

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

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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 10 th 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 \geq 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	0.57 <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	0.54 <0.001 1073
Log_e NNAL				1	0.58 <0.001 1076	0.64 <0.001 1070	0.55 <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 smoking¹. 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					
<i>p-value</i>					
N					
	Age	BMI	Parity	Education	SHS
Age	1 1253	-0.03 0.11 1233	0.22 <0.0001 1240	0.20 <0.0001 1212	-0.23 <0.0001 1253
BMI ¹		1 1233	0.01 0.58 1221	-0.04 0.12 1206	0.04 0.06 1233
Parity ²			1 1240	-0.03 0.37 1200	-0.14 <0.0001 1240
Education ³				1 1212	-0.22 <0.0001 1212
SHS ⁴					1 1254

Footnotes1 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 analyses¹

	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 BMI³ (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	393	1.00	53		36		45		12		0.51
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:											
High school or less	69	1.00	10		12		40		8		<0.0001
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 status¹

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	43.0	(-137.9, 223.8)	0.0263
	Active current smoking vs non-smokers	-127.7	(-223.9, -31.6)	
Birthweight z-score	SHS vs non-smokers	0.07	(-0.27, 0.41)	0.0176
	Active current smoking vs non-smokers	-0.25	(-0.43, -0.07)	
Gestational age, weeks	SHS vs non-smokers	0.02	(-0.59, 0.62)	0.0483
	Active current smoking vs non-smokers	-0.40	(-0.72, -0.08)	
Small-for-gestational age (< 10th centile)	SHS vs non-smokers	OR=1.19	(0.36, 4.07)	0.0056
	Active current smoking vs non-smokers	OR=2.56	(1.48, 4.42)	
Preterm birth (<37wks)	SHS vs non-smokers	OR=1.20	(0.35, 4.04)	0.0187
	Active current smoking vs non-smokers	OR=2.30	(1.32, 3.99)	
Crown-heel length z-score	SHS vs non-smokers	-0.002	(-0.52, 0.52)	0.1223
	Active current smoking vs non-smokers	-0.29	(-0.56, -0.01)	
Head circumference z-score	SHS vs non-smokers	0.02	(-0.35, 0.39)	0.2222
	Active current smoking vs non-smokers	-0.17	(-0.37, 0.03)	
	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+)

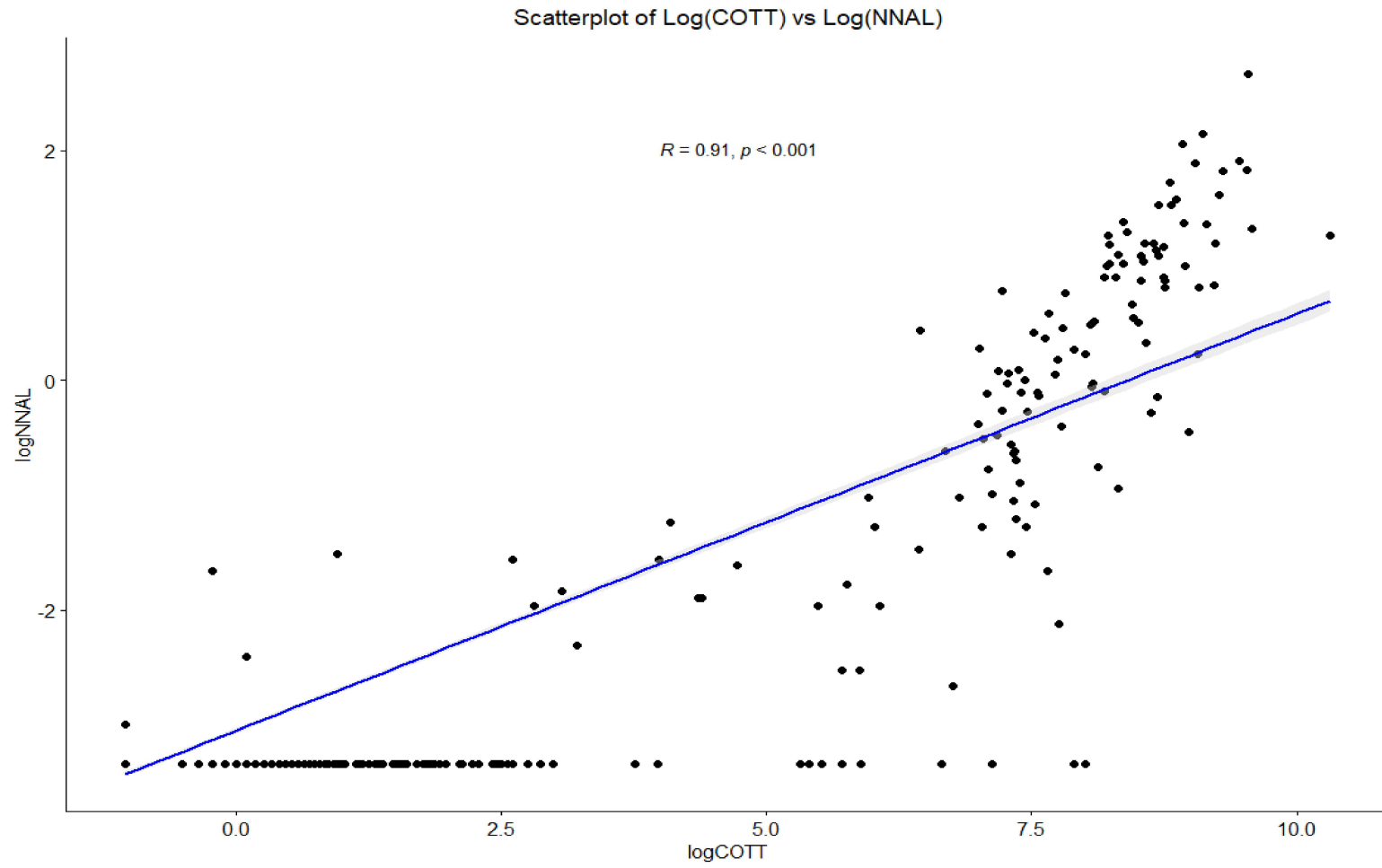
Figure S1: Scatterplot of Cotinine by NNAL (\log_e transformed)

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

