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Research Article

Cancer Epidemiology, Biomarkers & Prevention

Plasma Fibrinogen and sP-Selectin are Associated with the Risk of Lung Cancer in a Prospective Study

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Abstract

Background: While enhanced platelet activation and a procoagulant state may drive lung cancer progression and metastases, less is known about their role in earlier phases of cancer development. Thus, we evaluated whether prediagnostic biomarkers of platelet activation and coagulation are related to the risk of lung cancer in the prospective EPIC-Heidelberg Study using a case–cohort design.

Methods: Levels of fibrinogen, soluble glycoprotein (sGP) IIb/IIIa, soluble P-selectin (sP-selectin), soluble thrombomodulin (sTM), and thrombopoietin (TPO) were measured in baseline plasma samples of a random subcohort ($n = 2{,}480$) and incident cases of lung cancer ($n = 190$). Multivariable-adjusted Cox proportional hazards regression analyses were used to obtain HRs of lung cancer across quartiles of biomarker levels.

Results: Fibrinogen [HR highest vs. lowest quartile: 1.91 (95% confidence interval: 1.09–3.34)] and sP-Selectin [HR: 2.51 (1.39–4.52)] were significantly associated with lung cancer risk in multivariable adjusted Cox regression models. Adding both biomarkers to the established $PLCO_{m2012}$ algorithm, which alone showed a C-statistic of 0.788, led to a slight increment in lung cancer risk prediction, with a C-statistic of 0.814.

Conclusion: Our findings indicate that enhanced platelet activation and a procoagulative state contribute to lung carcinogenesis.

Impact: The current prospective study supports the hypothesis of increased coagulation being a possible driver of lung carcinogenesis, as strong positive associations were found between two procoagulative markers, sP-Selectin and fibrinogen, with lung cancer risk. Both biomarkers could improve lung cancer risk prediction, but external validation of the results is needed.

Introduction

Activated platelets and a procoagulant state may drive lung cancer progression by different mechanisms, such as the interaction between platelet adhesion molecules and tumor cells resulting in facilitated arrest and immune evasion as well as by growth-stimulating and angiogenic effect of platelet releasates on tumor cells (1, 2). While experimental and clinical evidence linking altered hemostasis and increased coagulation, as represented by elevated level of platelets and hemostatic molecules, with lung tumor growth, metastases, and prognosis is compelling (3–5), less is known about the role of these alterations in earlier phases of lung cancer development.

Fibrinogen, a plasmatic coagulation factor, plays a crucial role in hemostasis as it induces platelet activation and aggregation and constitutes the precursor of insoluble fibrin, which is needed for clot stabilization (6). A positive association between elevated plasma fibrinogen and respiratory/intrathoracic organ cancer mortality was first observed in a prospective study in 2007 (7). More recently, increased fibrinogen levels were shown to be positively associated with the risk of lung cancer in three prospective cohort studies (8–10). Further indirect indications for a role of activated platelets and a procoagulant state in tumorigenesis come from cohort studies and randomized trials, which have shown lower risks for some cancers among users of low-dose aspirin (11–13), where the pharmacologic effect of aspirin is thought to be due to its inhibition of platelet aggregation (14). However, the possible chemo-preventive effect of aspirin is moderate regarding lung cancer, and associations between aspirin use and lung cancer risk in prospective studies are less consistent than associations between aspirin use and risks for other cancers, particularly colorectal cancer (13, 15). An alternative explanation for the abovementioned associations between fibrinogen and lung cancer risk could be that increased fibrinogen is a consequence of smoking rather than an independent lung cancer risk factor (7, 9). At the same time, it is conceivable that smoking-induced hemostatic alterations may be one mechanistic link underlying the association between smoking and lung cancer (9).

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Although prospective epidemiologic studies are lacking, experimental and clinical studies suggest that beyond fibrinogen further molecules with distinct functions in platelet activation and coagulation may be interesting biomarkers of lung cancer risk. The adhesion factor P-Selectin is expressed on endothelial cells and platelets upon their activation and has been implicated in inflammation and platelet aggregation (16). P-Selectin has extensively been studied with respect to cancer progression, as it is assumed to enable tumor cell–platelet interactions, thereby facilitating tumor cell arrest and survival as well as metastatic spread (16, 17). In patients with cancer, high levels of soluble P-selectin (sP-selectin) have been related to poor prognosis (16). Like P-Selectin, glycoprotein IIb/IIIa (GPIIb/IIIa) is a platelet integral membrane protein (18). When platelets become activated, GPIIb/IIIa undergoes conformational change into a high-affinity receptor for fibrinogen, which explains its essential role in the regulation of platelet adhesion and aggregation (19). The GPIIb/IIIa receptor has been shown to mediate interactions between platelets and various tumor cell lines and its blockade resulted in decreased pulmonary metastasis *in vivo* (20). To our knowledge, there is a lack of studies with respect to its soluble form glycoprotein (sGPIIb/IIIa). Thrombopoietin (TPO) constitutes the main physiologic regulator of platelets as it stimulates the growth of megakaryocytes, that is, platelet progenitor cells (21). Increased plasma levels of the molecule have been related to poor survival in patients with cancer (22). Finally, thrombomodulin (TM), a transmembrane protein of the endothelium with anticoagulative properties (23). TM expression in squamous cell carcinoma of the lung (24) and other tumor types (25) has been related to better prognosis. In contrast, increased blood levels of its soluble form thrombomodulin (sTM) are indicative of vascular damage (26) and may correlate with cancer progression (27).

In this study, we analyzed the biomarkers of platelet activation and coagulation described above (fibrinogen, sP-Selectin, sGPIIb/IIIa, thrombopoietin and sTM) in relation to lung cancer risk in the prospective EPIC-Heidelberg cohort. We first obtained HRs of lung cancer risk across quartiles of biomarker concentrations in multivariable adjusted statistical models to assess whether enhanced platelet activation and a procoagulant state are independent risk factors for lung cancer. We further tested to which degree associations between smoking and lung cancer risk could be mediated by platelet activation and coagulation. Finally, we estimated whether the selected biomarkers improved lung cancer risk prediction.

Materials and Methods

Study population

The European prospective Investigation into Cancer and Nutrition (EPIC)-Heidelberg is a population-based cohort that was set up to investigate dietary, lifestyle, and metabolic factors in relation to risks of cancer and other chronic diseases. Overall, 25,540 individuals (53.3% women) aged 35–65 from the local general population were recruited for EPIC-Heidelberg between 1994 and 1998. At baseline, questionnaire and interview assessments of participants' health status, habitual diet, lifestyle, socioeconomic status, and reproductive health were carried out, a blood sample was obtained, and anthropometric measurements were taken (28). During follow-up, incident cancer cases were identified by active questionnaire follow-up and by record linkage with the federal cancer registry (29). All cases were then validated and coded by a study physician based on diagnostic records.

For this study, a case–cohort sample of the EPIC-Heidelberg cohort was selected including a random subcohort ($n = 2{,}480$) and all incident cases of lung cancer ($n = 190$; ICD-10: C34) that had occurred until December 31, 2012. The subcohort included 17 incident cases of lung cancer and 94 prevalent cases of cancer of any type. After exclusion of the prevalent cases, the subcohort consisted of 2,386 participants. As intended by design, participants in the subcohort showed highly similar characteristics compared with the full cohort (30).

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Heidelberg University Hospital (Heidelberg, Germany) and all participants gave written consent for the use of their data and blood samples. The study was performed in accordance with the Declaration of Helsinki.

Laboratory methods

At the baseline of EPIC-Heidelberg, blood samples were processed into serum, plasma, buffy coat, and erythrocyte samples, which were aliquoted and stored in gas-phase liquid nitrogen (−150°C). Aliquots were thawed for the first time for the current analyses. Electrochemiluminescence immunoassays were carried on the "Quickplex SQ 120″ instrument (Meso Scale Discoveries) to measure plasma concentrations of sP-selectin, sTM, and TPO, using MSD's "human vascular injury I" (sP-selectin and sTM) and "U-Plex TPO Assay" (TPO) kits. sGPIIb/IIIa and fibrinogen levels were measured by ELISAs using the assay kits "ab108851" from Abcam and "KA0475" from Abnova. Within-batch coefficients of variation (CV) (between-batch CVs) were 4.6% (19.5%), 3.8% (10.1%), 3.3% (9.1%), 5.5% (46.9%), and 5.7% (8.5%) for TPO, sTM, sP-selectin, sGPIIb/IIIa, and fibrinogen. The percentages of missing values for the biomarkers were below 1 % (TPO: $n = 3$, sTM: $n = 5$, sP-selectin: $n = 2$, fibrinogen: $n = 3$, and sGPIIb/IIIa: $n = 33$). Samples of individuals in the subcohort and later cases were randomly distributed across analytical batches, and the batch mean-centering method was used for batch standardization (31).

Statistical analyses

Baseline characteristics of the study population are presented as medians (percentiles 25th/75th) or proportions. Age- and sex-adjusted Spearman coefficients were calculated to evaluate correlations between TPO, sTM, sP-selectin, sGPIIb/IIIa, fibrinogen, and C-reactive protein (CRP). Age- and sex-adjusted generalized linear models were used to assess cross-sectional associations between biomarker levels and categorical covariates.

For analyses on lung cancer risk, biomarker levels were divided into sex-specific quartiles based on the distribution in the subcohort. Prentice-weighted (32) Cox proportional hazards regression models with age as the underlying timescale were used to obtain HRs of lung cancer across quartiles of TPO, sTM, sP-selectin, sGPIIb/IIIa, and fibrinogen. Participants were included with delayed entry from their age at recruitment until date of cancer diagnosis, or censoring for death or end of follow-up, whichever came first. The extended correlation test based on Schoenfeld residuals (33) did not indicate any violations of the proportional hazards assumption.

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The first Cox regression model was minimally adjusted, that is, for age and sex. In a second multivariable model, we additionally adjusted for smoking status (never smokers, long-term quitters $\geq$ 10 years, short-term quitters < 10 years, light smokers < 15 cigarettes per day, and heavy smokers $\geq$ 15 cigarettes per day), lifetime alcohol intake (g/d), current aspirin use (yes/no), physical activity (Cambridge physical activity index), height (cm), body mass index (BMI; kg/m^2), education level (primary school, secondary school, university degree), and CRP level (mg/dL) as a marker of inflammation. Potential confounders were identified by literature search. To test for linear trends, $\log_2$-transformed biomarker values on the continuous scale were entered into Cox regression models instead of quartiles. In sensitivity analyses, cases diagnosed within the first two years or within the first five years of follow-up were excluded from multivariable Cox regression models.

Mediation of associations between smoking and cancer risk by fibrinogen or sP-Selectin was assumed if the following four criteria were fulfilled (34): (i) smoking status associated with cancer risk, (ii) smoking status associated with fibrinogen and sP-Selectin levels, (iii) fibrinogen and sP-Selectin levels associated with cancer risk, and (iv) associations between smoking and cancer risk attenuated by adjustment for the markers. To assess the degree of mediation, we further calculated the proportions of lung cancer risk that might be mediated through our biomarkers using the mediation analysis developed by Vanderweele (35).

Improvement in absolute risk prediction was assessed with the concordance statistic (C-statistic) for the effect of the biomarkers alone, and in combination with the established $PLCO_{m2012}$ (the 2012 version of the Lung, Colorectal, and Ovarian Cancer Screening trial) risk prediction model as offset. The $PLCO_{m2012}$ includes age, smoking status, cigarettes smoked per day, smoking duration, years since cessation, chronic obstructive pulmonary disease (COPD), prior diagnosis of a malignant tumor, family history of lung cancer, BMI, and education level as predictors (36). We did not have information on family history of cancer and COPD and set zero values for these variables. Although such conservative coding might lead to underestimation, the $PLCO_{m2012}$ model was chosen as it had previously performed best in an evaluation of different published risk models in our cohort (37). The hypothesis of no improvement in prediction was tested along with the hypothesis of no association between predictor and outcome, as proposed by Pepe and colleagues (38). The frequency of improvement of prediction was evaluated with the net reclassification improvement (NRI; continuous). Analyses on C-statistic and NRI were internally validated on 1,000 boot-strap samples to correct for overfitting. All statistical analyses were performed with SAS 9.4.

Results

Characteristics of the study population

Characteristics of the EPIC-Heidelberg case–cohort sample are presented in Table 1. Lung cancer diagnosis was more common among men (72%) than women. As compared with the subcohort, there were more heavy smokers among individuals with lung cancer (10.5 vs. 53.2%). Median follow-up durations (min, max) were 15.6 (0.1–18.5) years among study participants in the random subcohort and 9.1 (0.1–18.1) years among incident cases of lung cancer. The median age at lung cancer diagnosis was 63.8 (38.1–82.0) years.

Plasma levels of sP-selectin and fibrinogen were higher among current smokers, as compared with former and never smokers. Aspirin users showed higher levels of fibrinogen (Supplementary Table S1). The prevalence of antithrombotic drug use beyond aspirin was low ($n = 15$). There were no inter-correlations at Spearman coefficients > 0.4 between TPO, sTM, sP-selectin, sGPIIb/IIIa, and fibrinogen. The only biomarker that showed a significant correlation with CRP was fibrinogen ($P = 0.45$, see Supplementary Fig. S1).

Biomarker levels and lung cancer risk

TPO, sTM, and sGPIIb/IIIa showed no significant associations with lung cancer risk, neither before nor after multivariable adjustment for smoking status, lifetime alcohol intake, current aspirin use, physical activity, height, BMI, education level, and CRP (see Table 2). sP-Selectin [HR, 95% confidence interval (CI): 5.04, (2.95–8.60)] and fibrinogen [HR: 3.88 (2.34–6.42)] were strongly and significantly associated with lung cancer in the age- and sex-adjusted Cox regression model. These associations were attenuated but remained statistically significant upon multivariable adjustment, with HRs of 2.77 (1.55–4.96) for sP-Selectin and 2.03 (1.16–3.55) for fibrinogen. Additional mutual adjustment for fibrinogen or sP-Selectin, respectively, resulted in HRs of 2.51 (1.39–4.52) for sP-Selectin and 1.91 (1.09–3.34) for fibrinogen. Sensitivity analyses excluding individuals diagnosed with lung cancer within two years ($n = 16$) or five years ($n = 45$) after blood collection showed results similar to those in our main analyses, with HRs of 2.40 (1.33–4.35) and 2.59 (1.33–5.05) for sP-Selectin, and HRs of 1.84 (1.04–3.25) and 1.87 (1.01–3.47) for fibrinogen, respectively. Analyses excluding never smokers did not lead to substantial changes in HRs [2.64 (1.42–4.93) for sP-Selectin and 2.28 (1.26–4.13) for fibrinogen].

Mediation analyses

All criteria for mediation of the association between smoking and lung cancer by sP-Selectin and fibrinogen according to Baron and Kenny (34) were met in our study: (i) smoking was significantly associated with lung cancer risk (Supplementary Table S2), (ii) smoking was associated with both sP-Selectin and fibrinogen levels (Supplementary Table S1), (iii) sP-Selectin and fibrinogen levels were significantly associated with lung cancer risk (Table 2), and (iv) the associations between smoking and lung cancer risk were attenuated by adjustment for either sP-Selectin or fibrinogen (Supplementary Table S2). Results of the mediation analysis with the method proposed by Vanderweele (35) are presented in Table 3. In the multivariable-adjusted Cox regression model, 17% of the lung cancer risk related to smoking was mediated through sP-Selectin and 13% through fibrinogen. After mutual adjustment for fibrinogen or sP-Selectin, mediated proportions were only slightly attenuated (15% for sP-Selectin and 9% for fibrinogen) and remained statically significant.

Absolute risk prediction

We further tested to which extent sP-Selectin and fibrinogen would improve absolute lung cancer risk prediction beyond the $PLCO_{m2012}$ prediction model. In our analyses, the $PLCO_{m2012}$ algorithm alone showed a C-statistic of 0.788. Adding either sP-Selectin or fibrinogen to the model, slightly improved the

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Table 1. Characteristics of the study population (EPIC-Heidelberg, case–cohort sample)

		Subcohort		
	Cases	**Women**	**Men**	**Total**
N	190	1,257	1,129	2,386
Age at recruitment (years)[a]	55.9 (36.3–65.4)	48.4 (35.2–66.0)	53.2 (40.3–65.4)	51.1 (35.2–66.0)
Age at diagnosis (years)[a]	63.8 (38.1–82.0)	–	–	–
Median follow-up time (years)[a]	9.1 (0.1–18.1)	15.6 (0.1–18.5)	15.4 (0.3–18.3)	15.6 (0.1–18.5)
Women (%)	28	100	–	52.7
Thrombopoietin (pg/mL)	323 (271–412)	349 (291–414)	331 (281–397)	339 (286–407)
Thrombomodulin (ng/mL)	2.9 (2.4–3.5)	2.7 (2.3–3.2)	3.1 (2.6–3.6)	2.9 (2.4–3.4)
sP-Selectin (ng/mL)	34.3 (27.7–41.6)	25.7 (20.5–31.7)	30.2 (24.0–37.5)	27.6 (22.0–34.4)
Glycoprotein IIb/IIIa (ng/mL)	400 (320–514)	384 (314–493)	382 (316–487)	383 (315–490)
Fibrinogen (µg/mL)	4,097 (3,734–4,577)	3,787 (3,399–4,277)	3,775 (3,394–4,266)	3,781 (3,398–4,270)
BMI (kg/m^2)	26.3 (23.7–29.0)	24.3 (21.9–27.7)	26.4 (24.3–28.9)	25.6 (22.9–28.4)
Height (cm)	171 (165–176)	164 (160–168)	176 (172–180)	169 (163–176)
CRP (mg/dl)	2.0 (0.9–4.3)	1.0 (0.5–2.5)	1.0 (0.5–2.6)	1.0 (0.5–2.5)
Aspirin use (%)	12.1	2.8	6.2	4.4
Smoking status (%)				
Never smokers	8.4	50.8	33.8	42.8
Long-term quitters (≥10 y)	13.2	18.0	28.1	22.8
Short-term quitters (<10 y)	11.1	9.8	12.1	10.9
Light smokers (<15 cig/d)	14.2	13.6	12.3	13.0
Heavy smokers (≥15 cig/d)	53.2	7.7	13.7	10.5

NOTE: Values are medians (p25, p75) or proportions.
[a]Median (min, max).

C-statistic to 0.805 (sP-Selectin) and 0.803 (fibrinogen). Adding both biomarkers resulted in a small but significant improvement of the risk prediction model, with a C-statistic of 0.814, $P = 2.5 \times 10^{-11}$ (Fig. 1). The respective net reclassification improvement with sP-Selectin and fibrinogen in addition to the $PLCO_{m2012}$ algorithm was 0.17, indicating improved prediction for 17% of the participants after correction for false changes. Statistical adjustment for CRP levels only marginally affected the reported associations.

Discussion

In this prospective study, we observed strong positive associations between prediagnostic plasma levels of sP-Selectin and fibrinogen with lung cancer risk in multivariable-adjusted Cox regression models, with HRs from extreme quartile comparisons with 2.77 (1.55–4.96) for sP-Selectin and 2.03 (1.16–3.55) for fibrinogen. Additional mutual adjustment for fibrinogen or sP-Selectin, respectively, resulted in HRs of 2.51 (1.39–4.52) for sP-Selectin and 1.91 (1.09–3.34) for fibrinogen. These

Table 2. Hazard Ratios for lung cancer across quartiles of plasma TPO, sTM, sP-Selectin, sGPIIb/IIIa and fibrinogen

		Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	$P_{val\ cont}$
TPO	Median[a]	254	304	365	446	
	n cases/subcohort	59/592	39/593	41/594	51/589	
Model 1		Ref	0.68 (0.44–1.04)	0.68 (0.45–1.04)	0.85 (0.57–1.27)	0.49
Model 2		Ref	0.69 (0.42–1.13)	0.89 (0.55–1.44)	0.86 (0.54–1.38)	0.79
sTM	Median[a]	2.26	2.81	3.17	3.96	
	n cases/subcohort	57/591	43/590	40/595	50/590	
Model 1		Ref	0.72 (0.48–1.10)	0.67 (0.44–1.03)	0.81 (0.54–1.21)	0.45
Model 2		Ref	0.79 (0.48–1.30)	0.80 (0.48–1.31)	1.05 (0.65–1.69)	0.46
sGPIIb/IIIa	Median[a]	275	350	422	585	
	n cases/subcohort	44/590	38/587	54/587	52/583	
Model 1		Ref	0.90 (0.57–1.42)	1.24 (0.81–1.88)	1.24 (0.81–1.88)	0.26
Model 2		Ref	0.95 (0.56–1.61)	1.06 (0.65–1.73)	1.27 (0.78–2.08)	0.45
sP-Selectin	Median[a]	20.2	25.2	32.7	42.6	
	n cases/subcohort	17/594	32/594	56/594	85/585	
Model 1		Ref	1.92 (1.05–3.50)	3.22 (1.84–5.64)	5.04 (2.95–8.60)	<0.001
Model 2		Ref	1.64 (0.85–3.18)	2.71 (1.48–4.97)	2.77 (1.55–4.96)	<0.001
Model 3		Ref	1.50 (0.77–2.95)	2.60 (1.41–4.78)	2.51 (1.39–4.52)	0.002
Fibrinogen	Median[a]	3,229	3,605	3,995	4,653	
	n cases/subcohort	21/597	34/594	52/590	83/587	
Model 1		Ref	1.55 (0.89–2.72)	2.31 (1.35–3.93)	3.88 (2.34–6.42)	<0.001
Model 2		Ref	1.21 (0.65–2.23)	1.49 (0.83–2.67)	2.03 (1.16–3.55)	<0.001
Model 3		Ref	1.22 (0.66–2.25)	1.48 (0.82–2.65)	1.91 (1.09–3.34)	0.002

NOTE: Results from Prentice-weighted Cox proportional hazards regression analyses. Model 1 adjusted for age and sex. Model 2 adjusted for age, sex, smoking (never smokers, long-term quitters ≥ 10 years, short-term quitters < 10 years, light smokers < 15 cigarettes per day, heavy smokers ≥ 15 cigarettes per day), lifetime alcohol intake (g/d), current aspirin use (yes/no), CRP (mg/dl), physical activity (Cambridge Index), BMI (kg/m^2), height (cm) and education level (primary school, secondary school, university degree). Model 3 additionally adjusted for sP-Selectin or fibrinogen, respectively. Numbers for the subcohort do not include incident cancer cases ($n = 17$).
[a]Median concentrations in pg/mL (thrombopoietin), in ng/mL (sTM, sP-Selectin, sGPIIb/IIIa) and in µg/mL (Fibrinogen).

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Table 3. Direct and indirect effects (mediated by sP-Selectin and fibrinogen) of smoking on lung cancer risk[a]

	Direct effect HR (95% CI)	*P*	Indirect effect HR (95% CI)	*P*	Proportion mediated
sP-Selectin					
Model 1	4.87 (3.48–6.82)	<0.001	1.16 (1.07–1.25)	<0.001	17%
Model 2	4.59 (3.31–6.35)	<0.001	1.14 (1.06–1.23)	<0.001	15%
Fibrinogen					
Model 1	4.96 (2.04–12.06)	<0.001	1.11 (1.05–1.18)	<0.001	13%
Model 2	4.41 (2.20–8.83)	<0.001	1.08 (1.02–1.14)	0.003	9%

NOTE: Mediated proportions calculated according to the Vanderweele method (35). Model 1: adjusted for age, sex, lifetime alcohol intake (g/d), current aspirin use (yes/no), CRP (mg/dl), physical activity (Cambridge Index), BMI (kg/m^2), height (cm) and education level (primary school, secondary school and university degree). Model 2: additionally adjusted for sP-Selectin or fibrinogen, respectively.

[a]Smoking modeled as continuous trend variable based on five scores: Never smokers (0), long-term quitters $\geq$ 10 years (1), short-term quitters <10 years (2), light smokers < 15 cigarettes per day (3), and heavy smokers $\geq$ 15 cigarettes per day (4).

associations did not depend on the lag time between blood draw and diagnosis. While they indicate that sP-Selectin and fibrinogen are independent risk factors for lung cancer, findings from mediation analyses in this study were also compatible with the hypothesis that both biomarkers partially mediate effects of smoking on lung carcinogenesis. In any case, adding sP-Selectin and fibrinogen to the $PLCO_{m2012}$ algorithm slightly improved lung cancer risk prediction (C-statistic of 0.788 without and 0.814 with both markers). No significant associations with lung cancer risk were found for sTM, TPO, and sGPIIb/IIIa, neither in age- and sex-adjusted nor in multivariable models.

Associations between fibrinogen and lung cancer risk have been shown in four previous prospective studies (7–10). Similar as in our study, these associations remained significant upon adjustment for potential confounders, particularly smoking. Thus, and given that the current association between fibrinogen and lung cancer risk was independent of lag time between blood draw and diagnosis, increased fibrinogen could be an independent risk factor for lung cancer. However, fibrinogen levels were higher with longer smoking duration and higher smoking intensity in EPIC-Heidelberg, the above mentioned previous studies, and further studies from the cardiovascular field (39). Moreover, associations between fibrinogen and lung cancer risk were clearly attenuated upon adjustment for smoking status in our study and previous studies (9). Considering that details on smoking status were interview-derived, it cannot be ruled out that higher fibrinogen levels reflect variations in smoking status not entirely covered by self-reported smoking history, and that residual confounding underlies the associations between fibrinogen and lung cancer risk (9).

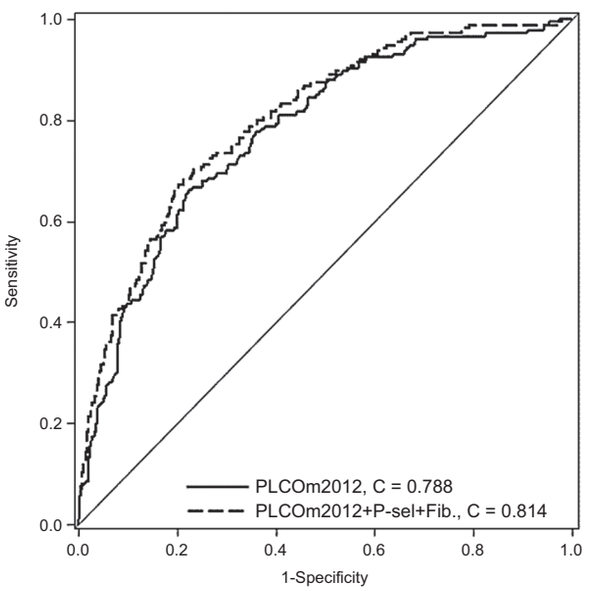

Figure 1.
ROC curves depicting discrimination of 6-year lung cancer risk estimates from $PLCO_{m2012}$-model (solid line) and for estimates additionally including sP-Selectin and fibrinogen (dotted line). The diagonal refers to the situation of no discrimination effect.

Rather than being a mere epiphenomenon of smoking, it has also been proposed that a procoagulant state may be a smoking-induced mechanism partially mediating the carcinogenic effects of smoking in lung cancer development (9). In our analyses, all formal criteria for mediation were fulfilled, and the Cox regression model indicated 9% of the statistical effect of smoking on lung cancer risk can be attributed to fibrinogen. Interestingly, our results on fibrinogen were paralleled by similar results on sP-Selectin, which showed slightly stronger associations with lung cancer risk than fibrinogen, and for which mediation analyses suggested that it may explain 15 % of the effect of smoking on lung cancer. These results are in line with well-known detrimental effects of cigarette smoke on the endothelium, and especially with evidence that smoking increases platelet activation and coagulation (40–42), which in turn may drive carcinogenesis (43). They are indirectly supported by our previous observation that neither fibrinogen nor sP-Selectin were associated with the risks of breast, prostate, or colorectal cancer, that is, cancers with a much lower fraction of cases attributable to smoking compared with lung cancer (30).

Notwithstanding the possible roles of sP-Selectin and fibrinogen as either risk factors for lung cancer (independent and/or smoking-induced) or epiphenomena of smoking, our analyses indicate that both markers may slightly improve lung cancer risk prediction by 2%–3% percentage points (C statistic). While this increment in predictive capacity and the improved net-reclassification of 17 % of the study participants achieved by adding the two biomarkers to established risk factors is modest, both biomarkers can easily be measured at low costs. Thus, they may be interesting candidates for future multibiomarker panels for the identification of individuals eligible for imaging-based lung cancer screening (44), provided that sP-Selectin, which can be measured with rather simple assays, is integrated into routine diagnostic panels.

The availability of only one blood draw in this study reflects the situation in lung cancer prescreening, but may be considered a limitation regarding our analyses on etiologic associations; however, we have demonstrated good reproducibility of thrombopoietin, sTM, sP-selectin, and sGPIIb/IIIa levels over time in a previous study (45), and good reproducibility has also been reported for fibrinogen levels (46). External validation of our findings was not possible and is needed before fibrinogen and sP-Selectin can be integrated into lung cancer risk prediction models. Moreover, we have to acknowledge that two predictors (COPD and family history of lung cancer), which are part of the

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$PLCO_{m2012}$ were not assessed at the baseline examination of EPIC-Heidelberg, and we had to set zero values for both variables in our risk prediction models. Strengths of our study include the population-based and prospective study design, the use of a broader set of biomarkers, and the comprehensive statistical adjustment.

In summary, we observed strong positive associations between sP-Selectin and fibrinogen with lung cancer risk in the EPIC-Heidelberg Study, which supports our predefined hypothesis of enhanced platelet activation and coagulation being possible drivers of early lung cancer development. Our findings may also suggest that fibrinogen and sP-Selectin are potential mediators of smoking in lung carcinogenesis. Both biomarkers slightly improve prediction of absolute lung cancer risk, but external validation of our results is needed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M. Grafetstätter, P. Bugert, R, Kaaks, T. Kühn
Development of methodology: M. Grafetstätter, T.S. Johnson, T. Kühn
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): T.S. Johnson, R, Kaaks, T. Kühn
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Grafetstätter, A. Hüsing, S.G. Maldonado, T.S. Johnson, R, Kaaks, T. Kühn
Writing, review, and/or revision of the manuscript: M. Grafetstätter, A. Hüsing, S.G. Maldonado, T.S. Johnson, L. Pletsch-Borba, V. Katzke, M. Hoffmeister, P. Bugert, T. Kühn
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D. Sookthai, M. Hoffmeister, R, Kaaks, T. Kühn
Study supervision: P. Bugert, T. Kühn
Other (carried out laboratory measurements): M. Grafetstätter

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