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Alterations in the Coagulation System of Active Smokers from the Ludwigshafen Risk and Cardiovascular Health (LURIC) Study

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Abstract

Smoking is an important and preventable risk factor of cardiovascular diseases with effects on blood coagulation. Our aim was to analyze the influence of smoking on coagulation parameters. Concentrations or activities of blood coagulation factors were compared in 777 active smokers and 1,178 lifetime non-smokers of the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. The association with mortality was examined using Cox regression. The findings show that AS had a tendency toward thrombosis. They displayed significantly higher values for fibrinogen, soluble fibrinogen, factor XIII, and tissue factor pathway inhibitor; whereas FVII, FVIII, FXII, von Willebrand factor (vWF), and thrombomodulin were decreased. The Cox regression analysis showed fibrinogen, FVIII, vWF, thrombomodulin, and tissue factor pathway inhibitor to be independent risk factors for mortality in active smokers with hazard ratios of 1.16 (95 % CI: 1.02–1.31), 1.40 (1.22–1.59), 1.37 (1.22–1.56), 1.19 (1.07–1.31), and 1.22 (1.06–1.40) per increase of one standard deviation. We conclude that active smokers have an increased

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thrombogenic potential associated with significant changes in the coagulation system. Individual parameters of the coagulation system are independent predictors of mortality. Therefore, parameters of the coagulation system, apart from other risk factors for cardiovascular disease (e.g., lipids or lifestyle) should be determined for risk prediction in active smokers.

Keywords
Cardiovascular disease • Hemostasis • Mortality • Smoking • Thrombosis

1 Introduction

Smoking is an important and preventable risk factor of cardiovascular diseases, as it is associated with increased inflammation, oxidative stress, thrombosis and atherosclerosis (Danaei et al. 2009). Cigarette smoke exposure seems to interfere with hemostasis through multiple pathways by affecting the functions of endothelial cells, platelets, and coagulation factors (Barua and Ambrose 2013). While a number of different hypotheses has been put forward to explain the harmful effect of tobacco smoke on the coagulation system, e.g., altered clot structure (Pretorius et al. 2010), decreased NO availability (Barua et al. 2001), increased oxidative stress or the generation of procoagulant microvesicles (Li et al. 2010), the underlying pathological mechanisms are still far from being fully understood. Therefore, the aim of our study was to analyze the influence of smoking on the subtle balance of antithrombotic and prothrombotic factors in a cohort with moderate to high risk for coronary heart disease.

2 Methods

2.1 Study Population

The study was approved by the Ethics Committee at the Ärztekammer Rheinland-Pfalz in Germany. All patients signed informed written consent at study onset. The LUdwigshafen RIsk and Cardiovascular Health (LURIC) study is an ongoing prospective study of 3,316 patients of German ancestry who had an indication for coronary angiography and were recruited between June 1997 and May 2001 at the Ludwigshafen Cardiac Center (Winkelmann et al. 2001). All patients were clinically stable (except for acute coronary syndromes). The information on vital status was obtained from local registries. Death certificates were obtained in 97 % of dead participants. Of the persons studied, 523 deaths (26.8 %) occurred during a median follow-up of 10 years. Cardiovascular death included the following categories: sudden death, fatal myocardial infarction, death due to congestive heart failure, death immediately after intervention to treat CHD, fatal stroke, and other causes of death due to CHD. Smoking status was assessed based on a questionnaire and verified by measurement of serum cotinine concentration.

2.2 Laboratory Procedures

Fasting blood samples were taken by venipuncture in the early morning prior to angiography. Aliquots were frozen at −80 °C. Coagulation factors were analyzed at the hemostaseology laboratory of the Ludwigshafen Heart Center at the same day. Endogenous thrombin potential (ETP) was determined from frozen aliquots of baseline samples using Innovance ETP on a BCS coagulation analyzer (Siemens Healthcare Diagnostics Inc., Munich, Germany).

2.3 Statistical Analysis

All continuous variables were checked for normality and the variables showing a skewed

distribution were logarithmically transformed to get a normal distribution. Continuous variables were compared between groups by Student's *t*-test. Associations between categorical variables were examined by chi-square testing. To examine the relationship of coagulation factors with mortality, we calculated hazard ratios (HR) and 95 % confidence intervals (95 % CI) using the Cox proportional hazards model. Multivariable adjustment was carried out as indicated. IBM SPSS Statistics v. 20.0 (IBM Corporation, USA) was used for all analyses.

3 Results

Active smokers (AS) were significantly younger, predominantly male, had a higher concentration of triglycerides, lower concentrations of LDL-C and HDL-C, and showed a higher percentage of coronary artery disease and hypertension compared with lifetime nonsmokers (NS) (Table 1). Their international normalized ratio (INR) of prothrombin time was significantly lower and endogenous thrombin potential (ETP) was higher, which speaks for a higher thrombogenic potential. We therefore investigated the differences in the concentration of coagulation factors in AS and NS.

In AS we observed significantly higher values for fibrinogen, soluble fibrinogen, factor XIII, and tissue factor pathway inhibitor; whereas the concentration of the factors VII, FXII, von Willebrand (vWF), and thrombomodulin were decreased (Table 2). No significant differences were found for the factors, activated prothrombin fragments 1 and 2, and antithrombin III (AT3).

We next examined whether parameters showing different values in AS and NS were associated with mortality by Cox regression analysis adjusted for other cardiovascular risk factors and found fibrinogen, factor VIII, vWF, thrombomodulin, and TFPI to be independent risk factors for mortality in AS with HRs of 1.16 (95 % CI: 1.02–1.31), 1.40 (1.22–1.59), 1.37 (1.22–1.56), 1.19 (1.07–1.31), and 1.22 (1.06–1.40) per increase of one standard deviation, respectively (Table 3). The HRs were similar in NS.

Table 1 Selected anthropometric data of study patients at study onset

	Never-smokers	Active smokers	p
Number	1,178	777	
Smoking (pack years)	0	30.0 (15.0–43.2)	
Age	65.3 ± 10.1	56.2 ± 10.3	<0.001[a]
Male Gender (%)	45.4	77.9	<0.001[b]
BMI	27.4 ± 4.2	27.0 ± 4.2	0.833[a]
LDL-C (mg/dl)	119.1 ± 36.4	117.5 ± 32.1	0.012[a]
HDL-C (mg/dl)	41.2 ± 11.1	36.2 ± 10.2	0.002[a]
Triglycerides (mg/dl)	136 (102–192)	154 (112–218)	<0.001[a]
Coronary artery disease (%)	68.1	80.1	<0.001[b]
Diabetes mellitus (%)	38.3	36	0.314[b]
Hypertension (%)	76.6	63.3	<0.001[b]
Lipid lowering drugs (%)	42.4	52.8	<0.001[b]
INR	1.05 (1.00–1.10)	1.03 (0.98–1.08)	<0.001[a]
ETP (%)	94.4 ± 28.5	99.1 ± 24.5	<0.001[a]

Data are means ± SD or median and 25th + 75th percentiles
BMI body mass index, *LDL-C* low density lipoprotein cholesterol, *HDL-C* high density lipoprotein cholesterol, *INR* International Normalized Ratio, *ETP* endogenous thrombin potential
[a]*t*-test
[b]chi-square test

Table 2 Concentration or activity of coagulation factors in never-smokers (NS) and active smoker (AS)

	Never-smokers		Active smokers		
	n		n		p[a]
Fibrinogen (mg/dl)	1,178	384.1 ± 99.7	775	417.0 ± 113.9	<0.001
Soluble fibrin (u/ml)	1,006	55.8 (34.6–83.9)	673	63.3 (41.5–95.8)	<0.001
Factor II (u/dl)	962	107 (93–122)	611	109 (95–122)	0.195
Factor V (u/dl)	680	111 (98–126)	435	115 (99–131)	0.172
Factor VII (u/dl)	1,173	125 (108–139)	775	120 (106–135)	0.023
Activated factor VII (u/l)	1,174	34.0 (21.8–51.0)	773	35.0 (22.0–52.0)	0.341
Factor VIII (u/dl)	1,175	172 (132–220)	775	154 (116–204)	<0.001
Factor XII (u/dl)	960	119 (87–145)	611	94 (80–131)	<0.001
Activated factor XII (µg/l)	1,176	2.64 ± 1.11	776	2.71 ± 1.24	0.214
Factor XIII (u/dl)	967	115 (100–131)	619	124 (109–141)	<0.001
Prothrombin fragments 1 + 2 (nmol/l)	1,176	0.63 (0.41–0.99)	777	0.64 (0.44–1.01)	0.182
Thrombomodulin (µg/l)	1,139	46.0 (35.0–59.0)	750	43.0 (33.0–56.0)	0.006
Antithrombin III (%)	1,169	97.7 ± 13.6	768	97.4 ± 13.3	0.575
Tissue factor pathway inhibitor (µg/l)	1,172	1.23 ± 0.37	773	1.30 ± 0.37	<0.001
Von Willebrand factor antigen (u/dl)	1,174	156 (122–202)	773	150 (112–196)	0.040

Data are means ± SD or median and 25th + 75th percentiles
[a]*t*-test for normally distributed variables, *t*-test of log transformed values for non-normally distributes variables

Table 3 Cox regression analysis of all-cause mortality per 1-SD increase adjusted for age, sex, LDL-C, HDL-C, BMI, diabetes, and hypertension

	Never-smokers		Active smokers	
	HR (95 % CI)	p	HR (95 % CI)	p
von Willebrand factor	1.37 (1.24–1.51)	<0.001	1.37 (1.22–1.56)	<0.001
Factor VIII	1.21 (1.11–1.32)	<0.001	1.40 (1.22–1.59)	<0.001
Tissue factor pathway inhibitor	1.20 (1.08–1.34)	0.001	1.22 (1.06–1.40)	0.005
Thrombomodulin	1.15 (1.03–1.29)	0.012	1.19 (1.07–1.31)	0.001
Fibrinogen	1.24 (1.10–1.39)	<0.001	1.16 (1.02–1.31)	0.026

4 Discussion

In participants of the LURIC study we found significant changes in individual parameters of the coagulation system in active smokers compared with lifetime nonsmokers. Elevated concentrations of fibrinogen in smokers have been reported before (Dotevall et al. 1994) as well as elevated levels of the A-subunit of factor XIII (Ariens et al. 1999) which covalently cross-links fibrin clots. Both parameters are also elevated in AS in the LURIC study. The concentration of soluble fibrin, which is created through the cleavage of fibrinogen by thrombin, also was increased in AS. These results point toward increased fibrin formation in AS. This is supported by the observed decrease in thrombomodulin levels in AS, which is a potent inhibitor of coagulation (Anastasiou et al. 2012). While there was a decrease in factor XII concentration (which initiates the intrinsic coagulation cascade) and factor VII (involved in the initiation of extrinsic coagulation cascade), we did not detect any differences in the activated factor XII or activated factor VII concentrations. In contrast to previous studies that reported an increase in vWF concentration through smoking (Price et al. 1999), we found a slight decrease in it, which was marginally significant. In cell culture assays, the serum from smokers induced a decrease in tissue factor pathway inhibitor (TFPI) secretion by endothelial cells accompanied

by a relative increase in tissue factor (TF) to TFPI ratio (Barua et al. 2002), while we observed a highly significant increase of TFPI in AS. TFPI is the most important inhibitor of the TF-mediated coagulation pathway, but TF itself was unfortunately not available in the LURIC study. However, there have been multiple reports of increased TFPI concentration in diseases like atherosclerosis and coronary artery disease, which has recently been discussed by Winckers et al. (2013). This might explain the observed increase of TFPI in AS of the LURIC study, since the majority of LURIC participants suffer from coronary artery disease.

Several prothrombotic factors, namely fibrinogen, vWF, and factor VIII were independent predictors of all-cause mortality in the LURIC study. Regarding TFPI, there also was a positive association with mortality, which might result from the association of this factor with coronary artery disease. We found that thrombomodulin also was associated with increased mortality in AS. Thrombomodulin, beside its antithrombotic functions, has antifibrinolytic activity (Anastasiou et al. 2012), which could be responsible for the increased risk of death in this study which comprises coronary artery disease patients.

5 Conclusions

The present study shows significant changes in individual parameters of the coagulation system in active smokers. These alterations point toward an increased thrombogenic potential. Individual parameters of the coagulation system were independent predictors of mortality in the LURIC study. Therefore, beside other risk factors for cardiovascular disease (e.g., lipids or life-style) parameters of the coagulation system should additionally be determined for risk prediction in active smokers.

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Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

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