

# Increased Plasma Soluble uPAR Level Is a Risk Marker of Respiratory Cancer in Initially Cancer-Free Individuals

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## Abstract

**Background:** Soluble urokinase plasminogen activator receptor (suPAR) is a stable plasma biomarker associated with inflammation and disease. This study tested the association between suPAR levels and incident respiratory, gastrointestinal, or other types of cancer in initially cancer-free individuals from a general population-based prospective study.

**Methods:** Baseline plasma samples, baseline characteristics, and follow-up data were available from 2,656 individuals from the population-based Danish MONICA10 (MONItoring trends and determinants of Cardiovascular disease) study, followed for a median of 12.6 years. Cancer was diagnosed according to international classification of diseases (ICD) 8 and ICD-10 codes and suPAR levels were measured using a commercially available ELISA. The association of suPAR levels with incident cancer during follow-up was analyzed using Cox regression, adjusted for established risk factors and the inflammatory markers C-reactive protein (CRP) and leukocyte numbers.

**Results:** suPAR levels ranged from 0.6 to 22 ng/mL and median suPAR level was 4.01 ng/mL. An increase of 1 ng/mL in baseline suPAR was associated with adjusted HR of 1.61 (95% CI: 1.23–2.11,  $P < 0.001$ ), 0.92 (95% CI: 0.69–1.24,  $P = 0.59$ ), and 1.33 (95% CI: 1.13–1.58,  $P < 0.001$ ) of being diagnosed with respiratory, gastrointestinal, and other cancer types, respectively.

**Conclusion:** Elevated suPAR levels were associated with increased risk of incident respiratory cancer and other types of cancer, but not gastrointestinal cancers, independently of established risk factors, CRP, and leukocyte numbers.

**Impact:** These findings suggest that inflammation is involved in cancer development. Risk algorithms based on established risk factors and risk-associated biomarkers should be developed and evaluated in large, general population-based studies. We suggest suPAR as a candidate for evaluation in cancer risk algorithms. *Cancer Epidemiol Biomarkers Prev*; 20(4); 609–18. ©2011 AACR.

## Introduction

Biomarkers of low-grade inflammation may predict incident cancer development, and thereby improve cancer prognosis by identifying individuals at high risk (1). We recently reported increased plasma-soluble urokinase

plasminogen activator receptor (suPAR) levels to be associated with increased risk of cardiovascular diseases, type 2 diabetes, cancer of all types, and mortality in the general population (2).

Increased plasma suPAR levels have been found in patients with various cancer types and have been identified as an adverse prognostic factor (3). Moreover, elevated plasma suPAR levels have been observed in various infectious, inflammatory, and autoimmune diseases and have predicted mortality in several of these diseases (4, 5). suPAR correlates positively with proinflammatory biomarkers such as leukocyte numbers, TNF- $\alpha$ , and high sensitive C-reactive protein (CRP; refs. 2, 6). In recent years, slightly elevated levels of CRP and TNF- $\alpha$  have been reported to be markers of low-grade inflammation in apparently healthy individuals (7–9). These findings suggest that suPAR may be a marker of low-grade inflammation, which is believed to be part of the etiology of diseases as diverse as cardiovascular diseases, type 2 diabetes, and cancer (7, 8, 10–12).

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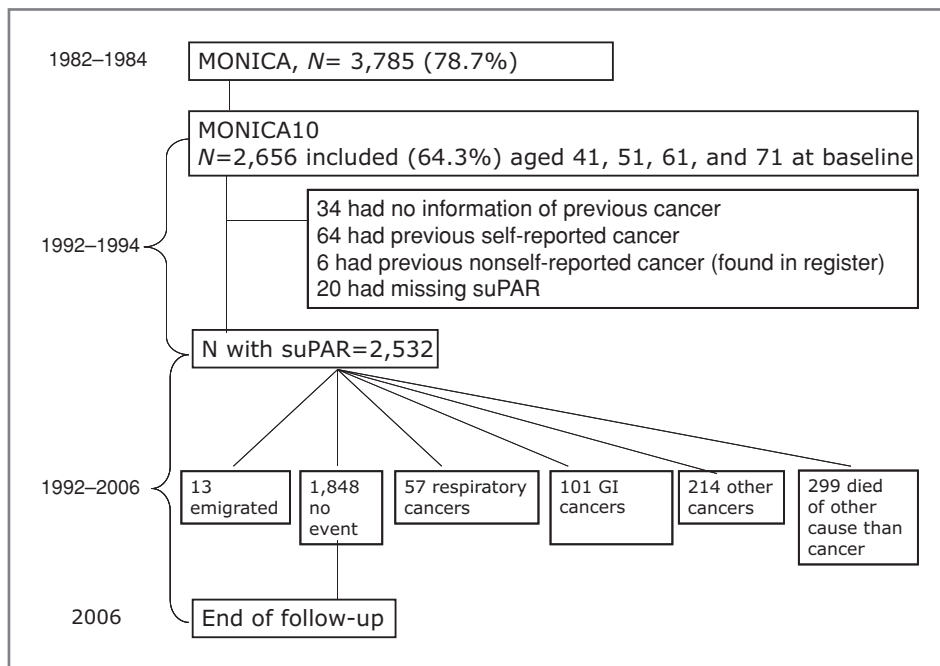
**Note:** Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (<http://cebp.aacrjournals.org/>).

The study was partly presented at the Second European Lung Cancer Conference in April–May 2010 at a poster presentation discussion.

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**Figure 1.** Study design.

Participants with cancer before or at baseline and/or missing suPAR measurements were excluded. During follow-up, 372 participants developed cancer. Individuals who emigrated were excluded at date of emigration. The endpoints were analyzed as competing, thus individuals exited analysis after they encountered an endpoint (cancer or death of other cause than cancer) and were therefore not available for analysis of the other endpoints.

The membrane-bound form of suPAR, uPAR/CD87, is involved in cell adhesion, migration, proliferation, and metastasis, that is, processes essential for tumor development and dissemination. suPAR can compete with uPAR function, but suPAR also possesses intrinsic biological activity (4, 13), indicating that suPAR could have an active role in carcinogenesis.

We investigated whether increased baseline suPAR levels were associated with the development of respiratory, gastrointestinal (GI), or other types of cancer in initially cancer-free individuals from a general population.

## Methods

### Cohort description

In 1982 to 1984, 3,785 randomly selected individuals aged 30, 40, 50, and 60 years, from 11 municipalities within Copenhagen County accepted the invitation for the Danish MONICA (MONItoring trends and determinants of Cardiovascular disease) study (78.7%; ref. 14). In 1992 to 1994, all individuals invited for the MONICA study, who were alive and had not emigrated, were reinvited for the MONICA10 study; 2,656 (64.3%) agreed to participate (15). For this study, plasma samples and self-administered questionnaire data from participants of the MONICA10 study were used. Figure 1 presents a study flowchart.

We excluded 98 individuals excluded from the analyses due to no information of cancer before or at baseline ( $N = 34$ ) or self-reported previous diagnosis of cancer ( $N = 64$ ). Moreover, 6 individuals were excluded due to previous not self-reported cancer incidence found in

registries and 20 individuals did not have suPAR measurements and were excluded from analyses. Thus, the final study population consisted of 2,532 individuals followed for a median of 12.6 (4.9–13.4 years 5th–95th percentile, minimum: 20 days, maximum: 13.6 years). Thirteen participants emigrated before event. Information of cause of death is 100% complete, information of diagnoses before death is 99.5% complete.

### Endpoints

Information of cancer diagnoses and death were obtained from the National Danish Civil Registration System and the Danish Patient Registry, using WHO International Classification of Diseases, 8th (ICD-8) and 10th edition (ICD-10) codes for discharge in December 2006. Cancer cases were placed in 3 predefined major groups: respiratory, GI, or other cancer types, since the total of 372 cancer cases was too small a number for elaborate cancer subgroup analyses. The predefined cancer subgroups were based on physiology, anatomy, and design of previous population-based cancer risk studies (11, 16). Only the first cancer diagnosis was included (ICD-8 codes: 140-207; ICD-10 codes: C00-C96, D00-D09), thus subjects are placed in just one cancer group. If the first cancer diagnosis was within C76-C80, C97, the diagnosis was considered unspecific. If the participant received a specific cancer diagnosis (C00-C75, C81-C96, D00-D09) within 90 days of the unspecific cancer diagnosis, the participant was assigned the specific cancer diagnosis with the date of the unspecific. The respiratory cancer group contained ICD-8 codes 161-163 and ICD-10 codes C32-34, C38-C39, C45.0, D02.0-2, D02.4; the GI cancer group contained ICD-8 codes 140-159 and

ICD-10 codes C00-C26, D0.0-D0.1, and other cancer types, ICD codes for cancer diagnoses in this cohort, not included in the respiratory or GI cancer groups. The cancer ICD codes constituting the cancer cases in this cohort are seen in Supplementary Table A1.

### Ethics

The Ethics Committee for Copenhagen County approved the study, which was conducted in accordance with the second Helsinki declaration. All participants gave written informed consent.

### Methods

suPAR was measured as described in the work of Eugen-Olsen and colleagues (2). The suPARnostic Kit was validated to measure suPAR concentrations within 0.6 to 22 ng/mL; intra-assay variance was 2.75%, and inter-assay variance was 9.17%.

CRP was measured using a highly sensitive CRP assay (Roche/Hitachi) with a range of 0.1 to 20 mg/L and a detection limit of 0.03 mg/L, as previously described (17). Body mass index (BMI) was measured as described in the work of Olsen and colleagues (17).

### Statistical analysis

The statistical software R, version 2.8.1 was applied for statistical analysis (18). Values of *P* less than 0.05 were considered statistically significant. Analysis of the association of increased plasma suPAR level with baseline characteristics was performed using 2-sided Kruskal-Wallis test (19). The association of increased plasma suPAR level with cancer development was analyzed using Cox regression analysis (20). All analyses used age as underlying time and were adjusted for time since blood sampling, sex, alcohol consumption [ $\leq 168$  (women)/252 (men) g/wk;  $>168$  (women)/252 (men) g/wk], BMI ( $<20$ , 20–24, 25–29, or  $>30$ ), and smoking (never, former, current). Using age as underlying time, and not time since blood sampling, orders the events by age and takes into account that age is a more important risk factor than time since blood sampling. Respiratory cancer analyses were adjusted for current smoking or current nonsmoking, since only current and former smokers developed respiratory cancer. Individuals with missing covariate information were excluded from multivariate analyses.

The proportional hazard assumption was controlled by examining plots of the Schoenfeld Residuals. This showed that linear scoring of suPAR and leukocyte numbers was appropriate for Cox regression analyses. The HRs were reported for an increase of 1 ng/mL in plasma suPAR level and an increase of  $1 \times 10^6$  leukocytes/mL in leukocyte level. The association of CRP with cancer development was assessed as CRP 1 to 3 mg/L and CRP greater than 3 compared with plasma CRP less than 1 mg/L, based on controls and previous reports (11).

Cox regression analyses were similarly used for mutually adjusted analyses of the independent association

of suPAR, CRP, and leukocyte numbers with cancer development. Analyses of CRP and leukocyte numbers and risk of cancer were performed identically to suPAR analyses.

The association of suPAR and incident respiratory cancer among current smokers was analyzed in a cumulative incidence model, using sex- and age-specific suPAR quartiles. suPAR increases with age and is higher in women than in men. Thus, to examine the highest suPAR levels independently of the effect of age and sex on suPAR levels, the sex- and age-specific suPAR quartiles were constructed by calculating suPAR quartiles for men and women and for the 4 age groups and distributing these to the age- and sex-specific quartiles. Cumulative incidence plots for suPAR and development of GI; other types of cancer; and mortality among all participants were produced equally. Moreover, the association of suPAR with subgroups of cancer was assessed by investigating the distribution of cancer cases in the highest age- and sex-specific suPAR quartile analyzed with Fisher's exact test.

Furthermore, the association of increased plasma suPAR levels with respiratory cancer development among smokers was analyzed using Cox regression analysis, adjusted for age, sex, BMI, alcohol consumption, and pack-years. The association of suPAR levels and pack-years among current smokers was analyzed using Kendall  $\tau$  correlation analysis of age-specific pack-year quartiles. We analyzed the association using age-specific pack-year quartiles, since pack-years are expected to increase with age and suPAR also increases with age. Hereby, we aimed to avoid the association between suPAR and pack-years to reflect the association between suPAR and age. Pack-years were calculated as follows: pack-year = [(number of cigarettes smoked per day)  $\times$  (number of years of smoking)]/20.

The endpoints of the analyses, respiratory, GI, and other types of cancer, were analyzed as competing endpoints. Therefore, individuals exited the analyses if an endpoint other than the endpoint of the analysis was encountered first, that is, individuals were only available for the analysis of their first endpoint (endpoints are shown in boxes in Fig. 1, individuals are only assigned to 1 box). Individuals were censored due to end of follow-up or emigration at date of emigration.

### Results

During follow-up, 372 participants developed cancer as follows: 57 respiratory, 101 GI, and 214 other types of cancer. The median suPAR concentration was 4.01 ng/mL (First quartile: 3.35 ng/mL, third quartile: 4.89 ng/mL, minimum: 1.34 ng/mL, maximum: 17.8 ng/mL).

Table 1 summarizes baseline characteristics. Plasma suPAR levels were significantly higher for women than for men and increased significantly with age, smoking status, CRP, leukocyte numbers, BMI  $< 20$  and BMI  $> 30$  for women, BMI  $< 20$  for men and alcohol consumption for men.

**Table 1.** Baseline characteristics of study cohort

	Women			Men		
	Distribution, %	Median suPAR, ng/mL (iqt)	<i>P</i>	Distribution, %	Median suPAR, ng/mL (iqt)	<i>P</i>
Age, y			<0.0001			<0.0001
41	29.4	3.91 (3.32–4.73)		26.5	3.33 (2.85–4.27)	
51	27.7	4.08 (3.49–5.03)		28.5	3.53 (2.98–4.25)	
61	24.7	4.38 (3.72–5.19)		25.9	4.07 (3.48–4.96)	
71	18.1	4.65 (4.07–5.37)		19.1	4.42 (3.74–5.43)	
BMI, kg/m <sup>2</sup>			<0.0001			0.14
<20	6.8	4.45 (3.72–5.57)		2.2	4.25 (3.35–5.72)	
20–24	48.4	4.04 (3.46–4.95)		35.2	3.84 (3.16–4.71)	
25–29	29.9	4.26 (3.67–5.00)		46.2	3.77 (3.10–4.64)	
>30	14.9	4.69 (3.98–5.52)		16.3	3.84 (3.20–4.60)	
Alcohol consumption, g/wk			0.73			<0.0001
<168/252	86.6	4.27 (3.61–5.14)		76.7	3.73 (3.10–4.54)	
>168/252	13.4	4.18 (3.52–5.02)		23.3	4.08 (3.37–4.96)	
Smoking status			<0.0001			<0.0001
Never	33.9	3.79 (3.38–4.53)		18.8	3.20 (2.83–3.82)	
Former	22.1	3.97 (3.39–4.58)		32.4	3.59 (3.02–4.39)	
Current	44.0	4.76 (4.04–5.68)		48.8	4.18 (3.55–5.11)	
CRP, mg/L			<0.0001			<0.0001
<1	31.9	3.85 (3.28–4.57)		28.7	3.41 (2.90–4.09)	
1–3	34.3	4.29 (3.68–5.11)		39.1	3.71 (3.13–4.42)	
>3	33.7	4.66 (3.83–5.58)		32.3	4.49 (3.65–5.51)	
Leukocytes			<0.0001			<0.0001
First quartile	24.6	3.73 (3.26–4.33)		25.7	3.30 (2.80–3.89)	
II quartile	24.5	4.04 (3.50–4.79)		26.4	3.61 (3.06–4.38)	
III quartile	26.0	4.36 (3.74–5.21)		23.9	3.95 (3.34–4.84)	
IV quartile	24.9	4.96 (4.14–5.82)		24.0	4.57 (3.85–5.55)	

NOTE: The association of suPAR and baseline characteristics was analyzed using 1-way Kruskal–Wallis analysis. The alcohol limit of 168 (women)/252 (men) g/wk is based on the recommendations concerning alcohol consumption from the Danish National Board of Health.

Abbreviation: iqt, interquartile range.

### suPAR and risk of cancer

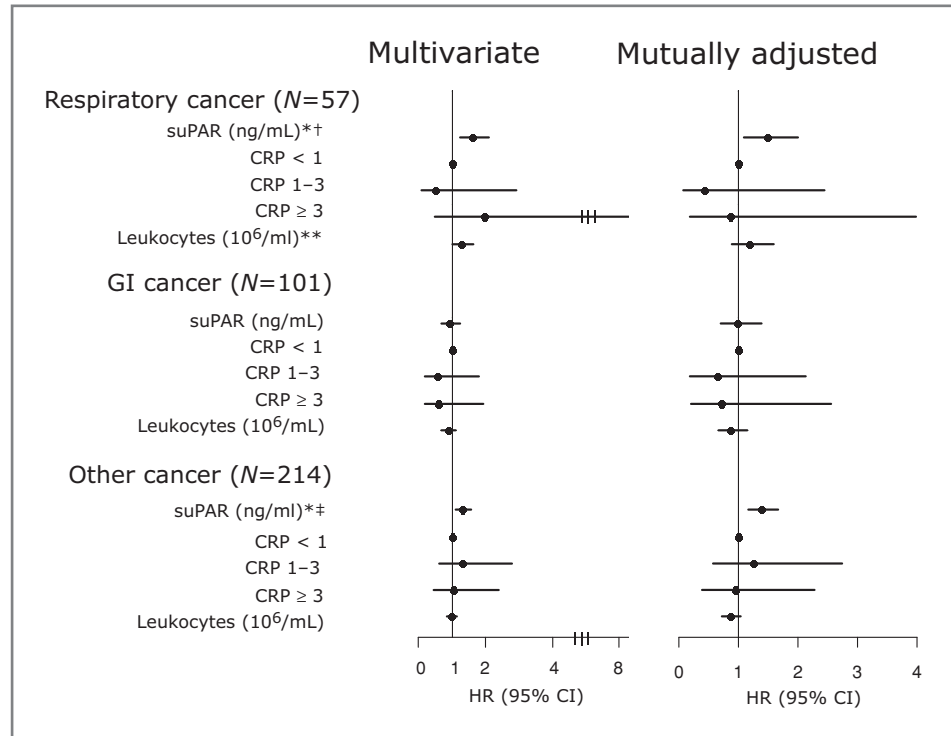
Increased plasma suPAR levels were significantly associated with being diagnosed with respiratory cancer and other cancer types during follow-up, but not with GI cancer in Cox regression analyses (Fig. 2, left and Supplementary Table A2). The multifactorially adjusted HRs for 1 ng/mL increase in plasma suPAR was 1.61 (95% CI 1.23–2.11,  $P < 0.001$ ) for respiratory, 0.92 (95% CI: 0.69–1.24,  $P = 0.59$ ) for GI, and 1.33 (95% CI: 1.13–1.58,  $P < 0.001$ ) for other cancer types. To investigate whether the increased suPAR levels originated from undiagnosed cancer at baseline, cancer cases diagnosed within 6 months of study entry (respiratory:  $n = 0$ , GI:  $n = 1$ , other:  $n = 9$ ) were excluded from analysis. This did not change the associations of increased suPAR levels and cancer development described above (data not shown). The association of suPAR and incident cancer of the 3 cancer groups is also

demonstrated in cumulative incidence plots according to sex- and age-specific suPAR quartiles as shown in Figure 4 and Supplementary Figure A1.

### Risk of cancer: suPAR versus the inflammatory markers CRP and leukocyte numbers

Increased baseline plasma CRP levels were not significantly associated with being diagnosed with cancer in any of the groups during follow-up. However, increased leukocyte numbers were significantly associated with increased risk of being diagnosed with respiratory cancer during follow-up (Fig. 2, left). The multifactorially adjusted HR for an increase of  $1 \times 10^6$  leukocytes/mL was 1.30 (95% CI: 1.01–1.66,  $P = 0.04$ ). Furthermore, it was analyzed whether increased suPAR and leukocyte numbers remained significantly associated with cancer development after additional adjustment for the other

**Figure 2.** HR plot of risk of cancer development by increase in suPAR, CRP, and leukocyte numbers. The Cox regression analyses use age as underlying time and are adjusted for time since blood sampling, sex, BMI, smoking, and alcohol consumption.  $N = 2,430$  for the suPAR analyses,  $N = 2,304$  for the CRP analyses, and  $N = 2,426$  for the leukocyte analyses. The mutually adjusted Cox regression analyses ( $N = 2,301$ ) are furthermore adjusted for suPAR, CRP, and leukocyte numbers respectively. \*,  $P < 0.001$ ; \*\*,  $P = 0.04$  in multivariate analysis and †,  $P = 0.008$ ; ‡,  $P < 0.001$  in mutually adjusted analysis.



inflammatory markers (CRP, suPAR, and leukocyte numbers). Only increased suPAR levels remained significantly associated with being diagnosed with respiratory cancer and other types of cancer (Fig. 2) during follow-up. The mutually and multifactorially adjusted HR for an increase of 1 ng/mL in plasma suPAR was 1.50 (95% CI: 1.11–2.03,  $P = 0.008$ ) and 1.39 (95% CI: 1.16–1.67,  $P < 0.001$ ) for being diagnosed with respiratory and for other types of cancer, respectively, during follow-up.

### Modeling the effect of baseline suPAR during follow-up

The HR for the association of baseline suPAR levels with being diagnosed with respiratory cancer during follow-up was modeled as a function of follow-up time (Fig. 3), to explore whether the informative value of baseline suPAR declines, during follow-up. The HR remained almost constant during the entire follow-up period in the model, but the 95% CI started to increase after 6 years of follow-up. Results for all cancer groups are given as  $\beta$ -values in Supplementary Table A2.

### suPAR and respiratory cancer among current smokers

The association between increased suPAR levels and increased risk of respiratory cancer was investigated further among current smokers ( $N = 1,174$ ). Current smokers in the highest age- and sex-specific suPAR quartile had a cumulative incidence of 18% of incident respiratory cancer, whereas those in the lowest had a cumulative incidence of 6% (Fig. 4).

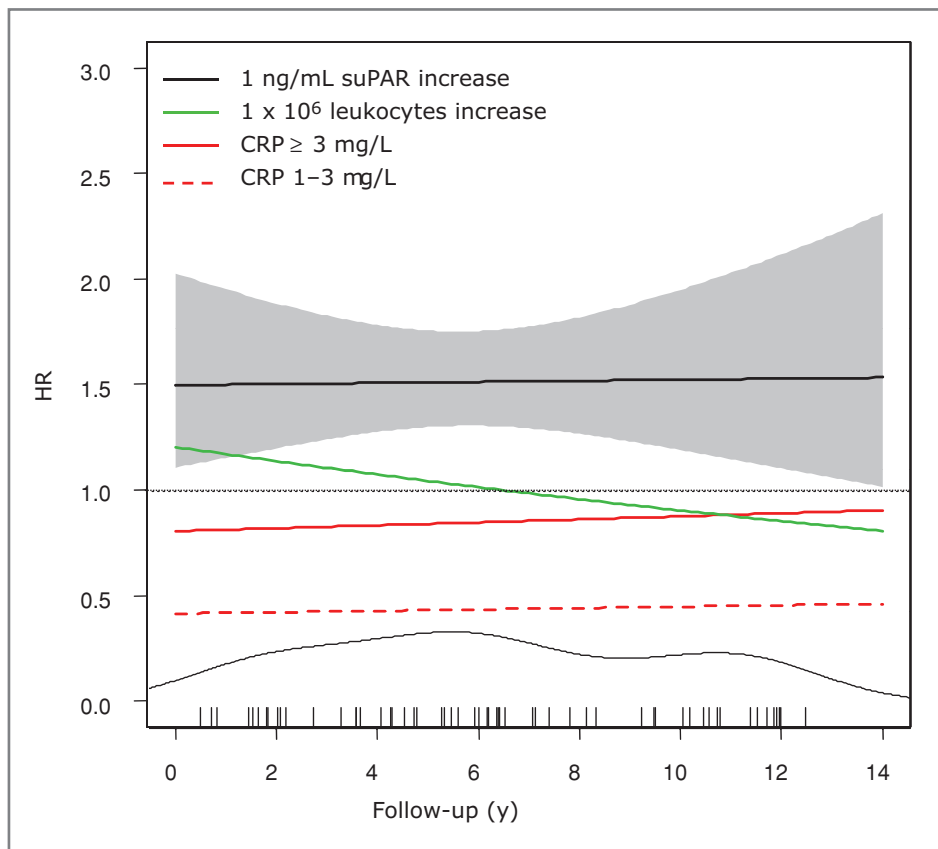
Furthermore, we examined the association between suPAR and pack-years among current smokers. suPAR was significantly, but weakly, positively correlated with age-specific pack-years (Kendall  $\tau$ -b = 0.15,  $P < 0.0001$ ), as seen in Figure 5. Moreover, we investigated the association between suPAR and respiratory cancer among current smokers in a Cox regression analysis adjusted for sex, age, alcohol consumption, BMI, and pack-years. The multifactorially adjusted HR for an increase of 1 ng/mL in plasma suPAR was 1.57 (95% CI: 1.24–1.98,  $P < 0.001$ ) for current smokers to be diagnosed with respiratory cancer during follow-up.

### suPAR and cancer subgroup analysis

The GI and the other types of cancer groups included a large variety of cancer types. To explore whether increased suPAR levels were associated with specific types of cancer within the 3 groups, cancer cases were assigned to specific subgroups. The association of suPAR and subgroups of cancer was assessed by analyzing the number of cases in the highest age- and sex-specific suPAR quartile (Supplementary Table A1). Only the lung cancer subgroup had a significantly larger number of cases in the highest age- and sex-specific suPAR quartile.

### Discussion

The recent finding of elevated suPAR levels as risk marker of incident cancer in initially cancer-free individuals was examined further. This study reports for the



**Figure 3.** Model-based effect of baseline suPAR for development of respiratory cancer during follow-up. The effects of a baseline increase of 1 ng/mL suPAR,  $1 \times 10^6$  leukocytes/mL, and CRP levels  $\geq 3$  and 1–3 mg/L during follow-up are measured as the HR using age as underlying time, adjusted for sex, BMI, smoking, and alcohol consumption. An HR of 1 is indicated by the black dotted line, the grey shaded area represent the 95% CI for the HR associated with an increase of 1 ng/mL suPAR. The 95% CI is not indicated for baseline leukocyte numbers or CRP levels, since neither of the HRs are significant during follow-up. Each respiratory cancer case is indicated with a line at the x-axis and the black curve above the cancer cases indicates the density of respiratory cancer cases during follow-up.

first time that plasma suPAR was significantly increased before diagnosis of respiratory cancer and cancers of the other types but not before diagnosis of GI cancer. In addition, the association of the inflammatory markers, CRP and leukocyte numbers, and risk of cancer development was evaluated. Increased leukocyte numbers were associated with increased risk of respiratory cancer (Fig. 3). In contrast to recent reports (11, 21), the present study did not find increased CRP levels to be significantly associated with being diagnosed with respiratory cancer during follow-up (Fig. 3). This could be due to a lower number of participants in the present study. When analyzing the inflammatory markers in the same model, only elevated plasma suPAR, but not CRP or leukocyte numbers, was independently associated with an increased risk of respiratory cancers and cancers of the other cancer group (Fig. 3).

The mechanisms underlying the association between elevated suPAR levels and increased risk of respiratory cancer in particular are not well understood. Overall, the elevated suPAR levels described in this study can result from undetected tumor at baseline, initiated or preneoplastic cells and/or inflammation.

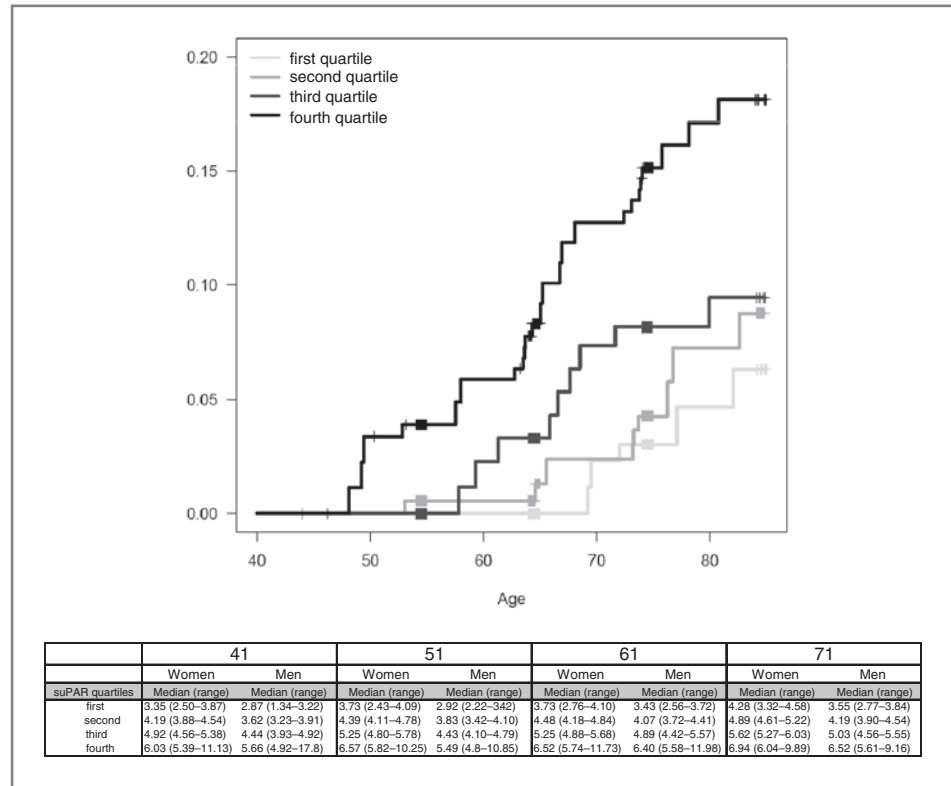
The membrane-bound counterpart of suPAR, uPAR, is expressed on leukocytes, and many types of cancer cells exhibit elevated uPAR expression (3, 4). Moreover, increased levels of suPAR have been found in plasma

from patients with various forms of cancer (3). Thus, the increased plasma suPAR levels found in this study could originate from an undetected tumor at baseline and/or tumor-associated leukocytes. However, no cases of respiratory cancer were diagnosed during the first 6 months of follow-up and increased suPAR levels remained significantly associated with respiratory cancer development throughout follow-up (Fig. 4). Thus, it seems unlikely that the increased baseline plasma suPAR levels originate only from undetected tumors or tumor associated leukocytes at baseline.

Initiated and preneoplastic cells can be present years before tumor detection (22). It is therefore possible that the increased baseline suPAR levels originate from initiated or preneoplastic cells. Supporting this hypothesis, overexpression of the epidermal growth factor receptor (EGFR) is found in 50% to 90% of non-small cell lung cancers (23, 24). Increased signaling through EGFR induces increased protein lipase C (PLC- $\gamma$ ) activity, which has been demonstrated to cleave uPAR, thereby releasing suPAR. Furthermore, EGF signaling was shown to induce uPAR expression (25).

The findings of several studies suggest that inflammation is involved in lung cancer development (11, 21, 26–28). Increased inflammation is associated with elevated proteolysis and oxidative stress and affects signal transduction, gene transcription, and cell turnover, processes

**Figure 4.** Cumulative incidence of respiratory cancer among smokers. Of 57 cases of respiratory cancer, 47 were registered among smokers. The single lines and boxes represent end of follow-up or individuals censored because of emigration. The suPAR distribution in the age- and sex-specific suPAR quartiles for smokers is shown in the Table. suPAR is measured in ng/mL.



central for initiation and carcinogenesis (10). Moreover, inflammation is induced by smoking (27) but is also associated with other factors affecting cancer risk such as age, BMI, and exercise (9, 29, 30). The increased suPAR levels and leukocyte numbers detected in the participants of this study could therefore reflect inflammation, explaining the association of increased suPAR levels and leukocyte numbers with respiratory cancer risk. Notably, only suPAR was independently associated with respiratory cancer, indicating that suPAR reflects pathogenic mechanisms of respiratory cancer development more specifically than do leukocyte numbers and CRP.

Since smoking is the primary cause of respiratory cancer, we investigated whether suPAR levels are markers of smoking history among current smokers. suPAR levels were weakly, but significantly positively correlated with pack-years, thereby not only reflect smoking history measured as pack-years. Moreover, when analyzing the association of suPAR with respiratory cancer among current smokers in a multifactorially adjusted Cox regression analysis including pack-years, increased suPAR levels were still significantly associated with respiratory cancer. These findings show that suPAR is not merely a marker of smoking history measured by pack-years; thereby not excluding that increased suPAR levels may reflect smoking-induced damage in respiratory cancer development.

suPAR might not only reflect the underlying pathology but might also play an active role in disease development. suPAR binds urokinase plasminogen activator and can

function as a scavenger receptor, thereby modulating uPAR activity (31). suPAR also associates with integrins, extracellular matrix molecules, and the 7-transmembrane receptor FPR-like receptor 1 (FPRL). suPAR might thereby affect cell migration, proliferation, bone marrow cell mobilization, and chemotaxis, processes important for cancer development (4, 32–34). Increased mobilization of hematopoietic progenitors is associated with smoking-induced carcinogenesis (35). suPAR may affect cancer development directly by recruiting leukocytes and hematopoietic stem cells to the lung (34). Finally, suPAR induces multiple intracellular signaling cascades and may potentially also influence cell growth, differentiation, apoptosis, and inflammation (13, 32, 36).

None of the inflammatory markers CRP, leukocyte numbers, or suPAR were significantly associated with GI cancer development in Cox regression analysis. However, the cumulative incidence of GI cancer was the highest in the fourth sex- and age-specific suPAR quartile (Supplementary Fig. A1), but not significantly, and we can therefore not exclude that other studies will find an association.

The GI and other types of cancer groups are very heterogeneous. Various etiologies likely underlie development of the different cancer types; therefore, cancer subgroup analyses were performed. No significant association between the highest sex- and age-specific suPAR quartiles and any of the subgroup cancers, besides lung and bronchus cancers (Supplementary Table A1), was found. A low number of cases in some cancer subgroups

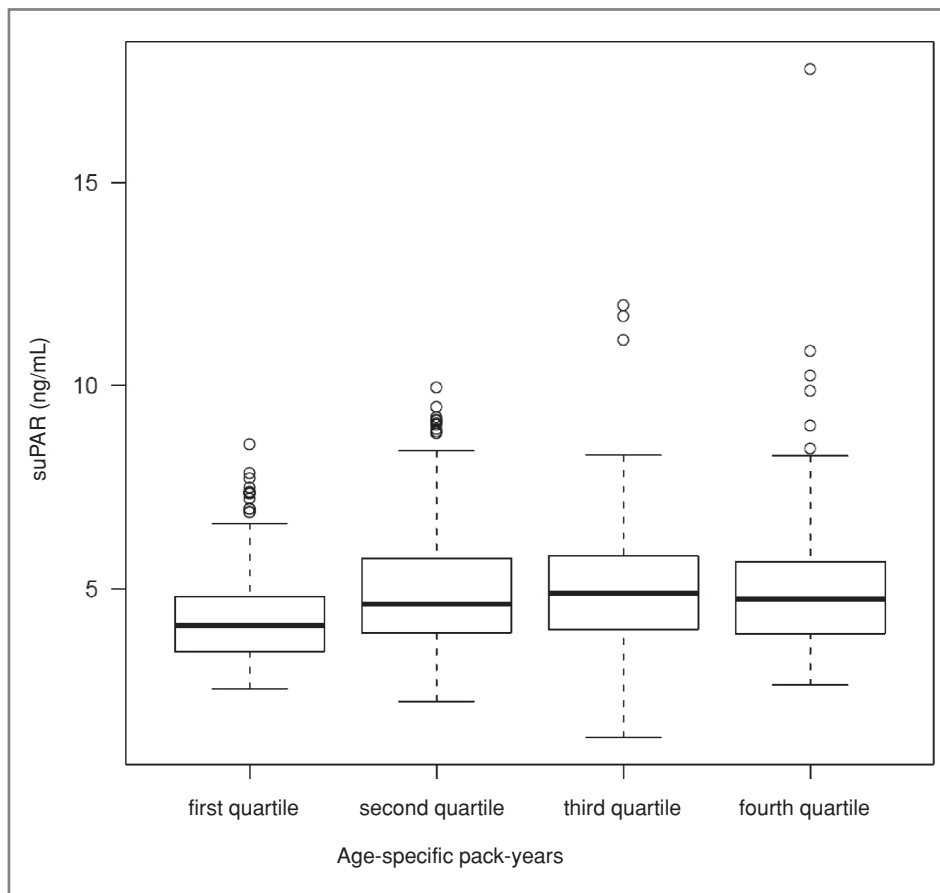


Figure 5. suPAR levels for age-specific pack-year quartiles among current smokers. Boxes represent 75% CI, black lines represent median suPAR level, and whiskers represent 95% CI. Circles represent suPAR levels for individuals not included in the 95% CIs.  $N = 1,126$ .

could explain this. However, it can also be explained by the effect of covariants, not included in Fisher's exact test of cancer subgroups such as age. Supporting this, increased suPAR levels seem to be associated with development of cancers of the other types of cancer group among younger people but not among older persons (Supplementary Fig. A1) in accordance with our recently published study (2). Moreover, increased suPAR levels are not only associated with cancer; they are also associated with various diseases such as type 2 diabetes and cardiovascular diseases and overall mortality (2). It should therefore be kept in mind that the association of increased suPAR levels and development of respiratory, GI, and other cancer types are analyzed as competing risks. Some individuals with high suPAR are therefore only available for analysis until they die of other causes than cancer, such as cardiovascular disease, or develop another type of cancer than that the cancer group examined.

Potential limitations of this study include self-selection bias and whether the MONICA10 participants are representative of the general population. The smoking prevalence in this study is similar to smoking prevalences found in other Danish population-based studies recruiting in the same period (37, 38). Moreover, we compared

MONICA10 cancer incidences with cancer incidences from the Danish National Cancer Registry published online through the Nordcan initiative (39, 40), as described in the Supplementary Section. Similar respiratory cancer incidences were found in MONICA10 ( $N = 57$ ) and in the Danish National Cancer Registry ( $N = 55$ ); however, total MONICA10 cancer incidence ( $N = 372$ ) was somewhat higher than that expected from the Danish National Cancer Registry ( $N = 322$ ).

Self-selection bias may have increased the cancer incidence of this study, if individuals at increased risk exhibited higher participation frequencies. On the contrary, individuals with any kind of illness may not find the energy to participate. In any case, if we expect the total cancer incidence to be slightly lower in the general population (as indicated by from Nordcan data), the cumulative incidence of cancers in all suPAR quartiles is expected to be lower than that reported in our study. However, this study reports on associations between biomarkers and incident cancer and not on Danish cancer incidence. Therefore, we expect that the associations observed in this study will be observed also in the general population.

Additional limitations of this study include that only individuals of Caucasian ethnicity participated; thus, the



results might not be applicable to other ethnic groups. Another limitation was that the population was too small to make elaborate cancer subgroup analyses. Former smokers and never smokers were grouped when adjusting for smoking in respiratory cancer analyses since no respiratory cancer cases were found in the never smoking group. However, this is a population-based study and not patient-based. Therefore, former smokers are expected to have a lower risk of developing lung cancer than do current smokers.

In conclusion, suPAR levels were significantly increased at baseline in individuals diagnosed with respiratory or other types of cancer during follow-up. The findings of this study and other recent studies of biomarkers and cancer risk suggest that inflammation is part of the etiology underlying cancer (11, 21, 28). Measuring inflammation in addition to established risk factors, such as age, sex, smoking, and alcohol consumption, could improve cancer risk stratification. More specifically, suPAR could be considered for lung cancer risk assessment in smokers. Large prospective or screening studies should aim at establishing cancer risk algorithms, as has been done for cardio-

vascular diseases (41, 42). We suggest suPAR to be a candidate for evaluation in risk algorithms.

### Disclosure of Potential Conflicts of Interest

ViroGates A/S, Denmark, donated the ELISA kits for measuring suPAR in plasma samples free of charge. The company had no influence on study design, results, and the decision to publish results. J. Eugen-Olsen is cofounder, shareholder, and board member of ViroGates A/S, Denmark, the company that developed the suPARnostic ELISA. J. Eugen-Olsen, O. Andersen, and S.B. Haugaard are inventors on a patent on suPAR and risk of diseases. Copenhagen University Hospital Hvidovre holds the patent, which is licensed to ViroGates A/S.

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