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Inverse association of the endogenous thrombin potential (ETP) with cardiovascular death: The Ludwigshafen Risk and Cardiovascular Health (LURIC) study

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ABSTRACT

Background: Coagulation and prothrombotic potential have genuinely been associated with increased cardiovascular risk. However, not all studies in this regard are conclusive. Some clinical trials have shown an increased frequency of cardiovascular complications in patients receiving direct thrombin inhibitors. Previous data from human subjects after acute cardiovascular events showed an inverse association between the thrombin generation marker F1+2 and cardiovascular endpoints indicating that not the lowest, but a slightly elevated propensity for thrombin generation is associated with a lower risk of cardiovascular events. This observation has been supported by findings in animal models of atherosclerosis. Hence, we evaluated the association between the endogenous thrombin potential (ETP) and cardiovascular death (CVD) and markers of vascular dysfunction in a large prospective study with long-term follow up.

Method: After excluding patients receiving anticoagulants we tested ETP in 2196 participants (median follow-up 10 years) for its ability to predict vascular death (CVD). In addition, the association between ETP and sVCAM-1, sICAM-1, $LpPLA_2$, hsCRP and SAA was determined.

Results: We observed an inverse association between ETP and CVD with the lowest hazard ratio in the 4th ETP quartile. The nadirs of sICAM-1 or sVCAM-1 were observed in the 3rd, for $LpPLA_2$ in the 4th ETP quartile. Conversely, hsCRP and SAA were highest in the 4th quartile.

Conclusions: These results demonstrate that not the lowest ETP possible, but slightly higher levels are associated with a reduced risk of CVD and lower markers of endothelial dysfunction, suggesting a more complex role of thrombin in cardiovascular disease.

Abbreviations: aPC, activated protein C; BMI, body mass index; CI, confidence intervals; CVD, cardiovascular death; DM, diabetes mellitus; ETP, endogenous thrombin potential; F1+2, prothrombin fragment 1 + 2; HR, hazard ratio; hsCRP, high sensitivity C-reactive protein; hsTNT, high sensitivity troponin T; LURIC, Ludwigshafen Risk and Cardiovascular health study; $LpPLA_2$, lipoprotein-associated phospholipase A_2, also known as platelet-activating factor acetylhydrolase (PAFAH); MI, myocardial infarction; MOR, all cause mortality; PAR, protease activated receptor; PC, protein C; SAA, serum amyloid A; sICAM-1, soluble ICAM-1; sVCAM-1, soluble VCAM-1; t-PA, tissue plasminogen activator.

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1. Introduction

Myocardial infarction (MI) is an acute outcome of the chronic atherosclerotic process in large arteries. This process, which may remain asymptomatic for decades, is a continuous chronic inflammatory process characterized by foam cell formation and the formation of atherosclerotic plaques in the vascular wall [1]. Acute events such as unstable angina, MI, or stroke subsequently result from an erosion of the endothelium or the rupture of an established atherosclerotic plaque. The latter processes acutely expose thrombogenic surfaces (e.g. negatively charged phospholipids) and tissue factor to the circulating blood [2], activating soluble blood coagulation factors, which trigger the formation of a fibrin-platelet aggregate and produce a potentially occlusive vascular thrombus. Thus, local thrombin generation during an acute cardiovascular event is potentially lethal. Consequently, anticoagulation is the standard of care in acute coronary syndromes [3].

Due to its detrimental effects in the acute phase thrombin is generally perceived as a disease-promoting protease in atherogenesis. Consequently, the use of new direct thrombin inhibitors has already been discussed in this context [4]. Unexpectedly, some prospective clinical studies evaluating new direct thrombin inhibitors for venous diseases showed a trend to or a significantly increased frequency of MI associated with the use of direct thrombin inhibitors [5–9]. This effect remained significant in two recent meta-analyses comprising 7 and 11 trials, respectively, evaluating the direct thrombin inhibitor dabigatran [10,11]. While MI was not a primary endpoint in these studies, the observed increased incidence rates of MI raise the question whether thrombin has an unanticipated protective role in cardiovascular disease.

Potential beneficial effects mediated by thrombin seem to be paradox at first glance, but are in agreement with results from the comparably small Italian GUSTO study, which evaluated the thrombin activation marker F1+2 in 319 consecutive patients with acute coronary syndromes and a median follow-up of 29 months [12]. In this study the risk for cardiac death or myocardial (re)infarction was lowest in patients with intermediate plasma levels of F1+2 [12]. This observation suggests that not the lowest thrombin generation possible, but slightly higher levels are associated with the lowest risk for cardiovascular events, at least after a first cardiovascular event. However, this observation, which the authors deemed "unexpected", has not been confirmed in a larger cohort or with a different marker so far. While the thrombin activation marker F1+2 is a molecular marker specific for thrombin generation in vivo, reflecting the acute situation, the endogenous thombin potential (ETP) reflects the thrombin-forming capacity in a specific individual. Associations between these markers of thrombin generation and clinical endpoints are partly discordant [13–15], indicating that they reflect differential information regarding thrombin generation.

The aim of the current study was to determine the association between the ETP and cardiovascular death (CVD) as endpoint in a large prospective study (3156 individuals, median follow-up 10 years). In addition, we included markers of endothelial dysfunction to gain insights into potential pathophysiological mechanisms

2. Methods

2.1. Study design and participants

We studied the participants of the Ludwigshafen Risk and Cardiovascular (LURIC) health study, a prospective cohort study of persons undergoing coronary angiography. The study protocol and baseline characteristics of patients have been previously published in detail [16]. Briefly, the inclusion criteria were: German ancestry, clinical stability and the availability of a coronary angiogram. Individuals included within the study had a status post-acute MI (3%, time interval between MI and blood sampling 1 day to 4 weeks), unstable angina pectoris (26%, time interval 1 day to 4 weeks), or a history of MI (41%, time interval at least 4 weeks). In patients with a status post-acute MI enrollment and blood sampling were conducted after the patient had been transferred to a regular ward and was clinically stable. Of those patients diagnosed as having unstable angina pectoris 62% were troponin negative (corresponding to ~16% of the total study population), while 38% were troponin positive (corresponding to non-ST-elevation MI, ~10% of the total study population). The indications for angiography in individuals in clinically stable condition (e.g. no history of MI or unstable angina pectoris, 30% of the total study population) were a history of chest pain and/or noninvasive test results suggestive of myocardial ischemia. Individuals suffering from acute illness other than acute coronary syndromes (e.g. infection, autoimmune disease, or recent accident/surgery), chronic non-cardiac diseases (e.g. chronic renal failure, severe rheumatic arthritis), or malignancy within the past 5 years and those unable to understand the purpose of the study were excluded. The study was approved by the ethics committee of the "Landesärztekammer Rheinland-Pfalz" (no. 1997-203). Informed written consent was obtained from all participants.

Measurements for ETP were complete in 3156 out of 3316 individuals. Patients receiving anticoagulants (e.g. heparinoids, vitamin K antagonists) were excluded in the current analyses since ETP is influenced by anticoagulant treatment [17,18]. After exclusion of patients receiving anticoagulants 2196 patients remained eligible for the current study.

Information regarding mortality (MOR) was obtained from local registries. CVD was defined as death from MI, death after an intervention to treat cardiovascular disease, death from heart failure, or sudden cardiac death.

Diabetes mellitus was diagnosed if plasma glucose was ≥7.0 mmol/L in the fasting state or ≥11.1 mmol/L 2 h after an oral glucose load or if individuals were receiving antidiabetic treatment. Hypertension was diagnosed if the systolic and/or diastolic blood pressure exceeded 140 and/or 90 mmHg or if there was a history of hypertension, evident through the use of antihypertensive drugs. Weight and height of all study participants were obtained at inclusion. Information regarding smoking habits was obtained in a standardized questionnaire [16].

2.2. Laboratory procedures

Fasting blood samples were collected before administration of any medication. The standard laboratory methods have been described [16]. The following assays were performed with reagents from Siemens Healthcare Diagnostics Inc., Germany: ETP was determined using INNOVANCE ETP on a BCS coagulation analyzer and F1+2 (Prothrombin fragment 1 + 2) was analyzed using the Enzygnost® F1+2 micro test on an automated platform (SLT Spectra TECAN, Männedorf, Switzerland). Plasma levels of sVCAM-1 and sICAM-1 were measured using ELISA with specific monoclonal antibodies to corresponding human proteins (R&D systems GmbH, Wiesbaden, Germany) using an automated system (Rosys Plato, Immucor, Norcross, GA, USA). $LpPLA_2$ (lipoprotein associated phospholipase A_2, also referred to as platelet-activating factor acetyl-hydrolase, PAFAH) was measured using a spectrophotometric activity assay (Azwell Auto PAF-AH kit, Azwell Inc., Osaka, Japan) on a Hitachi 912 autoanalyzer.

2.3. Statistical analysis

All authors had access to the clinical data and results obtained. Characteristics of individuals within the four quartiles of ETP (Table 1) are presented as percentages for categorical variables and as means (±SD) or medians (25th and 75th percentiles) for continuous variables. Associations of categorical and continuous variables were analyzed by logistic regression and univariate ANOVA, respectively, with covariables as indicated. All continuous variables were checked for normality and skewed data were transformed logarithmically. To examine the relationship of ETP with mortality from cardiovascular causes (CVD) we calculated hazard ratios and 95% confidence intervals (95% CI) using the Cox proportional hazards model. The time to CVD variable was defined as the time period between enrollment and CVD or the time to the last follow-up (May 27, 2009) for the censored subjects. We analyzed the effects of ETP on markers of endothelial dysfunction (sICAM-1, sVCAM-1) in an Analysis of Covariance (ANCOVA) according to the general linear model (GLM) using those factors not under examination as covariates.

Multivariable adjustment was carried out for age, gender, DM, BMI, hsCRP, vessel score, history of smoking, hypertension, or MI, use of platelet inhibition (ASS or Clopidogrel), ACE-inhibitors, beta blockage, statins, kidney function as well as LDL cholesterol, HDL cholesterol, and triglycerides. The SPSS 19.0 statistical package (SPSS Inc.) was used for all analyses. All tests were two-sided. Analyses were corrected for multiple hypotheses testing by applying the Bonferroni equation to adjust the P values. Adjusted P-values are reported. $P < 0.05$ was considered significant.

3. Results

3.1. Intermediate levels of ETP are associated with a lower risk of future CVD

Clinical and biochemical characteristics of the study participants following stratification ETP-quartiles are shown in Table 1. During the follow-up period (median follow-up: 10 years) 345 CVD were recorded in the current cohort (2196 study participants). Various baseline characteristics of the four groups differed significantly (Table 1). The average age, the prevalence rate of diabetes mellitus, and systolic blood pressure decreased significantly across the ETP quartiles, being lowest within the 4th ETP quartile (Table 1). However, other established or potential risk factors for cardiovascular disease, such as the BMI, total cholesterol, LDL cholesterol, or triglycerides were increased within the 4th ETP quartile (Table 1). Thus, individuals in the 4th ETP quartile did not generally