BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-054535
Article Type:	Original research
Date Submitted by the Author:	16-Jun-2021
Complete List of Authors:	Peacock, Janet; Dartmouth College Geisel School of Medicine, Epidemiology Palys, Thomas; Dartmouth College Geisel School of Medicine, Epidemiology Halchenko, Yuliya; Dartmouth College Geisel School of Medicine, Epidemiology Sayarath, Vicki; Dartmouth College Geisel School of Medicine, Epidemiology Takigawa, Cindy; Dartmouth College Geisel School of Medicine, Epidemiology Murphy, Sharon; University of Minnesota System, Masonic Cancer Center Peterson , Lisa; University of Minnesota System, Masonic Cancer Center Baker, Emily; Dartmouth College Geisel School of Medicine, Obstetrics and Gynecology Karagas, Margaret R.; Dartmouth College Geisel School of Medicine, Epidemiology
Keywords:	EPIDEMIOLOGY, PERINATOLOGY, STATISTICS & RESEARCH METHODS

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Self-reported smoking, cotinine and NNAL levels in pregnancy and outcome of pregnancy in the New Hampshire Birth Cohort Study

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Abstract

Objectives: Accurate assessment of tobacco smoke exposure is key to evaluate its effects and its interactions with other environmental exposures. We sought to validate and establish cut-offs for self-reported smoking and secondhand smoke (SHS) exposure during pregnancy using urinary cotinine and NNAL in a large contemporary prospective study from the USA, with lower smoking prevalence than has previously been evaluated.

Setting: Pregnancy clinics in New Hampshire and Vermont, US.

Participants: 1396 women enrolled in the New Hampshire Birth Cohort Study with self-reported smoking, urinary cotinine, NNAL and pregnancy outcomes.

Primary and secondary outcome measures: Cut-offs for urinary cotinine and NNAL concentrations were estimated from logistic regression models using Youden's method to predict SHS and active smoking. Cotinine and NNAL were each used as the exposure in separate multifactorial models for pregnancy outcomes.

Results: Self-reported maternal smoking was: 72% non-smokers, 5.7% ex-smokers, 6.4% SHS exposure, 6.2% currently smoked, 10% unreported. Cotinine and NNAL levels were low and highly inter-correlated (r=0.91). Geometric mean cotinine, NNAL were 0.99ng/ml, 0.05pmol/ml respectively. Cotinine cut-offs for SHS, current smoking were 1.2ng/ml and 1.8ng/ml (area under curve (AUC) 95% CI: 0.52 (0.47, 0.57), 0.90 (0.85, 0.94)). NNAL cut-off for current smoking was 0.09pmol/ml (AUC=0.82 (0.77, 0.87)). Using cotinine and NNAL cut-offs combined gave similar AUC to cotinine alone, 0.87 (0.82, 0.91). Cotinine and NNAL gave almost identical effect estimates when modelling pregnancy outcomes.

Conclusions: In this population we observed high concordance between self-complete questionnaire smoking data and urinary cotinine and NNAL. With respect to biomarkers, either cotinine or NNAL can be used as a measure of tobacco smoke exposure overall but only cotinine can be used to detect SHS.

Strengths and limitations of this study

- Compares the utility of a two tobacco biomarkers, urinary cotinine and NNAL to identify smoking in pregnant women
- Set within a large contemporary birth cohort in rural US with low prevalence of smoking
- Relies upon the availability of both urinary biomarker values and self-reported smoking

Introduction

The adverse effects of maternal smoking on birthweight and other pregnancy outcomes have been known for over 40 years with reports consistently showing that women who smoke cigarettes in pregnancy have smaller babies and are at greater risk of preterm delivery and other adverse outcomes[1] [2]. In more recent years, reports have focused on the effects of secondhand smoke (SHS) exposure in pregnancy and have shown evidence for small but statistically significant adverse effects on birthweight, stillbirth and congenital anomalies[3].

Alongside these outcome-focused studies there has been a growing interest in the accuracy of the assessment of the exposure, tobacco smoke intake. Many epidemiological studies have used interviews or self-complete questionnaires to ascertain smoking in pregnancy. Questionnaires continue to be widely used since they are relatively easy and inexpensive to administer but biomarkers are increasingly used to validate self-reported smoking. Cotinine, a metabolite of nicotine, can be measured in urine, saliva and plasma samples and was shown in the late 1980s to discriminate well between smokers and non-smokers with high sensitivity and specificity for data-derived cut-offs[4]. Since then many studies have derived cotinine cut-offs to discriminate between smokers and non-smokers for a range of patient groups including pregnant women[5], and these cut-offs have been used to identify participants whose reported smoking was inconsistent with their cotinine level i.e. they were 'misclassified' [6]. Thus, the advantage of cotinine over questionnaire measures has been demonstrated in the presence of smoking misclassification.

The tobacco-specific nitrosamine metabolite 4-(methylnitrosamino) -1-(-3-pyridyl) -1 -butanol (NNAL) can be measured in urine samples and has been associated with smoking-related cancers[7]. Urinary NNAL has been compared with cotinine to assess SHS exposure in adolescents by Benowitz and colleagues who reported that both biomarkers detected high percentages with SHS exposure among adolescents[8]. Postpartum urinary NNAL was reported to be correlated with cotinine (rho=0.78), and associated with neonatal NNAL level (rho=0.71)[9]. A few studies have used both questionnaires and biomarkers to assess exposure to tobacco smoke in pregnancy: a cohort study from Korea explored the use of NNAL to assesses tobacco smoke exposure and concluded that it added to the information provided by self-report or cotinine[10]; a mother-child cohort from Greece found that cotinine did not fully summarize exposure to NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) uptake[11]; a study from Poland explored relationships between maternal NNAL and cotinine in women reporting SHS or active smoking, and concluded that NNAL was a useful biomarker of pre-natal exposure to carcinogens in newborns[12]. Optimal cut-off points for detecting active and passive smoke exposure in pregnancy have been reported from the INMA Spanish cohort (18% active smokers)[6] and from the Hokkaido Japanese cohort (19% active smokers)[13].

We had the opportunity to validate and establish cut-offs for self-reported smoking and SHS exposure during pregnancy using urinary cotinine in a large, contemporary prospective study from the USA, with lower smoking exposure prevalence rates than was evaluated historically. We hypothesized that NNAL would be strongly positively correlated with cotinine and that the two biomarkers would be similarly predictive of SHS and active smoking, and that self-reported smoking would be shown to be reliable. Hence we explored relationships between reported smoking and NNAL, and between NNAL and cotinine to extend the knowledge base regarding the utility of this biomarker in pregnant women who currently smoke or are exposed to second hand smoke.

Methods

Study population

The New Hampshire Birth Cohort Study (NHBCS) is a prospective study that aims to examine the associations between environmental exposures and other factors, and maternal-child health outcomes[14]. Participants provided written informed consent and all study procedures were approved by the Committee for the Protection of Human Subjects at Dartmouth College. Beginning in January 2009, pregnant women between 24 and 28 weeks of gestation were recruited from prenatal clinics in New Hampshire. Criteria for eligibility included ages 18 to 45 years old, English literacy, the use of a private, unregulated water system (e.g., private well) at home, not planning to move, and a singleton pregnancy. The current analyses includes all women recruited until January 2017.

Patients and public involvement

There was no patient involvement in this specific study but the New Hampshire Birth Cohort Study has an active dissemination program for the community (https://geiselmed.dartmouth.edu/childrenshealth/quick-links/).

Data obtained

Demographic and lifestyle data including educational attainment and tobacco smoke exposure were obtained using NHBCS administered at enrollment. Smoking status and number of cigarettes smoked per day were assessed during the three months prior to pregnancy, as well as during the first and second trimesters of pregnancy. Additionally, exposure to secondhand smoke was assessed through the number of hours per day and days per week while the pregnant mother was in areas where others were smoking during the three months prior to pregnancy and during the first and second trimesters of pregnancy. Maternal smoking status was categorized from participants' reports in five groups: 1) current smoker 2) ex-smoker 3) non-smoker, secondhand smoke exposure 4) non-smoker, no secondhand smoke exposure 5) not reported. Number of cigarettes smoked per day was asked for current smokers. See supplement 'Methods - additional information' for details of how smoking was classified.

Maternal and infant anthropometry and birth outcome data were ascertained from prenatal and delivery medical records and included: mother's height, preconception weight, infant sex, birthweight, gestational age, head circumference and crown-heel length. Infant measurements were normalized using z-scores to adjust for sex and gestation.

Biospecimens

Spot urine samples were collected by the subject at the time of enrollment, at approximately 24 to 28 weeks gestation and transported on ice packs and stored in a 4°C refrigerator. Processing of urines into aliquots occurred within 24 hours of collection. During processing 10ml aliquots were transferred into 15ml trace-free metal tubes and immediately stored at -80°C. 10ml aliquots were thawed once to obtain aliquots for trace metal analysis and thawed again to obtain a 2ml aliquot for cotinine and NNAL analysis. 2ml aliquots were frozen at -80°C and referred to the Minnesota Children's Health Exposure Analysis Resource (CHEAR) Exposure Assessment Hub at the Masonic Cancer Center at the University of Minnesota. To assess urinary dilution, specific gravity was measured using digital refractometer.

Cotinine and NNAL

Two biomarkers of exposure to tobacco smoke, total cotinine and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) were quantified in maternal urine samples at 24-28 weeks gestation. "Total" refers to the sum of the compound and its glucuronide conjugates. The analysis was by LC-MS/MS as described previously[15 16]. Lab reported limit of quantitation values for cotinine and NNAL were 0.5 ng/ml and 0.05 pmol/ml respectively with inter-assay coefficients of variation, 5% and 12%.

Statistics

The study population included all NHBCS women who provided a 24 to 28 gestational week urine sample from which cotinine and NNAL levels could be obtained. Since some women did not provide a urine sample, we compared maternal characteristics in those with and without these urines (included/excluded) using the t-test and chi-squared or Fisher's exact test. We summarized the characteristics of the study population mothers and their babies using means and standard deviations for continuous data and frequencies and percentages for categorical data. Some variables are reported as both continuous and categorical (age, BMI) to aid interpretation and comparison with other studies. We cross-classified reported smoking by cotinine and NNAL levels in groups to explore the inter-relationships, and calculated the correlation between cotinine, NNAL and number of cigarettes smoked using Pearson's coefficient. A participant's reported smoking was defined as 'misclassified' (yes/no) if they reported not being a current smoker but had a urinary cotinine level at or above 30ng/ml, the lowest cotinine cut-off value for active smoking reported in a recent review [5].

We used logistic regression to model the relationship between being a current smoker and both cotinine and NNAL concentrations to determine the cotinine and NNAL level cut-offs that best identified current smoking. Youden's method[17] was used with the receiver operating characteristic (ROC) curve to choose the cut-off that gave the best combination of sensitivity and specificity. A similar analysis was conducted to determine the best cut-off for each of cotinine and NNAL to identify SHS. These cut-offs were used to categorize the participant's smoking status into groups for each of cotinine and NNAL: unexposed/exposed to SHS only/smoker.

We used cotinine and NNAL to assess the relationship between smoking and the outcome of pregnancy in multivariable regression models. We modelled each biomarker as a continuous variable, $\log_e$-transformed with values below the limit of detection (LOD) replaced by LOD/sqrt(2), i.e. 0.3536 for cotinine and 0.0354 for NNAL . The following outcomes of pregnancy were analyzed: birthweight, birthweight z-score, gestational age, small-for-gestational age (<10th percentile), preterm birth, head circumference z-score, and crown-heel length z-score. Results are given as regression coefficients for continuous outcomes and odds ratios for binary outcomes scaled to a one standard deviation change in cotinine or NNAL as appropriate (with 95% confidence intervals) to aid interpretation. All birth outcome models were adjusted for the following covariates: maternal age (continuous), BMI ($\log_e$-transformed), maternal education (beyond high school, yes/no) and parity (0 vs 1+).

In a sensitivity analysis, we separately modelled the effects of cotinine and NNAL on pregnancy outcome using the cut-offs derived previously to define smoking. In a post-hoc change for this sensitivity analysis only, we re-categorized women who reported being smokers but had low urinary cotinine level when assessed (i.e. below the data derived cut-off value) as active current smokers. We did this since we judged it likely that they were generally smoking in pregnancy.

Factors associated with self-reported smoking status during pregnancy (categorized as current smoker, ex-smoker, non-smoker) and being misclassified (yes/no) were explored using unifactorial and multivariable multinomial logistic regression. The following factors were included as possible predictors: maternal age at enrollment (continuous), BMI (log_e-transformed), education (education beyond high school, yes/no), and parity (0 vs 1+). Statistical analyses were conducted using SAS v 9.4, R v 3.6.3.

Power calculations

An indicative power calculation was conducted according to the available cohort size, approximately 1300, varying slightly for different analyses due to missing data. Assuming two-sided significance 5%, mean birthweights 3500g, 3470g, 3300g in the unexposed, SHS exposed only and current smokers, and standard deviation 500[18], power is over 90% both for 94% for a one-way analysis of variance and for testing the trend across groups (Stata 'power oneway').

Ethical approval

This study was approved by Trustees of Dartmouth College Committee For The Protection Of Human Subjects #STUDY00020844.

Results

A total 1739 women were enrolled in the NHBCS as of January 2017, of whom 1396 have cotinine and NNAL data and so comprise the study population for the current analyses. Table S1 (supplement) compares the characteristics for the study population with the 494 excluded (no cotinine/NNAL data) and indicates that the study population had a slightly lower mean BMI, were more likely to be primiparous, had more education, were less likely to smoke and were more likely to be of white race than those not included. Other characteristics were not appreciably different.

Overall, the study population had a mean age 31 years, mean BMI 25, 43% were nulliparous and 88% had been educated beyond high school. Seventy two percent of women self-reported as non-smokers, a further six percent were not active smokers but exposed to secondhand smoke (SHS) pre-conception or prenatal, six percent were ex-smokers and six percent were current smokers. Ten percent of women did not report smoking status. Twenty seven women (2%) reported not currently smoking but had cotinine levels consistent with active smoking and so their reported smoking was assumed to be 'misclassified'. Among smokers, the number of cigarettes smoked was relatively low with most women reporting to smoke less than 10 cigarettes per day. Geometric mean cotinine and NNAL levels were 0.99ng/ml and 0.05pmol/ml respectively and both distributions were positively skewed (figure 1, figure 2). For the newborns: mean birthweight was 3421g, 52% male, 9.2% were preterm and 9.8% were small-for-gestational age (table 1). The full table is given in the supplement (table S2).

There was generally good agreement between reported smoking and urinary cotinine level (table 2). The majority of self-reported non-smokers had very low (undetectable) cotinine levels and the majority of current smokers had cotinine above 30ng/ml, although some current smokers had very low cotinine levels (table S3). NNAL levels were undetectable in almost all women; only 8% (107/1396) overall had NNAL at or above 0.1 pmol/ml (table S3). Cotinine and NNAL levels were very similar among ex-smokers who reported smoking in the three months prior to conception compared to those who reported

smoking earlier (table S3). There were positive inter-correlations between the two tobacco biomarkers and the reported number of cigarettes smoked (table S4). In particular we noted a very strong correlation, r=0.91, between $\log_e$ cotinine and $\log_e$ NNAL (figure S1).

The data-derived cotinine cut-offs for SHS and current active smoking were 1.2ng/ml and 1.8ng/ml respectively. The cotinine cut-off for SHS had high specificity but very low sensitivity whereas the cut-off for active current smoking had high sensitivity and high specificity (table 2). NNAL levels could not be used to detect SHS but an NNAL cut-off of 0.09 detected active current smoking in this population with very high specificity and moderate sensitivity (table 2). A post-hoc analysis was conducted to determine whether cotinine plus NNAL improved the separation between smokers and non-smokers. This showed that using the criterion that either cotinine or NNAL were above their respective previously derived cut-offs, produced similar sensitivity and specificity and AUC to using cotinine alone (table 2).

Non-smokers without SHS exposure tended to be older and nearly all educated beyond high school (table S5). In contrast, current smokers were younger and less than one half were educated beyond high school (table S5). The associations between age, BMI, parity and education were weak (table S6), allowing mutual adjustment in multivariable analyses. While the associations between smoking group and age, parity and BMI were weaker after mutual adjustment and not statistically significant, the association with education remained strong (table S7).

The estimated effects of cotinine level and NNAL level on outcome of pregnancy were adjusted for maternal age, BMI, parity, and education, and scaled to a standard deviation increase in cotinine or NNAL. The scaled estimates for birth outcomes are very similar for the two biomarkers (table 3). Statistically significant inverse associations were observed for: birthweight (cotinine: -55.5g, NNAL: -57.8g), birthweight z-score (cotinine: -0.11, NNAL: -0.11), crown-heel length z-score (cotinine: -0.11, NNAL: -0.10). In the sensitivity analysis using the previously derived cotinine and NNAL cut-offs to define smoking groups, (non-smokers/SHS/smokers for cotinine; smokers/non-smokers for NNAL), there was a mean reduction in birthweight of 43g in those with SHS exposure, and 128g in active smokers compared to non-smokers. P values tended to be bigger (less significant) in the analyses with smoking biomarkers modelled in categories compared to as continuous (table S8, table 3).

Discussion

In this paper we have reported on the validation of self-reported smoking in an ongoing cohort study of pregnant women in rural USA, the New Hampshire Birth Cohort Study (NHBCS), using two biomarkers, cotinine and NNAL. The prevalence of maternal smoking among NHBCS is 6.2% and among those who smoked, the number smoked is low with the majority of NHBCS smokers reporting smoking less than 10 cigarettes per day. This prevalence of maternal smoking is lower than the overall US average, 7.2%, and the New Hampshire prevalence, 11%, reported for 2016 from the National Vital Statistics System[19]. These low levels of smoking in NHBCS were borne out by the cotinine and NNAL levels and contrast the higher prevalence of maternal smoking in other cohorts such as the Boston Cohort that reports that 10% women smoked in pregnancy[20], INMA study from Spain where 19% women self-reported smoking in pregnancy[6], the DEMOCOPHES study from Romania, Portugal and Poland with 25%, 30%, 19% respectively[21], and the Hokkaido Japanese cohort (19%)[13].

Our study shows broad agreement between questionnaire reports and both biomarkers. The use of questionnaires is cheaper and easier to collect as can be done without invasive and expensive laboratory analyses and potentially more representative of a woman's smoking as questions usually ask about smoking over a period of time. In contrast, as well as being objective and not subject to reporting bias, biomarker levels relate to recent tobacco smoke exposure - the half-life of cotinine in urine of pregnant smokers has been estimated to be about eight hours[22], shorter than in non-pregnant women due to accelerated metabolism in pregnancy[23]. The biomarker levels may be especially useful to determine effects of recent exposure.

Very few women mis-reported their smoking: under two percent of women (n=27) reported themselves as non-smoking but had tobacco biomarker levels consistent with active smoking. Just over one percent of women (n=17) reported being active smokers but their biomarker levels were very low. This shows the value of having both questionnaire and biomarker data if possible, but here the low level of discordance provides reassurance that the choice of which to use in analyses may be taken according to the question and nature of the modelling required.

The derived cut-off for urinary cotinine to define active smoking was low, 1.8ng/ml, reflecting the low number of cigarettes smoked by NHBCS women. This means that among NHBCS women and other similar populations where women smoke very little in pregnancy, cotinine levels are very reliable for predicting active smoking (sensitivity=80%, specificity=93%; AUC=0.90). However, cotinine levels are poor predictors of SHS (area under the curve: 0.52). A review article reported study-specific cut-offs for urinary cotinine varying between 31.5 and 550ng/ml[5] which is substantially higher than ours. The INMA study also reports a higher cut-off than ours, 82ng/ml[6], although the DEMOCOPHES study reported cut-offs of 4.4ng/ml (Poland), 7.9 (Portugal) and 254.2 (Romania)[21].

NNAL was able to distinguish between active smoking and non-smoking or SHS exposure with high specificity (98%) and moderate sensitivity (66%). However, given the low urinary NNAL values among our women NNAL levels were not able to be used to define SHS. The NNAL cut-off derived using NHBCS data, 0.09pmol/ml, was higher than that reported by Benowitz in adolescents, 0.05[8] (after conversion to SI units). There was a very high correlations between cotinine and NNAL (0.91) and so it is unsurprising that using cotinine plus NNAL gave no additional predictive ability beyond using cotinine alone, in our population.

When we used biomarker data to define tobacco exposure, we observed the expected relationships with pregnancy outcomes. Of particular note is that the estimated mean reductions in birthweight, Birthweight z-score and crown-heel length were very similar using cotinine compared to using NNAL, reflecting the concordance of the two biomarkers as measures of maternal tobacco smoke intake. When we used the data-derived cut-offs to define smoking groups we were able to estimate the effect of SHS as a mean reduction of 43g or 0.07 z-score units. The birthweight reduction falls within the 95% confidence interval from the pooled value reported in the Nieuwenhuijsen review of the literature with a pooled mean reduction of 60 g and 95% CI of 39 to 80[3]. This is reassuring given our data are from a relatively recent cohort with relatively low rates of current smoking.

Our population sample study data come from a large ongoing birth cohort from Northern New England where smoking data were carefully collected using detailed self-complete questionnaires supplemented by urinary tobacco biomarker data. This is one of only a few studies to examine NNAL in a pregnant population: Lee and colleagues[10] studied 251 pregnant women (8.4% smokers) in South Korea and

reported that positive NNAL, defined as NNAL greater than the lowest limit of detection, 2.0pg/ml, and not urinary cotinine, was an independent predictor of spontaneous abortion, preterm birth and small-for-gestational age. Florek and colleagues detected raised levels of cotinine and NNAL in newborn urine whose mothers had been exposed to tobacco in Poland (N=121)[12], and Vardavas and colleagues found that exposure to tobacco smoke correlated with cotinine and NNAL in Greece (N=1317)[11].

The limitations of our study are that urines were obtained at one time point only, 24-28 weeks gestation, and while identified misclassification of smoking was low, 10 percent of women did not report smoking. For these women, the biomarker data suggested that around a quarter were active smokers compared to 6.2% among those who responded to smoking questions and so using questionnaire data alone will underestimate the true prevalence of smoking.

Overall, we observed good concordance between our self-complete questionnaire smoking data and tobacco biomarker levels, suggesting that the percentage of misclassified non-smokers is small. Further we have found that an NNAL data-derived cut-off can be used to separate smokers from non-smokers with high specificity and moderate sensitivity, although in our population cotinine was a better predictor of reported smoking overall, with high sensitivity and specificity. We conclude from this relatively recent pregnancy cohort of USA women from rural Northern New England that either the self-completed questionnaire smoking data or biomarker data from may be used in further analyses of the effects of tobacco smoke on health outcomes in children. However, due to the lower smoking prevalence rates and frequency of smoking, the cut-offs to classify smokers are lower than have been used historically.

Table 1: Characteristics of the women and their babies (full version in supplement Table S2)

Total		1,396
Maternal Characteristics		
Maternal age, years	Mean (SD)	31.3 (4.9)
Pre-pregnancy maternal weight (lb)	Mean (SD)	153.9 (34.1)
Pre-pregnancy maternal height (in)	Mean (SD)	64.9 (2.7)
Pre-pregnancy maternal BMI	Mean (SD)	25.6 (5.6)
Parity: primiparous		43% (586)
Mother's race: white		97% (1357)
Maternal level of education		
	High School or less	12% (140)
	Junior college / college	57% (696)
	Postgraduate	31% (377)
Maternal Tobacco Exposure		
Reported smoking at 24 weeks		
	Non-smoker, no SHS	72% (999)
	SHS exposure pre-conception/prenatal	6.4% (90)
	Ex-smoker	5.7% (79)
	Current smoker	6.2% (86)
	Not reported	10% (142)
Urinary Cotinine, ng/ml	Mean (SD)	339.54 (1621.98)
	Geometric mean (geometric SD)	0.99 (12.43)
NNAL, pmol/ml	Mean (SD)	0.19 (0.81)
	Geometric mean (geometric SD)	0.05 (2.75)
Infant Characteristics		
Gestational age, weeks	Mean (SD)	38.96 (1.82)
Birthweight, grams	Mean (SD)	3421.2 (552.3)
Birthweight z-score	Mean (SD)	-0.05 (1.03)
Small-for-gestational age (below 10th centile for age)	% (n)	9.8% (132)
Preterm birth (gestational age <37wks)	% (n)	9.2% (129)
Infant sex – male	% (n)	52% (716)

Table 2: Optimal cutoff points for urinary cotinine and urinary NNAL to define active smoking and SHS

	Cut-point	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)	Youden Index
Cotinine (ng/ml)					
To detect SHS	1.2	11% (6.7, 17%)	95% (86, 99%)	0.52 (0.47, 0.57)	0.06
To detect active current smoking	1.8	80% (70, 88%)	93% (91, 94%)	0.90 (0.85, 0.94)	0.73
NNAL (pmol/ml)					
To detect current active smoking	0.09	66% (55, 76%)	98% (97, 99%)	0.82 (0.77, 0.87)	0.64
Cotinine and NNAL combined					
To detect current active smoking	Cotinine>1.8 or NNAL>0.09	81% (72, 89%)	89% (87, 91%)	0.87 (0.82, 0.91)	Not applicable

Table 3: Outcome of pregnancy by log_e transformed urinary cotinine and log_e transformed NNAL level in pregnancy N=1396

Outcome	Regression coefficient/Odds ratio (95% CI) P value	
	Cotinine, ng/ml (loge)	**NNAL, pmol/ml (loge)**
Birthweight[1], gram	-55.5 (-93.2, -17.8) P=0.0040	-57.8 (-96.6, -18.5) P=0.0039
Birthweight z-score[1]	-0.11 (-0.18, -0.04) P=0.0027	-0.11 (-0.18, -0.04) P=0.0035
Gestational age[1], weeks	-0.11 (-0.24, 0.01) P=0.0795	-0.06 (-0.19, 0.07) P=0.3904
Small-for-gestational age (< 10th centile)	OR=1.22 (0.98, 1.52) P=0.0884	OR=1.15 (0.92, 1.44) P=0.2326
Preterm birth (<37wks)	OR=1.21 (0.98, 1.50) P=0.1002	OR=1.07 (0.84, 1.36) P=0.6066
Crown-heel length z-score[1]	-0.11 (-0.22, -0.003) P=0.0433	-0.10 (-0.21, 0.02) P=0.0991
Head circumference z-score[1]	-0.04 (-0.12, 0.04) P=0.2915	-0.03 (-0.11, 0.05) P=0.5055

Footnotes

1 regression coefficients and odds ratios scaled to 1 standard deviation increase in log_e cotinine (2.520) or log_e NNAL (1.012) as appropriate

2 All models include the following covariates: maternal age, log_e BMI, maternal education (high school vs beyond high school), parity (0 vs 1+)

3 Totals vary due to missing or unreported data

Figure legends

Figure 1

Distribution of urinary cotinine level (ng/ml)

Figure 2

Distribution of urinary NNAL level (pmol/ml)

Acknowledgements

We would like to extend our deepest appreciation of the participants and staff of the New Hampshire Birth Cohort and Center for Molecular Epidemiology. We gratefully acknowledge Alexandra Roy, an MPH candidate at the University of New England for her assistance with literature review for the analyses described in this paper. Finally we thank Karin Vevang for her key role in managing the logistics of the CHEAR project at the University of Minnesota.

Funding

Research supported in this publication was supported in part by the National Institutes of Health: P20GM104416, P42ES007373, P01 ES022832, UC2ES026533 and US EPA RD83544201.

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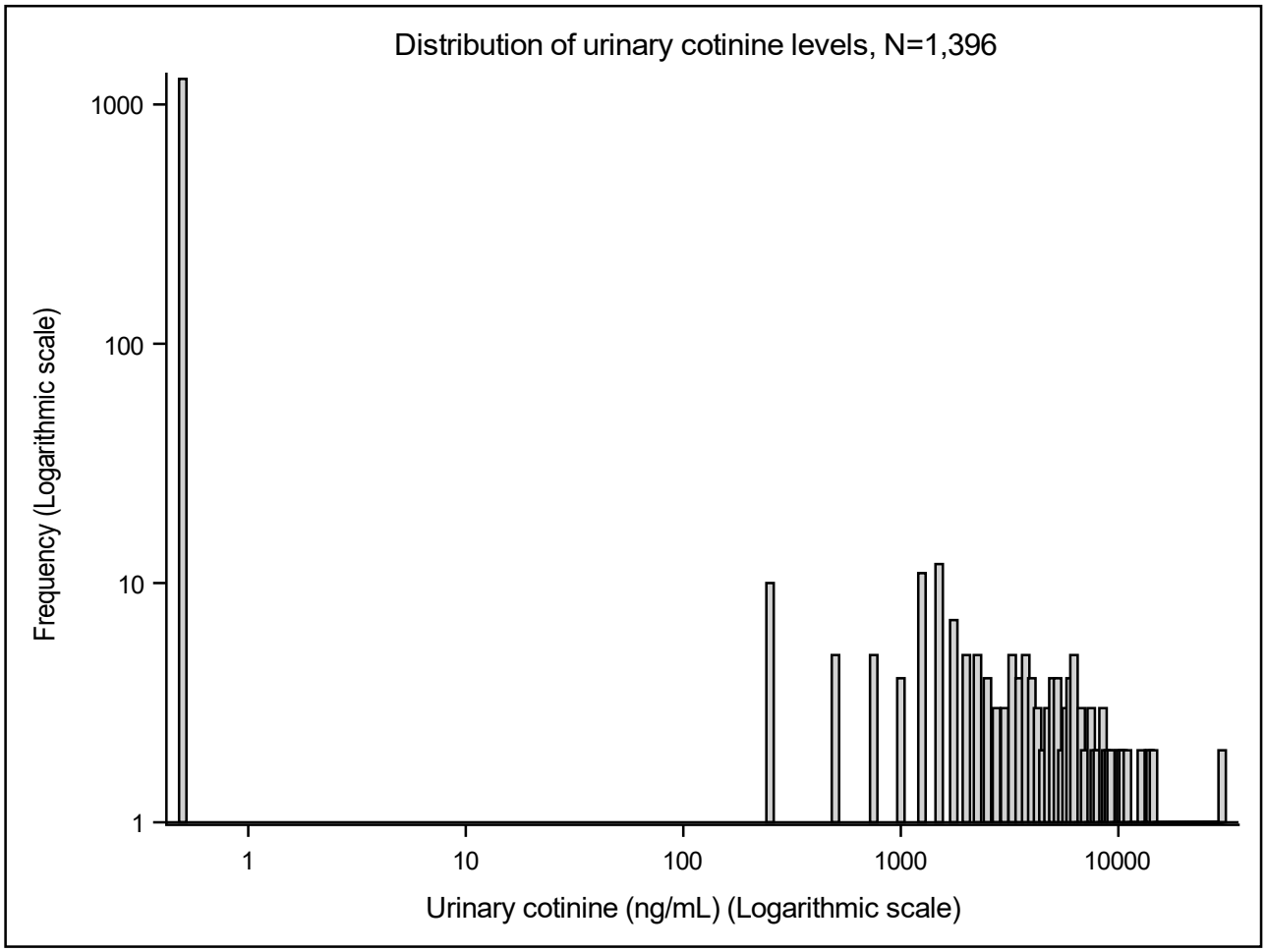
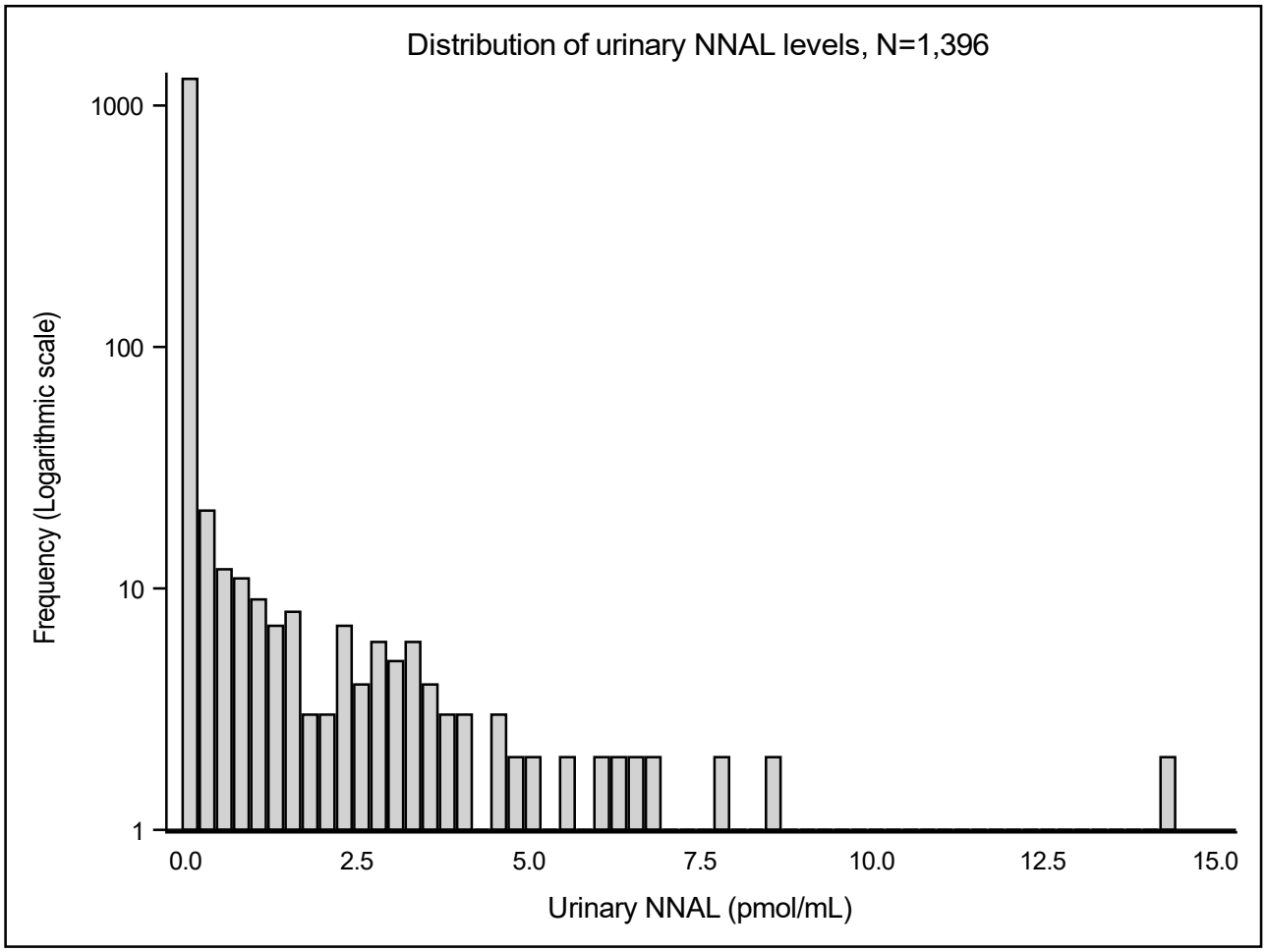




SUPPLEMENTARY MATERIAL

Self-reported smoking, cotinine and NNAL levels in pregnancy and outcome of pregnancy in the New Hampshire Birth Cohort Study

Methods: Additional information. Definition of smoking categories used in this paper

Women in the study population were assigned to one of the following mutually exclusive categories based on the NHBCS Prenatal Questionnaire's and NHBCS Postpartum Questionnaire's smoking related questions: 1) current smoker 2) ex-smoker 3) non-smoker, secondhand smoke exposure 4) non-smoker, no secondhand smoke exposure 5) not reported.

Current smoker was defined as anyone who reported smoking at any point during the pregnancy or smoking more than zero cigarettes per day.

If a participant did not report active smoking during the pregnancy but reported smoking prior to getting pregnant, she was assigned to the ex-smoker category. A sub-category of ex-smoker, ex-smoker in 3 months pre-conception was defined as an ex-smoker who smoked within 3 months prior to conception.

If a participant was not defined as a current smoker or ex-smoker but reported secondhand smoke exposure at any point during the pregnancy or within 3 months prior to getting pregnant or residing with at least one person that regularly smoked inside their home during pregnancy, this participant was assigned to the non-smoker, prenatal SHS category. A sub-category 'ex-smoker, smoked in 3 months pre-conception' was defined as a participant who experienced SHS within 3 months prior to getting pregnant but not during the pregnancy.

If a participant did not report smoking history or secondhand smoking exposure 3 months prior or during the pregnancy and answered at least one question in either questionnaire as a non-smoker, such participant was defined as a non-smoker.

Those who did not answer any of the smoke related questions were assigned to 'not reported' category.

Table S1: Characteristics of the Study population (women with 24 wk urine samples) and women not included (no 24wk urine)

		Study population N=1396	Not included N=494	p-value
Maternal age, years	Mean (SD)	31.25 (4.88)	31.06 (5.01)	0.440
	18-24	10% (140)	12% (60))	0.406
	25-34	68% (946)	66% (324)	
	35-45	22% (310)	22% (110)	
Maternal height, in	Mean (SD)	64.86 (2.65)	64.53 (2.54)	0.018
Pre-pregnancy maternal weight, lb		153.85 (34.10)	161.22 (43.00)	<0.001
Pre-pregnancy maternal BMI	Geometric mean (Geometric SD)	25.03 (1.22)	26.31 (1.27)	<0.001
	<18.5	2.4% (34)	1.8% (9)	0.013
	18.5 to <25	50% (694)	42% (208)	
	25+	40% (558)	46% (225)	
Parity	0	42% (586)	37% (182)	<0.001
	1	36% (506)	32% (157)	
	2+	20% (275)	21% (104)	
Mother's race	White	97% (1357)	91% (450)	<0.001
Maternal level of education	High School or less	10% (140)	11% (53)	<0.001
	Junior college / college	50% (696)	44% (218)	
	Postgraduate	27% (377)	19% (96)	
Reported smoking	Non-smoker, no SHS	72% (999)	59% (292)	<0.001
	Non-smoker, SHS exposure	6.4% (90)	7.5% (37)	

	Ex-smoker	5.7% (79)	5.7% (28)	
	Current smoker	6.2% (86)	6.1% (30)	
	Not reported	10% (142)	22% (107)	

Table S2: Characteristics of the women and their babies (full version)

Total		1,396
Maternal Characteristics		
Maternal age, years	Mean (SD)	31.3 (4.9)
	18-24	10% (140)
	25-34	68% (946)
	35-45	22% (310)
Pre-pregnancy maternal weight (lb)	Mean (SD)	153.9 (34.1)
Pre-pregnancy maternal height (in)	Mean (SD)	64.9 (2.7)
Pre-pregnancy maternal BMI	Mean (SD)	25.6 (5.6)
	GM (GSD)	25.0 (1.2)
	<18.5	2.6% (34)
	18.5 to <25	54% (694)
	25+	43% (558)
Parity	0	43% (586)
	1	37% (506)
	2+	20% (275)
Mother's race		
American Indian/Alaska Native		0.1% (2)
Asian		0.6% (9)
Native Hawaiian or Other Pacific Islander		0.1% (2)

White	97% (1357)
Mixed	1.6% (23)
Unknown	0.1% (2)
Maternal level of education	
High School or less	12% (140)
Junior college / college	57% (696)
Postgraduate	31% (377)
Maternal Exposure	
Reported smoking at 24 weeks	
Non-smoker, no SHS	72% (999)
SHS exposure pre-conception/prenatal	6.4% (90)
Ex-smoker	5.7% (79)
Current smoker	6.2% (86)
Not reported	10% (142)
Misclassified smoking habit[2]	1.9% (27/1396)
1st trimester number of cigarettes smoked per day in current smokers (%), N=86	
0	0% (0)
0.5 to < 5	37% (32)
5 to < 10	26% (22)
10+	35% (30)
Unreported	2.3% (2)
2nd trimester number of cigarettes smoked per day in current smokers (%), N=86	
0	26% (22)

	0.5 to < 5	19% (16)
	5 to < 10	16% (14)
	10+	15% (13)
	Unreported	24% (21)
3rd trimester number of cigarettes smoked per day in current smokers (%), N=86		
	0	26% (22)
	0.5 to < 5	19% (16)
	5 to < 10	16% (14)
	10+	15% (13)
	Unreported	24% (21)
Urinary Cotinine, ng/ml	Mean (SD)	339.54 (1621.98)
	GM (GSD)[1]	0.99 (12.43)
	Undetectable	76% (1,063)
	0.6 to <30	15% (208)
	30+	9.0% (125)
NNAL, pmol/ml	Mean (SD)	0.19 (0.81)
	GM (GSD)	0.05 (2.75)
	Undetectable	92% (1,274)
	0.05 to <0.1	0% (0)
	0.1+	8.4% (117)
Characteristics of smokers[3]: age	Mean (SD)	
Non-smoker (n=1088)		31.8 (4.5)

Ex-smoker (n=79)		30.5 (5.4)
Current smoker (n=86)		28.0 (5.7)
Characteristics of smokers[3]: Pre-pregnancy BMI[1]	GM (GSD)	
Non-smoker (n=1076)		25.1 (1.2)
Ex-smoker (n=78)		26.0 (1.2)
Current smoker (n=86)		24.8 (1.2)
Characteristics of smokers[3]: Parity 1+	% (n)	
Non-smoker (n=1071)		57% (612)
Ex-smoker (n=70)		49% (34)
Current smoker (n=86)		47% (40)
Characteristics of smokers[3]: education beyond high school	% (n)	
Non-smoker (n=1071)		90% (963)
Ex-smoker (n=70)		83% (57)
Current smoker (n=86)		47% (35)
Infant Characteristics		
Gestation age, weeks	Mean (SD)	38.96 (1.82)
Birthweight, grams	Mean (SD)	3421.2 (552.3)
Birthweight z-score	Mean (SD)	-0.05 (1.03)
Small-for-gestational age (below 10th centile for age)	% (n)	9.8% (132)
Preterm birth (gestational age <37wks)	% (n)	9.2% (129)
Infant sex – male	% (n)	52% (716)

Head circumference, cm	Mean (SD)	34.60 (1.74)
Crown-heel length, cm	Mean (SD)	50.63 (3.17)

Footnotes

1 Geometric mean and geometric standard deviation

2 Misclassified: Self-reported non-smoker with cotinine≥30ng/ml

3 Full tables are in supplement (S3, S4)

Table S3: Relationship between reported smoking habit, urinary cotinine and urinary NNAL

		Non-smoker, no SHS N=999	Non-smoker, SHS pre-conception N=14	Non-smoker, prenatal SHS N=76	[1]Ex-smoker, smoked in 3 months pre-conception N=56	[2]Ex-smoker N=79	Current smoker N=86	Not reported N=142
COTININE LEVEL (ng/ml)								
Undetectable	% (n)	87% (870)	86% (12)	64% (49)	57% (32)	57% (45)	15% (13)	52% (74)
0.6-29.99	% (n)	11% (112)	14% (2)	34% (26)	30% (17)	32% (25)	14% (12)	22% (31)
≥ 30	% (n)	1.7% (17)	0% (0)	1.3% (1)	13% (7)	11% (9)	71% (61)	26% (37)
NNAL LEVEL (pmol/ml)								
Undetectable	% (n)	99% (984)	100% (14)	96% (73)	86% (48)	89% (70)	34% (29)	72% (104)
0.05-0.09	% (n)	0.2% (2)	0% (0)	0% (0)	0% (0)	1.3% (1)	1.2% (1)	0.7% (1)
≥0.1	% (n)	1.3% (13)	0% (0)	4.0% (3)	14% (8)	10% (8)	65% (56)	26% (37)

Footnotes

1,2: 'ex-smoker in 3 months preconception' (1) is a subset of all ex-smokers (2)

Table S4: Correlation matrix for relationships between cotinine, log$_e$ Cotinine, NNAL, log$_e$ NNAL (Max N=1396)

Data are correlation coefficient, P value, N

	Cotinine	NNAL	Log$_e$ Cotinine	Log$_e$ NNAL	Cigs/day T1	Cigs/day T2	Cigs/day T3
Cotinine	1	0.82 <0.001 1396	0.72 <0.001 1396	0.80 <0.001 1396	0.54 <0.001 1076	0.61 <.001 1072	0.47 <0.001 1073
NNAL		1	0.67 <0.001 1396	0.82 <0.001 1396	0.45 <0.001 1076	0.50 <0.001 1072	0.39 <0.001 1073
Log$_e$ Cotinine			1	0.91 <0.001 1396	0.59 <0.001 1076	0.63 <0.001 1072	0.44 <0.001 1073
Log$_e$ NNAL				1	0.58 <0.001 1076	0.64 <0.001 1070	0.45 <0.001 1055
Cigarettes/day T1					1	0.91 <0.001 1072	0.83 <0.001 1056
Cigarettes/day T2						1	0.88 <0.001 1073
Cigarettes/day T3							1

Table S5: Maternal factors associated with self-reported smoking and misclassification of smoking[1]. Unifactorial analyses

	Non-smoker, no SHS N=982	Non-smoker, SHS exposure N=89	Ex-smoker N=70	Current smoker N=86	Misclassified[2] N=27	p-value
	Mean (SD) or % (n)					
Age at enrolment (years)	32.1 (4.4)	29.2 (4.5)	30.8 (5.0)	28.0 (5.7)	30.1 (6.0)	<0.001
Pre-pregnancy BMI[3]	25.0 (1.2)	27.8 (1.2)	26.3 (1.2)	24.8 (1.2)	23.6 (1.2)	0.037
Parity: 1+	60% (577)	40% (35)	49% (34)	47% (40)	56% (15)	0.001
Education: beyond high school	93% (888)	88% (75)	83% (57)	47% (35)	69% (18)	<0.001

Footnotes

1 Maximum number included in analyses is all women for whom smoking status was available (N=1254)

2 Misclassified: participants who reported being a non-smoker at 24 weeks and had urinary cotinine level 30ng/ml or more or equivalently NNAL level greater than 0.1pmol/ml

3 Geometric mean and geometric standard deviation

Table S6: Correlation matrix for relationships between maternal age, BMI, parity, education and SHS exposure

Kendall's tau-b Correlation coefficient ***p-value*** **N**					
	Age	**BMI**	**Parity**	**Education**	**SHS**
Age	1 1253	-0.03 0.11 1233	0.22 <0.0001 1240	0.20 <0.0001 1212	-0.27 <0.0001 1254
BMI[1]		1 1233	0.01 0.58 1221	-0.04 0.12 1206	0.04 0.06 1234
Parity[2]			1 1240	-0.03 0.37 1200	-0.14 <0.0001 1240
Education[3]				1 1212	-0.25 <0.0001 1212
SHS[4]					1 1254

Footnotes

1 BMI $\log_e$ transformed for analysis

2 Parity: 0 vs 1+

3 Education: high school vs beyond high school

4 SHS: Second-hand smoke exposure

Table S7: Maternal factors associated with self-reported smoking and misclassification of smoking. Multivariable analyses[1]

	N	Non-smoker, no SHS N=982	N	Non-smoker, SHS exposure N=88	N	Ex-smoker N=70	N	Current smoker N=86	N	Misclassified[2] N=27	p-value
	OR (95% CI)										
Age at enrolment (OR per 5 years)	982	1.00	88	1.15 (0.78, 1.72)	70	1.09 (0.80, 1.47)	86	1.00 (0.68, 1.44)	27	0.88 (0.55, 1.40)	0.86
Pre-pregnancy BMI[3] (OR per unit BMI)	972	1.00	86	0.75 (0.13, 4.28)	69	2.38 (0.68, 8.35)	79	0.27 (0.05, 1.49)	27	0.10 (0.01, 1.06)	0.062
Parity:											0.51
0	393	1.00	53		36		45		12		
1+	577		35	0.63 (0.31, 1.30)	34	0.69 (0.40, 1.19)	40	1.00 (0.49, 2.05)	15	0.87 (0.38, 2.02)	
Education:											<0.0001
High school or less	69	1.00	10		12		40		8		
Beyond high school	888		75	0.73 (0.28, 1.96)	57	0.39 (0.19, 0.83)	35	0.09 (0.04, 0.20)	18	0.19 (0.07, 0.50)	

Footnotes

1 Analyses include all women for whom the following were available: smoking status, age, BMI, parity, education (N=1253); reference category is non-smoker, no SHS apart from SHS exposed in pregnancy where the reference category is current smokers; all analyses adjusted for all other predictor variables. Table S1 includes the same material as table 4 but with the addition of subgroup total numbers.

2 Misclassified: participants who reported being a non-smoker at 24 weeks and had urinary cotinine level 30ng/ml or more

3 BMI $\log_e$ transformed for analysis

Table S8: Outcome of pregnancy by urinary cotinine and NNAL level in pregnancy using cut-points derived from self-reported smoking status[1]

Outcome		Mean difference between categories (continuous outcome) or OR (binary outcome)	95% CI	Overall P value
	Cotinine (ng/ml)			
Birthweight, gram	SHS vs non-smokers Active current smoking vs non-smokers	43.0 -127.7	(-137.9, 223.8) (-223.9, -31.6)	0.0263
Birthweight z-score	SHS vs non-smokers Active current smoking vs non-smokers	0.07 -0.25	(-0.27, 0.41) (-0.43, -0.07)	0.0176
Gestational age, weeks	SHS vs non-smokers Active current smoking vs non-smokers	0.02 -0.40	(-0.59, 0.62) (-0.72, -0.08)	0.0483
Small-for-gestational age (< 10th centile)	SHS vs non-smokers Active current smoking vs non-smokers	OR=1.19 OR=2.56	(0.36, 4.07) (1.48, 4.42)	0.0056
Preterm birth (<37wks)	SHS vs non-smokers Active current smoking vs non-smokers	OR=1.20 OR=2.30	(0.35, 4.04) (1.32, 3.99)	0.0187
Crown-heel length z-score	SHS vs non-smokers Active current smoking vs non-smokers	-0.002 -0.29	(-0.52, 0.52) (-0.56, -0.01)	0.1223
Head circumference z-score	SHS vs non-smokers Active current smoking vs non-smokers	0.02 -0.17	(-0.35, 0.39) (-0.37, 0.03)	0.2222
	NNAL (pmol/ml)			
Birthweight, gram	Active smoking vs SHS and non-smokers	-112.2	(-230.2, 5.8)	0.0618
Birthweight z-score	Active smoking vs SHS and non-smokers	-0.21	(-0.43, 0.02)	0.0677
Gestational age, weeks	Active smoking vs SHS and non-smokers	-0.27	(-0.66, 0.12)	0.1793
Small-for-gestational age	Active smoking vs SHS and non-smokers	OR=1.89	(0.96, 3.70)	0.0742

(< 10th centile)				
Preterm birth (<37wks)	Active smoking vs SHS and non-smokers	OR=1.83	(0.92, 3.63)	0.0998
Crown-heel length z-score	Active smoking vs SHS and non-smokers	-0.30	(-0.64, 0.04)	0.0807
Head circumference z-score	Active smoking vs SHS and non-smokers	-0.13	(-0.37, 0.11)	0.2857

Footnotes

1 women reporting being active smokers but with low urinary cotinine levels, are classified as active smokers (see methods section)

2 All models include the following covariates: maternal age, $\log_e$ BMI, maternal education (high school vs beyond high school), parity (0 vs 1+)

Figure S1: Scatterplot of Cotinine by NNAL (log_e transformed)

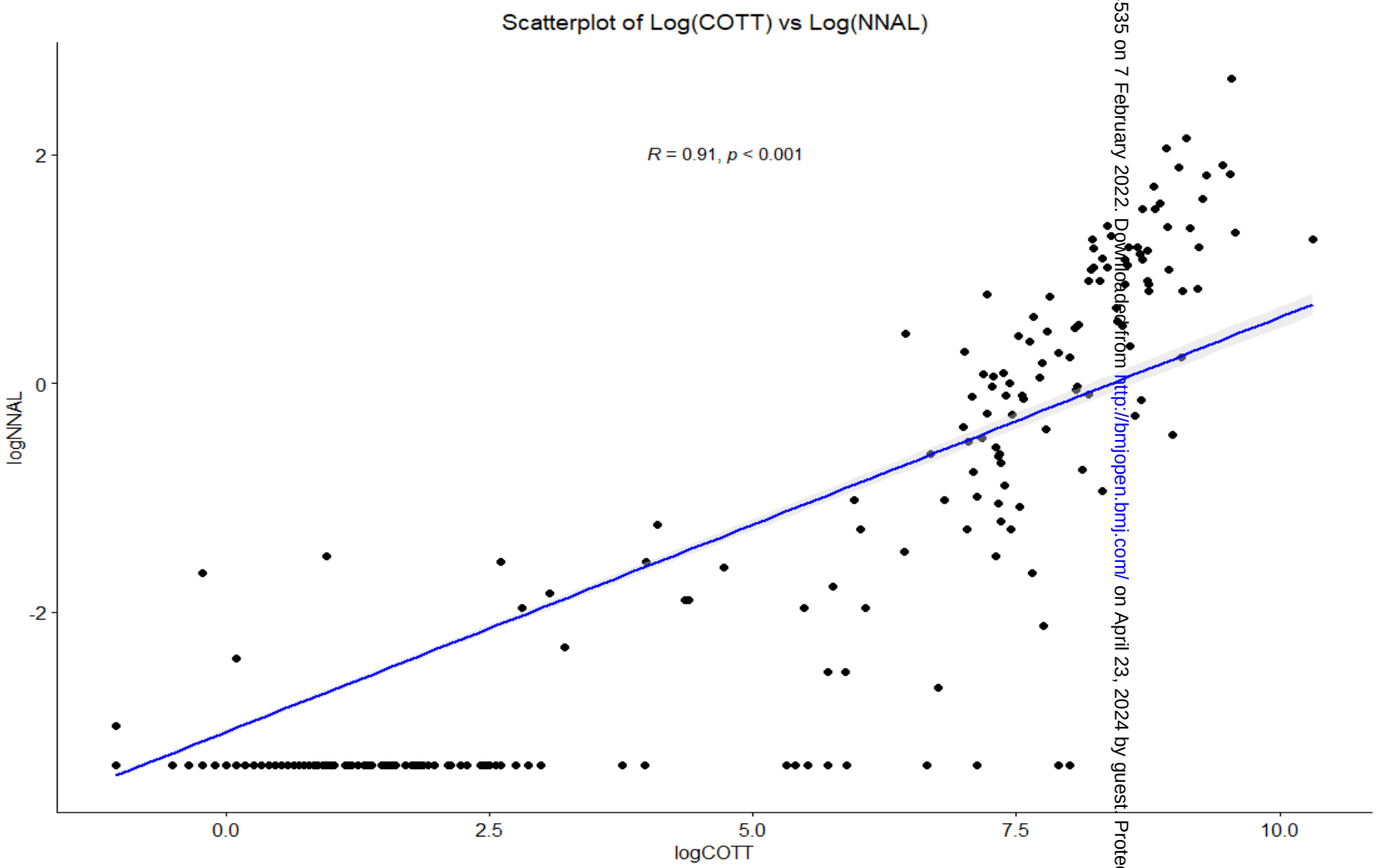
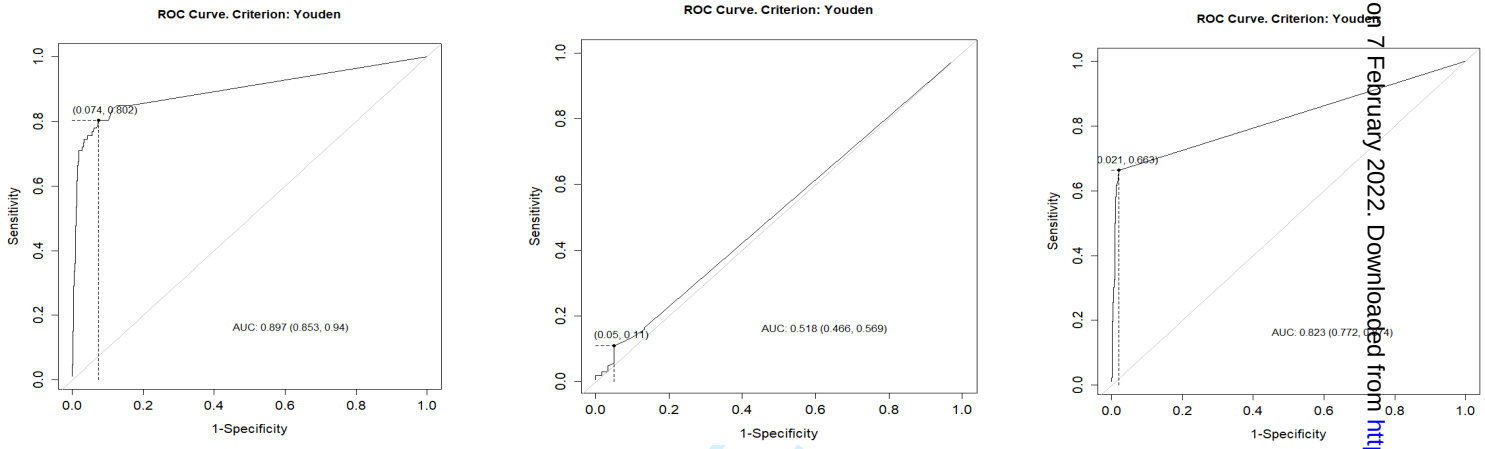


Figure S2 A,B,C: Receiver operating characteristic curves A) To detect active smoking using cotinine, B) To detect SHS using cotinine, C) to detect active smoking using NNAL



STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-5
Bias	9	Describe any efforts to address potential sources of bias	4-5
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	N/A
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	5
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	6
Outcome data	15*	Report numbers of outcome events or summary measures over time	Table 3

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Table 3 Table 3 N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Supplement
Discussion			
Key results	18	Summarise key results with reference to study objectives	7-8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9
Generalisability	21	Discuss the generalisability (external validity) of the study results	8-9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

Assessing tobacco smoke exposure in pregnancy from self-report, urinary cotinine and NNAL: a validation study using the New Hampshire Birth Cohort Study

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-054535.R1
Article Type:	Original research
Date Submitted by the Author:	08-Dec-2021
Complete List of Authors:	Peacock, Janet; Dartmouth College Geisel School of Medicine, Epidemiology Palys, Thomas; Dartmouth College Geisel School of Medicine, Epidemiology Halchenko, Yuliya; Dartmouth College Geisel School of Medicine, Epidemiology Sayarath, Vicki; Dartmouth College Geisel School of Medicine, Epidemiology Takigawa, Cindy; Dartmouth College Geisel School of Medicine, Epidemiology Murphy, Sharon; University of Minnesota System, Masonic Cancer Center Peterson , Lisa; University of Minnesota System, Masonic Cancer Center Baker, Emily; Dartmouth College Geisel School of Medicine, Obstetrics and Gynecology Karagas, Margaret R.; Dartmouth College Geisel School of Medicine, Epidemiology
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Smoking and tobacco, Paediatrics
Keywords:	EPIDEMIOLOGY, PERINATOLOGY, STATISTICS & RESEARCH METHODS

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Assessing tobacco smoke exposure in pregnancy from self-report, urinary cotinine and NNAL: a validation study using the New Hampshire Birth Cohort Study

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Abstract

Objectives: Accurate assessment of tobacco smoke exposure is key to evaluate its effects. . We sought to validate and establish cut-offs for self-reported smoking and secondhand smoke (SHS) exposure during pregnancy using urinary cotinine and NNAL in a large contemporary prospective study from the USA, with lower smoking prevalence than has previously been evaluated.

Design: Prospective birth cohort.

Setting: Pregnancy clinics in New Hampshire and Vermont, US.

Participants: 1396 women enrolled in the New Hampshire Birth Cohort Study with self-reported smoking, urinary cotinine, NNAL and pregnancy outcomes.

Primary and secondary outcome measures: Cut-offs for urinary cotinine and NNAL concentrations were estimated from logistic regression models using Youden's method to predict SHS and active smoking. Cotinine and NNAL were each used as the exposure in separate multifactorial models for pregnancy outcomes.

Results: Self-reported maternal smoking was: 72% non-smokers, 5.7% ex-smokers, 6.4% SHS exposure, 6.2% currently smoked, 10% unreported. Cotinine and NNAL levels were low and highly inter-correlated (r=0.91). Geometric mean cotinine, NNAL were 0.99ng/ml, 0.05pmol/ml respectively. Cotinine cut-offs for SHS, current smoking were 1.2ng/ml and 1.8ng/ml (area under curve (AUC) 95% CI: 0.52 (0.47, 0.57), 0.90 (0.85, 0.94)). NNAL cut-off for current smoking was 0.09pmol/ml (AUC=0.82 (0.77, 0.87)). Using cotinine and NNAL cut-offs combined gave similar AUC to cotinine alone, 0.87 (0.82, 0.91). Cotinine and NNAL gave almost identical effect estimates when modelling pregnancy outcomes.

Conclusions: In this population we observed high concordance between self-complete questionnaire smoking data and urinary cotinine and NNAL. With respect to biomarkers, either cotinine or NNAL can be used as a measure of tobacco smoke exposure overall but only cotinine can be used to detect SHS.

Strengths and limitations of this study

- Compares the utility of a two tobacco biomarkers, urinary cotinine and NNAL to identify smoking in pregnant women
- Set within a large contemporary birth cohort in rural US with low prevalence of smoking
- Relies upon the availability of both urinary biomarker values and self-reported smoking

Introduction

The adverse effects of maternal smoking on birthweight and other pregnancy outcomes have been known for over 40 years with reports consistently showing that women who smoke cigarettes in pregnancy have smaller babies and are at greater risk of preterm delivery and other adverse outcomes[1] [2]. In more recent years, reports have focused on the effects of secondhand smoke (SHS) exposure in pregnancy and have shown evidence for small but statistically significant adverse effects on birthweight, stillbirth and congenital anomalies[3].

Alongside these outcome-focused studies there has been a growing interest in the accuracy of the assessment of the exposure, tobacco smoke intake. Many epidemiological studies have used interviews or self-complete questionnaires to ascertain smoking in pregnancy. Questionnaires continue to be widely used since they are relatively easy and inexpensive to administer but biomarkers are increasingly used to validate self-reported smoking. Cotinine, a metabolite of nicotine, can be measured in urine, saliva and plasma samples and was shown in the late 1980s to discriminate well between smokers and non-smokers with high sensitivity and specificity for data-derived cut-offs[4]. Since then many studies have derived cotinine cut-offs to discriminate between smokers and non-smokers for a range of patient groups including pregnant women[5], and these cut-offs have been used to identify participants whose reported smoking was inconsistent with their cotinine level i.e. they were 'misclassified' [6]. Thus, the advantage of cotinine over questionnaire measures has been demonstrated in the presence of smoking misclassification.

The tobacco-specific nitrosamine metabolite 4-(methylnitrosamino) -1-(-3-pyridyl) -1 -butanol (NNAL) can be measured in urine samples and has been associated with smoking-related cancers[7]. Urinary NNAL has been compared with cotinine to assess SHS exposure in adolescents by Benowitz and colleagues who reported that both biomarkers detected high percentages with SHS exposure among adolescents[8]. Postpartum urinary NNAL was reported to be correlated with cotinine (rho=0.78), and associated with neonatal NNAL level (rho=0.71)[9]. A few studies have used both questionnaires and biomarkers to assess exposure to tobacco smoke in pregnancy: a cohort study from Korea explored the use of NNAL to assesses tobacco smoke exposure and concluded that it added to the information provided by self-report or cotinine[10]; a mother-child cohort from Greece found that cotinine did not fully summarize exposure to NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) uptake[11]; a study from Poland explored relationships between maternal NNAL and cotinine in women reporting SHS or active smoking, and concluded that NNAL was a useful biomarker of pre-natal exposure to carcinogens in newborns[12]. Optimal cut-off points for detecting active and passive smoke exposure in pregnancy have been reported from the INMA Spanish cohort (18% active smokers)[6] and from the Hokkaido Japanese cohort (19% active smokers)[13].

The New Hampshire Birth Cohort Study is a large ongoing prospective study from USA with lower smoking prevalence than has been evaluated historically and that has obtained detailed self-reported smoking data and urinary cotinine/NNAL levels. We sought to establish biomarker cut-offs for smoking and SHS exposure and to validate the use of self-reported smoking against the biomarkers to extend the knowledge base for the utility of NNAL. We hypothesized that NNAL would be strongly positively correlated with cotinine and that the two biomarkers would be similarly predictive of smoking and SHS, and that self-reported smoking would be shown to be reliable.

Methods

Study population

The New Hampshire Birth Cohort Study (NHBCS) is a prospective study that aims to examine the associations between environmental exposures and other factors, and maternal-child health outcomes[14]. Participants provided written informed consent and all study procedures were approved by the Committee for the Protection of Human Subjects at Dartmouth College. Beginning in January 2009, pregnant women between 24 and 28 weeks of gestation were recruited from prenatal clinics in New Hampshire. Criteria for eligibility included: age 18 to 45 years, English literacy, use a private, unregulated water system at home (e.g., private well), not planning to move residence, and a singleton pregnancy. The current analyses includes all women recruited until January 2017.

Patients and public involvement

There was no patient involvement in this specific study but the New Hampshire Birth Cohort Study has an active dissemination program for the community (https://geiselmed.dartmouth.edu/childrenshealth/quick-links/).

Data obtained

Demographic and lifestyle data including educational attainment and tobacco smoke exposure were obtained using NHBCS administered at enrollment. Smoking status and number of cigarettes smoked per day were assessed during the three months prior to pregnancy, as well as during the first and second trimesters of pregnancy. Additionally, exposure to secondhand smoke was assessed through the number of hours per day and days per week while the pregnant mother was in areas where others were smoking during the three months prior to pregnancy and during the first and second trimesters of pregnancy. Maternal smoking status was categorized from participants' reports in five groups: 1) current smoker 2) ex-smoker 3) non-smoker, secondhand smoke exposure 4) non-smoker, no secondhand smoke exposure 5) not reported. Number of cigarettes smoked per day was asked for current smokers. See supplement 'Methods - additional information' for details of how smoking was classified.

Maternal and infant anthropometry and birth outcome data were ascertained from prenatal and delivery medical records and included: mother's height, preconception weight, infant sex, birthweight, gestational age, head circumference and crown-heel length. Infant measurements were normalized using z-scores to adjust for sex and gestation.

Biospecimens

Spot urine samples were collected by the subject at the time of enrollment, at approximately 24 to 28 weeks gestation and transported on ice packs and stored in a 4°C refrigerator. Processing of urines into aliquots occurred within 24 hours of collection. During processing 10ml aliquots were transferred into 15ml trace-free metal tubes and immediately stored at -80°C. 10ml aliquots were thawed once to obtain aliquots for trace metal analysis and thawed again to obtain a 2ml aliquot for cotinine and NNAL analysis. 2ml aliquots were frozen at -80°C and referred to the Minnesota Children's Health Exposure Analysis Resource (CHEAR) Exposure Assessment Hub at the Masonic Cancer Center at the University of Minnesota. To assess urinary dilution, specific gravity was measured using digital refractometer.

Cotinine and NNAL

Two biomarkers of exposure to tobacco smoke, total cotinine and total 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) were quantified in maternal urine samples at 24-28 weeks gestation. "Total" refers to the sum of the compound and its glucuronide conjugates. The analysis was by LC-MS/MS as described previously[15 16]. Lab reported limit of quantitation values for cotinine and NNAL were 0.5 ng/ml and 0.05 pmol/ml respectively with inter-assay coefficients of variation, 5% and 12%.

Statistics

The study population included all NHBCS women who provided a 24 to 28 gestational week urine sample from which cotinine and NNAL levels could be obtained. Since some women did not provide a urine sample, we compared maternal characteristics in those with and without these urines (included/excluded) using the t-test and chi-squared or Fisher's exact test. We summarized the characteristics of the study population mothers and their babies using means and standard deviations for continuous data and frequencies and percentages for categorical data. Some variables are reported as both continuous and categorical (age, BMI) to aid interpretation and comparison with other studies. We cross-classified reported smoking by cotinine and NNAL levels in groups to explore the inter-relationships, and calculated the correlation between cotinine, NNAL and number of cigarettes smoked using Pearson's coefficient. A participant's reported smoking was defined as 'misclassified' (yes/no) if they reported not being a current smoker but had a urinary cotinine level at or above 30ng/ml, the lowest cotinine cut-off value for active smoking reported in a recent review [5].

We used logistic regression to model the relationship between being a current smoker and both cotinine and NNAL concentrations to determine the cotinine and NNAL level cut-offs that best identified current smoking. Youden's method[17] was used with the receiver operating characteristic (ROC) curve to choose the cut-off that gave the best combination of sensitivity and specificity. A similar analysis was conducted to determine the best cut-off for each of cotinine and NNAL to identify SHS. These cut-offs were used to categorize the participant's smoking status into groups for each of cotinine and NNAL: unexposed/exposed to SHS only/smoker.

We used cotinine and NNAL to assess the relationship between smoking and the outcome of pregnancy in multivariable regression models. We modelled each biomarker as a continuous variable, $\log_e$-transformed with values below the limit of detection (LOD) replaced by LOD/sqrt(2), i.e. 0.3536 for cotinine and 0.0354 for NNAL . The following outcomes of pregnancy were analyzed: birthweight, birthweight z-score, gestational age, small-for-gestational age (<10th percentile), preterm birth, head circumference z-score, and crown-heel length z-score. Results are given as regression coefficients for continuous outcomes and odds ratios for binary outcomes scaled to a one standard deviation change in cotinine or NNAL as appropriate (with 95% confidence intervals) to aid interpretation. All birth outcome models were adjusted for the following covariates: maternal age (continuous), BMI ($\log_e$-transformed), maternal education (beyond high school, yes/no) and parity (0 vs 1+).

In a sensitivity analysis, we separately modelled the effects of cotinine and NNAL on pregnancy outcome using the cut-offs derived previously to define smoking. In a post-hoc change for this sensitivity analysis only, we re-categorized women who reported being smokers but had low urinary cotinine level when assessed (i.e. below the data derived cut-off value) as active current smokers. We did this since we judged it likely that they were generally smoking in pregnancy.

Factors associated with self-reported smoking status during pregnancy (categorized as current smoker, ex-smoker, non-smoker) and being misclassified (yes/no) were explored using unifactorial and multivariable multinomial logistic regression. The following factors were included as possible predictors: maternal age at enrollment (continuous), BMI (log_e-transformed), education (education beyond high school, yes/no), and parity (0 vs 1+). Statistical analyses were conducted using SAS v 9.4, R v 3.6.3.

Power calculations

An indicative power calculation was conducted according to the available cohort size, approximately 1300, varying slightly for different analyses due to missing data. Assuming two-sided significance 5%, mean birthweights 3500g, 3470g, 3300g in the unexposed, SHS exposed only and current smokers, and standard deviation 500[18], power is over 90% both for 94% for a one-way analysis of variance and for testing the trend across groups (Stata 'power oneway').

Ethical approval

This study was approved by Trustees of Dartmouth College Committee For The Protection Of Human Subjects #STUDY00020844.

Results

A total 1739 women were enrolled in the NHBCS as of January 2017, of whom 1396 have cotinine and NNAL data and so comprise the study population for the current analyses. Table S1 (supplement) compares the characteristics for the study population with the 494 excluded (no cotinine/NNAL data) and indicates that the study population had a slightly lower mean BMI, were more likely to be primiparous, had more education, were less likely to smoke and were more likely to be of white race than those not included. Other characteristics were not appreciably different.

Overall, the study population had a mean age 31 years, mean BMI 25, 43% were nulliparous and 88% had been educated beyond high school. Seventy two percent of women self-reported as non-smokers, a further six percent were not active smokers but exposed to secondhand smoke (SHS) pre-conception or prenatal, six percent were ex-smokers and six percent were current smokers. Ten percent of women did not report smoking status. Twenty seven women (2%) reported not currently smoking but had cotinine levels consistent with active smoking and so their reported smoking was assumed to be 'misclassified'. Among smokers, the number of cigarettes smoked was relatively low with most women reporting to smoke less than 10 cigarettes per day. Geometric mean cotinine and NNAL levels were 0.99ng/ml and 0.05pmol/ml respectively and both distributions were positively skewed (figure 1, figure 2). For the newborns: mean birthweight was 3421g, 52% male, 9.2% were preterm and 9.8% were small-for-gestational age (table 1). The full table is given in the supplement (table S2).

There was generally good agreement between reported smoking and urinary cotinine level (table 2). The majority of self-reported non-smokers had very low (undetectable) cotinine levels and the majority of current smokers had cotinine above 30ng/ml, although some current smokers had very low cotinine levels (table S3). NNAL levels were undetectable in almost all women; only 8% (107/1396) overall had NNAL at or above 0.1 pmol/ml (table S3). Cotinine and NNAL levels were very similar among ex-smokers who reported smoking in the three months prior to conception compared to those who reported

smoking earlier (table S3). There were positive inter-correlations between the two tobacco biomarkers and the reported number of cigarettes smoked (table S4). In particular we noted a very strong correlation, r=0.91, between $\log_e$ cotinine and $\log_e$ NNAL (figure S1).

The data-derived cotinine cut-offs for SHS and current active smoking were 1.2ng/ml and 1.8ng/ml respectively. The cotinine cut-off for SHS had high specificity but very low sensitivity whereas the cut-off for active current smoking had high sensitivity and high specificity (table 2). NNAL levels could not be used to detect SHS but an NNAL cut-off of 0.09 detected active current smoking in this population with very high specificity and moderate sensitivity (table 2). A post-hoc analysis was conducted to determine whether cotinine plus NNAL improved the separation between smokers and non-smokers. This showed that using the criterion that either cotinine or NNAL were above their respective previously derived cut-offs, produced similar sensitivity and specificity and AUC to using cotinine alone (table 2).

Non-smokers without SHS exposure tended to be older and nearly all educated beyond high school (table S5). In contrast, current smokers were younger and less than one half were educated beyond high school (table S5). The associations between age, BMI, parity and education were weak (table S6), allowing mutual adjustment in multivariable analyses. While the associations between smoking group and age, parity and BMI were weaker after mutual adjustment and not statistically significant, the association with education remained strong (table S7).

The estimated effects of cotinine level and NNAL level on outcome of pregnancy were adjusted for maternal age, BMI, parity, and education, and scaled to a standard deviation increase in cotinine or NNAL. The scaled estimates for birth outcomes are very similar for the two biomarkers (table 3). Statistically significant inverse associations were observed for: birthweight (cotinine: -55.5g, NNAL: -57.8g), birthweight z-score (cotinine: -0.11, NNAL: -0.11), crown-heel length z-score (cotinine: -0.11, NNAL: -0.10). In the sensitivity analysis using the previously derived cotinine and NNAL cut-offs to define smoking groups, (non-smokers/SHS/smokers for cotinine; smokers/non-smokers for NNAL), there was a mean reduction in birthweight of 43g in those with SHS exposure, and 128g in active smokers compared to non-smokers. P values tended to be bigger (less significant) in the analyses with smoking biomarkers modelled in categories compared to as continuous (table S8, table 3).

Discussion

Tobacco smoke contains many constituents including nicotine and carbon monoxide which are known to adversely affect the mother and fetus through vasoconstriction (nicotine) and hypoxia (carbon monoxide)[19] and hence the accurate assessment of tobacco smoke exposure in pregnancy is critical. In this paper we have reported on the validation of self-reported smoking in an ongoing cohort study of pregnant women in rural USA, the New Hampshire Birth Cohort Study (NHBCS), using two biomarkers, cotinine and NNAL. The prevalence of maternal smoking among NHBCS is 6.2% and among those who smoked, the number smoked is low with the majority of NHBCS smokers reporting smoking less than 10 cigarettes per day. This prevalence of maternal smoking is lower than the overall US average, 7.2%, and the New Hampshire prevalence, 11%, reported for 2016 from the National Vital Statistics System[20]. These low levels of smoking in NHBCS were borne out by the cotinine and NNAL levels and contrast the higher prevalence of maternal smoking in other cohorts such as the Boston Cohort that reports that 10% women smoked in pregnancy[21], INMA study from Spain where 19% women self-reported smoking in

pregnancy[6], the DEMOCOPHES study from Romania, Portugal and Poland with 25%, 30%, 19% respectively[22], and the Hokkaido Japanese cohort (19%)[13].

Our study shows broad agreement between questionnaire reports and both biomarkers. The use of questionnaires is cheaper and easier to collect as can be done without invasive and expensive laboratory analyses and potentially more representative of a woman's smoking as questions usually ask about smoking over a period of time. In contrast, as well as being objective and not subject to reporting bias, biomarker levels relate to recent tobacco smoke exposure - the half-life of cotinine in urine of pregnant smokers has been estimated to be about eight hours[23], shorter than in non-pregnant women due to accelerated metabolism in pregnancy[24]. The biomarker levels may be especially useful to determine effects of recent exposure.

Very few women mis-reported their smoking: under two percent of women (n=27) reported themselves as non-smoking but had tobacco biomarker levels consistent with active smoking. Just over one percent of women (n=17) reported being active smokers but their biomarker levels were very low. The low level of tobacco biomarker in these 17 self-reported smokers is likely due to infrequent smoking which resulted in abstinence in the period prior to collection of the urine sample. These observations

show the value of having both questionnaire and biomarker data and alerts us to a limitation of using short-term biomarkers alone to quantify tobacco exposure. In our study, the low level of discordance between self-reported smoking and biomarker level provides reassurance that the choice of which to use in analyses may be taken according to the question and nature of the modelling required.

The derived cut-off for urinary cotinine to define active smoking was low, 1.8ng/ml, reflecting the low number of cigarettes smoked by NHBCS women. This means that among NHBCS women and other similar populations where women smoke very little in pregnancy, cotinine levels are very reliable for predicting active smoking (sensitivity=80%, specificity=93%; AUC=0.90). However, cotinine levels are poor predictors of SHS (area under the curve: 0.52). A review article reported study-specific cut-offs for urinary cotinine varying between 31.5 and 550ng/ml[5] which is substantially higher than ours. The INMA study also reports a higher cut-off than ours, 82ng/ml[6], although the DEMOCOPHES study reported cut-offs of 4.4ng/ml (Poland), 7.9 (Portugal) and 254.2 (Romania)[22].

NNAL was able to distinguish between active smoking and non-smoking or SHS exposure with high specificity (98%) and moderate sensitivity (66%). However, given the low urinary NNAL values among our women NNAL levels were not able to be used to define SHS. The NNAL cut-off derived using NHBCS data, 0.09pmol/ml, was higher than that reported by Benowitz in adolescents, 0.05[8] (after conversion to SI units). There was a very high correlations between cotinine and NNAL (0.91) and so it is unsurprising that using cotinine plus NNAL gave no additional predictive ability beyond using cotinine alone, in our population.

When we used biomarker data to define tobacco exposure, we observed the expected relationships with pregnancy outcomes. Of particular note is that the estimated mean reductions in birthweight, Birthweight z-score and crown-heel length were very similar using cotinine compared to using NNAL, reflecting the concordance of the two biomarkers as measures of maternal tobacco smoke intake. When we used the data-derived cut-offs to define smoking groups we were able to estimate the effect of SHS as a mean reduction of 43g or 0.07 z-score units. The birthweight reduction falls within the 95% confidence interval from the pooled value reported in the Nieuwenhuijsen review of the literature with

a pooled mean reduction of 60 g and 95% CI of 39 to 80[3]. This is reassuring given our data are from a relatively recent cohort with relatively low rates of current smoking.

Our population sample study data come from a large ongoing birth cohort from Northern New England where smoking data were carefully collected using detailed self-complete questionnaires supplemented by urinary tobacco biomarker data. This is one of only a few studies to examine NNAL in a pregnant population: Lee and colleagues[10] studied 251 pregnant women (8.4% smokers) in South Korea and reported that positive NNAL, defined as NNAL greater than the lowest limit of detection, 2.0pg/ml, and not urinary cotinine, was an independent predictor of spontaneous abortion, preterm birth and small-for-gestational age. Florek and colleagues detected raised levels of cotinine and NNAL in newborn urine whose mothers had been exposed to tobacco in Poland (N=121)[12], and Vardavas and colleagues found that exposure to tobacco smoke correlated with cotinine and NNAL in Greece (N=1317)[11].

The limitations of our study are that urines were obtained at one time point only, 24-28 weeks gestation, and while identified misclassification of smoking was low, 10 percent of women did not report smoking. For these women, the biomarker data suggested that around a quarter were active smokers compared to 6.2% among those who responded to smoking questions and so using questionnaire data alone will underestimate the true prevalence of smoking. Most of our self-reported smoking questions were related to current habit and so were not subject to recall bias but we did enquire about SHS exposure pre-conception and so those responses may have been affected by errors in recall.

Overall, we observed good concordance between our self-complete questionnaire smoking data and tobacco biomarker levels, suggesting that the percentage of misclassified non-smokers is small. Further we have found that an NNAL data-derived cut-off can be used to separate smokers from non-smokers with high specificity and moderate sensitivity, although in our population cotinine was a better predictor of reported smoking overall, with high sensitivity and specificity. We suggest on the basis of this relatively recent pregnancy cohort of USA women from rural Northern New England that either detailed self-completed questionnaire smoking data or biomarker data may be used in analyses of the effects of tobacco smoke on health outcomes in children. We further suggest that cotinine levels rather than NNAL levels be used to detect SHS exposure.

Table 1: Characteristics of the women and their babies (full version in supplement Table S2)

Total		1,396
Maternal Characteristics		
Maternal age, years	Mean (SD)	31.3 (4.9)
Pre-pregnancy maternal weight (lb)	Mean (SD)	153.9 (34.1)
Pre-pregnancy maternal height (in)	Mean (SD)	64.9 (2.7)
Pre-pregnancy maternal BMI	Mean (SD)	25.6 (5.6)
Parity: primiparous		43% (586)
Mother's race: white		97% (1357)
Maternal level of education		
	High School or less	12% (140)
	Junior college / college	57% (696)
	Postgraduate	31% (377)
Maternal Tobacco Exposure		
Reported smoking at 24 weeks		
	Non-smoker, no SHS	72% (999)
	SHS exposure pre-conception/prenatal	6.4% (90)
	Ex-smoker	5.7% (79)
	Current smoker	6.2% (86)
	Not reported	10% (142)
Urinary Cotinine, ng/ml	Mean (SD)	339.54 (1621.98)
	Geometric mean (geometric SD)	0.99 (12.43)
NNAL, pmol/ml	Mean (SD)	0.19 (0.81)
	Geometric mean (geometric SD)	0.05 (2.75)
Infant Characteristics		
Gestational age, weeks	Mean (SD)	38.96 (1.82)
Birthweight, grams	Mean (SD)	3421.2 (552.3)
Birthweight z-score	Mean (SD)	-0.05 (1.03)
Small-for-gestational age (below 10th centile for age)	% (n)	9.8% (132)
Preterm birth (gestational age <37wks)	% (n)	9.2% (129)
Infant sex – male	% (n)	52% (716)

Table 2: Optimal cutoff points for urinary cotinine and urinary NNAL to define active smoking and SHS

	Cut-point	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)	Youden Index
Cotinine (ng/ml)					
To detect SHS	1.2	11% (6.7, 17%)	95% (86, 99%)	0.52 (0.47, 0.57)	0.06
To detect active current smoking	1.8	80% (70, 88%)	93% (91, 94%)	0.90 (0.85, 0.94)	0.73
NNAL (pmol/ml)					
To detect current active smoking	0.09	66% (55, 76%)	98% (97, 99%)	0.82 (0.77, 0.87)	0.64
Cotinine and NNAL combined					
To detect current active smoking	Cotinine>1.8 or NNAL>0.09	81% (72, 89%)	89% (87, 91%)	0.87 (0.82, 0.91)	Not applicable

Table 3: Outcome of pregnancy by log_e transformed urinary cotinine and log_e transformed NNAL level in pregnancy N=1396

Outcome	Regression coefficient/Odds ratio (95% CI) P value	
	Cotinine, ng/ml (loge)	**NNAL, pmol/ml (loge)**
Birthweight[1], gram	-55.5 (-93.2, -17.8) P=0.0040	-57.8 (-96.6, -18.5) P=0.0039
Birthweight z-score[1]	-0.11 (-0.18, -0.04) P=0.0027	-0.11 (-0.18, -0.04) P=0.0035
Gestational age[1], weeks	-0.11 (-0.24, 0.01) P=0.0795	-0.06 (-0.19, 0.07) P=0.3904
Small-for-gestational age (< 10th centile)	OR=1.22 (0.98, 1.52) P=0.0884	OR=1.15 (0.92, 1.44) P=0.2326
Preterm birth (<37wks)	OR=1.21 (0.98, 1.50) P=0.1002	OR=1.07 (0.84, 1.36) P=0.6066
Crown-heel length z-score[1]	-0.11 (-0.22, -0.003) P=0.0433	-0.10 (-0.21, 0.02) P=0.0991
Head circumference z-score[1]	-0.04 (-0.12, 0.04) P=0.2915	-0.03 (-0.11, 0.05) P=0.5055

Footnotes

1 regression coefficients and odds ratios scaled to 1 standard deviation increase in log_e cotinine (2.520) or log_e NNAL (1.012) as appropriate

2 All models include the following covariates: maternal age, log_e BMI, maternal education (high school vs beyond high school), parity (0 vs 1+)

3 Totals vary due to missing or unreported data

Figure legends

Figure 1

Distribution of urinary cotinine level (ng/ml)

Figure 2

Distribution of urinary NNAL level (pmol/ml)

Acknowledgements
We would like to extend our deepest appreciation of the participants and staff of the New Hampshire Birth Cohort and Center for Molecular Epidemiology. We gratefully acknowledge Alexandra Roy, an MPH candidate at the University of New England for her assistance with literature review for the analyses described in this paper. Finally we thank Karin Vevang for her key role in managing the logistics of the CHEAR project at the University of Minnesota.

Funding
Research supported in this publication was supported in part by the National Institutes of Health: P20GM104416, P42ES007373, P01 ES022832, UC2ES026533, UH30D023275, UG3OD023275 and US EPA RD83544201.

Data Sharing
These data form part of the NIH HHEAR program. The data will be uploaded to their portal at a future date. https://www.niehs.nih.gov/research/supported/exposure/hhear/index.cfm

Competing Interests
The authors have no financial relationships to disclose.

Contributors
MRK, TJP, VS, ERB conceptualized and designed this study, and assisted with methodology. SEM, LAP, TJP conducted and quality-assured all laboratory assessments. CAT conducted the literature review. JLP, YH conceived and conducted the statistical analyses. JLP drafted the manuscript including all tables and figures. All authors reviewed the manuscript and agreed the final submission.

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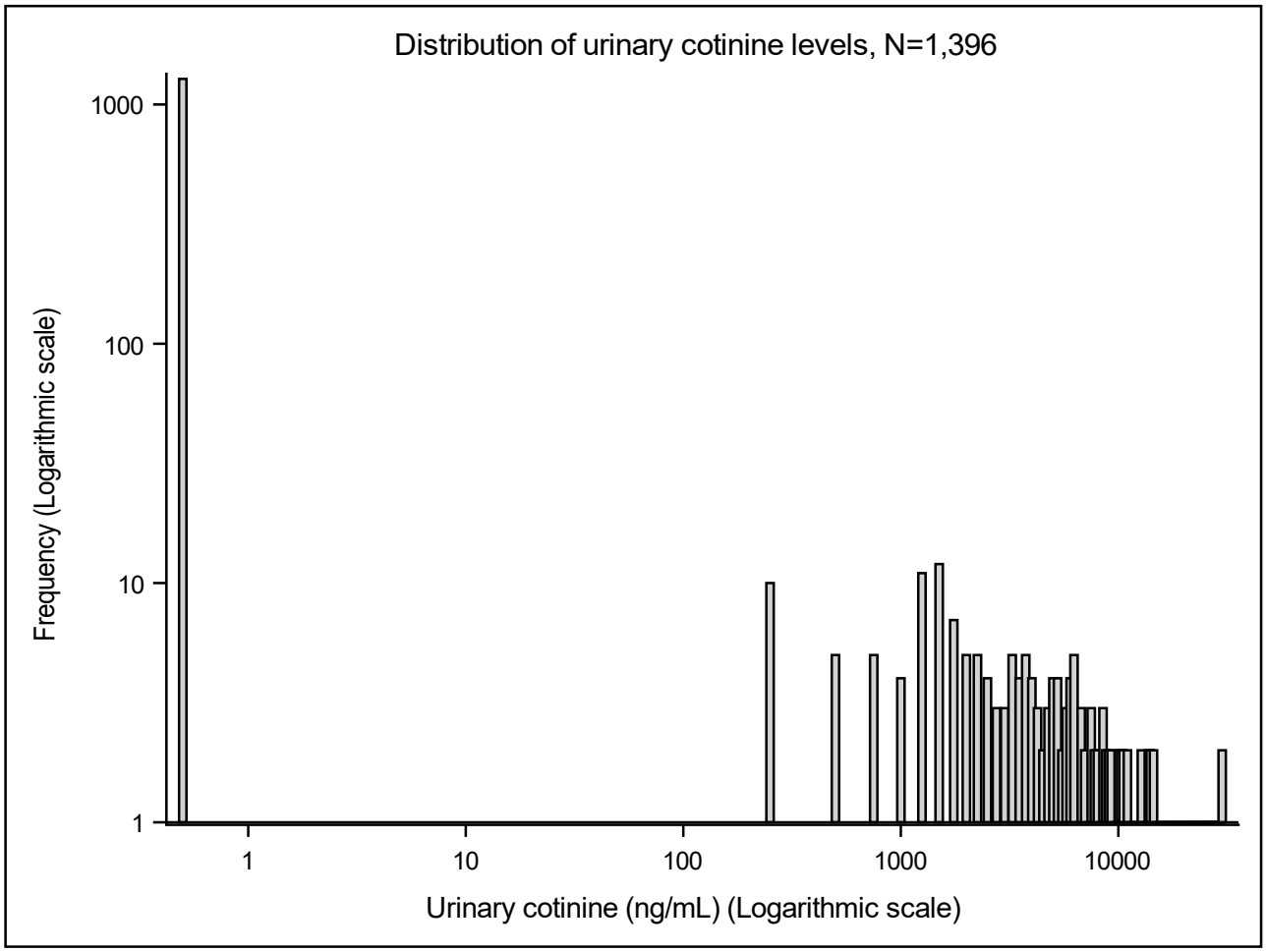
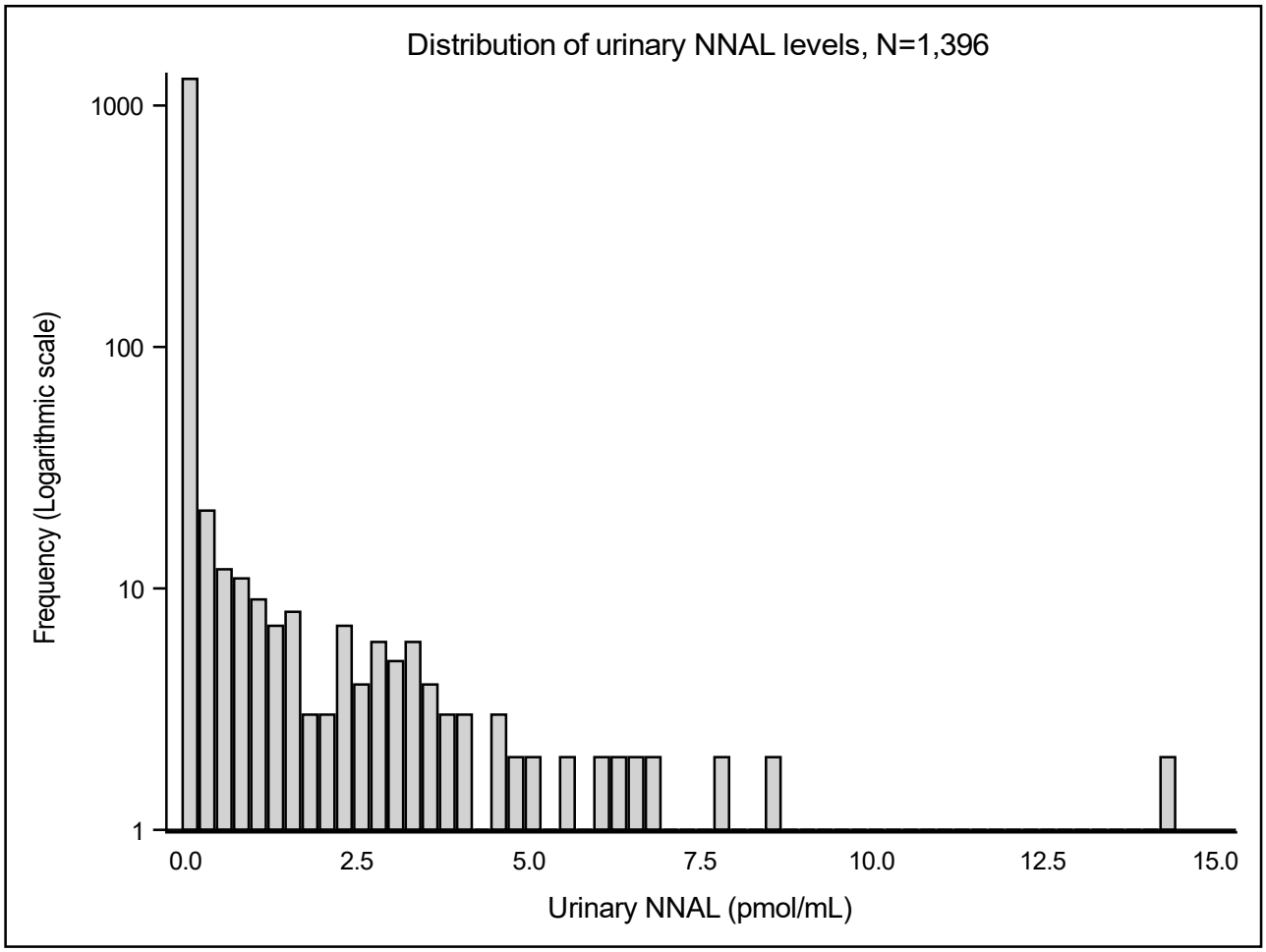




SUPPLEMENTARY MATERIAL

Assessing tobacco smoke exposure in pregnancy from self-report, urinary cotinine and NNAL: a validation study using the New Hampshire Birth Cohort Study

Methods: Additional information. Definition of smoking categories used in this paper

Women in the study population were assigned to one of the following mutually exclusive categories based on the NHBCS Prenatal Questionnaire's and NHBCS Postpartum Questionnaire's smoking related questions: 1) current smoker 2) ex-smoker 3) non-smoker, secondhand smoke exposure 4) non-smoker, no secondhand smoke exposure 5) not reported.

Current smoker was defined as anyone who reported smoking at any point during the pregnancy or smoking more than zero cigarettes per day.

If a participant did not report active smoking during the pregnancy but reported smoking prior to getting pregnant, she was assigned to the ex-smoker category. A sub-category of ex-smoker, ex-smoker in 3 months pre-conception was defined as an ex-smoker who smoked within 3 months prior to conception.

If a participant was not defined as a current smoker or ex-smoker but reported secondhand smoke exposure at any point during the pregnancy or within 3 months prior to getting pregnant or residing with at least one person that regularly smoked inside their home during pregnancy, this participant was assigned to the non-smoker, prenatal SHS category. A sub-category 'ex-smoker, smoked in 3 months pre-conception' was defined as a participant who experienced SHS within 3 months prior to getting pregnant but not during the pregnancy.

If a participant did not report smoking history or secondhand smoking exposure 3 months prior or during the pregnancy and answered at least one question in either questionnaire as a non-smoker, such participant was defined as a non-smoker.

Those who did not answer any of the smoke related questions were assigned to 'not reported' category.

Table S1: Characteristics of the Study population (women with 24 wk urine samples) and women not included (no 24wk urine)

		Study population N=1396	Not included N=494	p-value
Maternal age, years	Mean (SD)	31.25 (4.88)	31.06 (5.01)	0.440
	18-24	10% (140)	12% (60))	0.406
	25-34	68% (946)	66% (324)	
	35-45	22% (310)	22% (110)	
Maternal height, in	Mean (SD)	64.86 (2.65)	64.53 (2.54)	0.018
Pre-pregnancy maternal weight, lb		153.85 (34.10)	161.22 (43.00)	<0.001
Pre-pregnancy maternal BMI	Geometric mean (Geometric SD)	25.03 (1.22)	26.31 (1.27)	<0.001
	<18.5	2.4% (34)	1.8% (9)	0.013
	18.5 to <25	50% (694)	42% (208)	
	25+	40% (558)	46% (225)	
Parity	0	42% (586)	37% (182)	<0.001
	1	36% (506)	32% (157)	
	2+	20% (275)	21% (104)	
Mother's race	White	97% (1357)	91% (450)	<0.001
Maternal level of education	High School or less	10% (140)	11% (53)	<0.001
	Junior college / college	50% (696)	44% (218)	
	Postgraduate	27% (377)	19% (96)	
Reported smoking	Non-smoker, no SHS	72% (999)	59% (292)	<0.001
	Non-smoker, SHS exposure	6.4% (90)	7.5% (37)	

	Ex-smoker	5.7% (79)	5.7% (28)	
	Current smoker	6.2% (86)	6.1% (30)	
	Not reported	10% (142)	22% (107)	

Table S2: Characteristics of the women and their babies (full version)

Total		1,396
Maternal Characteristics		
Maternal age, years	Mean (SD)	31.3 (4.9)
	18-24	10% (140)
	25-34	68% (946)
	35-45	22% (310)
Pre-pregnancy maternal weight (lb)	Mean (SD)	153.9 (34.1)
Pre-pregnancy maternal height (in)	Mean (SD)	64.9 (2.7)
Pre-pregnancy maternal BMI	Mean (SD)	25.6 (5.6)
	GM (GSD)	25.0 (1.2)
	<18.5	2.6% (34)
	18.5 to <25	54% (694)
	25+	43% (558)
Parity	0	43% (586)
	1	37% (506)
	2+	20% (275)
Mother's race		
American Indian/Alaska Native		0.1% (2)
Asian		0.6% (9)
Native Hawaiian or Other Pacific Islander		0.1% (2)

White	97% (1357)
Mixed	1.6% (23)
Unknown	0.1% (2)
Maternal level of education	
High School or less	12% (140)
Junior college / college	57% (696)
Postgraduate	31% (377)
Maternal Exposure	
Reported smoking at 24 weeks	
Non-smoker, no SHS	72% (999)
SHS exposure pre-conception/prenatal	6.4% (90)
Ex-smoker	5.7% (79)
Current smoker	6.2% (86)
Not reported	10% (142)
Misclassified smoking habit[2]	1.9% (27/1396)
1st trimester number of cigarettes smoked per day in current smokers (%), N=86	
0	0% (0)
0.5 to < 5	37% (32)
5 to < 10	26% (22)
10+	35% (30)
Unreported	2.3% (2)
2nd trimester number of cigarettes smoked per day in current smokers (%), N=86	
0	26% (22)

	0.5 to < 5	19% (16)
	5 to < 10	16% (14)
	10+	15% (13)
	Unreported	24% (21)
3rd trimester number of cigarettes smoked per day in current smokers (%), N=86		
	0	26% (22)
	0.5 to < 5	19% (16)
	5 to < 10	16% (14)
	10+	15% (13)
	Unreported	24% (21)
Urinary Cotinine, ng/ml	Mean (SD)	339.54 (1621.98)
	GM (GSD)[1]	0.99 (12.43)
	Undetectable	76% (1,063)
	0.6 to <30	15% (208)
	30+	9.0% (125)
NNAL, pmol/ml	Mean (SD)	0.19 (0.81)
	GM (GSD)	0.05 (2.75)
	Undetectable	92% (1,274)
	0.05 to <0.1	0% (0)
	0.1+	8.4% (117)
Characteristics of smokers[3]: age	Mean (SD)	
Non-smoker (n=1088)		31.8 (4.5)

Ex-smoker (n=79)		30.5 (5.4)
Current smoker (n=86)		28.0 (5.7)
Characteristics of smokers[3]: Pre-pregnancy BMI[1]	GM (GSD)	
Non-smoker (n=1076)		25.1 (1.2)
Ex-smoker (n=78)		26.0 (1.2)
Current smoker (n=86)		24.8 (1.2)
Characteristics of smokers[3]: Parity 1+	% (n)	
Non-smoker (n=1071)		57% (612)
Ex-smoker (n=70)		49% (34)
Current smoker (n=86)		47% (40)
Characteristics of smokers[3]: education beyond high school	% (n)	
Non-smoker (n=1071)		90% (963)
Ex-smoker (n=70)		83% (57)
Current smoker (n=86)		47% (35)
Infant Characteristics		
Gestation age, weeks	Mean (SD)	38.96 (1.82)
Birthweight, grams	Mean (SD)	3421.2 (552.3)
Birthweight z-score	Mean (SD)	-0.05 (1.03)
Small-for-gestational age (below 10th centile for age)	% (n)	9.8% (132)
Preterm birth (gestational age <37wks)	% (n)	9.2% (129)
Infant sex – male	% (n)	52% (716)

Head circumference, cm	Mean (SD)	34.60 (1.74)
Crown-heel length, cm	Mean (SD)	50.63 (3.17)

Footnotes

1 Geometric mean and geometric standard deviation

2 Misclassified: Self-reported non-smoker with cotinine≥30ng/ml

3 Full tables are in supplement (S3, S4)

Table S3: Relationship between reported smoking habit, urinary cotinine and urinary NNAL

		Non-smoker, no SHS N=999	Non-smoker, SHS pre-conception N=14	Non-smoker, prenatal SHS N=76	[1]Ex-smoker, smoked in 3 months pre-conception N=56	[2]Ex-smoker N=79	Current smoker N=86	Not reported N=142
COTININE LEVEL (ng/ml)								
Undetectable	% (n)	87% (870)	86% (12)	64% (49)	57% (32)	57% (45)	15% (13)	52% (74)
0.6-29.99	% (n)	11% (112)	14% (2)	34% (26)	30% (17)	32% (25)	14% (12)	22% (31)
≥ 30	% (n)	1.7% (17)	0% (0)	1.3% (1)	13% (7)	11% (9)	71% (61)	26% (37)
NNAL LEVEL (pmol/ml)								
Undetectable	% (n)	99% (984)	100% (14)	96% (73)	86% (48)	89% (70)	34% (29)	72% (104)
0.05-0.09	% (n)	0.2% (2)	0% (0)	0% (0)	0% (0)	1.3% (1)	1.2% (1)	0.7% (1)
≥0.1	% (n)	1.3% (13)	0% (0)	4.0% (3)	14% (8)	10% (8)	65% (56)	26% (37)

Footnotes

1,2: 'ex-smoker in 3 months preconception' (1) is a subset of all ex-smokers (2)

Table S4: Correlation matrix for relationships between cotinine, log$_e$ Cotinine, NNAL, log$_e$ NNAL (Max N=1396)

Data are correlation coefficient, P value, N

	Cotinine	NNAL	Log$_e$ Cotinine	Log$_e$ NNAL	Cigs/day T1	Cigs/day T2	Cigs/day T3
Cotinine	1	0.82 <0.001 1396	0.72 <0.001 1396	0.80 <0.001 1396	0.54 <0.001 1076	0.61 <.001 1072	0.47 <0.001 1073
NNAL		1	0.67 <0.001 1396	0.82 <0.001 1396	0.45 <0.001 1076	0.50 <0.001 1072	0.39 <0.001 1073
Log$_e$ Cotinine			1	0.91 <0.001 1396	0.59 <0.001 1076	0.63 <0.001 1072	0.44 <0.001 1073
Log$_e$ NNAL				1	0.58 <0.001 1076	0.64 <0.001 1070	0.45 <0.001 1055
Cigarettes/day T1					1	0.91 <0.001 1072	0.83 <0.001 1056
Cigarettes/day T2						1	0.88 <0.001 1073
Cigarettes/day T3							1

Table S5: Maternal factors associated with self-reported smoking and misclassification of smoking[1]. Unifactorial analyses

	Non-smoker, no SHS N=982	Non-smoker, SHS exposure N=89	Ex-smoker N=70	Current smoker N=86	Misclassified[2] N=27	p-value
	Mean (SD) or % (n)					
Age at enrolment (years)	32.1 (4.4)	29.2 (4.5)	30.8 (5.0)	28.0 (5.7)	30.1 (6.0)	<0.001
Pre-pregnancy BMI[3]	25.0 (1.2)	27.8 (1.2)	26.3 (1.2)	24.8 (1.2)	23.6 (1.2)	0.037
Parity: 1+	60% (577)	40% (35)	49% (34)	47% (40)	56% (15)	0.001
Education: beyond high school	93% (888)	88% (75)	83% (57)	47% (35)	69% (18)	<0.001

Footnotes

1 Maximum number included in analyses is all women for whom smoking status was available (N=1254)

2 Misclassified: participants who reported being a non-smoker at 24 weeks and had urinary cotinine level 30ng/ml or more or equivalently NNAL level greater than 0.1pmol/ml

3 Geometric mean and geometric standard deviation

Table S6: Correlation matrix for relationships between maternal age, BMI, parity, education and SHS exposure

Kendall's tau-b Correlation coefficient ***p-value*** **N**					
	Age	**BMI**	**Parity**	**Education**	**SHS**
Age	1 1253	-0.03 0.11 1233	0.22 <0.0001 1240	0.20 <0.0001 1212	-0.27 <0.0001 1254
BMI[1]		1 1233	0.01 0.58 1221	-0.04 0.12 1206	0.04 0.06 1234
Parity[2]			1 1240	-0.03 0.37 1200	-0.14 <0.0001 1240
Education[3]				1 1212	-0.25 <0.0001 1212
SHS[4]					1 1254

Footnotes

1 BMI $\log_e$ transformed for analysis

2 Parity: 0 vs 1+

3 Education: high school vs beyond high school

4 SHS: Second-hand smoke exposure

Table S7: Maternal factors associated with self-reported smoking and misclassification of smoking. Multivariable analyses[1]

	N	Non-smoker, no SHS N=982	N	Non-smoker, SHS exposure N=88	N	Ex-smoker N=70	N	Current smoker N=86	N	Misclassified[2] N=27	p-value
	OR (95% CI)										
Age at enrolment (OR per 5 years)	982	1.00	88	1.15 (0.78, 1.72)	70	1.09 (0.80, 1.47)	86	1.00 (0.68, 1.44)	27	0.88 (0.55, 1.40)	0.86
Pre-pregnancy BMI[3] (OR per unit BMI)	972	1.00	86	0.75 (0.13, 4.28)	69	2.38 (0.68, 8.35)	79	0.27 (0.05, 1.49)	27	0.10 (0.01, 1.06)	0.062
Parity:											0.51
0	393	1.00	53		36		45		12		
1+	577		35	0.63 (0.31, 1.30)	34	0.69 (0.40, 1.19)	40	1.00 (0.49, 2.05)	15	0.87 (0.38, 2.02)	
Education:											<0.0001
High school or less	69	1.00	10		12		40		8		
Beyond high school	888		75	0.73 (0.28, 1.96)	57	0.39 (0.19, 0.83)	35	0.09 (0.04, 0.20)	18	0.19 (0.07, 0.50)	

Footnotes

1 Analyses include all women for whom the following were available: smoking status, age, BMI, parity, education (N=1253); reference category is non-smoker, no SHS apart from SHS exposed in pregnancy where the reference category is current smokers; all analyses adjusted for all other predictor variables. Table S1 includes the same material as table 4 but with the addition of subgroup total numbers.

2 Misclassified: participants who reported being a non-smoker at 24 weeks and had urinary cotinine level 30ng/ml or more

3 BMI $\log_e$ transformed for analysis

Table S8: Outcome of pregnancy by urinary cotinine and NNAL level in pregnancy using cut-points derived from self-reported smoking status[1]

Outcome		Mean difference between categories (continuous outcome) or OR (binary outcome)	95% CI	Overall P value
	Cotinine (ng/ml)			
Birthweight, gram	SHS vs non-smokers Active current smoking vs non-smokers	43.0 -127.7	(-137.9, 223.8) (-223.9, -31.6)	0.0263
Birthweight z-score	SHS vs non-smokers Active current smoking vs non-smokers	0.07 -0.25	(-0.27, 0.41) (-0.43, -0.07)	0.0176
Gestational age, weeks	SHS vs non-smokers Active current smoking vs non-smokers	0.02 -0.40	(-0.59, 0.62) (-0.72, -0.08)	0.0483
Small-for-gestational age (< 10th centile)	SHS vs non-smokers Active current smoking vs non-smokers	OR=1.19 OR=2.56	(0.36, 4.07) (1.48, 4.42)	0.0056
Preterm birth (<37wks)	SHS vs non-smokers Active current smoking vs non-smokers	OR=1.20 OR=2.30	(0.35, 4.04) (1.32, 3.99)	0.0187
Crown-heel length z-score	SHS vs non-smokers Active current smoking vs non-smokers	-0.002 -0.29	(-0.52, 0.52) (-0.56, -0.01)	0.1223
Head circumference z-score	SHS vs non-smokers Active current smoking vs non-smokers	0.02 -0.17	(-0.35, 0.39) (-0.37, 0.03)	0.2222
	NNAL (pmol/ml)			
Birthweight, gram	Active smoking vs SHS and non-smokers	-112.2	(-230.2, 5.8)	0.0618
Birthweight z-score	Active smoking vs SHS and non-smokers	-0.21	(-0.43, 0.02)	0.0677
Gestational age, weeks	Active smoking vs SHS and non-smokers	-0.27	(-0.66, 0.12)	0.1793
Small-for-gestational age	Active smoking vs SHS and non-smokers	OR=1.89	(0.96, 3.70)	0.0742

(< 10th centile)				
Preterm birth (<37wks)	Active smoking vs SHS and non-smokers	OR=1.83	(0.92, 3.63)	0.0998
Crown-heel length z-score	Active smoking vs SHS and non-smokers	-0.30	(-0.64, 0.04)	0.0807
Head circumference z-score	Active smoking vs SHS and non-smokers	-0.13	(-0.37, 0.11)	0.2857

Footnotes

1 women reporting being active smokers but with low urinary cotinine levels, are classified as active smokers (see methods section)

2 All models include the following covariates: maternal age, $\log_e$ BMI, maternal education (high school vs beyond high school), parity (0 vs 1+)

Figure S1: Scatterplot of Cotinine by NNAL (log_e transformed)

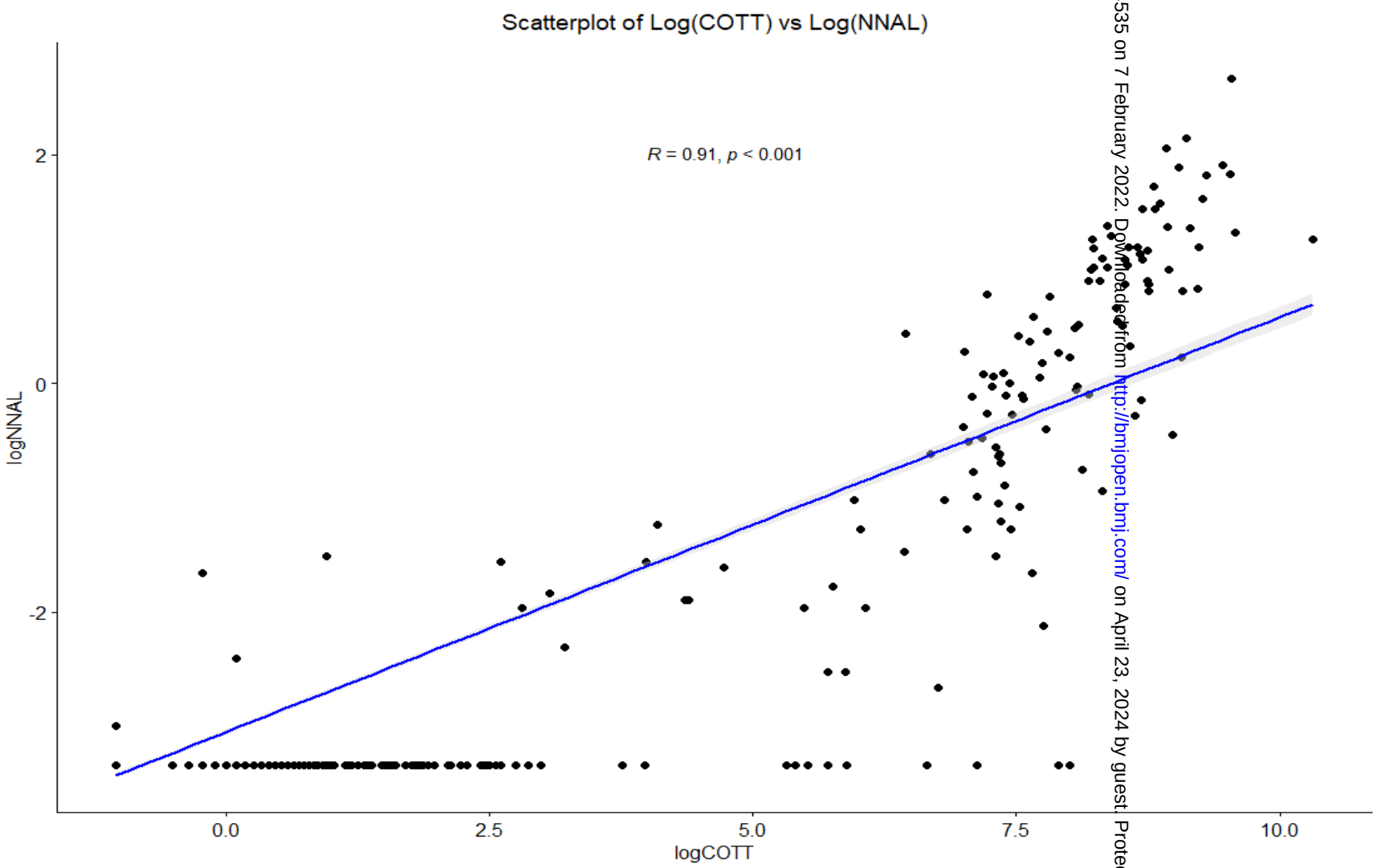
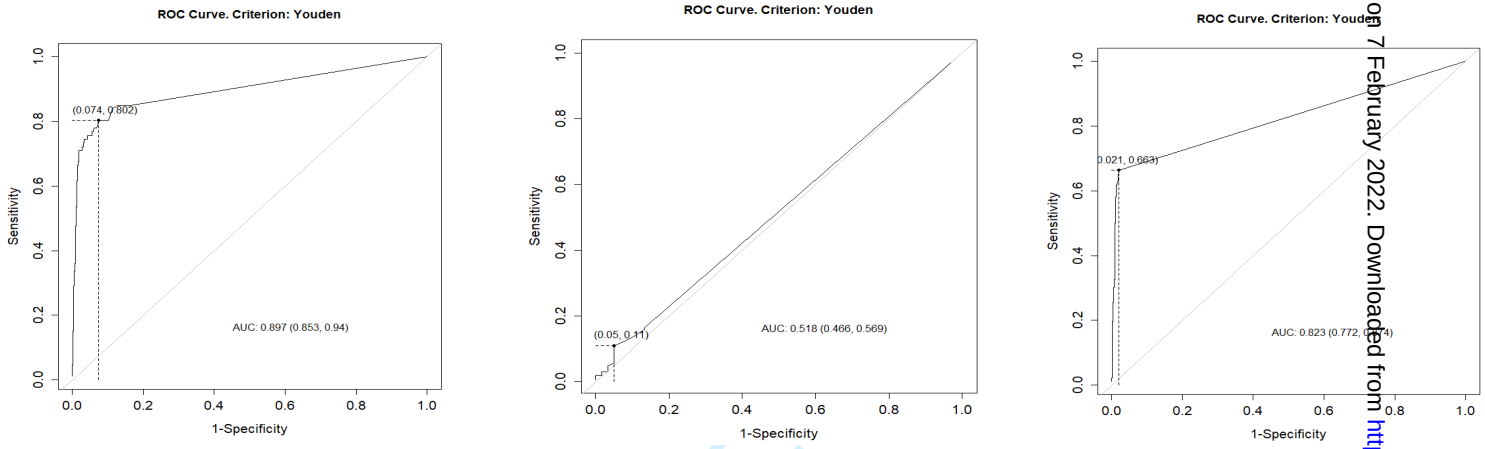


Figure S2 A,B,C: Receiver operating characteristic curves A) To detect active smoking using cotinine, B) To detect SHS using cotinine, C) to detect active smoking using NNAL



STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-5
Bias	9	Describe any efforts to address potential sources of bias	4-5
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	N/A
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	5
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	6
Outcome data	15*	Report numbers of outcome events or summary measures over time	Table 3

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Table 3 Table 3 N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Supplement
Discussion			
Key results	18	Summarise key results with reference to study objectives	7-8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9
Generalisability	21	Discuss the generalisability (external validity) of the study results	8-9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.