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ORIGINAL ARTICLE

Relationship of the oxidative damage biomarker 8-*epi*-prostaglandin $F_{2\alpha}$ to risk of lung cancer development in the Shanghai Cohort Study

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Abstract

It has been hypothesized that the pathogenesis of lung cancer induced by cigarette smoking involves oxidative damage by free radicals. Epidemiological data on biomarkers of oxidative damage and risk of lung cancer development are sparse. A nested case–control study of 610 lung cancer cases and 610 matched controls was conducted within a prospective cohort of 18 244 Chinese men in Shanghai, China. The concentrations of 8-*epi*-prostaglandin $F_{2\alpha}$ (8-*epi*$PGF_{2\alpha}$), a biomarker of oxidative stress, were determined in baseline urine samples using a validated mass-spectrometry assay. Current smokers had significantly higher level of 8-*epi*$PGF_{2\alpha}$ than former smokers or never smokers ($P < 0.001$). 8-*epi*$PGF_{2\alpha}$ levels were significantly higher in lung cancer cases than their smoking-matched controls in former and current smokers, but not different in never smokers (P for interaction = 0.019). The relative risks of developing lung cancer for former and current smokers in the highest relative to the lowest quartile of 8-*epi*$PGF_{2\alpha}$ were 5.25 ($P_{trend} = 0.035$) and 1.99 ($P_{trend} = 0.007$), respectively. The effect of 8-*epi*$PGF_{2\alpha}$ and biomarkers of cigarette smoke exposure on lung cancer risk was additive; the relative risk was 5.33 (95% confidence interval = 2.65–7.51) for current smokers with the highest thirds of 8-*epi*$PGF_{2\alpha}$ and total cotinine compared with their lowest thirds. Smokers with a heightened state of oxidative stress in response to the insults of cigarette smoking may be more susceptible to smoking-induced lung carcinogenesis.

Introduction

Lung cancer is the leading cause of cancer death in both men and women worldwide (1.5 million deaths per year) and in the USA (158 000 deaths per year) (1,2). Tobacco smoking is the most important causal factor for lung cancer. It is estimated that 90% of all lung cancer deaths in the USA are attributable to cigarette smoking, while it accounts for 80% of the worldwide lung cancer burden in males and at least 50% in females (3,4). However, there is considerable variation in susceptibility to lung cancer among smokers; only 24% of male lifelong smokers and 12% of female lifelong smokers die from lung cancer by 85 years of age after taking into account competing risks of death (5). This large inter-individual variation in smoking-related lung cancer risk may be determined in part by variability in the uptake and metabolism of tobacco smoke carcinogens. There are 72 established carcinogens in cigarette smoke (6). Among these, polycyclic aromatic hydrocarbons and the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are widely considered to be among the most important causative agents for lung cancer (7,8). We previously reported that the urinary levels of total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), a metabolite of NNK, phenanthrene tetraol (PheT), a biomarker of polycyclic aromatic hydrocarbon and

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Abbreviations

BMI	body mass index
CI	confidence interval
8-epiPGF2α	8-epi-prostaglandin F2α
NNAL	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol
OR	odds ratio
PheT	phenanthrene tetraol

total cotinine, a metabolite of nicotine and biomarker of overall tobacco constituent uptake, were associated with an increased risk of lung cancer in two prospective cohorts of Chinese smokers (9,10).

Decades of research have firmly established that cigarette smoke and its condensate have tumor promoting and co-carcinogenic activity, which collaborate with the multiple carcinogens in smoke to cause cancer. While the mechanisms by which these tumor promoting and co-carcinogenic agents act are complex and not fully understood, there is little doubt that oxidative damage and inflammation play a significant role (11–17). Epidemiologic studies of lung cancer have not previously investigated this combination using appropriate biomarkers reflecting both carcinogen exposure and oxidative stress.

8-*epi*-Prostaglandin $F_{2\alpha}$ (8-epiPGF$_{2\alpha}$) is a product of lipid peroxidation and a reliable biomarker of *in vivo* oxidative stress (18,19). 8-*epi*PGF$_{2\alpha}$ is excreted in urine and chemically stable (20). Urinary levels of 8-*epi*PGF$_{2\alpha}$ are elevated in smokers, and are associated with number of cigarettes smoked per day (21). Epidemiological studies on the relationship of 8-*epi*PGF$_{2\alpha}$ to the risk of lung cancer are sparse. A cross-sectional study reported that serum levels of 8-isoprostane were elevated in lung cancer patients than control subjects, and were higher in patients with advanced disease (22). A nested case–control study within the Multiethnic Cohort found that baseline urinary levels of 15-isoprostane $F_{2\alpha}$ (the same as 8-*epi*PGF$_{2\alpha}$) were related to lung cancer risk in men, but not in women (23).

Utilizing the resources of the Shanghai Cohort Study, the present study examined the association between urinary levels of 8-*epi*PGF$_{2\alpha}$ and risk of lung cancer in all subjects, and in never, former and current smokers separately. Among current smokers, we further assessed the independent association between urinary levels of 8-*epi*PGF$_{2\alpha}$ and lung cancer risk with adjustment for biomarkers of cigarette smoke exposure in addition to self-reported smoking history.

Material and methods

Study population

Subjects were drawn from the Shanghai Cohort Study (24). Briefly, the Shanghai Cohort consisted of 18 244 men enrolled from 1 January 1986 through 30 September 1989, who were 45–64 years of age and resided in the city of Shanghai, China. In addition to in-person interviews eliciting information on use of tobacco and alcohol, a 10-ml blood sample and a single-void urine specimen were collected from each participant at baseline. The Shanghai Cohort Study has been approved by the Institutional Review Boards at the Shanghai Cancer Institute and the University of Pittsburgh. The present study has been approved by the Institutional Review Boards at the University of Minnesota and the University of Pittsburgh.

Nested case–control study

Identification of incident lung cancer cases was accomplished through annual in-person re-interviews of all surviving cohort members and routine review of reports from the population-based Shanghai Cancer Registry. To date, losses to follow-up totaled 839 individuals (4.6%) of the entire cohort after 25 years of study. The nested case–control design was previously described (10,25). As of 31 December 2006, 706 cohort participants developed lung cancer. Among them, 574 were current smokers, 43 were former smokers and 89 were never smokers at baseline. To increase sample sizes for never and former smokers, we included 42 never smokers and 16 former smokers who were diagnosed with lung cancer during 2007 through 2013. For each case, we randomly selected one control subject from all cohort members who were free of cancer and alive at the time of cancer diagnosis of the index case. Controls were matched to the index case by age at enrollment (±2 years), date of biospecimen collection (±1 month), neighborhood of residence and smoking status (never, former and current smokers) at recruitment.

Laboratory measurements

Urine samples of all study subjects were retrieved from the biospecimen bank. Specimens from matched control subjects and their index cases were always assayed in the same batch. All urine aliquots were identified only by unique codes, and laboratory personnel had no knowledge of the case/control status of the test samples. The assay for quantifying 8-*epi*PGF$_{2\alpha}$ in urine was described previously (26). Briefly, urine (0.2 ml) was mixed with the internal standard [D_4]8-*epi*PGF$_{2\alpha}$ (2 ng) and the sample was acidified with 20 µl 88% formic acid, then applied to Agilent Bond Elut C18 solid phase extraction plates, previously conditioned with 1 ml methanol, 1 ml acetonitrile and 3 ml 50 mM phosphate buffer, pH 3. The plates were washed with 3 ml 50 mM phosphate buffer and 3 ml hexanes, then 8-*epi*-PGF$_{2\alpha}$ was eluted with 1 ml ethyl acetate, collected in 96-well Tru-Taper plates, dried and stored at −20°C until analysis by LC-ESI-MS/MS. For the analysis, the samples were redissolved in 30 µl 80:20:25 H_2O:MeOH:NH_4OH and injected on a 50 × 1.0 mm Waters X-Bridge BEH C18 column with elution by a program from 5% B to 18% B in 11 min at a flow rate of 45 µL/min where solvent A was 0.15% NH_4OH and Solvent B was 95:5:0.15 acetonitrile: methanol: NH_4OH. LC-ESI-MS/MS was carried out on a Thermo Dionex Ultimate 3000 LC coupled to a Thermo Scientific TSQ Vantage triple quadrupole mass spectrometer, in the negative ESI mode, monitoring *m/z* 193 → *m/z* 173 for the analyte and *m/z* 197 → *m/z* 177 for the internal standard.

The measurements of serum concentrations of retinol, alpha- and gamma-tocopherols, and specific carotenoids including alpha-carotene, beta-carotene, beta-cryptoxanthin, lycopene and lutein/zeaxanthin by HPLC methods were described in our previous report (25). The measurements of urinary total NNAL, PheT and total cotinine were also previously reported (9,10). Urinary creatinine was assayed using a Kodak Ektachem 500 chemistry analyzer.

Of the 764 case–control pairs, 103 cases (92 current smokers and 11 never smokers) and 66 controls (60 current smokers, 1 former smoker and 5 never smokers) did not have urinary 8-*epi*PGF$_{2\alpha}$ measurements because their urine samples collected at baseline were depleted after quantitation of other urinary biomarkers in previous studies (9,10,27–29). In addition, we excluded 51 cases without their matched controls and 88 controls without their index cases. The present study included the remaining 610 cases of lung cancer and 610 their individually matched control subjects.

Statistical analysis

Urinary 8-*epi*PGF$_{2\alpha}$ was expressed in pg per mg creatinine (Cr) to correct for varying water contents of individual spot urine samples. Given the markedly skewed distributions of the urinary biomarkers, formal statistical testing was performed on logarithmically transformed values, and geometric means and 95% confidence intervals (CIs) are presented.

The χ^2 test and the *t*-test were used to compare the distributions of selected variables between lung cancer cases and controls. The analysis of covariance method was used to examine the difference in the Cr-adjusted concentration of urinary 8-*epi*PGF$_{2\alpha}$ across different smoking status/dose groups among control subjects as well as between lung cancer cases and controls with adjustment for potential confounders. The two-way analysis of variance method was used to simultaneously examine the effect of cigarette smoking and lung cancer status on levels of 8-*epi*PGF$_{2\alpha}$.

The relationship between urinary 8-*epi*PGF$_{2\alpha}$ and lung cancer risk was assessed by odds ratios (ORs) and their 95% CIs and *P* values using the conditional logistic regression method that took into account for the effect of matching factors including age, year of biospecimens collection and neighborhood of residence at enrollment on lung cancer risk. In the preliminary analysis, we noticed that urinary levels of 8-*epi*PGF$_{2\alpha}$ were

significantly higher in current smokers than never and former smokers. Thus study subjects were grouped into quartiles according to the distributions of 8-*epi*$PGF_{2\alpha}$ among separate groups of controls by smoking status. In the analysis for the association between 8-*epi*$PGF_{2\alpha}$ and risk of lung cancer among current smokers, we further adjusted for biomarkers of exposure to cigarette smoke including total cotinine, total NNAL and PheT, the identified biomarkers that were independently associated with lung cancer in this cohort (9,10). Unconditional logistic regression models were used for all stratified analyses. An ordinal value of 8-*epi*$PGF_{2\alpha}$ in quartile was used for all linear trend tests for its relation to lung cancer risk.

We examined the potential interaction effects between 8-*epi*$PGF_{2\alpha}$ and levels of self-reported smoking history or biomarkers of cigarette smoke constituents on lung cancer risk. When examining whether the combined effect of two factors was greater than the multiplicative product of their individual effects, we used multivariate logistic regression models with the two main effects and their product term as covariates. When assessing if the combined effect of two factors on lung cancer risk was greater than the sum of the individual effects, we used the method described by Rothman (30). Under the null hypothesis of additivity, the synergy index would take on the value one. A lower 95% CI of the synergy index that is greater than one is indicative of a statistically significant synergistic effect between the two factors.

Statistical analyses were carried out using SAS software version 9.3 (SAS Institute, Cary, NC). All *P*-values reported are two-sided, and those that were less than 0.05 were considered to be statistically significant.

RESULTS

Of the 610 cases, 424 (69.5%) were histopathologically confirmed as lung cancer while the remaining 186 (30.5%) were diagnosed based on radiography or computer-assisted tomography evidence. Among the histopathologically confirmed cases, 171 (40.3%) were squamous cell cancers, 150 (35.4%) adenocarcinomas, 25 (5.9%) small cell cancers and 78 (18.4%) other cell types. The mean age (standard deviation) at cancer diagnosis of all case patients was 69.0 (7.6) years. The mean (SD) time interval between baseline biospecimen collection and cancer diagnosis was 11.3 (6.1) years, ranging from 1 month to 26.8 years.

Compared with controls, cases had lower body mass index (BMI) (kg/m^2). Smoking status was matched between cases and controls. Among ever smokers, cases had greater numbers of cigarettes smoked per day, years of smoking and pack-years of smoking than controls. The percentage of regular drinkers of alcohol was comparable between cases and controls. Among drinkers of alcohol, cases consumed more drinks per day than their controls (Table 1). As previously reported (10), the geometric means of urinary total cotinine, total NNAL and PheT in lung cancer cases were significantly higher than those in controls among current smokers (Table 1).

Cigarette smoking had a significant impact on urinary levels of 8-*epi*$PGF_{2\alpha}$. In both lung cancer cases and controls, current smokers had significantly higher levels of urinary 8-*epi*$PGF_{2\alpha}$ than never and former smokers (Table 2). Among controls of current smokers, levels of 8-*epi*$PGF_{2\alpha}$ were positively associated with number of cigarettes per day ($\rho = 0.20$, $P < 0.001$), urinary total cotinine ($\rho = 0.33$, $P < 0.001$), urinary total NNAL ($\rho = 0.26$, $P < 0.001$) and urinary PheT ($\rho = 0.20$, $P < 0.001$), but not associated with years of smoking ($\rho = 0.02$, $P = 0.687$). Among former smokers, the 8-*epi*$PGF_{2\alpha}$ levels were not significantly correlated with number of years since quitting smoking in cases ($\rho = -0.14$, $P = 0.311$) and controls ($\rho = -0.20$, $P = 0.133$), respectively. Among all controls, BMI had a positive association with 8-*epi*$PGF_{2\alpha}$ in former smokers ($\rho = 0.22$, $P = 0.098$), and an inverse association in current smokers ($\rho = -0.21$, $P < 0.001$), but no association in never smokers ($\rho = 0.008$, $P = 0.934$). Of the 610 control subjects, 128

Table 1. Baseline demographic and lifestyle characteristics and urinary biomarkers of cigarette smoke exposure in lung cancer cases and matched control subjects, The Shanghai Cohort Study 1986–2013

Characteristics or biomarkers	Cases	Controls	P*
Number of subjects	610	610	
Mean age (SD), years	57.3 (5.2)	57.1 (5.0)	0.513[a]
Men, *N* (%)	610 (100)	610 (100)	1.000[a]
Mean body mass index (SD), kg/m^2	21.6 (2.8)	22.1 (3.1)	0.001
Level of education, *N* (%)			
No formal education	51 (8.4)	41 (6.7)	0.225
Primary school (1–6 years)	204 (33.4)	186 (30.5)	
Secondary school or higher	355 (58.2)	383 (62.8)	
Smoking status, *N* (%)			
Non-smokers	116 (19.0)	116 (19.0)	1.000[a]
Former smokers	58 (9.5)	58 (9.5)	
Current smokers	436 (71.5)	436 (71.5)	
Mean cigarettes/day (SD)[b]	20.2 (8.6)	15.4 (7.6)	<0.001
Mean years of smoking (SD)[b]	34.7 (8.5)	30.6 (10.5)	<0.001
Mean pack-years of smoking (SD)[b]	35.5 (18.0)	24.3 (15.3)	<0.001
Alcohol drinking status, *N* (%)			
Non-drinkers	292 (47.9)	278 (45.6)	0.421
Regular drinkers	318 (52.1)	332 (54.4)	
Mean drinks/day (SD)[c]	3.0 (2.7)	2.5 (2.2)	0.005
Urinary biomarkers of cigarette smoking			
Total cotinine (nmol/mg Cr)[d]	13.38 (12.16, 14.76)	6.98 (6.32, 7.68)	<0.001
Total NNAL (pmol/mg Cr)[d]	0.28 (0.26, 0.30)	0.20 (0.18, 0.20)	<0.001
PheT (pmol/mg Cr)[d]	32.38 (30.66, 34.18)	28.10 (26.62, 29.68)	<0.001

Cr, creatinine; SD, standard deviation.[a]Age, sex and smoking status were matched between cases and controls.
[b]Among ever smokers.
[c]Among regular alcohol drinkers only.
[d]Among current smokers only; geometric means (95% confidence intervals) are shown.
*Two-sided *P*'s were based on *t* test for continuous variables or chi-square test for categorical or nominal variables.

Table 2. Geometric means of 8-*epi*-prostaglandin $F_{2\alpha}$ (8-*epi*$PGF_{2\alpha}$) in lung cancer cases and control subjects by smoking status, The Shanghai Cohort Study 1986–2013

	Cases		Controls		
Subject group	*N*	8-*epi*$PGF_{2\alpha}$[a] (pg/mg creatinine)	*N*	8-*epi*$PGF_{2\alpha}$[a] (pg/mg creatinine)	*P* for cases versus controls
Total subjects	610	456 (434–479)	610	402 (382–422)	<0.001
By smoking status					
Never smokers	116	248 (226–273)	116	254 (231–280)	0.743
Former smokers	58	293 (256–335)	58	222 (194–255)	0.005
Current smokers	436	573 (545–602)	436	488 (464–512)	<0.001
P for current versus never smokers		<0.001		<0.001	*P* for interaction = 0.019[†]
P for current versus former smokers		<0.001		<0.001	
P for former versus never smokers		0.113		0.051	

[a]Geometric means (95% confidence intervals) and *P* values were derived from the two-way analysis of variance with log (8-*epi*$PGF_{2\alpha}$) as dependent variable and lung cancer status and smoking status as the two main factors with the following covariates: age, year of biospecimen collection, neighborhood of residence at baseline, level of education and body mass index (kg/m^2).

[†]Two-sided *P* for interaction between smoking status (current versus never) and lung cancer status on log (8-*epi*$PGF_{2\alpha}$).

had serum measurements of antioxidants from a previous study (25). Urinary levels of 8-*epi*$PGF_{2\alpha}$ were significantly or borderline significantly associated with lower levels of serum α-tocopherol (ρ = –0.21, *P* = 0.018), serum α-carotene (ρ = –0.16, *P* = 0.069) and serum lycopene [(ρ = –0.18, *P* = 0.049), but not associated with other serum antioxidants including retinol, β-carotene, β-cryptoxanthin or lutein/zeaxanthin (data not shown)].

The geometric mean of creatinine-corrected 8-*epi*$PGF_{2\alpha}$ in lung cancer cases was significantly higher than that in controls after adjustment for age, level of education, BMI, smoking status, number of cigarettes per day and years of smoking (Table 2). The differences in geometric means of 8-*epi*$PGF_{2\alpha}$ between cases and controls within each category of smoking status were different, with the largest case–control difference being seen in current smokers (*P* < 0.001), a moderate difference in former smokers (*P* = 0.005), but no difference in never smokers (*P* = 0.743). The interaction between smoking status (current versus never) and lung cancer status on urinary concentrations of 8-*epi*$PGF_{2\alpha}$ was statistically significant (*P* for interaction = 0.019) after adjustment for multiple potential confounding factors (Table 2).

Higher levels of urinary 8-*epi*$PGF_{2\alpha}$ were associated with a higher risk of lung cancer. Among all subjects, the risk of developing lung cancer was doubled for individuals in the highest quartile of 8-*epi*$PGF_{2\alpha}$ relative to the lowest quartile after adjustment for history of smoking and other potential confounders (*P* for trend = 0.007) (Table 3). This positive association was present in both current and former smokers. Compared with the lowest quartile of 8-*epi*$PGF_{2\alpha}$, the multivariable-adjusted ORs of developing lung cancer for the highest quartile in former and current smokers were 5.25 (95% CI = 0.94–29.41, *P* for trend = 0.035) and 1.99 (95% CI = 1.26–3.14, *P* for trend = 0.007), respectively. However, there was no positive relationship of 8-*epi*$PGF_{2\alpha}$ to lung cancer risk among never smokers (*P* for trend = 0.538).

Among former smokers, more than half of cases but less than a quarter of controls were in the highest quartile of 8-*epi*$PGF_{2\alpha}$ at baseline, yielding an OR of 5.29 (95% CI = 1.58–17.72), comparing with the first, second and third quartiles combined. Among the 32 cases of former smokers with the highest quartile of 8-*epi*$PGF_{2\alpha}$, the median time interval from quitting smoking to the study enrollment (i.e. collection of biospecimen) was 15.6 years. Thus, the elevation of urinary 8-*epi*$PGF_{2\alpha}$ for these cases was less likely related to recent smoking cessation.

Among current smokers, additional adjustment for biomarkers of cigarette smoke exposure including total cotinine, total NNAL and PheT, diminished the OR of lung cancer associated with higher levels of 8-*epi*$PGF_{2\alpha}$. Compared with the lowest quartile of 8-*epi*$PGF_{2\alpha}$, the biomarker-adjusted OR of lung cancer for the highest quartile was 1.44 (95% CI = 0.88–2.36) (data not shown). We further analyzed for the association for the joint levels of 8-*epi*$PGF_{2\alpha}$ and these biomarkers of cigarette smoke exposure with lung cancer risk among current smokers. Compared

Table 3. Urinary levels of 8-*epi*-prostaglandin $F_{2\alpha}$ (8-*epi*$PGF_{2\alpha}$) in relation to lung cancer risk in total and subgroups by smoking status, The Shanghai Cohort Study 1986–2013

8-*epi*$PGF_{2\alpha}$ in quartile (pg/mg Cr)[a]	Cases	Controls	OR (95% CI)[b]
All subjects			
First quartile[b]	93	150	1.00
Second quartile	139	151	1.31 (0.88, 1.94)
Third quartile	147	156	1.18 (0.80, 1.74)
Fourth quartile	231	153	1.79 (1.22, 2.63)
P for trend			0.007
Never smokers			
First quartile (<172)	23	29	1.00
Second quartile (172–241)	32	27	1.62 (0.70, 3.78)
Third quartile (242–338)	37	30	1.54 (0.68, 3.52)
Fourth quartile (>338)	24	30	0.71 (0.29, 1.75)
P for trend			0.528
Former smokers			
First quartile (<165)	11	15	1.00
Second quartile (165–210)	7	14	1.04 (0.19, 5.75)
Third quartile (211–267)	8	15	0.83 (0.21, 3.34)
Fourth quartile (>267)	32	14	5.25 (0.94, 29.41)
P for trend			0.035
Current smokers			
First quartile (<340)	59	106	1.00
Second quartile (340–487)	100	110	1.33 (0.83, 2.12)
Third quartile (488–650)	102	111	1.13 (0.70, 1.82)
Fourth quartile (>650)	175	109	1.99 (1.26, 3.14)
P for trend			0.007

[a]Quartile cut-off values were based on the distribution of urinary 8-*epi*$PGF_{2\alpha}$ among control subjects stratified by smoking status.

[b]Odds ratios (95% confidence intervals) were derived from conditional logistic regression models with adjustment for level of education, body mass index (kg/m^2), smoking status, number of cigarettes/day, years of smoking and years since quitting smoking (for former smokers only).

with the lowest tertiles of both urinary 8-*epi*$PGF_{2\alpha}$ and total cotinine, the OR of developing lung cancer for the highest tertiles of both 8-*epi*$PGF_{2\alpha}$ and total cotinine was 5.33 (95% CI = 2.64–7.51) (Table 4). Similarly, ORs (95% CIs) for 8-*epi*$PGF_{2\alpha}$ in combination with total NNAL and PheT were 3.84 (95% CI = 1.93–7.65) and 2.90 (95% CI = 1.42–5.92), respectively, after adjustment for smoking intensity and duration and other potential confounders (Table 4). However, these combined effects were not more than the sum of the two individual effect of 8-*epi*$PGF_{2\alpha}$ and total cotinine (or other biomarkers of cigarette smoking) on the estimates of lung cancer risk.

We also examined association between the levels of 8-*epi*$PGF_{2\alpha}$ and risk of lung cancer among current smokers stratified by levels of cigarette smoking. The association between 8-*epi*$PGF_{2\alpha}$ and lung cancer risk was comparable for <20 cigarettes/day with ≥20 cigarettes/day (*P* for interaction = 0.203) or for smokers with different pack-years of smoking—<20, 20–39 and ≥40 pack-years of smoking (*P* for interaction = 0.722). The duration of smoking had a borderline significant modifying effect on the association between 8-*epi*$PGF_{2\alpha}$ and lung cancer risk (*P* for interaction = 0.056) (Supplementary Table 1, available at *Carcinogenesis* Online).

The potential impact of disease progression on 8-*epi*$PGF_{2\alpha}$ was examined among 610 cases by the time interval from biospecimen collection to lung cancer diagnosis. Urinary level of 8-*epi*$PGF_{2\alpha}$ was not correlated with the time interval (ρ = –0.007, *P* = 0.859). The geometric means of 8-*epi*$PGF_{2\alpha}$ were similar for patients whose urine samples for 8-*epi*$PGF_{2\alpha}$ were collected within 2 years as for those collected more than 20 years before cancer diagnosis in all cases (Supplementary Table 2, available at *Carcinogenesis* Online). The associations between quartile levels of urinary 8-*epi*$PGF_{2\alpha}$ and lung cancer risk among ever smokers did not attenuate for longer than shorter duration of follow-up from biospecimens collection to lung cancer diagnosis. A statistically significant, positive association for urinary 8-*epi*$PGF_{2\alpha}$ was still present for lung cancer cases diagnosed 10 or more years after the collection of urine samples for measurement of 8-*epi*$PGF_{2\alpha}$ (Table 5).

The associations between urinary 8-*epi*$PGF_{2\alpha}$ and risk of lung cancer differed by histological subtype. The levels of 8-*epi*$PGF_{2\alpha}$ were significantly associated with increased risk lung squamous cell carcinoma (*P* for trend = 0.016), but not with risk of lung adenocarcinoma (*P* for trend = 0.354) (Supplementary Table 3, available at *Carcinogenesis* Online). However, the difference between the two risk associations was not statistically significant (*P* = 0.194).

Of the 610 lung cancer cases and 610 control subjects included in the present study, 182 cases and 128 controls had measurements of serum antioxidants from our previous study (25). In this subset, ORs of lung cancer for highest relative to the lowest quartile of urinary 8-*epi*$PGF_{2\alpha}$ were 1.63 (95% CI = 0.74–3.59, *P* for trend = 0.202) after adjustment for matching factors (age, year of biospecimens collection and neighborhood of residence), level of education, BMI and history of smoking (smoking status, number of cigarettes/day, years of smoking and years since quitting smoking for former smokers). Further adjustment for serum antioxidants including α- and β-carotenes, β-cryptoxanthin, lycopene, lutein/zeaxanthin, α- and γ-tocopherols and retinol did not materially change the association between 8-*epi*$PGF_{2\alpha}$ and risk of lung cancer; the antioxidants-adjusted OR was 1.79 (95% CI = 0.78–4.11, *P* for trend = 0.163).

Discussion

In this nested case–control study within the Shanghai Cohort Study, we found a statistically significant, independent association between the urinary level of 8-*epi*$PGF_{2\alpha}$ and risk of lung cancer after adjustment for smoking intensity and duration and other potential confounding factors. The overall risk of lung cancer development was doubled for subjects in the highest quartile of 8-*epi*$PGF_{2\alpha}$. The positive association remained statistically significant even after further adjustment for multiple biomarkers of cigarette smoke exposure and serum antioxidants. The significant association between urinary 8-*epi*$PGF_{2\alpha}$ and lung cancer risk was present in both former and current smokers. The number of years since quitting smoking did not confound the 8-*epi*$PGF_{2\alpha}$-lung cancer risk association among former smokers. For current smokers, there was evidence supporting an additive effect of 8-*epi*$PGF_{2\alpha}$ and biomarkers of cigarette smoke constituents including total cotinine, total NNAL and PheT. This is the first prospective study that examined the joint effect of 8-*epi*$PGF_{2\alpha}$ and these biomarkers of cigarette smoking on lung cancer risk.

8-*epi*$PGF_{2\alpha}$ is a reliable marker and considered a gold standard for oxidative damage via lipid peroxidation. Several epidemiological studies have reported positive associations between urinary 8-*iso*-$PGF_{2\alpha}$ levels and risk of multiple cancers including

Table 4. Joint tertile levels of urinary 8-*epi*-prostagla ndin $F_{2\alpha}$ with urinary biomarkers of cigarette smoke exposure in relation to risk of lung cancer among current smokers, The Shanghai Cohort Study 1986–2013

		Urinary 8-*epi*-prostaglandin $F_{2\alpha}$ in tertile					
		First tertile		Second tertile		Third tertile	
Biomarkers of cigarette smoke exposure	Levels in tertile	Ca/Co[a]	OR (95% CI)[b]	Ca/Co[a]	OR (95% CI)[b]	Ca/Co[a]	OR (95% CI)[b]
Total cotinine	First tertile	15/75	1.00 (ref)	13/43	1.15 (0.43, 3.03)	14/28	1.95 (0.75, 5.08)
	Second tertile	32/37	3.70 (1.65, 8.28)	37/61	1.77 (1.65, 8.28)	52/45	3.32 (1.56, 7.04)
	Third tertile	42/29	2.93 (1.33, 6.42)	71/47	3.55 (1.68, 7.51)	160/71	5.33 (2.64, 7.51)
Total NNAL	First tertile	21/60	1.00 (ref)	17/51	0.78 (0.35, 1.76)	21/35	1.24 (0.53, 2.89)
	Second tertile	37/52	1.52 (0.72, 3.21)	47/52	1.37 (0.65, 2.91)	64/44	2.10 (1.05, 4.19)
	Third tertile	31/29	1.95 (0.84, 4.53)	57/47	2.03 (0.96, 4.28)	141/65	3.84 (1.93, 7.65)
PheT	First tertile	29/57	1.00 (ref)	28/59	0.67 (0.30, 1.46)	48/28	2.61 (1.19, 5.75)
	Second tertile	26/45	1.30 (0.61, 2.79)	43/50	1.59 (0.77, 3.28)	67/49	2.16 (1.04, 4.49)
	Third tertile	34/39	1.67 (0.72, 3.90)	50/42	2.05 (0.93, 4.53)	111/67	2.90 (1.42, 5.92)

[a]Ca/Co: number of case and control.

[b]Odds ratios (95% confidence intervals) were derived from conditional logistic regression models with adjustment for level of education, body mass index (kg/m^2), number of cigarettes per day and years of smoking.

Table 5. Urinary levels of 8-*epi*-prostaglandin $F_{2\alpha}$ (8-*epi*$PGF_{2\alpha}$) in relation to lung cancer risk by years between biospecimen collection and cancer diagnosis among ever smokers, The Shanghai Cohort Study 1986–2013

		<5 years		5–<10 years		10–<15 years		≥15 years	
8-*epi*$PGF_{2\alpha}$	Controls	Cases	OR (95% CI)[a]	Cases	OR (95% CI)[a]	Cases	OR (95% CI)[a]	Cases	OR (95% CI)[a]
First quartile	121	15	1.00	17	1.00	15	1.00	23	1.00
Second quartile	124	27	1.59 (0.77, 3.25)	29	1.82 (0.90, 3.68)	27	1.37 (0.67, 2.77)	24	1.04 (0.54, 2.00)
Third quartile	126	23	1.30 (0.62, 2.72)	31	1.58 (0.79, 3.18)	27	1.27 (0.62, 2.58)	29	1.11 (0.58, 2.10)
Fourth quartile	123	35	1.96 (0.97, 3.98)	58	2.70 (1.39, 5.24)	57	2.73 (1.40, 5.33)	57	1.88 (1.04, 3.40)
P for trend			0.109		0.006		0.002		0.024

[a]Odds ratios (95% confidence intervals) were derived from unconditional logistic regression models with adjustment for matching factors (age, years of biospecimen collection, neighborhood of residence at baseline), level of education, body mass index (kg/m^2), smoking status, number of cigarettes/day and years of smoking.

lung cancer (23), breast cancer (31), liver cancer (32) and prostate cancer (33). In the only reported study on lung cancer, higher levels of 15-isoprostane F_{2t} (the same as 8-*epi*$PGF_{2\alpha}$) in prediagnostic urine samples were associated with a statistically non-significant, 2-fold risk of lung cancer in men (*P* for trend = 0.12), but were not associated with lung cancer risk in women (23). The non-significant results could be due to the relatively small sample size of the study that included only 136 male cases and 71 female cases. Another limitation of that study was the use of an enzyme-linked immunosorbent assay (ELISA) to quantify urinary 15-F_{2t}-isoP, which may have cross-reactivity with related compounds and result in low specificity and an overestimation (26). In this study, we used the LC-MS/MS method that provides high specificity, accuracy and precision (26). Furthermore, other biomarkers of cigarette smoke exposure including total cotinine, total NNAL and PheT were measured on all study subjects from a previous study (10), allowing for investigating the independent effect and joint effect of 8-*epi*$PGF_{2\alpha}$ on lung cancer risk.

The increased risk of lung cancer with high levels of 8-*epi*$PGF_{2\alpha}$ was present in former and current smokers, but not in never smokers. Urinary levels of 8-*iso*-$PGF_{2\alpha}$ were significantly higher in current smokers than never and former smokers in the present study populations. These findings are similar to those of previous studies (21,26,34–36). In both former and current smokers, urinary 8-*epi*$PGF_{2\alpha}$ levels at baseline, on average, 11 years before cancer diagnosis, were significantly higher than their controls. These data are fully consistent with previous mechanistic studies of tobacco carcinogenesis which clearly demonstrate that tumor promotion and co-carcinogenesis, mediated by oxidative damage and inflammation, are critical events in cancer development caused by cigarette smoking (11–17). Thus, this is the first study to demonstrate the joint effect of carcinogen exposure and oxidative damage in increasing the risk for lung cancer in cigarette smokers. The consistency of our data on exposure biomarkers representing tobacco-specific nitrosamines and polycyclic aromatic hydrocarbon and effect biomarkers such as 8-*epi*-PGF_{2a} is remarkable and supports the multiple mechanistic studies that implicate these factors in tobacco carcinogenesis.

The present study has several strengths. First, the LC-MS/MS method for quantification of 8-*epi*$PGF_{2\alpha}$ provides high quality data. Second, we used a comprehensive approach that simultaneously quantified 8-*epi*$PGF_{2\alpha}$, a biomarker of oxidative stress and biomarkers of cigarette smoke exposure, and incorporated these biomarkers into the statistical analysis, and demonstrated an additive effect of 8-*epi*$PGF_{2\alpha}$ on lung cancer risk. Third, the prospective study design eliminated potential effects of lung cancer development and progression on 8-*epi*$PGF_{2\alpha}$. Fourth, the long term follow-up of study subjects after baseline urine collection allowed for a detailed analysis for the potential impact of subclinical stage and progression of disease (i.e. lung cancer) on urinary levels of 8-*epi*$PGF_{2\alpha}$ and on the association between 8-*epi*$PGF_{2\alpha}$ and lung cancer risk. Fifth, the study has the largest sample size so far with 610 lung cancer cases and 610 individually matched controls allowing for statistical analyses for the association between 8-*epi*$PGF_{2\alpha}$ and lung cancer risk in never, former and current smokers, and among current smokers by different levels of cigarette smoking or smoking biomarkers. The major limitation is that the present study included men only.

In summary, using prospectively collected urine samples from participants in the Shanghai Cohort Study, we demonstrated that high urinary levels of 8-*epi*$PGF_{2\alpha}$ at baseline were associated with a statistically significant, more than doubled risk of lung cancer in former and current smokers. The positive association remained after comprehensive adjustment for smoking intensity and duration, and multiple biomarkers of cigarette smoke exposure including total cotinine, total NNAL and PheT, suggesting an important effect of 8-*epi*$PGF_{2\alpha}$ on the risk of lung cancer development, most likely due to tumor promotion, co-carcinogenesis and/or inflammation. Thus, smokers who are at a heightened state of oxidative stress in response to the insults of tobacco smoking may be more susceptible to smoking-induced lung carcinogenesis.

Supplementary material

Supplementary materials can be found at *Carcinogenesis* online.

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