified as major contributors to cardiac damage and outcome in the setting of myocardial infarction [4,20,21]. Coagulation activation is a result of plaque rupture or erosion, the concomitant exposure of subendothelial tissue factor and platelet activation [22]. Subsequent thrombus formation impairs blood flow, oxygen and nutrient supply and may result in myocardial necrosis.

Previous studies have shown that elevated markers of thrombin activation, including TAT and F1 + 2, are elevated in patients with acute myocardial infarction [23–25]. In addition, surrogate markers of APC activation (for example, APC-protein C inhibitor complex) have also been shown to be elevated in patients with AMI [12]. Our data support these findings and add that beyond indirect markers of thrombin activation, both catalytically active enzymes thrombin and APC can also be quantified in the circulation of patients with AMI. This indicates that beyond the activation of the local coagulation, plaque disruption may contribute to systemic coagulation activation. This is supported by previous studies that have indicated that AMI is associated with sustained alterations in systemic coagulation [4].

In addition, coagulation activation has been linked to the extent of myocardial infarction. Hansen et al. reported that markers of thrombin generation (for example, F1 + 2) were associated with myocardial necrosis and left ventricular impairment [10]. Our data support this notion particularly for thrombin, albeit the correlations in our study were weak and not consistent across the markers CK, CK-MB, troponin and the delta

of troponins measured on day 0 and day 1.

Lastly, previous studies reported significant associations between coagulation activation and outcome. Hansen et al. reported that F1 + 2 are associated with short and long term outcome in patients with STEMI [11], while Fellner et al. demonstrated that declining APC levels compared to baseline could predict worse outcomes in patients with cardiogenic shock after AMI [26]. Chiba et al. found that in patients with STEMI, lower APC levels were associated with in-hospital mortality [27]. In this study, APC levels were measured upon arrival at the emergency room, but not after revascularization therapy.

Our study supports the finding that changes in plasma levels of APC are associated with outcome. Albeit we found that elevated and not decreasing APC levels predict worse outcome, these data indicate that APC may play a role in AMI. The main differences between the mentioned studies and our study are differences in the study populations (particularly cardiogenic shock) and the assays used to determine APC plasma concentrations. In the studies by Chiba et al. and Fellner et al., APC was measured with the biological activity assay method (Chiba et al.) or an enzyme-linked immunoassay (Fellner et al.). Both assays feature a different design compared to the OECAs used in the present study that are based on aptamers with high specificity for the target enzyme. In addition, we also added aprotinin to the blood collection tubes to prevent the formation of APC-PCI and other APC-inhibitor complexes ex-vivo. Although MACCE rates were higher in patients with elevated thrombin plasma levels, we did not find statistically significant differences compared to those without such elevations. This could be attributable to the limited study size. Given the short half-life of circulating thrombin below one minute [28], it is also feasible that thrombin levels are highly dynamic and may have thus less predictive potential compared to degradation products with longer half-life in the range of hours (e.g. F1 + 2, TAT, D-Dimer) [29].

Prior studies have explored the addition of plasmatic coagulation inhibitors (e.g. Rivaroxaban, and Apixaban) and the PAR-1 antagonist Vorapaxar to the standard of care in patients with established atherosclerosis with the goal to improve secondary prevention [30–33]. ATLAS ACS 2–TIMI 51 (Rivaroxaban) and TRA 2P–TIMI 50 (Vorapaxar) both demonstrated outcome benefits while all studies reported increased bleeding events. This indicates that benefits and risks of such an intervention need to be carefully balanced. Whether the measurement of thrombin and APC may help to identify patients that would benefit from prolonged or intensified coagulation after AMI needs to be assessed in future studies.

4.1. Limitations

There are some limitations that need to be acknowledged. First, we did not distinguish between Type I and Type II myocardial infarction in patients with NSTEMI. Second, blood samples were only collected immediately at the start of the PCI and the day after. In addition, a structured assessment of the extent of myocardial infarction was not available and correlations with APC and troponin were only performed through the analysis of biomarkers and echocardiographic data. Third, follow up data was only available for 30 days. Forth, outcome may not only be associated with APC and thrombin levels but may also depend on *peri*- and postinterventional factors (e.g. onset-to-balloon time, presence of prodromal angina etc.) for which no adjustment was performed. Fifth, it is currently unknown whether patients with a cardiovascular risk profile comparable to the study population may exhibit measurable thrombin and APC values in the absence of myocardial infarction. Lastly, this is also unknown for individuals with stable CAD.

5. Conclusion

In conclusion, we demonstrate that both active coagulation enzymes thrombin and APC are quantifiable in a subset of patients with myocardial infarction and correlate with surrogate markers of

myocardial injury, while APC also correlated with outcome. Future studies should examine for how long and under what circumstances thrombin and/or APC elevations are sustained and whether patients with elevated thrombin and/or APC plasma levels may potentially benefit from individualized plasmatic coagulation inhibition.

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Disclosures

Tobias Becher is currently an employee of Roche Diagnostics, TRotkreuz, Switzerland.

CRediT authorship contribution statement

Tobias Becher: Conceptualization, Methodology, Funding acquisition, Formal analysis, Writing. **Robert Schimanski:** Data curation, Formal analysis, Validation. **Jens Müller:** Methodology, Data curation, Resources, Writing. **Stefan Baumann:** Validation, Resources, Review. **Selina Klenantz:** Validation, Review, Editing. **Bernd Pötzsch:** Methodology, Data curation, Resources, Review. **Dirk Lossnitzer:** Formal analysis, Funding acquisition, Supervision, Resources, Writing and Review.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcha.2022.101097.

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