Markers of Thrombin Generation Are Associated With Long-Term Clinical Outcome in Patients With ST-Segment Elevation Myocardial Infarction

Charlotte Holst Hansen, MN[1,2], **Vibeke Ritschel, MD**[1,2,3],
Geir Øystein Andersen, MD, PhD[1,2], **Sigrun Halvorsen, MD, PhD**[2,3],
Jan Eritsland, MD, PhD[2], **Harald Arnesen, MD, PhD**[1,3],
and **Ingebjørg Seljeflot, PhD**[1,2,3]

Abstract

Hypercoagulability in ST-segment elevation myocardial infarction (STEMI) as related to long-term clinical outcome is not clarified. We aimed to investigate whether prothrombin fragment 1+2 (F1+2), D-dimer, and endogenous thrombin potential (ETP) measured in the acute phase of STEMI were associated with outcome. Blood samples were drawn median 24 hours after symptom onset in 987 patients with STEMI. Median follow-up time was 4.6 years. Primary outcome was a composite of all-cause mortality, reinfarction, stroke, unscheduled revascularization, or rehospitalization for heart failure; secondary outcome was total mortality. The number of combined end points/total mortality was 195/79. Higher levels of D-dimer and F1+2 were observed with both end points (all $P < .005$), whereas ETP was significantly lower ($P < .01$). Dichotomized at medians, increased risk was observed for levels above median for F1+2 and D-dimer (combined end point $P = .020$ and $P = .010$ and total mortality $P < .001$, both), while an inverse pattern was observed for ETP ($P < .02$, both). Adjusting for covariates, D-dimer was still associated with reduced risk of total mortality ($P = .034$) and receiver operating characteristic curve analyses showed area under the curve of 0.700 (95% confidence interval, 0.640-0.758). The hypercoagulable state in acute STEMI seems to be of importance for clinical outcome.

Introduction

The most important underlying mechanism of coronary artery disease is a progressive atherosclerotic process, which, along with intracoronary thrombus formation, may lead to an acute myocardial infarction (AMI).[1-4] During thrombus formation, both platelet activation and thrombin generation with fibrin formation play important roles.[2,5,6] Thrombin generation in vivo is indicated by the conversion of prothrombin to thrombin with formation of fragments 1 + 2 (F1+2) and the formation of fibrin. Activation of the fibrinolytic system with conversion of plasminogen to plasmin degrades fibrin, resulting in degradation products like D-dimer, being an indicator of both ongoing coagulation and fibrinolysis. The endogenous thrombin potential (ETP) is a measure of the potential to generate thrombin ex vivo and has been suggested to be an informative method to determine the degree of hypercoagulability.[7]

Studies have shown increased levels of prothrombotic markers in patients with myocardial infarction, being associated with myocardial injury and function[8,9] as well as clinical outcome.[10-13] There is, however, limited knowledge on the association between the acute prothrombotic state and long-term clinical outcome in patients with ST-segment elevation

[1] Center for Clinical Heart Research, Oslo University Hospital, Ullevål, Oslo, Norway
[2] Department of Cardiology, Oslo University Hospital, Ullevål, Oslo, Norway
[3] Faculty of Medicine, University of Oslo, Oslo, Norway

Corresponding Author:
Charlotte Holst Hansen, Department of Cardiology and Center for Clinical Heart Research, Oslo University Hospital, Ullevål, PB 4956 Nydalen, N-0424 Oslo, Norway.
Email: uxchho@ous-hf.no

myocardial infarction (STEMI). The aim of the present study was therefore to investigate whether plasma levels of the pro-thrombotic markers F1+2, D-dimer, and ETP measured in the acute phase of STEMI are associated with long-term clinical outcome.

Material and methods

Study Population and Design

In a cross-sectional cohort study performed at Oslo University Hospital, Ullevål, a total of 987 patients with STEMI were included between 2007 and 2011, as previously described.[8,14] When admitted to the hospital, they were treated with primary percutaneous coronary intervention (PCI) according to the standard operating procedures. Patients below the age of 18 years and patients unwilling or unable to give written informed consent were not included, and patients on warfarin treatment were excluded from the present investigation. Of the patients included, 43% presented with an anterior infarction and significant stenosis was observed in 1, 2, and 3 vessels in 54%, 29%, and 17%, respectively.

Blood samples were collected between 8 and 10 AM in the morning at a median time of 24 hours after onset of symptoms, 18 hours after the PCI procedure. All samples were taken after an overnight fast in order to standardize blood sampling and also to avoid any influence of diurnal variations and food intake. Routine blood samples were drawn at hospital admission, and samples for troponin T were measured after standardized time intervals. Clinical information was collected from hospital records and questionnaires acquired at the time of inclusion. The study was approved by the regional ethics committee, and all patients gave written informed consent.

Laboratory Methods

Citrated blood (0.129 M trisodium citrate in dilution 1:10) was centrifuged within 30 minutes at 2500g at 4°C and plasma kept frozen at −80°C until analyzed. Both F1+2 and D-dimer were determined by enzyme-linked immunosorbent assay (Asserachrom D-dimer; Stago Diagnostica, Ansiere, France and Enzygnost F1+2; Siemens, Marburg, Germany, respectively). Interassay coefficient of variation (CV) for F1+2 and D-dimer was 5.4% and 6.5%, respectively. The ETP was determined by the calibrated automated thrombogram assay according to the manufacturer's instruction as previously described in detail.[8] All experiments were run in duplicates, and the interassay CV was 6.0%. The C-reactive protein (CRP) was measured with kits from DRG Instruments (Marburg/Lahn, Germany), and CV was <5%.

Electrochemiluminescence technology for quantitative measurement was used for repeated measures of TnT (third-generation cTroponinT, Elecsys 2010; Roche, Mannheim, Germany), and NT-proBNP was measured by Elecsys proBNP sandwich immunoassay on Elecsys 2010 (Roche Diagnostics, Indianapolis, Indiana), with interassay CV of 7% for both. Left ventricular ejection fraction (LVEF) was measured by echocardiography within 3 months after the AMI (n = 767).

Clinical Outcome

The primary outcome was a composite of all-cause mortality, reinfarction, stroke, unscheduled revascularization, or rehospitalization for heart failure, whichever occurred first. Secondary outcome was total mortality. All patients were followed from date of inclusion until December 2013, giving a median follow-up time of 4.6 years. End points were recorded by telephone call and cross-checked with hospital records. The hospital records were evaluated and adjudicated by an end point committee for each end point, before analyzing the results. Mortality end points were obtained from the Cause of Death Registry, administered by the Norwegian Institute of Public Health.

Statistics

Continuous variables are presented as median values with 25, 75 percentiles and categorical variables as number or proportions. As most of the variables were skewed, nonparametric methods were used. Differences between groups were tested by Mann-Whitney U test for continuous variables and by Pearson χ^2 test for categorical variables. Multivariate analyses were performed by logistic regression, adjusting for relevant covariates as appears from Table 1 and variables associated with the coagulation markers, as well as troponin T, previously reported to be associated with the coagulation markers.[8] The relatively low number of deaths restricted the number of variables included. Receiver operating characteristic (ROC) curve analysis with the corresponding area under the curve (AUC) with 95% confidence interval (CI) was performed to determine the overall performance of D-dimer. P values <.05 were considered statistically significant. The SPSS software version 23.0 (SPSS Inc, Chicago, Illinois) was used.

Results

A total of 987 patients were included and clinical end points were obtained from 971 (98.4%) patients. Sixteen patients were not reached by telephone or mail. The number of combined end points was 195, consisting of 62 deaths, 61 reinfarctions, 6 strokes, 52 unscheduled revascularizations, and 14 hospitalizations for heart failure. The number of total mortality was 79, of which 17 died after first having experienced a nonfatal event.

Table 1 shows the baseline characteristics of the study population (n = 971) and according to groupings of with or without combined end point. Patients with combined end points were older, they were more frequent users of aspirin and statins at hospital admission, and they had higher levels of admission glucose and NT-proBNP and lower LVEF. In the Supplemental Table a, results on groupings according to total mortality are shown. We have previously reported on significant associations between the measured biomarkers and levels of NT-proBNP and troponin T.[8]

Table 1. Characteristics of the Total Study Population.[a]

	Total Population (n = 971)	Combined Events+ (n = 195)	Combined Events− (n = 776)	P
Age	61 (24-94)	65 (24, 94)	60 (24, 90)	<.001
Male sex	780 (80)	153 (79)	627 (81)	.463
Current smokers	459 (47)	91 (47)	368 (47)	.826
Previous CVD	222 (23)	53 (27)	169 (22)	.112
Treated hypertension	329 (34)	74 (38)	255 (33)	.180
Treated diabetes mellitus	122 (12)	32 (16)	90 (12)	.070
BMI (kg/m^2)	26.6 (24.3, 29.2)	26.3 (24.2, 29.4)	26.6 (24.3, 29.1)	.779
Prehospital thrombolysis	109 (11)	15 (8)	94 (12)	.080
Aspirin	222 (23)	63 (32)	159 (21)	<.001
Statins	217 (22)	55 (28)	162 (21)	.028
Total cholesterol (mmol/L)	4.9 (4.1, 5.6)	4.7 (3.9, 5.7)	4.9 (4.2, 5.6)	.056
HDL (mmol/L)	1.07 (0.88, 1.30)	1.08 (0.88, 1.34)	1.06 (0.88, 1.27)	.392
Triglycerides (mmol/L)	1.25 (0.89, 1.78)	1.22 (0.81, 1.72)	1.25 (0.90, 1.79)	.160
CRP (mg/L)	13.5 (7.0, 32.0)	14.8 (6.9, 36.2)	13.2 (7.0, 31.5)	.789
Admission glucose (mmol/L)	7.4 (6.3, 9.0)	7.8 (6.5, 9.4)	7.3 (6.3, 8.8)	.007
HbA$_{1c}$ (%)	5.9 (5.6, 6.3)	6.0 (5.7, 6.4)	5.9 (5.6, 6.2)	.085
Peak troponin T (ng/L)	3850 (1730, 7160)	3900 (1440, 7530)	3845 (1815, 7135)	.635
NT-proBNP (pmol/L)	31 (10, 118)	50 (12, 181)	28 (10, 107)	.004
LV ejection fraction (%)	49.2 (±9.18)	46.7 (11.4)	49.8 (8.5)	.005
Time from onset of symptoms to blood sampling (hours) (range)	24 (5, 118)	24 (6, 96)	24 (2, 264)	.916

Abbreviations: BMI, body mass index; CRP, C-reactive protein; CVD, cardiovascular disease (previous myocardial infarction, percutaneous coronary intervention, or coronary artery bypass surgery); HDL, high-density lipoprotein cholesterol; LV, left ventricle.
[a]n = 971. Number (proportions) or median (25, 75 percentiles) are given. P values refer to differences between a group of patients with clinical events versus without.

Table 2. Levels of F1+2, D-dimer, and ETP in the Total Population and as Related to Clinical Events.[a]

	Total Population	Combined Events+	Combined Events−	P1	Total Mortality+	Total Mortality−	P2
F1+2 (pmol/L)	246 (178, 359)	265 (198, 395)	241 (173, 351)	.012	321 (206, 457)	242 (177, 353)	<.001
D-dimer (ng/mL)	456 (286, 801)	519 (319, 1012)	442 (285, 778)	.026	807 (470, 4695)	435 (280, 776)	<.001
ETP (nM·min)	1564 (1366, 1740)	1504 (1335, 1697)	1576 (1372, 1749)	.009	1464 (1250, 1642)	1570 (1371, 1743)	.009

Abbreviation: ETP, endogenous thrombin potential; F1+2, fragments 1 + 2.
[a]Medians (25, 75 percentiles) are given. P1 values refer to difference between patients with and without clinical events; P2 values refer to differences between those who died and not).

Prothrombotic Markers and Clinical Outcome

The prothrombotic markers as related to the clinical end points are shown in Table 2. The levels of F1+2 and D-dimer were significantly higher with regard to both the combined end points (*P* = .012 and *P* = .026, respectively) and patients who later died (*P* < .001, both). The levels of ETP were significantly lower with regard to both end points (*P* = .009, both; Table 2).

When dividing the levels of F1+2, D-dimer, and ETP in quartiles, we observed significant trends of increasing number of both end points through quartiles of F1+2 and D-dimer (Figure 1). When dichotomizing levels at median values of the total population (Table 2), significantly increased risk of events was observed in univariate analyses (combined end point *P* = .020 and *P* = .010, respectively, and total mortality *P* < .001 for both; Table 3). When adjusting for relevant covariates (age, sex, admission glucose, NT-proBNP, and peak troponin T,

aspirin, and statin), the significance was lost for the composite end points, whereas D-dimer was still significantly associated with total mortality with an odds ratio of 2.01 (95% CI, 1.06-3.83; *P* = .034) and F1+2 of borderline significance (*P* = .090; Table 3). The opposite picture was seen for ETP with reduced risk of both end points through quartiles (Figure 1). Dichotomized at median, significantly reduced risk was observed in patients with ETP levels above median, however, statistically significant only with respect to the primary end point (Table 3). The results did not change when adding CRP levels, prehospital thrombolysis, and the time from onset of symptoms to blood sampling to the models.

The ROC curve for D-dimer in the prognosis of mortality is visualized in Figure 2, showing an AUC of 0.700 (95% CI, 0.640-0.758; *P* < .001). The AUC of NT-proBNP, which is known to be a strong predictor of mortality, is shown in comparison (AUC: 0.702; 95% CI, 0.644-0.760).
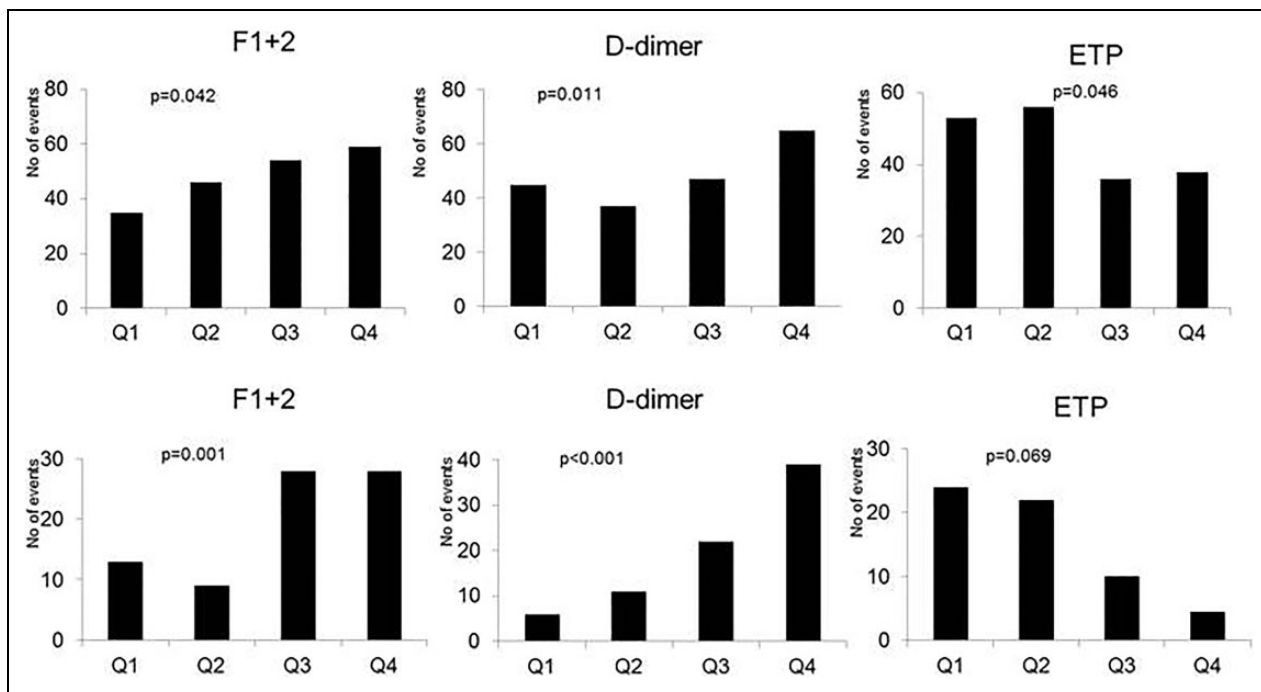
Figure 1. Levels of fragments 1 + 2 (F1+2), D-dimer, and endogenous thrombin potential (ETP) in quartiles as related to first clinical event (upper panel) and total mortality (lower panel). P values refer to trends across quartiles. F1+2: Q1 < 178, Q2 ≥ 179-246, Q3 ≥ 247-356, Q4 ≥ 357; D-dimer: Q1 < 287, Q2 ≥ 288-456, Q3 ≥ 457-796, Q4 ≥ 797; ETP: Q1 < 136, Q2 ≥ 1367-1564, Q3 ≥ 1565-1743, Q4 ≥ 1744.

Table 3. Crude and Multivariate Associations Between the Markers of Hypercoagulability and Clinical Events (A) or Total Mortality (B).

| | Crude | | | Adjusted[a] | | |
	OR	95% CI	P	OR	95% CI	P
Combined events						
F1+2	1.457	1.060-2.003	.020	1.337	0.936-1.909	.110
D-dimer	1.520	1.105-2.090	.010	1.179	0.814-1.706	.384
ETP	0.641	0.462-0.890	.008	0.688	0.484-0.980	.038
Total mortality						
F1+2	2.718	1.631-4.527	<.001	1.664	0.924-2.995	.090
D-dimer	3.972	2.284-6.908	<.001	2.010	1.055-3.829	.034
ETP	0.543	0.330-0.895	.017	0.824	0.469-1.449	.502

Abbreviation: CI, confidence interval; ETP, endogenous thrombin potential; F1+2, fragments 1 + 2; OR, odds ratio.
[a]Adjusted for age, gender, admission glucose, NT-ProBNP, troponin T markers, use of aspirin, and statins at hospital admission.

Discussion

The main findings in this study on patients with STEMI were that biomarkers of hypercoagulability, that is, F1+2, D-dimer, and ETP, were all associated with clinical outcome after 4.6 years in univariate analyses. When adjusting for relevant covariates, D-dimer was still significantly associated with a 2-fold increased risk of death and ETP with reduced risk of first composite end point. It should be emphasized that patients on oral anticoagulation were excluded from the study, and medication like aspirin and statins, which might have influenced the results, were included in the multivariate analyses.
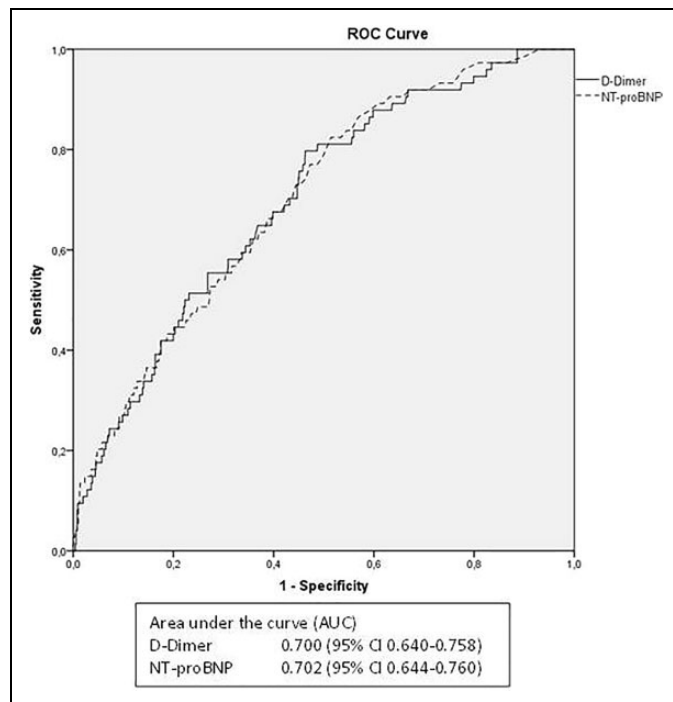
Figure 2. Receiver operating characteristic curves for D-dimer and NT-proBNP as related to total mortality.

D-dimer is known to be an acute-phase protein and thus unspecific; however, the association with mortality was still significant after adjustment for CRP. The AUC of 0.700 is

indicative of D-dimer to be related to the prognosis, although only fair, as good as for NT-proBNP.

The relationship between D-dimer and clinical outcome has previously been reported in patients with cardiovascular disease. In patients with unstable angina, some early reports in smaller cohorts showed D-dimer to be associated with a high risk of future events.[11,15] In patients with an AMI, there are limited reports, especially with long-term follow-up. Results are diverging, also for the time of blood sampling and follow-up time. In a study by Erkol et al, D-dimer assessed on admission in a relatively large AMI population was shown to have no independent predictivity for long-term outcome.[16] On the contrary, and in line with our findings, the Horizon study with long-term follow-up of patients with AMI over 3 years demonstrated that D-dimer measured very early did predict future clinical outcome, whereas D-dimer measured at discharge from hospital was not associated with long-term prognosis.[17] In another study, D-dimer measured in the stable phase 2 months after AMI was shown to be independently associated with cardiovascular outcome, including cardiovascular death after 26 months, indicating an increased prothrombotic state long term after the initial event.[17]

The mechanism behind the association between D-dimer and death may be related to the thrombogenic burden, represented by fibrin formation and turnover, clinically indicative of coronary thrombus and AMI. D-dimer has been reported to be of importance for myocardial necrosis and function after AMI. In the present population, we have previously reported that markers of thrombin generation were associated with myocardial necrosis and impaired left ventricular function.[8] Furthermore, Choi et al reported, in their study on 208 patients with STEMI, D-dimer levels on admission to be associated with larger infarct size, greater area at risk, and lower myocardial salvage index, evaluated by cardiac magnetic resonance 3 days after PCI treatment.[18]

As for F1+2 which more directly reflects thrombin generation in vivo, there are limited data with respect to clinical events. In our study, F1+2 was strongly associated with both the primary end point and total mortality; however, after adjusting for covariates, of which age seemed to be the most important (data not shown), the significance was lost. In a small study of 68 patients, F1+2 measured 24 hours after an index STEMI, similar to ours, was shown to predict clinical outcome after 6 weeks.[19]

In our study, ex vivo thrombin generation, assessed by ETP, was shown to be inversely associated with the primary end point, also after adjustments for covariates. We have previously shown an inverse relation between in vivo and ex vivo thrombin generation in the present population,[8] that is, as also reflected in the present findings. In accordance with our results, the large LURIC study on patients with AMI showed ETP to be inversely associated with cardiovascular events after a median of 10-year follow-up.[20] In that study, however, blood sampling was performed between 1 day and 4 weeks after AMI. Also in line with our result, Smid et al showed low ETP levels alone to be insignificantly associated with clinical end points; however,

in combination with high D-dimer, significantly elevated risk for thrombotic cardiovascular events after 12 months was shown.[21] This somewhat strange finding of ETP being inversely related to risk of new clinical events is not easily explained. However, the high in vivo thrombin generation, shown by the levels of F1+2 and D-dimer, may contribute to an exhaustion phenomenon, especially during an acute situation, implicating less potential to generate thrombin ex vivo. In addition, as discussed by Smid et al, several plasma coagulation factors may influence on the determination of ETP, of which tissue factor pathway inhibitor seems to be most important.[21] These assumptions are, however, in contrast to the findings in a small study on patients with ACS, in which ETP was shown not to be related to recurrent events,[22] and Attanasio et al showed ETP to be positively associated with cardiovascular death after 2 years in 292 patients initially having an ACS and blood sampling within 12 to 24 hours.[23]

Nevertheless, the hypercoagulable state shown to be present in the acute phase of STEMI might indicate a role for long-term anticoagulation as shown in earlier studies with warfarin[24,25] and the recent study with rivaroxaban in addition to aspirin.[26] However, confirmatory studies are needed before any cutoff value of D-dimer for potentially initiating treatment can be discussed.

Limitations

Single blood sampling prevented us from studying the initial time course of the measured markers. We do not know whether we have captured the real peak values of the variables or transient changes due to the variability in the time frame from onset of symptoms to blood sampling. However, the results did not change when time from symptom onset was included into the multivariate models. Our cohort of patients with STEMI was a low-risk population with few complications, and this fact may have influenced the results.

All patients in our study received heparin during the PCI procedure approximately 16 to 20 hours before blood sampling. Although any influence of heparin seems unlikely, we cannot completely exclude that small amounts of heparin were remaining.

Conclusion

The hypercoagulable state in the acute phase of a STEMI seems to be of significant importance for future clinical outcome, especially for mortality. Thus, anticoagulant treatment after AMI might still be discussed.

Authors' Note

Supplemental Material

Supplemental material for this article is available online.

References

1. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*. 2002;105(9):1135-1143.
2. Robbie L, Libby P. Inflammation and atherothrombosis. *Ann N Y Acad Sci*. 2001;947:167-179; discussion 179-180.
3. Corti R, Farkouh ME, Badimon JJ. The vulnerable plaque and acute coronary syndromes. *Am J Med*. 2002;113(8):668-680.
4. Brener SJ. Insights into the pathophysiology of ST-elevation myocardial infarction. *Am Heart J*. 2006;151(suppl 6):S4-S10.
5. Merlini PA, Bauer KA, Oltrona L, et al. Persistent activation of coagulation mechanism in unstable angina and myocardial infarction. *Circulation*. 1994;90(1):61-68.
6. James SK, Siegbahn A, Armstrong P, et al. Activation of the inflammation, coagulation, and fibrinolysis systems, without influence of abciximab infusion in patients with non-ST-elevation acute coronary syndromes treated with dalteparin: a GUSTO IV substudy. *Am Heart J*. 2004;147(2):267-274.
7. Hemker HC, Al Dieri R, De Smedt E, Béguin S. Thrombin generation, a function test of the haemostatic-thrombotic system. *Thromb Haemost*. 2006;96(5):553-561.
8. Hansen CH, Ritschel V, Halvorsen S, et al. Markers of thrombin generation are associated with myocardial necrosis and left ventricular impairment in patients with ST-elevation myocardial infarction. *Thromb J*. 2015;13:31.
9. Halvorsen S, Seljeflot I, Weiss T, Bøhmer E, Arnesen H. Inflammatory and thrombotic markers in patients with ST-elevation myocardial infarction treated with thrombolysis and early PCI: a NORDISTEMI substudy. *Thromb Res*. 2012; 130(3):495-500.
10. Christersson C, Oldgren J, Bylock A, Siegbahn A, Wallentin L. Early decrease in coagulation activity after myocardial infarction is associated with lower risk of new ischaemic events: observations from the ESTEEM Trial. *Eur Heart J*. 2007; 28(6):692-698.
11. Ardissino D, Merlini PA, Gamba G, et al. Thrombin activity and early outcome in unstable angina pectoris. *Circulation*. 1996; 93(9):1634-1639.
12. Seljeflot I, Hurlen M, Hole T, Arnesen H. Soluble tissue factor as predictor of future events in patients with acute myocardial infarction. *Thromb Res*. 2003;111(6):369-372.
13. Kikkert WJ, Claessen BE, Stone GW, et al. D-dimer levels predict ischemic and hemorrhagic outcomes after acute myocardial infarction: a HORIZONS-AMI biomarker substudy. *J Thromb Thrombolysis*. 2014;37(2):155-164.
14. Ritschel VN, Seljeflot I, Arnesen H, et al. Circulating levels of IL-6 receptor and gp130 and long-term clinical outcomes in ST-elevation myocardial infarction. *J Am Heart Assoc*. 2016; 5(6). pii: e003014. doi:10.1161/JAHA.115.003014.
15. Oldgren J, Linder R, Grip L, Siegbahn A, Wallentin L. Coagulation activity and clinical outcome in unstable coronary artery disease. *Arterioscler Thromb Vasc Biol*. 2001;21(6): 1059-1064.
16. Erkol A, Oduncu V, Turan B, et al. The value of plasma D-dimer level on admission in predicting no-reflow after primary percutaneous coronary intervention and long-term prognosis in patients with acute ST segment elevation myocardial infarction. *J Thromb Thrombolysis*. 2014;38(3):339-347.
17. Moss AJ, Goldstein RE, Marder VJ, et al. Thrombogenic factors and recurrent coronary events. *Circulation*. 1999;99(19): 2517-2522.
18. Choi S, Jang WJ, Song YB, et al. D-Dimer levels predict myocardial injury in ST-segment elevation myocardial infarction: a cardiac magnetic resonance imaging study. *PLoS One*. 2016;11(8):e0160955.
19. Ulrich-Mockel NV, Riehle M, Vollert J, et al. Thrombin activity throughout the acute phase of acute ST-elevation myocardial infarction and the relation to outcome. *Biomarkers*. 2009;14(5): 311-316.
20. Schneider JG, Isermann B, Kleber ME, et al. Inverse association of the endogenous thrombin potential (ETP) with cardiovascular death: the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. *Int J Cardiol*. 2014; 176(1):139-144.
21. Smid M, Dielis AW, Winkens M, et al. Thrombin generation in patients with a first acute myocardial infarction. *J Thromb Haemost*. 2011;9(3):450-456.
22. Loeffen R, van Oerle R, Leers MP, et al. Factor XIa and thrombin generation are elevated in patients with acute coronary syndrome and predict recurrent cardiovascular events. *PLoS One*. 2016; 11(7):e0158355.
23. Attanasio M, Marcucci R, Gori AM, et al. Residual thrombin potential predicts cardiovascular death in acute coronary syndrome patients undergoing percutaneous coronary intervention. *Thromb Res*. 2016;147:52-57.

24. Hurlen M, Abdelnoor M, Smith P, Erikssen J, Arnesen H. Warfarin, aspirin, or both after myocardial infarction. *N Engl J Med*. 2002;347(13):969-974.

25. van Es RF, Jonker JJ, Verheugt FW, Deckers JW, Grobbee DE; Antithrombotics in the Secondary Prevention of Events in Coronary Thrombosis-2 (ASPECT-2) Research Group. Aspirin and coumadin after acute coronary syndromes (the ASPECT-2 study): a randomised controlled trial. *Lancet*. 2002;360(9327): 109-113.

26. Eikelboom JW, Connolly SJ, Bosch J, et al. Rivaroxaban with or without aspirin in stable cardiovascular disease. *N Engl J Med*. 2017;377(14):1319-1330.