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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

Table 1
Baseline characteristics of individuals following stratification into ETP-quartiles.

	1st quartile	2nd quartile	3rd quartile	4th quartile	P^*
Number	551	545	552	548	
ETP (nmol*min)	78.6 (77.5–79.5)	99.0 (98.0–99.9)	107.0 (106.0–107.9)	120.8 (119.8–121.8)	<0.001
Age, years	64.7 ± 10.6	62.1 ± 10.6	61.0 ± 10.6	61.0 ± 10.1	<0.001
Male gender	392 (71.2)	383 (70.3)	368 (66.7)	347 (63.3)	0.019
BMI, kg/m^2	27.0 ± 4.1	26.9 ± 3.6	27.4 ± 4.1	28.3 ± 4.0	<0.001
Myocardial infarction					0.870
One	174	181	167	183	
Two or more	32	31	27	33	
CAD (vessel score)					0.160
0	160	179	204	194	
1	113	92	103	107	
2	107	96	100	92	
3	171	178	145	155	
Peripheral vascular disease	57	45	44	43	0.385
Stroke/TIA	51	41	30	41	0.122
Type 2 diabetes	119	87	68	72	<0.001
HbA1c, %	6.3 ± 1.2	6.2 ± 1.17	6.2 ± 1.10	6.3 ± 1.20	0.333
Fasting glucose, mg/dl	116 ± 37	112 ± 32	111 ± 34	112 ± 35	0.139
Systolic blood pressure, mmHg	145 ± 25	143 ± 23	141 ± 23	142 ± 24	0.031
Diastolic blood pressure, mmHg	82 ± 12	82 ± 11	82 ± 11	82 ± 11	0.921
Total cholesterol, mg/dl	185 ± 34	192 ± 37	198 ± 38	210 ± 42	<0.001
LDL cholesterol, mg/dl	110 ± 99	116 ± 33	119 ± 21	129 ± 38	<0.001
HDL cholesterol, mg/dl	40 ± 10	41 ± 11	41 ± 11	40 ± 11	0.268
Triglycerides, mg/dl	162 (154–169)	164.2 (156–171)	162.2 (154–170)	182.7 (175–190)	<0.001
Smoking, packyears	18.3 (16.2–20.4)	18.7 (16.7–20.8)	17.1 (15.0–19.1)	18.38 (16.3–20.4)	0.693
Therapy with beta-blocker, N	332	350	326	337	0.336
Therapy with statin, N	234	243	241	245	0.875
Therapy with ACE-inhibitor, N	292	250	243	269	0.024
Therapy with ASS, N	389	416	403	412	0.158

display a favorable risk profile with regard to CVD. No difference between the ETP quartiles was observed regarding the history of previous MI, peripheral vascular disease, stroke or TIA (Table 1). Likewise, the ETP quartiles did not differ in regard to the vessel score as determined by angiography at the time of enrollment (Table 1). Use of ACE-inhibitors differed significantly between the ETP-quartiles, but did not follow a constant trend across the ETP-quartiles. Medication with beta-blockers, statins, or platelet aggregation inhibitors did not differ between groups.

When evaluating the association between CVD and ETP we found the highest hazard ratio (HR) for CVD in the 1st ETP quartile and significantly lower HRs for CVD in the 2nd, 3rd, and 4th ETP quartiles with an uncorrected model approach (data not shown). After adjusting for potential confounders as indicated, the HR for CVD remained highest in the 1st ETP quartile. The HR for CVD was significantly lower in the 2nd, 3rd, and 4th ETP quartiles, being the lowest in the 4th quartile (HR 0.613, 95% CI 0.447–0.839, $P = 0.002$). Consistently, a survival analysis revealed the lowest survival rate among the individuals in the first ETP quartile, while survival was highest among individuals in the 4th ETP quartile (Fig. 1). When performing analyses with data obtained after a median follow-up of 5 years almost identical results were obtained (data not shown). Taken together, among patients with pre-existing or suspected coronary disease the risk for CVD is inversely associated with the propensity to activate thrombin, as reflected by the ETP.

3.2. Inverse association of the ETP with markers of endothelial dysfunction

Thrombin is commonly perceived as a pro-inflammatory and endothelial cell damaging protease. To determine whether ETP levels are positively or negatively associated with markers of endothelial cell activation (sVCAM-1, sICAM-1) or of vascular inflammation ($LpPLA_2$, lipoprotein-associated phospholipase A_2, also known as platelet-activating factor acetylhydrolase, (PAFAH)) [19], we next evaluated the relation between ETP and these markers of vascular disease.

After correction for the confounding factors as indicated, we observed an inverse association between ETP and $LpPLA_2$ (PAFAH) with the lowest $LpPLA_2$ (PAFAH) levels in the 4th ETP quartile (466.8 U/l, range 459.1–475.5, $P < 0.001$ for the overall trend, Fig. 2A). This distribution of the vascular inflammation marker $LpPLA_2$ across ETP quartiles resembles the inverse association between ETP and CVD. The endothelial cell activation markers sICAM-1 and sVCAM-1 showed a significant reduction of plasma concentrations in the 2nd and 3rd ETP quartile for both markers and in addition in the 4th ETP quartile for sVCAM-1. The

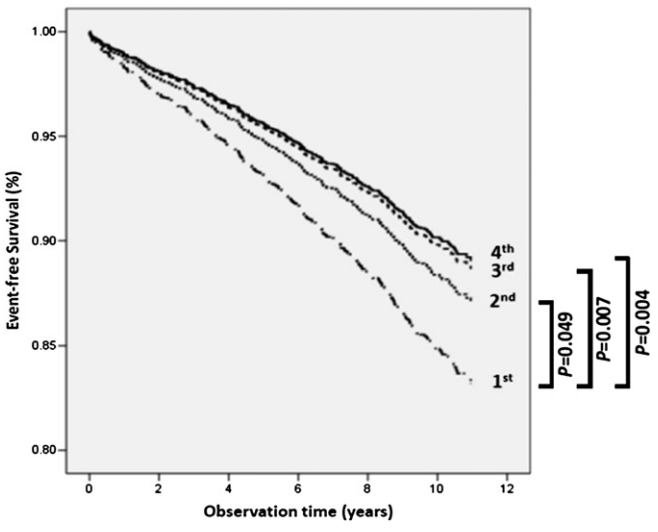

Fig. 1. Event-free survival of individuals stratified according to ETP-quartiles. Event-free survival is significantly reduced in the 1st ETP-quartile compared to the 2nd, 3rd, or 4th quartile. The *P*-value for the overall trend is 0.011. Data are adjusted for age, gender, diabetes mellitus, body mass index, smoking, hypertension, LDL cholesterol, HDL cholesterol, triglycerides, hsCRP, vessel score, history of myocardial infarction, and use of statins or ACE-inhibitor.

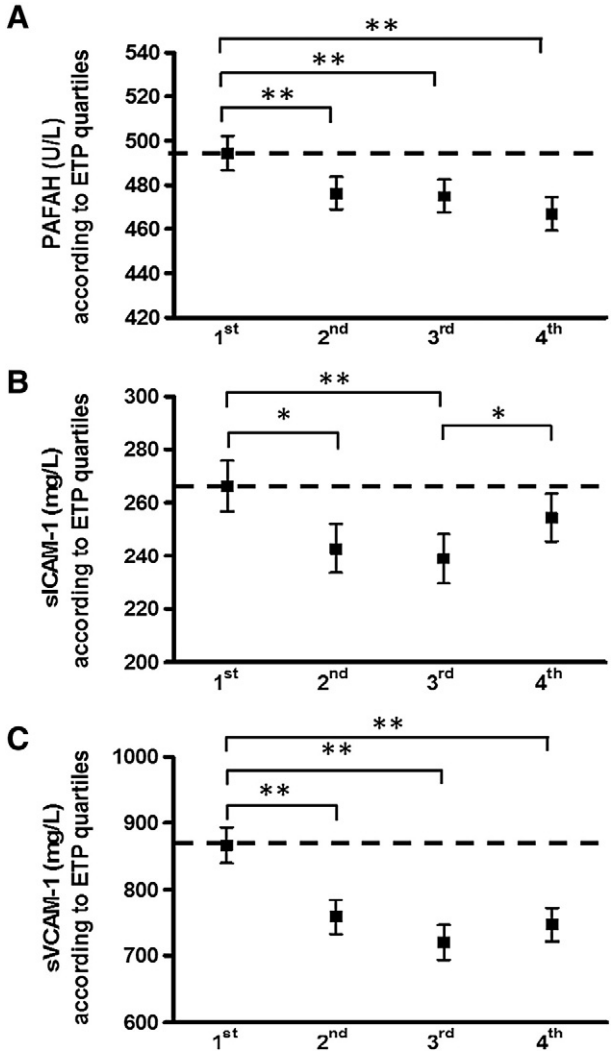

Fig. 2. Association of ETP with markers of endothelial dysfunction and inflammatory cell activation. Estimated marginal means (±95% CI) of sICAM-1 (A), sVCAM-1 (B), or $LpPLA_2$ (C) in study participants stratified according to ETP quartiles. Data are adjusted for age, gender, diabetes mellitus, body mass index, smoking, hypertension, LDL cholesterol, HDL cholesterol, and triglycerides, and study participants receiving anticoagulant treatment were excluded; $^{*}P < 0.05$; $^{**}P < 0.01$.

nadir of plasma levels for both soluble adhesion molecules was observed in the 3rd quartile, and levels of sICAM were significantly increased in the 4th ETP quartile as compared to the 3rd quartile (sICAM-1: 238.9 mg/l, range: 229.5–248.3; sVCAM-1: 719.7 mg/l, range 693.5–746.0, $P < 0.001$ for the overall trend, Fig. 2B,C). A non-significant trend for increased sVCAM levels was likewise observed in the 4th ETP quartile, indicating levels of ETP higher than those observed in the present cohort will eventually be associated with increased markers of endothelial cell dysfunction.

The association between CVD and systemic inflammation is well established [20,21]. Therefore, we also assessed the associations between high sensitivity c-reactive protein (hsCRP) and serum amyloid a (SAA) and ETP. Both, hsCRP and SAA were the lowest in the first ETP quartile (hsCRP: 4.46 mg/l, range 3.31–5.60; SAA: 10.4 mg/l, range 3.03–17.2) and markedly increased in higher quartiles, being significantly elevated in the 4th quartile (hsCRP: 10.4 mg/l, range 9.26–11.6; SAA: 35.1 mg/l, range 27.8–42.5, $P < 0.001$ for the overall trend (Fig. 3A,B)).

Together, within this study, markers of endothelial cell activation and vascular inflammation showed an inverse and markers of systemic inflammation a positive association with ETP as the incidence of CVD over a 10 year follow-up period.

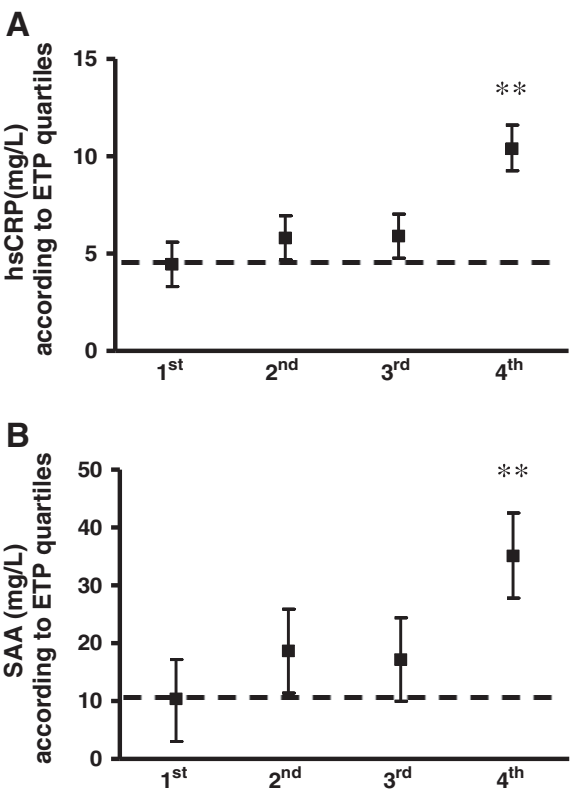

Fig. 3. Association of ETP with markers of systemic inflammation. Estimated marginal means (±95% CI) of hsCRP (A) and SAA (B) in study participants stratified according to ETP quartiles. Data are adjusted for age, gender, diabetes mellitus, body mass index, smoking, hypertension, LDL cholesterol, HDL cholesterol, and triglycerides, and study participants receiving anticoagulant treatment were excluded; $^{**}P < 0.01$.

4. Discussion

The role of coagulation activation in cardiovascular disease is being considered as detrimental in general. Trends toward a certain hypercoagulability have been reported for subjects with diabetes, hypertension or suffering from acute coronary events [22,23]. However, recent reports demonstrated that thrombin may not only be disease-promoting, since it can exert endothelial protective effects at low concentrations [24].

In the present study we have observed an inverse association of an indicator of thrombin generation, ETP, and CVD in a large study population (N = 2196) over a longer follow-up (10 years). While this seems to be conflicting in the context, there is also other data suggesting that elevated thrombin activation is associated with a reduced risk for CVD. In the Italian GUSTO study a U-shaped association between the thrombin activation marker F1+2 and CVD or myocardial (re)infarction was observed [12]. When using the same marker as analyzed in the GUSTO study (F1+2) we likewise observed a U-shaped association in relation to CVD in the current study (data not shown). The consistent results obtained from the GUSTO and the current study imply that the inverse or U-shaped association between cardiovascular endpoints and thrombin activation markers may be a genuine phenomenon. The data suggest that slightly higher levels of thrombin activation, rather than the lowest possible, may be associated with a reduced risk for cardiovascular complications and death.

The associative nature of the current study precludes any causal inference. Nonetheless, results from preclinical studies do support the notion of the so-called "thrombin paradoxon". The term "thrombin paradoxon" was initially coined to describe the two-faced properties of thrombin in regard to coagulation [25]. Thrombin has well established pro-coagulant functions, stemming from its ability to activate platelets,

fibrinogen, and various coagulation factors. Conversely, when bound to thrombomodulin, thrombin is a potent activator of protein C (PC), an important anti-coagulant [26]. In addition to being an anti-coagulant activated PC (aPC) procures mechanistically distinct cytoprotective functions [27], which may ameliorate the atherosclerotic process [28].

Recent studies propose that thrombin-dependent signaling itself may convey protective effects. In that context, atherosclerosis prone mice with genetically superimposed hypercoagulability and impaired PC activation were shown to have increased plaque stability [29]. In addition, low dose thrombin can mediate anti-inflammatory and endothelial protective effects in vitro by a reduction of adhesion molecule expression (ICAM-1, VCAM-1, E-selectin) and transendothelial migration of leucocytes in cytokine stimulated endothelial cells [30]. Other experimental studies provide further evidence for possible protective effects of thrombin in the context of cardiovascular disease [31–34]. It is, however, noteworthy that the ETP quartiles were positively associated with systemic markers of inflammation in our study. This finding suggests that thrombin exerts its protective function primarily locally at the vessel wall in a paracrine fashion.

A limitation of the current study is that blood sampling was only performed once in each patient. Although we have not found an association between ETP and acute events in our study (data not shown), ETP has been found to be elevated in the circumstance of acute coronary events [35], thus the distinction between acute versus chronic events may be very important in the context of coagulation [36].

In the GUSTO study, which enrolled a lower number of patients ($N = 319$), multiple blood samples were obtained during a follow-up of 12 months. The U-shaped association between F1+2 and the primary endpoint (cardiovascular death or myocardial (re)infarction) remained stable during follow-up in the GUSTO study, indicating that the association of thrombin activation markers and cardiovascular endpoints is not an artifact caused by an acute coronary syndrome. Along this line, the inverse association between ETP and CVD in the current study remained stable when excluding patients with an elevated hsTNT (larger 14 pg/ml, data not shown), supporting the notion that the inverse association is not an artifact related to an acute coronary event.

However, we cannot exclude the existence of other – and potentially unrecognized – confounders contributing to the observed "thrombin paradoxon". In order to determine whether the current observation can be generalized, other cohorts and populations will be required to be studied in that specific conditions/perturbations allow further insight into the coagulation system [37].

While the inverse association between markers of blood coagulation activation and cardiovascular endpoints appears to be unrelated to an acute coronary event, it cannot be generalized. In both studies (GUSTO and LURIC) patients were selected on the basis of an index event (proven or suspected acute coronary syndrome, respectively). Hence, an "index event bias" cannot be excluded [38]. Recruitment of individuals into a study based on an index event, such as proven or suspected acute coronary syndrome, may skew the risk profile of enrolled individuals in comparison to the general population.

In the current study individuals within the 4th ETP quartile had a lower age, a lower frequency of diabetes mellitus, and a reduced systolic blood pressure in comparison to individuals from the 1st ETP quartile. However, the baseline characteristics of individuals within the 4th ETP quartile were not consistently favorable. In particular, the lipid profile was disadvantageous among individuals of the 4th ETP quartile (increased total and LDL cholesterol and increased triglycerides). Hence, a selection bias or "index event" effect seems less likely.

The current and previous results [12] contribute data to the ongoing discussion regarding the efficacy and safety of novel anticoagulants (NOACs) [39]. Some [5,6,8], but not all [40,41] studies evaluating direct thrombin inhibitors revealed a significantly or tentatively increased frequency of cardiovascular events. Consequently, current data is inconclusive on the topic [42]. However, two recent meta-analyses confirmed that the direct thrombin inhibitor dabigatran is associated with an increased risk of MI or acute coronary syndrome [10,11]. In contrast, factor Xa inhibitors were consistently associated with a significantly or tentatively reduced frequency of cardiovascular events [8,43–46].

It is tempting to speculate that a difference in cardiovascular endpoints might indicate an unfavorable cardiovascular side effect profile of the direct thrombin inhibitors [47]. In that case the observed effect could be a dose-dependent phenomenon and the difference in relation to cardiovascular endpoints may not be specific for a substance, but rather for the respective dosages employed. A dose-dependent effect of direct thrombin inhibitors is likewise suggested by studies evaluating the direct thrombin inhibitor hirudin in patients with acute MI. In these studies hirudin did not only fail to show a benefit, but even tended to have a worse outcome at higher doses, prompting the authors to suggest that "too much thrombin inhibition may be harmful" [3]. Regarding NOACs the jury is still out on the case [42,47] and more clinical data are needed. It may therefore be instructive to determine the effect of different dosages of NOACs on cardiovascular endpoints or to define patient populations that have specific and increased benefit from such substances. Answering such questions may increase the beneficial use of NOACs.

5. Conclusion

We have provided evidence that not the lowest ETP possible, but slightly higher levels of ETP were associated with a reduced risk of CVD and lower markers of endothelial dysfunction in a well-defined cohort of patients, suggesting a more complex role of thrombin in cardiovascular disease.

Conflict of interest

Disclosure of conflicts: None.

Author's contributions

The authorship contribution is as follows: B.I., J.S. and M.E.K. performed data analyses and wrote the manuscript, B.O.B. and F.P. designed and conducted the study, T.B.G. helped with data interpretation and writing, H.W. performed data analyses, P.P.N. helped in writing the manuscript and discussed the data, W.M. designed and conducted the study and wrote the manuscript.

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