Clin. Cardiol. 29, 165–169 (2006)

Assessment of Markers of Thrombin Generation in Patients with Acute Myocardial Infarction Complicated by Ventricular Fibrillation

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Summary

Background: In most cases, sudden cardiac death is triggered by ischemia-related ventricular tachyarrhythmias and accounts for 50% of deaths from cardiovascular disease in developed countries. Chronic elevation of indicators of coagulation activation has been found in patients with coronary heart disease, but a role of coagulation activation as a potential risk factor for ventricular fibrillation (VF) during acute myocardial infarction (MI) has not been investigated.

Methods: We enrolled 50 patients with a history of MI, of whom 26 presented with VF in the acute phase of myocardial ischemia; 24 patients had an acute MI without ventricular tachyarrhythmias. Levels of thrombin-antithrombin complexes (TAT), prothrombin fragment F1 + 2 (F1 + 2), fibrinopeptide A (FPA), plasmin-antiplasmin complexes (PAP), protein C, antithrombin, activated partial thromboplastin time (aPTT), thromboplastin time, D-Dimer, fibrinogen, and high-sensitivity C-reactive protein (hs-CRP) were measured in plasma samples of all patients. Blood collection was obtained sequentially in two separate settings. Patients were studied at a median of 351 days after the acute coronary event.

Results: Higher levels of TAT complexes (13.4 ± 22.2 vs. 3.03 ± 4.3 µg/l; p = 0.02), FPA (79.7 ± 132.3 vs. 24.04 ± 41.3 ng/ml; p = 0.04), and F1+2 (1.89 ± 1.3 vs. 1.16 ± 0.5 nmol/l; p = 0.01) were observed in patients with VF compared with patients without ventricular tachyarrhythmias during the acute phase of MI. D-Dimer levels displayed a trend without reaching statistical significance (0.69 ± 0.48 vs. 0.48 ± 0.24 mg/l; p = 0.06). No differences were found in hs-CRP (3.25 ± 4.5 vs. 4.4 ± 8.8 mg/l; p = 0.5) and fibrinogen (2.8 ± 0.9 vs. 2.7 ± 0.9 g/l; p = 0.6) measurements. Repeat assessment of markers of coagulation activation at a median of 847 days revealed a highly significant decrease in patients with VF.

Conclusions: Markers of thrombin generation are transiently increased in patients with VF during the acute phase of MI. These findings have implications for risk assessment and genetic screening of patients prone to VF during acute myocardial ischemia.

Key words: acute myocardial infarction, ventricular fibrillation, coagulation

Introduction

Ventricular fibrillation (VF) triggered by acute myocardial ischemia is the most common cause of sudden death.[1] More than 30% of all sudden deaths due to coronary heart disease (CHD) occur as a first clinical event.[1] Approaches to predict sudden cardiac death (SCD) have been complicated by the heterogeneity in factors contributing to arrhythmogenesis. Based on population studies, Spooner *et al.*[2] indicated that a significant portion of the risk of SCD is supposed to be independent of traditional risk factors such as diabetes, hypertension, and atherosclerosis, and instead is more likely influenced by genetic variation and environmental interactions.[2] Useful markers were separated into three categories: (1) factors contributing to ischemia and infarction, including activators of plaque rupture and thrombogenesis; (2) changes in the sympathetic-parasympathetic pathway; and (3) changes of excitability of cardiomyocytes.[2] Myerburg recently suggested a number of cascades of risk that investigators have used to identify targets for preventive actions.[3] The cascade identifies four levels of evaluation of risk in coronary artery disease.[3] Initially,

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Received: November 25, 2005
Accepted with revision: January 10, 2006

there is lesion initiation (atherogenesis), followed by development and progression to onset of an active state, then to onset of ACS, and finally the expression of life-threatening ventricular arrhythmias. Once coronary plaque is in an active state, it is vulnerable to disruption, hemorrhage, and initiation of the thrombotic cascade leading to coronary occlusion.[3] This acute event leads to changes in myocardial electrophysiology triggering potential life-threatening ventricular tachyarrhythmias (VT).[3] Experimental data show that acute thrombotic coronary occlusion increases the probability of VT.[4, 5] Goldstein *et al.*[4] showed that VF occurred more frequently following a thrombotic coronary artery occlusion than after a balloon occlusion alone.[4] The incidence of VT associated with acute left anterior descending (LAD) coronary artery thrombosis was compared in open-chest anesthesized dogs after electrical injury or intracoronary stenting versus LAD balloon occlusion.[4] Nonsustained VT, sustained VT, or VF occurred more often in animals subjected to thrombotic occlusion than after nonthrombotic balloon occlusion.[4] Coronel *et al.*[5] observed profibrillatory effects of an intracoronary thrombus during acute regional ischemia in a blood-perfused animal model. Conduction velocity decreased more and ST elevation was greater during a thrombotic occlusion than following coronary artery ligation alone.[5] Myocardial excitation and conduction properties resulting in an enhancement of contractility are shown to be altered by thrombin by several mechanisms, possibly promoting VT or VF.[6–9] Therefore, we hypothesized that in patients with VF during the acute phase of myocardial ischemia, markers of thrombin generation may be elevated and contribute to arrhythmogenesis. We investigated the prothrombotic state in patients with ST-elevation myocardial infarction (STEMI) complicated by VF remote from the acute phase in order to assess possible intrinsic abnormalities promoting VF during the acute phase of myocardial infarction (MI).

Methods

Study Subjects

In all, 50 patients with a history of acute MI were enrolled. All patients were clinically asymptomatic at the time of blood sampling; 26 patients suffered from VF during acute STEMI, while in 24 patients ischemia-associated ventricular arrhythmias were absent. Diagnosis of acute MI was based on the following criteria: acute sustained chest pain, ST-segment elevation, and raised peak creatine kinase. Coronary angiography performed within 24 h after onset of symptoms revealed coronary artery occlusion of the target vessel. Ventricular fibrillation was documented outside the hospital by paramedics or in the intensive care unit during 24 h after onset of symptoms. Some of the patients with primary VF during documented STEMI who were resuscitated outside the hospital underwent thrombolysis before reaching the hospital. In 85% of the patients, acute MI was the primary manifestation of CHD.

Written informed consent for participation in the study was obtained from each subject. The study protocol was approved by the ethics committee of the University Hospital of Mannheim.

Laboratory Measurements

Patients were studied at a median of 351 days after acute MI. Repeat measurements were performed at a median of 847 days. Blood samples were collected by venipuncture into sample tubes containing 1/10 volume of 3.13% sodium citrate. Plasma samples were processed immediately by centrifugation at 2000 g for 10 min, snap frozen in liquid nitrogen, and stored at −70°C until being analyzed. Analyses included antithrombin (MDA® Antithrombin), protein C (MDA Protein C), fibrinogen level (MDA Fibriquick™), D-Dimer antigen (MDA D-Dimer), prothrombin time (MDA Simplastin®) and activated partial thromboplastin time (MDA Platelin® LS) using a MDA II coagulation analyzer (bioMérieux, Inc., Durham, N.C., USA). Measurements of thrombin-antithrombin complexes (Enzygnost® TAT micro, Dade Behring, Marburg, Germany; normal range: 1.0–4.1 µg/l), prothrombin fragment F1 + 2 (Enzygnost F1 + 2 micro, Dade Behring; normal range: 0.4–1.1 nmol/l), fibrinopeptide A (Novitec® FPA, HISS Diagnostics GmbH, Freiburg, Germany; 0–5 ng/ml) and plasmin-antiplasmin complexes (PAP micro, DRG Instruments GmbH, Marburg, Germany; normal range 120–700 mg/l) were performed by a microtiter plate enzyme-linked immunosorbent assay (ELISA).

High-sensitivity C-reactive protein (hsCRP) was measured with a latex-enhanced immunonephelometric assay on a Dimension® analyzer (Dade Behring; normal range < 5 mg/l).

Statistical Analysis

The calculations were performed using SAS, version 8.2 (SAS, Cary, N.C., USA). Numerical data were expressed as means ± standard deviation (SD). Means or proportions for baseline clinical and laboratory characteristics were computed and significant differences were tested using the Student's *t*-test or Mann-Whitney U-test, as appropriate. Categorical variables were compared by the Pearson chi-square test. A two-tailed probability value < 0.05 was considered significant

Results

Patients' demographics are outlined in Table I. In all, 50 patients with a mean age of 62 ± 10 years (88% male) were included in the study. Compared with the non-VF group, patients with VF did not differ regarding baseline characteristics and conventional risk factors. However, diabetes was more prevalent in VF survivors (p = 0.01). Regarding left ventricular function (LVF %), no difference was detected between the two patient groups (VF group: 57% ± 12, non-VF group 60% ± 13, p = 0.4)

In Table II, markers of coagulation activation are compared between patients with and without VF during acute MI. Higher levels of thrombin-antithrombin complexes (p = 0.02), FPA

TABLE I Patients' demographics

	VF during MI n = 26	MI without VF n = 24	p Value
Age (years)	61.8 ± 10.6	61.2 ± 8.9	0.8
Male (%)	88	87	0.9
Smoking (%)	50	67	0.2
Hypertension (%)	73	61	0.3
Diabetes mellitus (%)	23	0	0.01
Family history of CHD (%)	28	25	0.8
Hyperlipidemia (%)	73	70	0.8
Chronic treatment			
Aspirin (%)	23	29	0.6
Statins (%)	80	95	0.1
Beta blockers (%)	85	79	0.6
ACE inhibitors (%)	69	70	0.9
Site of myocardial infarction			
Anterior (%)	70	54	0.2
Posterior/inferior (%)	30	46	
Extent of CHD (%)			
Single-vessel disease (%)	19	42	0.2
Double-and triple-vessel disease (%)	81	58	

Abbreviations: CHD = coronary heart disease, MI = myocardial infarction, VF = ventricular fibrillation.

(p = 0.04), and prothrombin fragment F1 + 2 (p = 0.01) were detected in patients with VF at the first timepoint of blood sampling. D-Dimer antigen levels displayed a trend but did not reach statistical significance (p = 0.06). When comparing the change of parameters between patients with and without VF, it is remarkable that the changes of thrombin-antithrombin complexes (p = 0.04) and prothrombin fragment F1+2 (p = 0.01) are significant. Alterations of antithrombin levels (p = 0.06) show a trend without being statistically significant.

No differences in hs-CRP and fibrinogen levels were found between the two study groups (Table III). During follow-up, coronary bypass surgery occurred more frequently among patients with VF (p = 0.01). Regarding reinfarction, percutaneous coronary intervention (PCI), and death, there was no significant difference between patients with and without VF during follow-up (p > 0.05).

Figures 1 to 3 illustrate the change of parameters within the VF and the non-VF groups. Within the VF group, markers of coagulation and inflammation showed a significant decrease during long-term assessment. Thrombin-antithrombin complexes (p = 0.02), D-dimer (p = 0.03), prothrombin fragment F1+2 (p = 0.0), and antithrombin levels (p = 0.0) diminished during the blood sampling period in patients with VF over time; however, there was a significant increase in fibrinogen levels (p = 0.01). Among patients without VF, plasmin-antiplasmin complexes (p = 0.01) and prothrombin fragment F1 + 2 (p = 0.001) measurements displayed a significant decrease during both sampling timepoints, whereas other markers remained stable within this group.

TABLE II Comparison of markers of coagulation activation between patients with and without ventricular fibrillation

	VF during MI n = 26	MI without VF n = 24	p Value
TAT [1] (µg/l)	13.4 ± 22.2	3.03 ± 4.3	0.02
TAT [2] (µg/l)	2.4 ± 2.4	1.8 ± 0.8	0.2
Change TAT	−11.01 ± 22.86	−1.2 ± 4.4	0.04
F1+2 [1] (nmol/l)	1.89 ± 1.3	1.16 ± 0.5	0.01
F1+2 [2] (nmol/l)	0.89 ± 0.5	0.8 ± 0.35	0.4
Change F1+2	−1.0 ± 1.1	−0.36 ± 0.44	0.01
PAP [1] (µg/l)	1445 ± 865.8	1152 ± 677	0.19
PAP [2] (µg/l)	892 ± 690	738 ± 334	0.3
Change PAP	−552 ± 949	−414 ± 743	0.5
FPA [1] (ng/ml)	79.7 ± 132.3	24.04 ± 41.3	0.04
FPA [2] (ng/ml)	28.07 ± 58.06	22.04 ± 58.8	0.7
Change FPA	−51.7 ± 150.64	−1.9 ± 75.02	0.14
D-Dimer [1] (mg/l)	0.69 ± 0.48	0.48 ± 0.24	0.06
D-Dimer [2] (mg/l)	0.51 ± 0.43	0.39 ± 0.27	0.2
Change D-Dimer	−0.17 ± 0.38	−0.09 ± 0.28	0.4

Numbers in brackets refer to first [1] and second [2] blood sampling. *Abbreviations:* F1+2 = prothrombin fragment F1+2, FPA = fibrinopeptid A, PAP = plasmin-antiplasmin-complexes, TAT = thrombin-antithrombin complexes. Other abbreviations as in Table I.

Discussion

Prospective data suggest that parameters of chronic coagulation activation correlate positively with CHD and acute coronary syndromes.[10] Indicators of coagulation activation are also

TABLE III Baseline coagulation parameters and markers of inflammation

	VF during MI n = 26	MI without VF n = 24	p Value
Antithrombin [1] (%)	85.4 ± 21.0	90.6 ± 23.3	0.4
Antithrombin [2] (%)	96.4 ± 12.8	92.6 ± 13.2	0.3
Change antithrombin	12.4 ± 15.3	2.0 ± 22.6	0.06
aPTT [1] (s)	31.06 ± 14.01	27.18 ± 2.76	0.17
aPTT [2] (s)	28.9 ± 4.2	27.2 ± 2.9	0.1
Change aPTT	−2.16 ± 12.9	0.02 ± 1.8	0.4
Protein C [1] (%)	107 ± 25	114 ± 22.4	0.3
Protein C [2] (%)	105 ± 29.4	120 ± 27.05	0.06
Change protein C	−2.1 ± 25.4	6.2 ± 15.6	0.17
hs-CRP [1] (mg/l)	3.25 ± 4.5	4.4 ± 8.8	0.5
hs-CRP [2] (mg/l)	4.7 ±9.4	3.4 ± 6.6	0.5
Change hs-CRP	1.4 ± 6.7	−0.9 ± 3.5	0.1
Fibrinogen [1] (g/l)	2.8 ± 0.9	2.7 ± 0.9	0.6
Fibrinogen [2] (g/l)	3.3 ± 1.0	2.9 ± 0.9	0.2
Change fibrinogen	0.49 ± 0.9	0.3 ± 0.8	0.4

Numbers in brackets refer to first [1] and second [2] blood sampling. *Abbreviations:* aPTT = activated partial thromboplastin time, hs-CRP = high-sensitivity C-reactive protein. Other abbreviations as in Table I.

found to be useful for predicting future cardiovascular events, such as myocardial reinfarction or death.[10,11] However, in patients with acute MI complicated by VF, markers of coagulation activation have not as yet been investigated. The present study shows that patients with VF during the acute phase of MI have elevated levels of markers of thrombin formation at a timepoint remote from the acute coronary event. Blood sampling has not been obtained during the acute phase as there were patients receiving thrombolytic drugs. During long-term assessment, there was a significant decrease of coagulation parameters, especially within the VF group. These findings suggest that there is a transient active state leading to increase of markers of thrombin formation.[3] Comparing the change of parameters between patients with and without VF, it is conspicuous that the difference between thrombin-antithrombin complexes and prothrombin fragment F1 + 2 is statistically significant. One possible implication of these data is that markers of coagulation activation may be positively associated with VF. These data indicate that hypercoagulability in patients with CHD may be associated with an increased prevalence of ventricular tachycardia. Inflammation and thrombosis are the main processes contributing to atherosclerotic vascular lesions. In our study, survivors of SCD show an intrinsically increased systemic coagulation activation. This may be triggered by the coronary lesion or by systemic procoagulant stimuli. Genetic population studies dealing with probable inherited molecular risk for lethal tachyarrhythmia postulate mechanistic pathways which could contribute to the risk for SCD.[2] Potential markers of risk are atherothrombosis, electrogenesis, and neural regulation.[2] For example, in patients with CHD who have the PI^{A2} polymorphism in the IIIa gene, there is an increased platelet aggregation which is associated with enhanced mortality.[2]

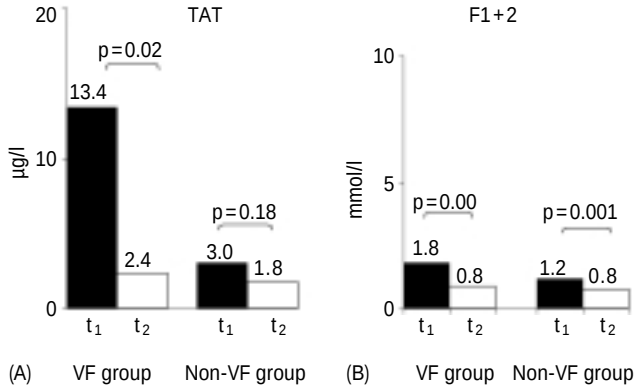

FIG. 1 (A) Comparison of thrombin-antithrombin complexes (TAT) within the groups; (B) comparison of prothrombin fragment F1+2 levels (F1+2) within the groups. ■ t_1 = First blood sampling, □ t_2 = second blood sampling.

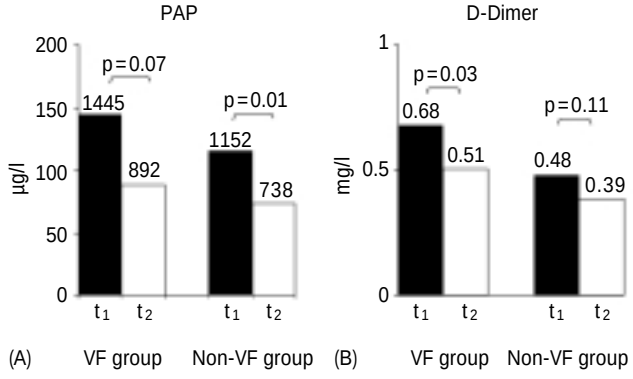

FIG. 2 (A) Comparison of plasmin-antiplasmin-complexes (PAP) within the groups; (B) comparison of D-Dimer levels within the groups. ■ t_1 = First blood sampling, □ t_2 = second blood sampling.

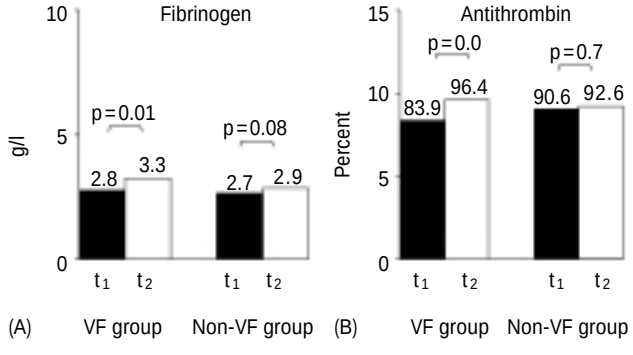

FIG. 3 (A) Comparison of fibrinogen levels within the groups; (B) comparison of antithrombin levels within the groups. ■ t_1 = First blood sampling, □ t_2 = second blood sampling.

Albert *et al.* found that higher hs-CRP levels correlate positively with an enhanced risk of SCD and were highly predictive in identifying SCD victims.[12] It is unclear, whether chronic increase of hs-CRP levels or cytokines which support its production are directly proarrhythmic. In our study, plasma hs-CRP levels were not different in patients with or without VF.

The most important clinical determinants of cardiac arrhythmias following ischemia are the presence of structural heart disease, the absence of collateral vessels, increased heart rate, and the size of the ischemic area.[13] Burke *et al.* investigated the relationship between plaque morphology and coronary risk factors in men with coronary disease who died suddenly.[14] They found that among these men abnormal serum cholesterol levels predispose to rupture of vulnerable plaques and cigarette smoking to acute thrombosis.[14] Experimental data by Goldstein *et al.*[4] display the arrhythmogenic influence of intracoronary thrombosis during acute myocardial ischemia in open-chest anesthetized dogs. They compared the incidence of malignant ventricular tachycardia (VT) associated with acute thrombosis of the LAD coronary artery, which was induced by electrical injury or intracoronary stenting, compared with LAD balloon occlusion.[4] Malignant VT was more prevalent in animals with thrombotic occlusion than in those with balloon occlusion, implying that intracoronary thrombosis per se promotes ventricular arrhythmia.[4] Coronel *et al.*[5] investigated profibrillatory effects of intracoronary thrombus in acute regional ischemia of the in situ porcine heart, showing that increased conduction slowing strengthens

"the occurrence of early VF in animals with intracoronary thrombosis compared with animals with cross-clamping of the coronary artery."[5] However, after repetitive episodes of ischemia and reperfusion, the electrophysiologic effects of the thrombus lasted for only several minutes, which suggests that this is associated with the release of lysophosphatidylcholine (LPC) from endothelial cells and from ventricular myocytes.[5] Lysophosphatidylcholine has been shown to alter electrical excitation and conduction properties, including a decrease of the resting membrane potential, the duration and amplitude of the action potential, shortening of the refractory period, and prolongation of the conduction time.[15] Thrombin can stimulate the synthesis of LPC in isolated ventricular myocytes.[15, 16] Experimental studies indicate that cardiomyocytes express G-protein-coupled protease-activated receptors (PAR) which are activated by thrombin.[16] Furthermore, stimulation of thrombin receptors on cardiomyocytes causes an enhanced activity of the sarcolemmal Na^+-H^+ exchanger,[15, 16] which plays an important role in the development of acute cardiac dysfunction and arrhythmias during acute ischemia.[16] Therefore, thrombin facilitates arrhythmogenesis during acute ischemia.

Limitations

Although the sample size was small, a significant change of markers of coagulation activation was found in this cohort of patients. Patients with VF during acute MI have a high mortality and the number of patients available in this group was limited. During acute MI, blood sampling was not obtained as some patients were being resuscitated or were receiving thrombolytic drugs which influence the coagulation cascade.

Conclusion

This study indicates that markers of thrombin formation are transiently increased in patients with VF during the acute phase of MI. Molecular and genetic mechanisms as well as genetic testing may substantiate an important pathway leading to sudden death during acute MI.

Acknowledgments

The authors thank A. Kirchner, N. Heim, C. Kehl for technical assistance and J. Brade for his statistical advice.

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