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Urinary levels of volatile organic carcinogen and toxicant biomarkers in relation to lung cancer development in smokers

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Besides polycyclic aromatic hydrocarbons (PAH) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which are established lung carcinogens, tobacco smoke also contains relatively large quantities of volatile organic carcinogens and toxicants, including 1,3-butadiene, ethylene oxide, benzene, acrolein and crotonaldehyde. Although animal experiments showed that some of these compounds can induce tumors in multiple organs including the lung, epidemiological studies of their relationship with lung cancer in smokers have not been reported. Therefore, in this study, we quantified urinary mercapturic acid metabolites of 1,3-butadiene, ethylene oxide, benzene, acrolein and crotonaldehyde in addition to urinary biomarkers for PAH, NNK and nicotine in 343 lung cancer cases and 392 matched controls among a cohort of 18 244 Chinese men in Shanghai, China, followed from 1986 to 2006. Compared with the lowest quartiles, highest quartiles of all measured mercapturic acids were associated with statistically significantly ∼2-fold increased risk for lung cancer (all *P*'s for trend <0.01) after adjustment for smoking intensity and duration. The positive associations between biomarkers of ethylene oxide, benzene or acrolein and lung cancer risk remained statistically significant after adjustment for biomarkers of PAH and NNK, whereas urinary total cotinine completely explained the mercapturic acid metabolites and lung cancer associations (all *P*'s for trend ≥0.39). We conclude that mercapturic acid metabolites of 1,3-butadiene, ethylene oxide, benzene, acrolein and crotonaldehyde may not be independent risk predictors of lung cancer among Shanghai smokers, in contrast to biomarkers of PAH, NNK and nicotine exposure.

Introduction

The annual worldwide death toll from lung cancer, the most common cause of cancer death, is 1.38 million, an average of well over 3000 deaths per day (1). Lung cancer is also the leading cause of cancer death in the USA, with nearly 157 000 deaths expected in 2011 (2). Cigarette smoking is the most important risk factor for lung cancer. It is estimated to account for 80% of the worldwide lung cancer burden in males and at least 50% in females (3). In the USA, 90% of lung cancer deaths are attributable to tobacco smoking (4).

Only a fraction of lifelong smokers develop lung cancer. It is estimated that ∼24% of male and 11% of female smokers in the USA would die from lung cancer by 85 years of age after taking into account the competing causes of death (5,6). Differences in susceptibility to lung cancer in smokers might be related in part to differences in the uptake and metabolism of tobacco smoke carcinogens and toxicants. There are >70 established carcinogens in cigarette smoke (4).

Abbreviations: BaP, benzo[a]pyrene; HBMA, 4-hydroxybut-2-yl mercapturic acid; HEMA, 2-hydroxyethyl mercapturic acid; HPMA, 3-hydroxypropyl mercapturic acid; MHBMA, monohydroxybutyl mercapturic acid; PAH, polycyclic aromatic hydrocarbons; RSD, relative standard deviation; SPMA, S-phenyl mercapturic acid.

Polycyclic aromatic hydrocarbons (PAH), typified by benzo[*a*]pyrene (BaP) and tobacco-specific nitrosamines, typified by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), are among the carcinogens that have been most extensively investigated as causative agents for lung cancer (7). Recent studies from our group demonstrated that urinary or serum metabolites—biomarkers of PAH and NNK uptake—were significantly associated with lung cancer incidence in smokers (8–10). But PAH and NNK are not the only compounds suspected to contribute to lung cancer development in smokers. Volatile carcinogens and toxicants, including 1,3-butadiene, ethylene oxide, benzene, acrolein and crotonaldehyde may also be involved. Levels in mainstream smoke of the routinely quantified compounds 1,3-butadiene (6.4–68.7 μg per cigarette), benzene (6.1–58.9 μg per cigarette), acrolein (54–155 μg per cigarette) and crotonaldehyde (11–17 μg per cigarette) are 100–1000 times greater than those of typical PAH and NNK, whereas less is known about ethylene oxide levels (11,12)

Mice exposed to 1,3-butadiene by inhalation developed bronchiolar/alveolar adenomas and carcinoma of the lung in addition to other tumors (13,14). But lung tumors were not observed in rats exposed to 1,3-butadiene (14). Epidemiologic studies suggested that workers exposed to 1,3-butadiene had an increased risk of leukemia and non-Hodgkin lymphoma but not lung cancer (14). 1,3-Butadiene is considered carcinogenic to humans by the International Agency for Research on Cancer (IARC) (14).

Inhalation studies demonstrate that ethylene oxide causes alveolar/bronchiolar adenomas and carcinomas of the lung in mice but not in rats (14). Ethylene oxide is considered carcinogenic to humans by International Agency for Research on Cancer (IARC), based on a combination of epidemiological evidence for associations between occupational exposure to ethylene oxide and lymphatic and hemoatopoietic malignancies and consistent mechanistic data demonstrating its alkylating and mutagenic effects in various test systems and humans. However, occupational exposure to ethylene oxide has not been related to lung cancer (14).

Benzene causes tumors at multiple sites including the lung when administered to mice by gavage, and in some cases, by inhalation (15,16). Occupational studies found increased risks of mortality from hematopoietic malignancies and sometimes lung cancer in workers who were exposed to benzene in the workplace (17–19). Benzene is considered to be a cause of various types of leukemia in humans (20).

Acrolein is toxic to the cilia of the lung and is an intense irritant (21,22). Acrolein–DNA adducts are present in the human lung (23), and it reacts with the *p53* gene at hot spots associated with lung cancer, leading some to propose that it is important in lung cancer etiology in smokers (24). Acrolein and crotonaldehyde are products of lipid peroxidation and may be involved in inflammation (25,26), but they are weak carcinogens.

Urinary mercapturic acids are well-established biomarkers of uptake of 1,3-butadiene, ethylene oxide, benzene, acrolein and crotonaldehyde, and all are found at higher levels in the urine of smokers than in non-smokers (21). We and others have developed assays for quantitation of urinary mercapturic acid metabolites for these volatile carcinogens and toxicants (11). All biomarkers have been validated analytically. Most have been used in multiple studies on hundreds or even thousands of smokers and non-smokers (22). However, there are no reports in the literature on the relationship of these biomarkers to lung cancer risk. In this paper, we prospectively examined the relationship between these biomarkers and the risk of developing lung cancer in smokers of the Shanghai Cohort Study that enrolled >18 000 middle-aged or older Chinese men with 20 years of follow-up. Given the simultaneously measured urinary *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT), total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides

(total NNAL) and cotinine and its glucuronides (total cotinine) in the same study subjects, we were able to examine the associations between mercapturic acid metabolites and risk of lung cancer alone and in combination with biomarkers of PAH, NNK and nicotine.

Materials and methods

Subjects

Details of the Shanghai Cohort Study have been previously published (27,28). In brief, the cohort consisted of 18 244 men (constituting 80% of eligible subjects) enrolled from 1 January 1986 through 30 September 1989 who were between 45 and 64 years of age and resided in one of four small geographically defined communities in Shanghai, China. In addition to in-person interviews eliciting information on use of tobacco and alcohol, usual diet and medical history, we collected a 10 ml blood sample and one single void urine sample from each participant at baseline. The Shanghai Cohort Study has been approved by the Institutional Review Boards at the University of Minnesota and the Shanghai Cancer Institute.

Identification of incident lung cancer cases and deaths 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 and from the Shanghai Municipal Vital Statistics Office. As of 31 December 2006, losses to follow-up totaled 839 individuals (4.6%) after 20 years of study.

As of 31 December 2006, 706 cohort participants developed lung cancer. Among them, 574 were smokers, 43 were former smokers and 89 were never-smokers at baseline. The present study focused on current smokers at the time of enrollment and collection of a spot urine sample. These samples were recently analyzed in our study of the relationship of PheT, total NNAL and total cotinine to lung cancer risk (29). For each case who smoked cigarettes at baseline, we randomly selected one control subject from all cohort members who were current smokers at enrollment, 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 biologic specimen collection (±1 month) and neighborhood of residence 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 randomly placed in any given batch by laboratory personnel who had no knowledge of the case/control status of the test samples.

Urinary mercapturic acid metabolites of the following were quantified: for 1,3-butadiene [1-hydroxy-2-(*N*-acetylcysteinyl)-3-butene and 1-(*N*-acetylcysteinyl)-2-hydroxy-3-butene, collectively called MHBMA for monohydroxybutyl mercapturic acid]; for ethylene oxide [(*N*-acetylcysteinyl)ethanol, also called 2-hydroxyethyl mercapturic acid (HEMA)]; for benzene [*S*-phenyl mercapturic acid (SPMA)]; for acrolein [3-hydroxypropyl mercapturic acid (HPMA)] and for crotonaldehyde [4-hydroxybut-2-yl mercapturic acid (HBMA)]. The analyses for the mercapturic acids were carried out essentially as described previously (11). The detection limits were 3.0 pmol/ml for MHBMA, 0.20 pmol/ml for HEMA, 0.025 pmol/ml for SPMA, 2.5 pmol/ml for HPMA and 0.20 pmol/ml for HBMA. The inter-day precision of the assays were 8.9% relative standard deviation (RSD) for MHBMA, 14% RSD for HEMA, 14% RSD for SPMA, 14% RSD for HPMA and 15% RSD for HBMA.

We also measured in the same samples levels of PheT, total NNAL and total cotinine, validated biomarkers of uptake of PAH, NNK and nicotine, respectively. Those data have been reported previously and were used in this study for statistical analyses (8,10).

Of the original 574 case–control pairs, urine samples were depleted for 225 cases and 170 controls after measurement of PheT, total NNAL, total cotinine and other urinary biomarkers. In addition, 6 cases and 12 controls had missing values for one or more of the five mecapturic acid metabolites. Thus, the present study included 343 cases and 392 controls with data on all five mercapturic acid metabolites.

Statistical analysis

All urinary biomarkers were expressed per mg creatinine (Cr) to correct for varying water content of individual spot urine samples. The distributions of all urinary biomarkers measured were markedly skewed toward high values, which were corrected to a large extent by transformation to logarithmic values. Therefore, formal statistical testing was performed on logarithmically transformed values, and geometric (as opposed to arithmetic) means are presented.

We used the analysis of covariance method (30) to examine the difference in the levels of urinary biomarkers between cases and controls with adjustment for age, neighborhood of residence and duration of biospecimen storage before laboratory analysis. Standard statistical methods were used for case–control studies (31). The original matched case–control pairs were broken to maximize the number of subjects to be included in the present analysis. Unconditional logistic regression models were used to calculate odds ratios and their corresponding 95% confidence intervals and *P*-values. For each urinary biomarker, study subjects were grouped into quartiles according to the distributions among control subjects. The linear trend test for the association between levels of biomarkers and lung cancer risk was based on ordinal values of quartile categories. To assess the independent effects of a specific biomarker on risk of lung cancer, we simultaneously included urinary mercapturic acid metabolites plus PheT, total NNAL, total cotinine, number of cigarettes smoked per day and number of years of smoking in the logistic regression models.

Statistical analyses were carried out using SAS software version 9.2 (SAS Institute, Cary, NC). All *P*-values reported are two-sided, and those that were <0.05 were considered to be statistically significant.

Results

Of the 343 cases, 224 (65%) were histopathologically confirmed, whereas the remaining 119 (35%) were based on clinical diagnosis, including radiography or computer-assisted tomography. Among the histopathologically confirmed cases, 104 (46%) were squamous cell cancers, 70 (31%) adenocarcinomas, 22 (10%) small cell cancers and 28 (13%) other cell types. The mean age (±standard deviation) of all case patients at cancer diagnosis was 69.4 (±6.3) years. The corresponding figure for control subjects was 69.1 (±6.0) years. The average time interval between baseline biospecimen collection and cancer diagnosis was 12.4 (±4.6) years, ranging from 1 month to 20.5 years.

Compared with controls, men who developed lung cancer had higher numbers of cigarettes smoked per day, years of smoking and pack years of smoking at baseline (Table I). Besides urinary PheT, total NNAL and total cotinine, lung cancer patients had significantly higher urinary levels of MHBMA, HEMA, SPMA, HPMA and HBMA (all *P*-values for the differences between cases and controls <0.01).

All urinary biomarkers except for PheT were statistically significantly, albeit moderately, associated with number of cigarettes per day (Spearman correlation coefficients $r = 0.16$–0.37), and all urinary biomarkers were significantly correlated with total cotinine ($r = 0.23$–0.52) among control subjects only (Table II). Urinary mercapturic acid metabolites were correlated each other ($r = 0.38$–0.83), with the highest correlation coefficient between HBMA and HPMA ($r = 0.83$), followed by those between MHBMA and SPMA ($r = 0.67$), HBMA and HEMA ($r = 0.64$) and HPMA and HEMA ($r = 0.64$). The lowest correlations were between MHBMA and HPMA ($r = 0.43$) and MHBMA and HBMA ($r = 0.38$) among control subjects only.

Levels of all mercapturic acid metabolites in urine collected before cancer diagnosis were associated with statistically significantly increased risk of developing lung cancer in smokers after taking into account number of cigarettes per day and number of years of smoking (Table III). Compared with the lowest quartile, men with the highest quartile of each mercapturic acid metabolite experienced approximately doubled risk of lung cancer (all *P*'s for trend <0.02). Adjustment for urinary PheT and total NNAL diminished the associations, but *P*-values for trend test for all urinary biomarkers except MHBMA were statistically significant or borderline significant. However, adjustment for urinary total cotinine significantly attenuated and resulted in a null association between all mercapturic acid metabolites and lung cancer risk. Further adjustment for urinary PheT and total NNAL in addition to total cotinine did not materially change the null association between urinary mercapturic acid metabolites and lung cancer risk.

We reanalyzed our data after excluding patients who were diagnosed with lung cancer <12 months after the collection of urine samples at enrollment ($n = 10$) to reduce the possibility of the impact of early disease symptoms on patient's smoking behavior. Exclusion of the early lung cancer cases did not materially change the association between mercapturic acid metabolites and lung cancer risk described above.

We also examined the association between urinary levels of mercapturic acid metabolites and risk of lung cancer by histology. Elevated levels of HBMA, HEMA and HPMA were associated with statistically significantly increased risk of squamous cell cancer ($n = 104$) after

Table I. Distributions of cigarette smoking and urinary biomarkers of tobacco smoke constituents among current smokers who developed lung cancer (cases) and those who remained cancer-free (controls), The Shanghai Cohort Study 1986–2007

		Controls (n = 392)	Cases (n = 343)	Two-sided P[a]
Cigarette smoking		Arithmetic mean (95% CI)		
No. of cigarettes per day		15.3 (14.2–14.0)	18.9 (17.7–20.0)	<0.001
No. of years of smoking		30.8 (29.8–31.9)	34.4 (33.3–35.4)	<0.001
No. of pack years of smoking		24.4 (22.1–26.6)	33.0 (30.8–35.3)	<0.001
Urinary biomarkers	Smoke constituents	Geometric mean (95% CI)		
Total cotinine (nmol/mg Cr)[b]	Nicotine	7.10 (6.14–8.20)	13.0 (11.2–15.1)	<0.001
Total NNAL (pmol/mg Cr)[c]	NNK	0.22 (0.20–0.24)	0.30 (0.28–0.34)	<0.001
PheT (pmol/mg Cr)	PAH	25.4 (23.6–27.3)	29.0 (26.9–31.2)	<0.001
MHBMA (pmol/mg Cr)	1,3-Butadiene	8.3 (7.2–9.7)	11.3 (9.8–13.1)	<0.001
HEMA (pmol/mg Cr)	Ethylene oxide	13.6 (12.0–15.5)	18.2 (15.9–20.7)	<0.001
SPMA (pmol/mg Cr)	Benzene	2.46 (2.16–2.80)	2.96 (2.60–3.36)	0.009
HPMA (pmol/mg Cr)	Acrolein	6712 (5845-7707)	8882 (7726-10210)	<0.001
HBMA (pmol/mg Cr)	Crotonaldehyde	5749 (5022-6581)	7915 (6906-9071)	<0.001

CI, confidence interval.
[a]P's and means and their 95% confidence intervals were derived from analysis of covariance with adjustment for matching factors, including age, neighborhood of residence and duration of biospecimen storage before laboratory analysis.
[b]Two cases and four controls with missing total cotinine values were excluded from this analysis.
[c]Eleven cases and 10 controls with missing total NNAL values were excluded from this analysis.

Table II. Spearman's correlation coefficients between cigarette smoking and urinary biomarkers of tobacco smoke constituents among current smokers (392 control subjects only), The Shanghai Cohort Study 1986–2007[a]

	Total cotinine[b]	Total NNAL[c]	PheT	MHBMA	HEMA	SPMA	HPMA	HBMA
No. of cigarettes per day	0.37	0.26	0.09	0.21	0.23	0.16	0.33	0.35
Total cotinine[b]		0.45	0.23	0.43	0.49	0.34	0.50	0.52
Total NNAL[c]			0.24	0.26	0.26	0.15	0.25	0.24
PheT				0.25	0.21	0.26	0.17	0.18
MHBMA					0.53	0.67	0.43	0.38
HEMA						0.56	0.64	0.64
SPMA							0.45	0.46
HPMA								0.83

[a]All P's <0.003 except for the coefficient between number of cigarettes per day and PheT (P = 0.074).
[b]Four control subjects with missing total cotinine were excluded from these analyses.
[c]Ten control subjects with missing total NNAL values were excluded from these analyses.

adjustment for smoking intensity and duration. Smoking-adjusted odds ratios (95% confidence intervals) for the highest versus the lowest tertile were 2.52 (1.23–5.10) for HBMA, 1.96 (1.06–3.60) for HEMA and 2.56 (1.30–5.05) for HPMA (all P's for trend <0.05). There was no statistically significant association between HPMA or SPMA and risk of squamous cell cancer (both P's for trend >0.13). Positive, albeit weak, associations were observed for all of the mercapturic acid metabolites with risk of adenocarcinoma after adjustment for self-reported history of smoking, but they were not statistically significant (all P's for trend >0.15), probably due to the small sample size (70 cases). Further adjustment for urinary total cotinine attenuated and resulted in null associations between all mercapturic acid metabolites measured and risk of either squamous cell cancer or adenocarcinoma (all P's for trend >0.10). There was no discernable difference in the mercapturic acid–cancer risk association between squamous cell cancer and adenocarcinoma (all P's for difference >0.20).

Discussion

This study demonstrates that elevated urinary levels of the mercapturic acids MHBMA, HEMA, SPMA, HPMA and HBMA, biomarkers of the tobacco smoke gas phase constituents 1,3-butadiene, ethylene oxide, benzene, acrolein and crotonaldehyde, respectively (11), were associated with statistically significantly increased risk of developing lung cancer among Shanghai smokers after adjustment for smoking intensity and duration. Urinary PheT and total NNAL, biomarkers of the established tobacco smoke carcinogens PAH and NNK, respectively, could not completely explain the positive associations between urinary levels of HEMA, SPMA and HPMA and lung cancer risk, whereas urinary total cotinine almost completely explained the effects of all the mercapturic acids measured here on lung cancer risk.

The null association between urinary mercapturic acid metabolites and lung cancer risk after adjustment for urinary total cotinine likely results from several factors. Cotinine is a metabolite of nicotine, the major known addictive constituent of cigarette smoke, and therefore an excellent biomarker of overall cigarette smoke exposure. In our previous study of this cohort, total cotinine was a better predictor of lung cancer risk than either total NNAL or PheT. The weaker relationship of total NNAL to lung cancer risk than total cotinine may have been due to the relatively low levels of NNK in the smoke of Chinese cigarettes consumed in Shanghai (29). The weaker relationship of PheT to lung cancer risk than total cotinine may have been due to the relatively high exposure to PAH among all residents of Shanghai (29). Thus, in the Shanghai cohort, total cotinine stands out as an excellent and unique biomarker of overall smoke exposure, thus encompassing the mercapturic acids measured here. Therefore, these mercapturic acid biomarkers have limited power, in addition to urinary total cotinine in prediction of lung cancer risk in Shanghai smokers. However, these results do not completely rule out the possibility of a potential role of these volatile tobacco smoke constituents in the development of lung cancer in humans. Future case–control studies nested in other prospective studies of smoking and lung cancer as well as in studies of lung cancer in never-smokers are warranted to clarify their role.

Table III. Urinary levels of mercapturic acid metabolites of tobacco smoke constituents in relation to risk of lung cancer among current smokers, The Shanghai Cohort Study 1986–2007

	First quartile	Second quatile	Third quartile	Fourth quartile	*P* for trend
MHBMA (pmol/mg creatinine)	<4.31	4.31–8.56	8.57–18.8	≥18.9	
No. of controls/No. of cases	98/58	98/82	98/81	98/122	
Smoking-adjusted OR (95% CI)[a]	1.00	1.25 (0.79–1.98)	1.20 (0.76–1.90)	1.75 (1.12–2.75)	0.018
NNAL- and PheT-adjusted OR (95% CI)[b]	1.00	1.15 (0.72–1.85)	1.03 (0.64–1.66)	1.47 (0.93–2.34)	0.129
Cotinine-adjusted OR (95% CI)[c]	1.00	1.03 (0.63–1.68)	0.84 (0.51–1.37)	1.08 (0.66–1.75)	0.893
HEMA (pmol/mg creatinine)	<8.90	8.90–15.7	15.8–26.6	≥26.7	
No. of controls/No. of cases	98/49	98/65	98/100	98/129	
Smoking-adjusted OR (95% CI)[a]	1.00	1.10 (0.68–1.80)	1.61 (1.01–2.56)	2.13 (1.34–3.37)	<0.001
NNAL- and PheT-adjusted OR (95% CI)[b]	1.00	1.03 (0.63–1.69)	1.38 (0.85–2.23)	1.73 (1.07–2.80)	0.008
Cotinine-adjusted OR (95% CI)[c]	1.00	0.81 (0.48–1.36)	0.97 (0.58–1.62)	1.12 (0.66–1.89)	0.393
SPMA (pmol/mg creatinine)	<1.39	1.39–2.34	2.35–4.60	≥4.61	
No. of controls/No. of cases	98/51	98/77	98/107	98/108	
Smoking-adjusted OR (95% CI)[a]	1.00	1.46 (0.91–2.35)	1.94 (1.23–3.07)	1.80 (1.14–2.85)	0.007
NNAL- and PheT-adjusted OR (95% CI)[b]	1.00	1.31 (0.81–2.19)	1.78 (1.12–2.85)	1.55 (0.97–2.50)	0.043
Cotinine-adjusted OR (95% CI)[c]	1.00	1.28 (0.78–2.09)	1.54 (0.95–2.48)	1.20 (0.74–1.96)	0.444
HPMA (pmol/mg creatinine)	<4232	4232–8660	8661–14077	≥14078	
No. of controls/No. of cases	98/49	98/74	98/92	98/128	
Smoking-adjusted OR (95% CI)[a]	1.00	1.39 (0.86–2.23)	1.60 (1.00–2.58)	2.00 (1.25–3.20)	0.004
NNAL- and PheT-adjusted OR (95% CI)[b]	1.00	1.26 (0.77–2.05)	1.38 (0.84–2.26)	1.64 (1.01–2.66)	0.046
Cotinine-adjusted OR (95% CI)[c]	1.00	0.98 (0.59–1.65)	1.02 (0.61–1.72)	1.06 (0.62–1.80)	0.772
HBMA (pmol/mg creatinine)	<3167	3168–7132	7133–11927	≥11927	
No. of controls/No. of cases	98/47	98/74	98/94	98/128	
Smoking-adjusted OR (95% CI)[a]	1.00	1.34 (0.83–2.17)	1.58 (0.98–2.56)	1.95 (1.22–3.12)	0.004
NNAL- and PheT-adjusted OR (95% CI)[b]	1.00	1.19 (0.73–1.95)	1.33 (0.81–2.18)	1.58 (0.96–2.57)	0.058
Cotinine-adjusted OR (95% CI)[c]	1.00	0.90 (0.53–1.52)	0.95 (0.56–1.62)	0.97 (0.56–1.66)	0.956

CI, confidence interval; OR, odds ratio.

[a]ORs were derived from unconditional logistic regression models that included matching factors (age, neighborhood of residence and duration of biospecimen storage before assays for urinary biomarkers), number of cigarettes smoked per day and number of years of smoking at baseline.

[b]In addition to matching factors and smoking variables, the logistic regression models included urinary total NNAL and PheT in quartile. A separate dichotomous variable was created to indicate the missing value for total NNAL (11 cases and 10 controls) to maximize the number of subjects for the analyses.

[c]In addition to matching factors and smoking variables, the logistic regression models included total cotinine in quartile. A separate dichotomous variable was created to indicate the missing value for total cotinine (two cases and four controls) to maximize the number of subjects for the analyses.

Why are PheT and total NNAL but not the mercapturic acids studied here, independent risk predictors for lung cancer, even after correcting for total cotinine? We hypothesize that this is due to the strong lung carcinogenicity of PAH and NNK, as opposed to the gas phase compounds considered here. The carcinogenicities of PAH and NNK to the lung are so strong that they cannot be captured in a study such as this simply by measuring urinary total cotinine as a surrogate tobacco smoke biomarker. In spite of their relatively low concentrations in cigarette smoke compared with 1,3-butadiene, benzene, acrolein and crotonaldehyde, PAH and NNK, as represented by the biomarkers PheT and total NNAL, respectively, stand out as potent lung carcinogens and important targets for cancer prevention.

Direct comparisons of the carcinogenicity of PAH and NNK with the volatile organics studied here are difficult because assays have been carried out under different conditions and in different species. BaP is a typical carcinogenic PAH. When administered to hamsters by inhalation, BaP induced significant incidences of respiratory tract tumors—nasal cavity, larynx, trachea but not lung—at doses of 9.5 or 46.5 mg/m^3 for 4.5 h per day for 10 weeks, then for 3 h per day for the rest of their life (32). Other studies demonstrate that BaP is a potent carcinogen, easily inducing lung, forestomach and liver tumors as well as tumors at other sites in various strains of mice or rats after oral administration, malignant lung tumors after intratracheal instillation to hamsters, rats and mice and malignant lung tumors in rats upon implantation in the lung (33). There are no reports in the literature of inhalation studies of NNK, but it readily induces lung tumors in rats after administration by multiple subcutaneous injections or in the drinking water (34). In one dose-response study, subcutaneous administration of 1 mg/kg NNK three times weekly for 20 weeks resulted in a 53% incidence of lung tumors after 104 weeks (35), whereas another study demonstrated that 1 p.p.m. in the drinking water for 2 years produced a 25% incidence of lung tumors (36). NNK also readily induces lung tumors in various strains of mice treated by different routes of administration (34). These findings attest to the strong respiratory tract carcinogenicity of BaP and NNK.

1,3-Butadiene, administered to mice by inhalation, induced tumors at multiple sites, including the lung, hematopoietic system, heart, forestomach, Harderian gland, preputial gland, liver, mammary gland, ovary and kidney, whereas in rats tumors were observed in pancreas, testis, thyroid gland, mammary gland, uterus and Zymbal gland but not the respiratory tract (14,16). Significant incidences of lung tumors were produced in mice by doses of 138 mg/m^3 (62.5 p.p.m.) and higher, administered 6 h/day, 5 days/week, for 2 years (14). Ethylene oxide, administered to mice by inhalation at doses of 92 mg/m^3 (50 p.p.m.) or 183 mg/m^3 (100 p.p.m.) 6 h/day, 5 days/week, for up to 102 weeks, induced tumors of the lung. Tumors of the Harderian gland, malignant lymphomas, uterine adenocarcinomas and mammary gland carcinomas were also observed (14). But similar studies in rats treated by inhalation did not result in lung or other respiratory tract tumors (14). Benzene (300 p.p.m., 6 h/day, 5 days/week, for 16 weeks and held 18 months) was tested by inhalation in CBA/Ca mice. Lung adenoma were observed in 36% of the mice compared with 14% of sham-exposed mice; malignant lymphoma and preputial gland squamous cell carcinoma were the main observations (15). Multiple types of tumors but not lung tumors were observed in rats treated by gavage with 50, 100 or 200 mg/kg body wt benzene for 103 weeks, whereas mice treated in this way had lung tumors at the mid and high doses, in addition to a number of other tumor types (37). Carcinogenicity studies of acrolein and crotonaldehyde have not produced any pulmonary tumors (22,38). Collectively, these results are consistent with the conclusion that BaP and NNK are substantially more carcinogenic to the rodent lung and respiratory tract than are the volatile carcinogens and toxicants 1,3-butadiene, ethylene oxide, benzene, acrolein and crotonaldehyde.

The biomarkers measured here are a result of dose plus metabolism. Although dose is determined in large part by the number of cigarettes smoked and the way in which they are smoked, different pathways are involved in the metabolism of these agents. PheT results from the diol epoxide metabolic activation pathway of phenanthrene, total NNAL results from carbonyl reduction of NNK to NNAL and glucuronidation of NNAL and total cotinine from the action of P450 2A6 on nicotine as well as cotinine glucuronidation (34,39,40). In contrast, the mercapturic acids all result mainly from the glutathione-*S*-transferase pathway. Even within the group of mercapturic acids, there are different metabolic processes. HEMA, HBMA and HPMA result mainly from direct interaction of substrate with glutathione followed by normal metabolic processing, whereas SPMA formation requires conversion of benzene to the unstable benzene oxide, followed by conjugation (11,41). These considerations indicate that differences in metabolism could affect our results. However, comparative studies demonstrate that levels of all of these metabolites are higher in smokers than non-smokers (21,42). Therefore, dose seems to be more important than metabolism in determining levels of these urinary metabolite biomarkers.

Some epidemiologic studies have examined the relationship between the volatile carcinogens examined here and lung cancer. The relationship between exposure to butadiene and cancer in humans mainly focused on working populations who were employed in butadiene monomer and styrene–butadiene rubber production. In the past 30 years, most studies focused on a possible increased risk for neoplasms of the lymphatic and hematopoietic system from exposure to butadiene (reviewed in ref. 14). These studies did not find a statistically elevated lung cancer mortality rate among workers who were exposed to butadiene (43,44). A meta-analysis including 36 published articles describing mortality/incidence of cancer in 31 different cohort groups of workers in the synthetic rubber-producing industry reported a null association between exposure to butadiene and lung cancer mortality or incidence (45). A recent analysis of 4101 women and 15 958 men employed in the synthetic rubber industry showed an increased lung cancer mortality rate in women but not in men (46). Furthermore, there was no dose-response relationship between estimated butadiene dose and lung cancer mortality in women (46). These data do not support a causal role of butadiene in the development of lung cancer in humans.

Numerous epidemiological studies examined and produced a null association between exposure to ethylene oxide in occupational settings and risk of cancer, mainly hematopoietic malignancies (reviewed in ref. 14). A meta-analysis of 10 cohort groups or ∼33 000 workers did not find an increased risk for hematopoietic cancer or cancers of the brain, pancreas and stomach associated with exposure to ethylene oxide (47). There was no epidemiological evidence in support of a positive association between exposure to ethylene oxide and risk of lung cancer in humans. There have been no epidemiological data on exposure to acrolein and crotonaldehyde and risk of lung cancer in humans.

Benzene is a widely used solvent and is found in gasoline, automobile emissions and other products besides tobacco smoke. Multiple occupational studies have shown that workers exposed to benzene are at increased risk of death from lung cancer and hematopoietic malignancies (17–19). Interestingly, the elevated risk of lung cancer death for exposure to benzene has been observed among non-smokers (17), suggesting an independent role of benzene from other tobacco carcinogens on the development of lung cancer. In a previous study, we found significantly increased levels of SPMA in the urine of women who frequently did wok cooking at home. Although the present study could not establish the biomarker of benzene to be an independent risk factor for lung cancer among male smokers after taking into account smoking intensity and duration and urinary total cotinine, exposure to benzene may be an independent risk factor for lung cancer in non-smokers, especially for Chinese women who have higher incidence rates of lung cancer and lower smoking prevalence in Asia than in many other places in the world (48). This requires further investigation.

The present study did not demonstrate statistically significant differences in the associations between urinary levels of mercapturic acid metabolites and risk of lung cancer by histology. This could be due to relative small sample sizes for specific histological subtypes of squamous cell cancer (104 cases) and adenocarcinoma (70 cases). Future studies with larger numbers of squamous cell cancer and adenocarcinoma of the lung are warranted to clarify the differential role of these volatile carcinogens and toxicants in the development of specific cell types of lung cancer.

One of the strengths of the present study is that biomarkers of volatile organic carcinogens and toxicants in tobacco smoke were measured in urine samples collected years before cancer diagnosis, thereby ruling out the possibility of a spurious association due to smoking behavior changes in lung cancer patients close to their time of clinical diagnosis. Another strength is simultaneously measured multiple urinary biomarkers, thus allowing for examining the biomarkers' independent effects on lung cancer risk. The relatively large sample size of the study provided sufficient statistical power to test the study hypotheses.

A potential limitation of the present study is that urine samples were collected only once, at baseline, from all subjects. Thus, single measurements might not adequately represent an individual's true exposure given the intra-individual variation in urinary biomarkers measured over a wide time period. Longitudinal studies demonstrate that levels of cotinine, total NNAL and PheT in urine are relatively stable over time (49,50), but similar data are not available for the mercapturic acids measured here.

In summary, using prospectively collected urine samples from participants of the Shanghai Cohort Study, we demonstrated a statistically significant, dose-dependent relationship of urinary concentrations of the mercapturic acids MHBMA, HEMA, SPMA, HPMA and HBMA, biomarkers of the tobacco smoke gas phase constituents 1,3-butadiene, ethylene oxide, benzene, acrolein and crotonaldehyde, respectively, to lung cancer risk among smokers with adjustment for smoking intensity and duration. The positive association for lung cancer risk with HEMA, SPMA and HPMA remained statistically significant after adjustment for urinary total NNAL and PheT, biomarkers of NNK and PAH, respectively. However, urinary total cotinine, a biomarker for nicotine, completely explained the association between these mercapturic acid metabolites and lung cancer. These results suggest that these mercapturic acid metabolites are less important than total cotinine as predictors for a smoker's risk of developing lung cancer, at least in Shanghai smokers. Given the non-tobacco sources of these volatile organic carcinogens and toxicants, a similar biomarker study in people who never smoked could help to clarify their role in lung carcinogenesis in humans.

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