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SECONDHAND TOBACCO SMOKE EXPOSURE AMONG CHILDREN UNDER 5 YEARS OLD; QUESTIONNAIRES VERSUS COTININE BIOMARKERS: A COHORT STUDY

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This paper is part of the PhD work of Nerea Mourino.

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ABSTRACT

Objectives: Cotinine is the gold standard to estimate prevalence of secondhand tobacco smoke exposure (SHS) and assay limit of detection (LOD) cut-points are typically used regardless of age. Our aim was to compare the concordance between mother-reported SHS exposure and serum cotinine categorizing children as exposed with the assay LOD or age-specific cut-points.

Design: Data from the Health Outcomes and Measures of the Environment (HOME) Study, a prospective pregnancy and birth cohort.

Setting: hospital or participant's homes.

Participants: 389 pregnant women aged 18 years and older, between 13 and 19 weeks of gestation, living in a 5 county region of the Cincinnati, OH metropolitan area and with follow-up on their children at birth and ages 12, 24, 36 and 48 months.

Primary and secondary outcome measures: Children´s serum cotinine, mother-reported active smoking and SHS exposure were available at birth and during follow-up visits. We used Cohen's Kappa index to assess concordance between maternal self-report and child´s serum cotinine concentrations. We estimated optimal age-specific cut-points, its sensitivity-specificity and positive-negative predictive values with receiver operating characteristic curves.

Results: Self-reported exposure and cotinine data were available for 280 women who gave birth to singleton child. When applying the assay LOD (0.015 ng/ml), concordance between maternal report and serum cotinine, without accounting for age, was below 0.23 at all times. When using age-specific cut-points (12 months: 0.11 ng/ml; 24 months: 0.08 ng/ml; 36 months: 0.05 ng/ml and 48 months: 0.04 ng/ml), concordance improved, being low at 12 months (0.39), moderate at 24 and 36 months (0.47 and 0.43), and high at 48 months (0.62).

Conclusions: Concordance between mother-reported SHS exposure among children under 5 years and serum cotinine improved considerably after applying the cohort- and age-specific cut-points. Future studies are necessary to verify these results.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- First study estimating serum cotinine age-specific cut-points for children under 5 years old.
- This study has one of the largest samples of children younger than 5 years to validate SHS exposure using parental report and biomarkers.
- We have longitudinal measures of SHS exposure derived from both maternal report and children's serum cotinine concentrations over the first four years of life
- We do not include questions to evaluate third-hand smoke exposure or dietary intake.
- To identify the optimal serum cotinine cut-points we assume maternal-report as the gold standard.

BACKGROUND

Exposure to secondhand tobacco smoke (SHS), or passive smoking, is the involuntary inhalation of a complex mixture of tobacco smoke produced by the consumption of tobacco (1). SHS exposure is a global public health concern and there is no safe threshold of exposure (2). Children are especially vulnerable to the effects of SHS exposure due to their narrower airways, faster respiratory rate, and undeveloped immune system (3). The detrimental effects of SHS exposure on children's health, which have been documented since the 1970s, include an increased risk of sudden infant death syndrome, acute respiratory tract infections (bronchitis and pneumonia), asthma exacerbation, respiratory symptoms (cough, phlegm, wheeze and breathlessness) and ear infections (4). Over the last 50 years, more than 100,000 infants exposed to SHS have already died in the United States (U.S.) (5).

Parent-report SHS exposure, which is often used to estimate SHS exposure, suffers from recall bias, social desirability bias and lack of knowledge about the child's exposure in the parents' absence (6). As a result, having access to sensitive and specific biomarkers of SHS exposure, such as cotinine, is generally considered a more valid and reliable method (1). Cotinine, which is the primary metabolite of nicotine, is considered the optimal biomarker for measuring SHS exposure. Cotinine has high specificity and sensitivity (7), as well as a prolonged half-life, relative to nicotine, which ranges from 16-20 hours in children (1). Cotinine can be quantified in serum, urine, hair, saliva, maternal milk, amniotic fluid and meconium (8, 9). While there are validated cotinine cut-points which can differentiate between active smokers and non-smokers among adults, such cut-points are not well established for distinguishing SHS exposure among children (10, 11, 12). Most studies use the analytical technique limit of detection (LOD) to define SHS exposure.

Various investigators who have evaluated the validity of information collected from questionnaires using cotinine as the gold standard, have found that the concordance between parental self-report and cotinine in children is inconsistent (13, 14, 15, 16). These inconsistencies could be related to poor validity of parental self-report, age-related differences or a lack of adequate cut-points for cotinine concentrations among children (17, 18). Given that assays for cotinine are so sensitive, the values above the LOD might be derived from other sources of nicotine, such as diet (19, 7). Thus, the establishment of valid cut-points for distinguishing SHS exposure from non-exposure among children could reduce exposure misclassification.

The purpose of this study was to characterize the concordance between mother-reported SHS exposure and serum cotinine concentrations in children younger than 5 years and assess the utility of age-specific serum cotinine cut-points to characterize children's SHS exposure compared with the serum cotinine assay LOD.

METHODS

Study participants

We used data from the Health Outcomes and Measures of the Environment (HOME) Study, a prospective cohort study that enrolled pregnant women from the Cincinnati, Ohio from 2003-2006 (20). The principal objective of the HOME Study was to evaluate the association of pre- and post-natal exposure to environmental toxicants with health and neurobehavioral outcomes in infants and children. The inclusion criteria were: ≥ 18 years old, between 13 to 19 weeks of gestation, single pregnancy, residing in a house built before 1978 within the study area, HIV-negative, not taking thyroid or epilepsy medication and not undergoing chemotherapy or radiation therapy. Of the 1,263 eligible pregnant women, 468 agreed to participate and 389 women remained in the study until the birth of their child. A detailed description of the cohort is published elsewhere (20).

Patient and Public Involvement

A community advisory board provided feedback on the original design of the HOME Study before the study began. Before initiating any new follow-up, we conducted pilot testing to ensure that the visit length and types of assessments were appropriate. At more recent childhood follow-up visits, we collected information regarding the visit length and experience from participants and used this to inform the development of subsequent visits. We previously reported back concentrations of environmental chemical biomarkers to participants while also providing contextual information. Finally, we reported clinically significant findings to participants and their medical providers.

Assessment of SHS exposure

Maternal-reported tobacco consumption and SHS exposure

Maternal-reported tobacco consumption and children's SHS exposure was obtained by using standardized face-to-face interviews administered by a trained interviewer. The questionnaire was administered at five different points during the follow-up: 4-weeks after birth and when children were ages 12, 24, 36 and 48 months. At each interview, trained research staff surveyed the women about their smoking of cigarettes, cigars and pipes as well as the smoking of these products by other members of the household. Women were also asked about their SHS exposure and that of their child at home (living with a smoker who smokes at home), in other frequently visited homes, and in the car. Each mother was classified as either a smoker, exposed (non-smoker with SHS exposure), or unexposed (non-smoker with no SHS exposure). Each child was classified as exposed if the mother reported either being a smoker or living with a smoker who smokes at home or if the mother reported that her child was exposed to SHS in any setting ever, sometimes or seldom. Otherwise, we classified children as unexposed.

Serum biomarkers of SHS exposure

We collected venous serum samples from children (umbilical cord) at delivery and at 12, 24, 36, and 48 months. The samples were stored at or below -80ºC until analysis. Serum cotinine concentrations were determined by the Centers for Disease Control and Prevention Environmental Health Laboratories using high performance liquid chromatography atmospheric pressure tandem mass spectrometry. The assay LOD threshold for cotinine was 0.015 ng/ml (19). We classified women as unexposed if the cotinine concentration from newborn´s umbilical cord blood after birth was < LOD, SHS exposed if the value was ≥ LOD but ≤ 3 ng/ml, and smokers if the cotinine concentration was > 3 ng/ml (11). Children were classified as unexposed if their cotinine concentration was < LOD and exposed if it was ≥ LOD. After calculating age-specific cut-points for children (described below), we classified them as unexposed if their cotinine concentration was lower than the new cut-points and exposed if it was equal or higher than the new cut-points.

Statistical Analysis

We calculated descriptive analysis for maternal sociodemographic characteristics, serum cotinine concentrations and SHS exposure prevalence in children using maternal-reported information and serum cotinine concentrations considering both assay LOD derived cut-point and age-specific cut-points. The concordance between maternal-reported SHS exposure and categories of serum cotinine concentrations were calculated using Cohen's Kappa index for two observers, considering 2 categories (exposed/unexposed). Correlation and agreement between children's log-base transformed serum cotinine concentrations at different moments between 12 and 48 months of age were estimated using Pearson correlation coefficients (r) and intraclass correlation coefficient (ICC).

We used receiver operating characteristic (ROC) curves to identify the optimal serum cotinine concentration to distinguish SHS exposure from non-exposure in children at 12, 24, 36 and 48 months. For this calculation, we considered several information reported by mothers regarding their children´s exposure to SHS as the gold standard. Exposed category comprised children whose mothers reported either being a smoker or living with a smoker who smokes at home and those whose mothers reported they were exposed in any setting ever, sometimes or seldom. Area under ROC curves (AUC) was calculated besides age-specific cut-points. The optimal serum cotinine cut-points at each age were the concentrations at which the difference between sensitivity and specificity was minimum. Specificity-sensitivity and positive-negative predictive values (PPV-NPV) were calculated for each age-specific cut-point. Estimations were accompanied by confidence intervals of 95% (CI 95%). Analysis is restricted to children with mother-reported information and serum sample. The analysis was performed by using Stata v14.2.

RESULTS

Sample characteristics

A total of 384 women had complete data on their tobacco smoke exposure at delivery, while 336 (87.5%) children had complete self-report data at 12 months, 280 (72.9%) at 24 months, 258 (67.2%) at 36 months, and 187 (48.7%) at 48 months. At baseline, 31% of women were between ages 30-34 years, 62% were non-Hispanic white, 75% had greater than high school education, 81% were employed, 78% lived with a spouse or partner and 71% had private health insurance. The attrition rate of women at 48 months was 51.3%; loss to follow-up was not related to any sociodemographic characteristics (Supplementary Table 1).

We restricted the analyses to children with both maternal self-reported and cotinine measures, information was available for 280 newborns, 270 children at 12 months, 197 at 24 months, 196 at 36 months, and 150 at 48 months (Supplementary Table 2). The attrition rate of these children at 48 months was 46.4% (Supplementary Table 1).

Serum cotinine distribution

Children's geometric mean (GM) serum cotinine concentrations from 12-48 months was higher than newborn´s GM umbilical cord serum concentrations (Table 1). Serial measures of children's serum cotinine concentrations from 12-48 months were highly correlated with correlation coefficients between log-transformed children´s serum cotinine concentrations in consecutive periods ranging from 0.81 (24-36 months) to 0.72 (12-36 months). The ICC between repeated serum cotinine concentrations (analysis restricted to 73 children with cotinine measures at 12, 24, 36 and 48 months) was 0.72 (CI 95% 0.63-0.80) reflecting good agreement between measurements.

Prevalence of exposure to SHS

The prevalence of children exposed to SHS based on maternal report varied between 26.8%-31.3%. (Figure 1). The prevalence of SHS exposure based on cord serum cotinine after applying the assay LOD derived cut-point of 0.015 ng/ml, was double the self-reported prevalence. The prevalence of SHS exposure based on children´s serum cotinine concentrations decreased from 86.7% at 12 months to 74.7% at 48 months (Figure 1 upper). The difference between maternal-reported prevalence of exposure and that estimated from children´s serum cotinine concentrations, excluding newborns and using LOD as cut-point, was nearly 50 percentage points at any age.

Children whose mothers reported SHS exposure had higher serum cotinine concentrations than children whose mothers reported no exposure to SHS (Figure 2). Fifty percent of the newborns born to self-reported smokers had cord serum cotinine concentrations > 3 ng/ml. At 12 and 24 months of age, 83% and 80% of children whose mothers reported that they were not exposed had cotinine values higher than the LOD, and at 36 and 48 months of age, the percentage was

66% and 65%. Moreover, the distribution of serum cotinine concentration was similar among children whose mothers were active smokers and non-smoking mothers who reported SHS exposure (Figure 2); for this reason, these categories were combined in further analysis.

Estimation of age-specific cut-points for distinguishing SHS exposure

The AUC of various serum cotinine thresholds ranged from 0.80 and 0.89 (Supplementary Figure 1). Cut-points for distinguishing SHS exposure from non-exposure decreased with child age and were set at 0.11 ng/ml at 12 months, 0.08 ng/ml at 24, 0.05 ng/ml at 36 and at 0.04 ng/ml at 48 months (Table 2). The sensitivity and specificity corresponding to these cut-points were above 72% and NPV was over 87%. Using the optimal serum cotinine age-specific cut-points, the prevalence of SHS was highest at 12 months (39.3%) and lowest at 48 months (38.0%). The greatest difference between maternal self-reported prevalence and serum cotinine estimated prevalence of SHS exposure is nearly 13 percentage points at 12 months (Figure 1 bottom).

Concordance between self-reported exposure and serum cotinine measures

The concordance between maternal-reported SHS exposure and serum cotinine improved considerably after applying age-specific cut-points. The Kappa coefficient between mother-reported exposure and child´s serum cotinine concentrations, using the LOD as threshold, was below 0.22 in each of the four time periods. In contrast, when age-specific cut-points were used, the kappa coefficient improved from 0.39 at 12 months to 0.62 at 48 months (Table 3). Taking Landis and Koch criteria into account in assessing the Kappa index, when using the assay LOD of 0.015 ng/ml as cut-point, the concordance between maternal-reported and children´s serum cotinine concentrations after delivery was insignificant at 12, 24 and 36 months and low at 48 months. When using the new age-specific cut-points, concordance improved with age, being low at 12 months, but moderate at 24, 36 and high at 48 months.

DISCUSSION

In this cohort, pre-school aged children whose mothers reported SHS exposure had higher serum cotinine concentrations than children whose mothers reported no SHS exposure. When using the serum cotinine assay LOD as the threshold for distinguishing SHS exposure from no exposure, concordance between serum cotinine concentrations and maternal-reported exposure was non-significant. In contrast, after deriving age-specific serum cotinine cut-points, the concordance between serum cotinine and mother-reported exposure to SHS improved, with increasing concordance as child age increased.

Various studies conclude that serum cotinine concentrations in children vary as a function of age, sex, or race (2, 21, 22). With respect to age, children seem to have higher cotinine concentrations than adults at similar exposures. This could be due to metabolic differences, or to the fact that children have a faster respiration rate and inhale larger quantities of SHS contaminants than adults (23). Previous studies found differences in cotinine concentrations among children in different age groups, with higher concentrations among the youngest children (1, 2, 24). Our results are consistent with this as GM serum cotinine concentrations increased from birth to 12 months and then declined again, possibly reflecting decreased respiratory rates and breathing zones as children aged. Differences between cord blood and later concentrations could be explained by the higher metabolism and faster elimination of cotinine in their mothers (25). Thus, results of prior studies and developmental appropriate changes in child behavior, anatomy, and physiology support the need for age-specific cut-points for children. Indeed, when the serum cotinine assay LOD threshold was used to distinguish SHS exposure, 65-83% of children classified as unexposed by mother report were re-classified as SHS exposed. In contrast, when we used the new age-specific cut-points, only 17-27% were re-classified as SHS exposed.

Other investigators have concluded that the prevalence of SHS exposure obtained from maternal-report consistently underestimates actual exposure. Presumably, some of this estimate is because mothers might not report SHS exposure because of recall bias, social desirability bias or ignorance about their children's exposure in other settings (12, 21). Children's exposure may be so negligible that mothers are not able to identify or quantify it (6). Our results indicate that some of the discordance was because of failure to account for age-related differences in exposure or metabolism.

Based on cotinine derived from the LOD threshold, the prevalence of SHS exposure was 73.5% and 74.7% at 3 and 4 years-old, respectively. Taking into account the estimates obtained in population studies, about 4 out of 10 U.S. children aged 3–11 years (40.6%) are exposed to secondhand smoke (2). These large differences, however, fail to assess the influence of other factors such as the sensitivity and suitability of the cut-points used to classify exposure to SHS. The assay LOD has become much more sensitive over time. Earlier studies set it at 0.05 ng/ml (4, 12, 17), while more recent studies, including ours, have a LOD of 0.015 ng/ml. This lower LOD could mean that low serum cotinine concentrations reflect transient SHS exposure or exposure from other sources, such as food (tomatoes, potatoes, cauliflower and black

tea). Food consumption levels of dietary nicotine are insignificant compared with moderate SHS exposure, but consumption of high quantities of nicotine-containing foodstuffs might contribute to low-level elevations in serum cotinine (e.g. 80 g of eggplant is equivalent to approximately 0.01 ng/ml of serum cotinine) (7). Future studies could verify this hypothesis using studies with detailed dietary information and sensitive cotinine biomarkers.

Despite the improvement in the concordance between maternal-reported SHS and children's serum cotinine concentrations using the age-specific cut-points, misclassification is still a problem. This misclassification has been observed in prior studies, including those of pregnant women and their children younger than 5 (26, 27, 28) and it could be due to maternal concealment to avoid social judgment or ignorance about negligible and transient low level nicotine exposures quantified with sensitive cut-points.

This study has some limitations worth nothing. First, we had a modest sample size. Still, it was one of the largest samples of children younger than 5 years to validate SHS exposure using parental report and biomarkers (12, 27, 29, 30). Another limitation is the attrition of study participants. Yet loss to follow-up was not associated with any measured sociodemographic characteristic. Third, we did not include questions to evaluate third-hand smoke exposure, such as involuntary inhalation or cutaneous absorption of nicotine particles deposited on clothing and furniture, or dietary intake. Fourth, we did not account for factors such as the size of the home, the intensity of exposure, or the proximity to smokers (7, 31, 32). Fifth, to identify the optimal serum cotinine cut-points we assumed maternal-report was the gold standard. We note that we expect any misreporting to predominately affect the sensitivity of maternal report and not the specificity; few women would report exposure in its absence. Also, it should be noted that the gold-standard does not refer solely to whether the mother declares that the child is exposed, but it also takes into consideration if the mother is a smoker or the child lives with other smokers who smoke at home. Finally, our findings may not be generalizable to other populations as our eligibility criteria were not designed to ensure that our cohort was representative of births in the study region. However, most chemical biomarker concentrations among HOME Study participants are similar to pregnant women and children in the USA during the time of enrolment and follow-up (20).

This study also has several strengths. First, we had longitudinal measures of SHS exposure derived from both maternal report and children's serum cotinine concentrations over the first four years of life. Second, our cohort was relatively higher SES, with 75% of the mothers having greater than high school education. Thus, misreporting of SHS exposure is likely reduced as previous studies have shown that higher educational level is associated with more accurate SHS exposure reporting (15).

CONCLUSIONS

Previous investigators have concluded that maternal reports dramatically underestimate children's SHS exposure. When we used age-specific cut-points, we found that many fewer children were re-classified as SHS exposed. Thus, maternal report may be a better indicator of children's SHS than previous estimates. The age specific cut-points should be validated in other cohorts.

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FOOTNOTES

- **Contributors:** MPR, MIS and JB planned the study and supervise it with contribution from all the other authors. MPR and MIS performed the analysis with substantive contribution from all the other authors, MPR and NM wrote the first draft of the manuscript and MIS, JB, BP and KY provided critical comments and contributed on the drafts to successive versions of the manuscript. All the authors approved the final version paper. MPR is the guarantor.

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- **Competing interests:** JMB received an honoraria from Quest Diagnostic for serving on an expert panel related to endocrine disrupting chemicals. JMB's institution was financially compensated for his services as an expert witness for plaintiffs in litigation related to PFAS-contaminated drinking water; these funds were not paid to JMB directly. The other authors declare no competing interests.

- **Patient consent for publication:** not required.

- **Ethics approval:** The institutional review boards of Cincinnati Children's Hospital Medical Center, the participating delivery hospitals, and the CDC approved this study. During face-to-face visit, research assistants explained study protocols to each prospective participant and completed a checklist to ensure women were fully informed about the study. All mothers provided written informed consent for both themselves and their children prior to enrolment. ID of approval: 01-8-5.

- **Provenance and peer review:** Not commissioned; externally peer reviewed.

- **Data availability statement:** data are available upon reasonable request.

The HOME Study Principal Investigators welcome new collaborations with other

investigators and have actively engaged in collaborative data sharing projects.

Interested investigators should contact Drs Joseph M. Braun (joseph_ braun_ 1@brown. edu) and Kimberly Yolton (kimberly. yolton@ cchmc. org) to obtain additional information about The HOME Study, discuss

collaborative opportunities, and request a project proposal form. The HOME Study Protocol Review Committee reviews proposed research projects to ensure that they do not overlap with extant projects and are an efficient use of scarce resources (eg, biospecimens).

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TABLES

Table 1. Descriptive statistics of serum cotinine concentrations (ng/ml) at birth and at 12, 24, 36 and 48 months: n, range, quartiles and geometric mean with CI 95%.

			Quartiles			Geometric mean		
	n	Range	P25	P50	P75	Mean	95% CI	
Newborn´s cotinine [a]	280	0.00000 - 261.0	0.003	0.017	0.088	0.022	0.015	0.032
Child´s cotinine [a]								
12 months	270	0.00030 - 35.3	0.023	0.063	0.357	0.093	0.073	0.118
24 months	197	0.00126 - 10.5	0.020	0.046	0.212	0.070	0.053	0.092
36 months	196	0.00032 - 21.6	0.012	0.033	0.199	0.046	0.034	0.064
48 months	150	0.00024 - 14.9	0.013	0.027	0.249	0.047	0.033	0.067

ng/ml, nanogram/milliliter; n, number of observations; CI, confidence interval; P, percentile.

[a] Analysis is restricted to participants with both maternal-reported data and cotinine measures.

Table 2. AUC, new age-specific cut-points for each age (ng/ml) with its sensitivity, specificity, PPV and NPV. Estimations are accompanied by 95% CI.

	12 months	24 months	36 months	48 months
n	270	197	196	150
Exposed to SHS	72 (26.7%)	54 (27.4%)	51 (26.0%)	47 (31.3%)
AUC (95% CI)	0.80 (0.74 - 0.86)	0.83 (0.76 - 0.90)	0.84 (0.77 - 0.91)	0.89 (0.82 - 0.95)
Cut-points (ng/ml)[a]	**0.11**	**0.08**	**0.05**	**0.04**
Sensitivity (95% CI)	72.20 (60.40 - 82.10)	75.90 (62.40 - 86.50)	74.50 (60.40 - 85.70)	83.00 (69.20 - 92.40)
Specificity (95% CI)	72.70 (66.00 - 78.80)	76.20 (68.40 - 82.90)	74.50 (66.60 - 81.40)	82.50 (73.80 - 89.30)
PPV (95% CI)	49.10 (39.20 - 59.00)	54.70 (42.70 - 66.20)	50.70 (38.90 - 62.40)	68.40 (54.80 - 80.10)
NPV (95% CI)	87.80 (81.80 - 92.40)	89.30 (82.50 - 94.20)	89.30 (82.30 - 94.20)	91.40 (83.80 - 96.20)

AUC, area under receiver operating characteristic curves; ng/ml, nanogram/milliliter; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; SHS, secondhand tobacco smoke.

[a] Age-specific cut-point values are those that maximized the AUC, that is to say, those which minimize the difference between sensitivity and specificity . These values were calculated with receiver operating characteristic curves and children´s SHS exposure reported by their mothers was considered the gold standard. Children´s serum cotinine concentrations above these cut-point values will reflect SHS exposure.

Table 3. Kappa concordance coefficient between maternal-reported SHS exposure (exposed/ unexposed) and child´s serum cotinine concentrations accompanied with the percentage of agreement when using the assay LOD threshold and age-specific cut-points at 12, 24, 36 and 48 months of age.

	12 months	24 months	36 months	48 months
Assay LOD threshold[a]				
Agreement (%)	38.52	42.13	49.49	54.00
Kappa (95% CI)	0.08 (0.04 - 0.13)	0.12 (0.07 - 0.17)	0.18 (0.10 - 0.25)	0.22 (0.13 - 0.32)
Age-specific cut-points[b]				
Agreement (%)	72.59	76.14	74.49	82.67
Kappa (95% CI)	0.39 (0.28 - 0.50)	0.47 (0.34 - 0.59)	0.43 (0.30 - 0.56)	0.62 (0.49 - 0.75)

LOD, limit of detection; CI, confidence interval.

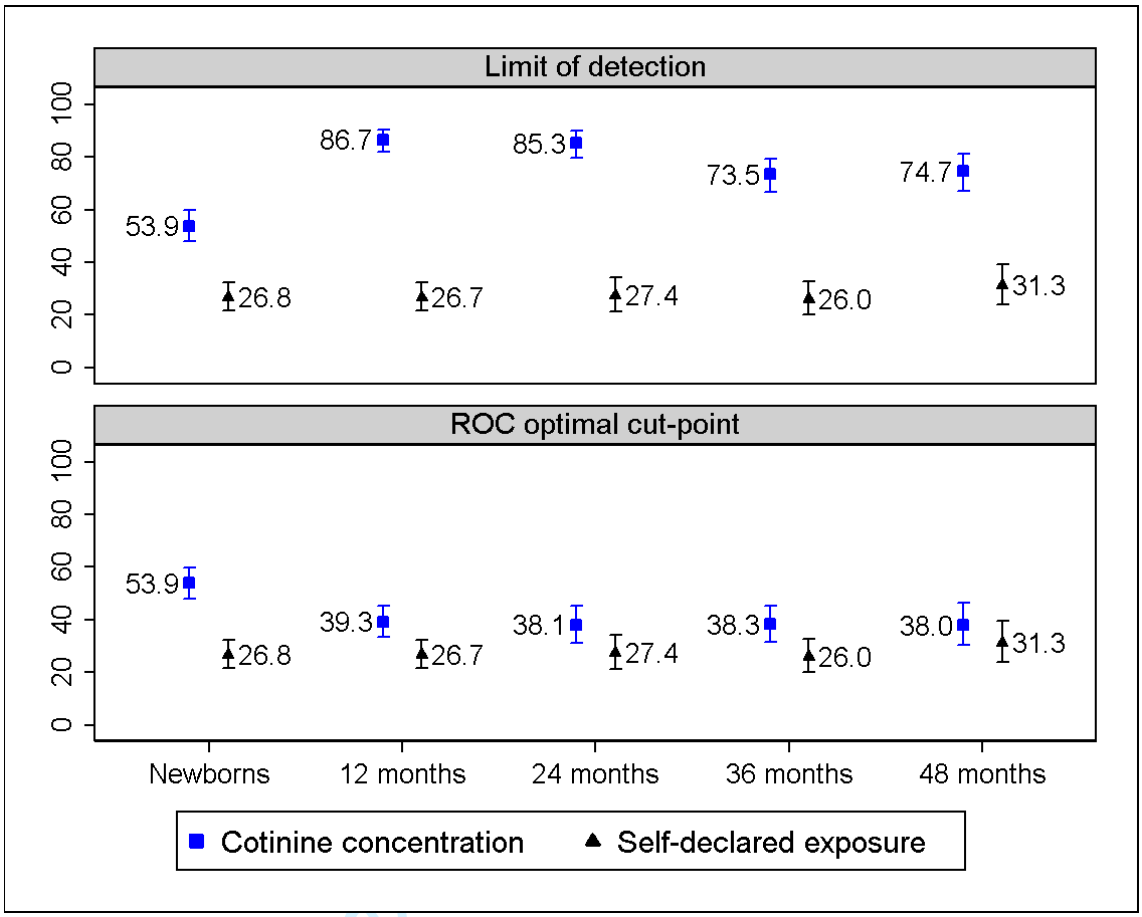
[a] Assay LOD threshold to discriminate between children exposed and unexposed to SHS: 0.015 ng/ml.

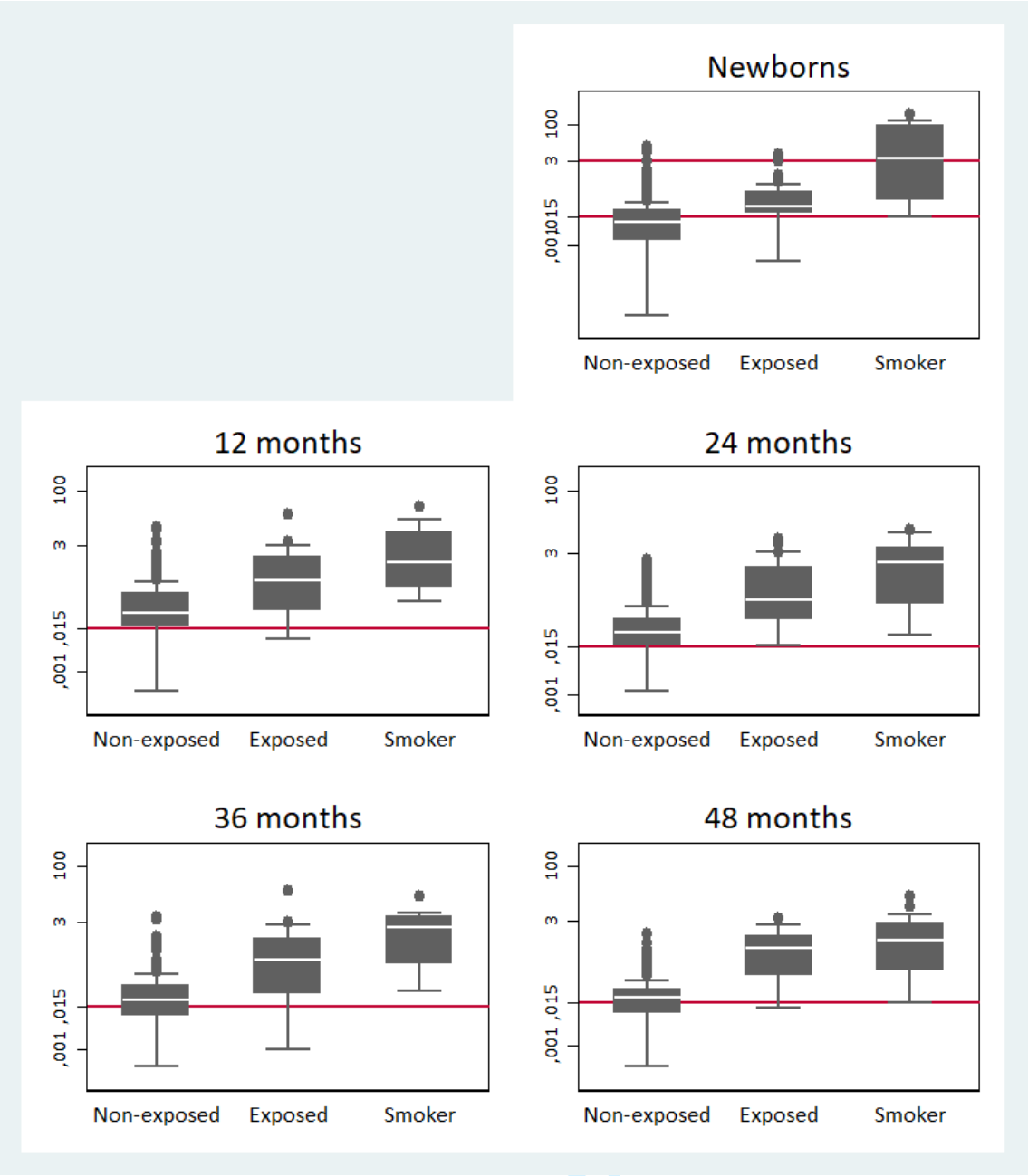
[b] Age-specific cut-points (ng/mL) to discriminate between children exposed and unexposed to SHS calculated with receiver operating characteristic curves, 12 months: 0.11; 24 months: 0.08; 36 months: 0.05; 48 months: 0.04.

FIGURE LEGENDS

Figure 1. Prevalence of SHS exposure among children is derived from maternal self-report (exposed/unexposed), depicted with a triangle, and also from serum cotinine concentrations, depicted with a square, applying assay LOD derived cut-point of 0.015 ng/ml (upper) and age-specific cut-points of 0.11 ng/ml at 12 months; 0.08 ng/ml at 24 months; 0.05 ng/ml at 36 months and 0.04 ng/ml at 48 months (bottom).

Figure 2. The box plots depict the distribution of serum cotinine concentrations (ng/ml), as logarithm, from neonatal umbilical cord (upper line=3ng/ml and bottom line=0.015 ng/ml) and child at 12, 24, 36 and 48 months (line=0.015 ng/ml) depending on children´s SHS exposure reported by mothers (unexposed/ exposed/ mother smoker). If using the LOD derived cut-point of 0.015 ng/ml to distinguish between SHS exposure/non-exposure, all the children, including those from the non-exposure category, had serum cotinine concentrations comparable to SHS exposure.




Newborns
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48 months
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Non-exposed
Exposed
Smoker

Supplementary Table 1. Baseline sociodemographic characteristics of HOME Study women at pregnancy and at 12, 24, 36 and 48 months after delivery accompanied with their children sex.

	Pregnancy	12 months	24 months	36 months	48 months
n	384(100.0)	336 (87.5)	280 (72.9)	258 (67.2)	187 (48.7)
Age group (years)[a]					
under 25	93 (24.2)	67 (19.9)	50 (17.9)	45 (17.4)	36 (19.3)
25-29	109 (28.4)	97 (28.9)	79 (28.2)	80 (31.0)	59 (31.6)
30-34	120 (31.3)	115 (34.2)	106 (37.9)	90 (34.9)	61 (32.6)
35 and over	62 (16.2)	57 (17.0)	45 (16.1)	43 (16.7)	31 (16.6)
Race/ethnicity[a]					
Non-Hispanic white	238 (62.0)	226 (67.3)	197 (70.4)	183 (70.9)	128 (68.5)
Non-Hispanic black	121 (31.5)	89 (26.5)	66 (23.6)	58 (22.5)	47 (25.1)
Other	25 (6.5)	21 (6.3)	17 (6.1)	17 (6.6)	12 (6.4)
Level of education[a]					
Less than high school/high school	95 (24.7)	67 (19.9)	50 (17.9)	49 (19.0)	34 (18.2)
Some college	98 (25.5)	85 (25.3)	66 (23.6)	60 (23.3)	49 (26.2)
College	191 (49.7)	184 (54.8)	164 (58.6)	149 (57.8)	104 (55.6)
Employment[b]					
Not working	74 (19.3)	74 (22.0)			
Working	310 (80.7)	262 (78.0)			
Living with partner					
Yes	300 (78.1)	273 (81.3)	234 (83.6)	218 (84.5)	155 (82.9)
No	84 (21.9)	63 (18.8)	46 (16.4)	40 (15.5)	32 (17.1)
Income ($/year)					
Until 25,000	102 (26.6)	69 (20.7)	48 (17.3)	47 (18.4)	37 (19.9)
Over 25,000	282 (73.4)	264 (79.3)	229 (82.7)	209 (81.6)	149 (80.1)
Health insurance[b]					
Public	102 (26.6)	87 (25.9)	59 (21.1)	57 (22.1)	
Private	272 (70.8)	245 (72.9)	216 (77.1)	198 (76.7)	
None	10 (2.6)	4 (1.2)	5 (1.8)	3 (1.2)	
Child[c]					
n	384 (100.0)	336 (87.5)	280 (72.9)	258 (67.2)	187 (48.7)
Male	178 (46.4)	151 (44.9)	129 (46.1)	119 (46.1)	78 (41.7)
Female	206 (53.7)	185 (55.1)	151 (53.9)	139 (53.9)	109 (58.3)
Child[d]					
n	280 (100.0)	270 (96.4)	197 (70.4)	196 (70.0)	150 (53.6)
Male	130 (46.4)	122 (45.2)	90 (45.7)	86 (43.9)	65 (43.3)
Female	150 (53.6)	148 (54.8)	107 (54.3)	110 (56.1)	85 (56.7)

n, number of observations.

[a] Unchanged sociodemographic characteristics obtained at baseline and further adjusted to the distribution of mothers remaining at each time period of the HOME Study.

[b] Data not available at all the time periods.

[c] Child with maternal self-reported data.

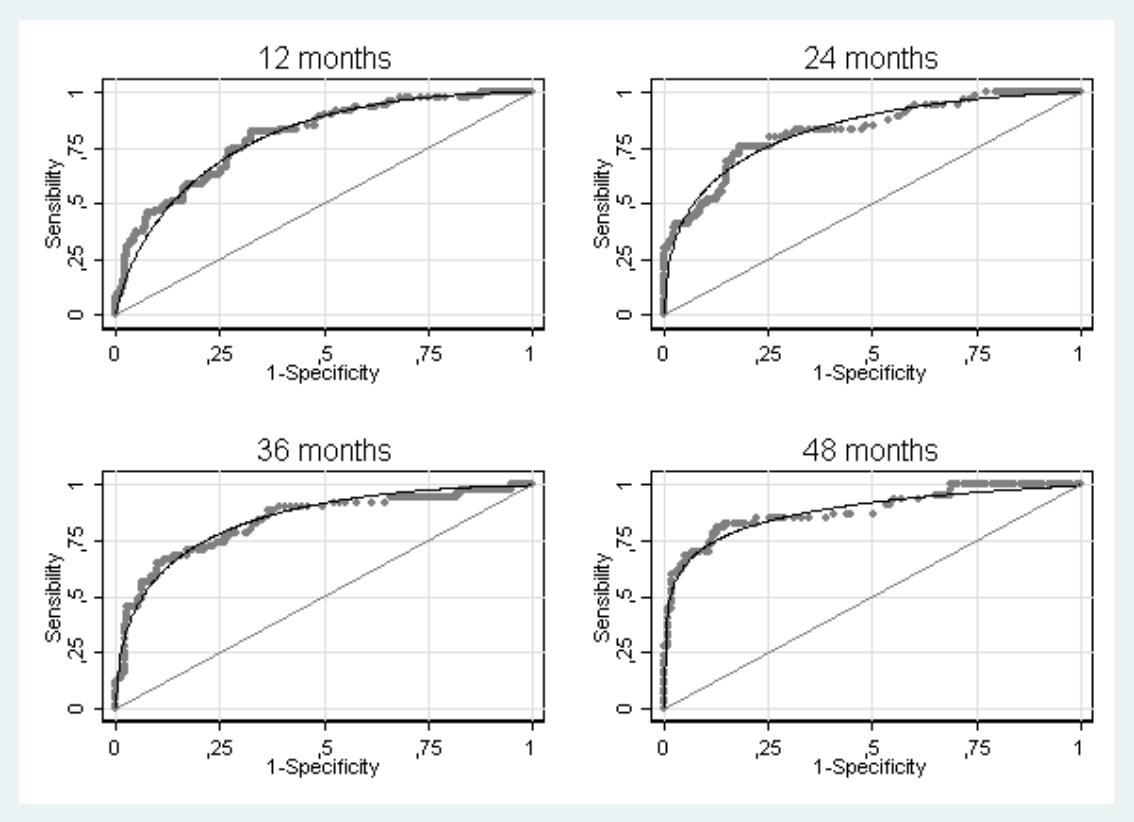
[d] Child with both maternal self-reported data and serum cotinine measures.

Supplementary Table 2. Prevalence of maternal tobacco consumption and exposure to SHS during pregnancy and at 12, 24, 36 and 48 months among participants with maternal-reported data and among participants with both maternal-reported data and cotinine measures.

	Pregnancy	12 months	24 months	36 months	48 months
Participants with self-reported data					
n	384	336	280	258	187
Mother active smoker	48 (12.5%)	35 (10.4%)	26 (9.3%)	23 (8.9%)	23 (12.3%)
Mother/Child Exposed to SHS	59 (15.4%)	55 (16.4%)	50 (17.9%)	41 (15.9%)	36 (19.3%)
Mother/Child Unexposed to SHS	277 (72.1%)	246 (73.2%)	204 (72.9%)	194 (75.2%)	128 (68.5%)
Participants with both self-reported data and cotinine measures					
n	280	270	197	196	150
Mother active smoker	34 (12.1%)	25 (9.3%)	17 (8.6%)	22 (11.2%)	21 (14.0%)
Mother/Child Exposed to SHS	41 (14.6%)	47 (17.4%)	37 (18.8%)	29 (14.8%)	26 (17.3%)
Mother/Child Unexposed to SHS	205 (73.2%)	198 (73.3%)	143 (72.6%)	145 (74.0%)	103 (68.7%)

SHS, secondhand tobacco smoke; n, number of observations.

Supplementary Figure 1. Receiver operating characteristic curves, empirical and binormal, for child´s serum cotinine concentrations and maternal-reported SHS exposure at each age.



Supplementary Figure 1. Sensitivity is represented on the y-axis and the complementary of specificity (1-specificity), which is the ratio of false positives, on the x-axis. The area under receiver operating characteristic curves was above 0.80 at all times. This value reflects the excellent diagnostic ability of the serum cotinine to classify SHS exposure among the participating children.

STROBE (Strengthening The Reporting of OBservational Studies in Epidemiology) Checklist

A checklist of items that should be included in reports of observational studies. You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

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 www.strobe-statement.org.

Section and Item	Item No.	Recommendation	Reported on Page No.
Title and Abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	
Introduction			
Background/Rationale	2	Explain the scientific background and rationale for the investigation being reported	
Objectives	3	State specific objectives, including any prespecified hypotheses	
Methods			
Study Design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	

Section and Item	Item No.	Recommendation	Reported on Page No.
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	
Bias	9	Describe any efforts to address potential sources of bias	
Study Size	10	Explain how the study size was arrived at	
Quantitative Variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical Methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
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	
		(b) Give reasons for non-participation at each stage	
		(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	
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome Data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	

Section and Item	Item No.	Recommendation	Reported on Page No.
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	
Other Analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key Results	18	Summarise key results with reference to study objectives	
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	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	
Generalisability	21	Discuss the generalisability (external validity) of the study results	
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	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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SECONDHAND TOBACCO SMOKE EXPOSURE AMONG CHILDREN UNDER 5 YEARS OLD; QUESTIONNAIRES VERSUS COTININE BIOMARKERS: A COHORT STUDY

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Primary Subject Heading:	Smoking and tobacco
Secondary Subject Heading:	Paediatrics, Public health, Epidemiology
Keywords:	EPIDEMIOLOGY, Child protection < PAEDIATRICS, PUBLIC HEALTH

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SECONDHAND TOBACCO SMOKE EXPOSURE AMONG CHILDREN UNDER 5 YEARS OLD; QUESTIONNAIRES VERSUS COTININE BIOMARKERS: A COHORT STUDY

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This paper is part of the PhD work of Nerea Mourino.

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Supplementary Tables/Figures: 3

ABSTRACT

Objectives: Cotinine is the gold standard to estimate prevalence of secondhand tobacco smoke exposure (SHS), and assay limit of detection (LOD) cut-points are typically used regardless of age. Our aim was to compare the concordance between mother-reported SHS exposure and serum cotinine categorizing children as exposed with the assay LOD or age-specific cut-points.

Design: Data from the Health Outcomes and Measures of the Environment (HOME) Study, a prospective pregnancy and birth cohort.

Setting: hospital or participant's homes.

Participants: 389 pregnant women aged 18 years and older, between 13 and 19 weeks of gestation, living in a 5 county region of the Cincinnati, OH metropolitan area and with follow-up on their children at birth and ages 12, 24, 36 and 48 months.

Primary and secondary outcome measures: Children´s serum cotinine, mother-reported active smoking and SHS exposure were available at birth and during follow-up visits. We used Cohen's Kappa index to assess concordance between maternal self-report and child´s serum cotinine concentrations. We estimated optimal age-specific cut-points, its sensitivity-specificity and positive-negative predictive values with receiver operating characteristic curves.

Results: Self-reported exposure and cotinine data were available for 280 women who gave birth to singleton child. When applying the assay LOD (0.015 ng/ml), concordance between maternal report and serum cotinine, without accounting for age, was below 0.23 at all times. When using age-specific cut-points (12 months: 0.11 ng/ml; 24 months: 0.08 ng/ml; 36 months: 0.05 ng/ml and 48 months: 0.04 ng/ml), concordance improved, being low at 12 months (0.39), moderate at 24 and 36 months (0.47 and 0.43), and high at 48 months (0.62).

Conclusions: Concordance between mother-reported SHS exposure among children under 5 years and serum cotinine improved considerably after applying the cohort- and age-specific cut-points. Future studies are necessary to verify these results.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- First study estimating serum cotinine age-specific cut-points for children under 5 years old.
- This study has one of the largest samples of children younger than 5 years to validate SHS exposure using parental report and biomarkers.
- We have longitudinal measures of SHS exposure derived from both maternal report and children's serum cotinine concentrations over the first four years of life.
- We do not include questions to evaluate third-hand smoke exposure or dietary intake.
- We use concordance as another way of validating the discriminatory capacity of the ROC curve.

BACKGROUND

Exposure to secondhand tobacco smoke (SHS), or passive smoking, is the involuntary inhalation of a complex mixture of tobacco smoke produced by the consumption of tobacco (1). SHS exposure is a global public health concern and there is no safe threshold of exposure (2). Children are especially vulnerable to the effects of SHS exposure due to their narrower airways, faster respiratory rate, and undeveloped immune system (3). The detrimental effects of SHS exposure on children's health, which have been documented since the 1970s, include an increased risk of sudden infant death syndrome, acute respiratory tract infections (bronchitis and pneumonia), asthma exacerbation, respiratory symptoms (cough, phlegm, wheeze and breathlessness) and ear infections (4). Over the last 50 years, more than 100,000 infants exposed to SHS have already died in the United States (U.S.) (5).

Parent-report SHS exposure, which is often used to estimate SHS exposure, suffers from recall bias, social desirability bias and lack of knowledge about the child's exposure in the parents' absence (6). As a result, having access to sensitive and specific biomarkers of SHS exposure, such as cotinine, is generally considered a more valid and reliable method for estimating exposure (1). Cotinine, which is the primary metabolite of nicotine, is considered the optimal biomarker for measuring SHS exposure. Cotinine has high specificity and sensitivity (7), as well as a prolonged half-life, relative to nicotine, which ranges from 16-20 hours in children (1). Cotinine can be quantified in serum, urine, hair, saliva, maternal milk, amniotic fluid and meconium (8, 9). While there are validated cotinine cut-points which can differentiate between active smokers and non-smokers among adults, such cut-points are not well established for distinguishing SHS exposure among children (10-12). Most studies use the analytical technique limit of detection (LOD) to define SHS exposure.

Various investigators who have evaluated the validity of information collected from questionnaires using cotinine as the gold standard, have found that the concordance between parental self-report and cotinine measured in child serum is inconsistent (13-16). These inconsistencies could be related to poor validity of parental self-report, age-related differences or a lack of adequate cut-points for cotinine concentrations among children (17, 18). Given that assays for cotinine are so sensitive, the values above the LOD might be derived from other sources of nicotine, such as diet (7, 19). Thus, the establishment of valid cut-points for distinguishing SHS exposure from non-exposure among children could reduce exposure misclassification.

The purpose of this study was to characterize the concordance between mother-reported SHS exposure and serum cotinine concentrations in children younger than 5 years and assess the utility of age-specific serum cotinine cut-points to characterize children's SHS exposure compared with the serum cotinine assay LOD.

METHODS

Study participants

We used data from the Health Outcomes and Measures of the Environment (HOME) Study, a prospective cohort study that enrolled pregnant women from the Cincinnati, Ohio from 2003-2006 (20). The principal objective of the HOME Study was to evaluate the association of pre- and post-natal exposure to environmental toxicants with health and neurobehavioral outcomes in infants and children. The inclusion criteria were: ≥ 18 years old, between 13 to 19 weeks of gestation, single pregnancy, residing in a house built before 1978 within the study area, HIV-negative, not taking thyroid or epilepsy medication and not undergoing chemotherapy or radiation therapy. From March 2003 to January 2006, we recruited 468 pregnant women living in a five county region of the Cincinnati, OH metropolitan area (Butler, Clermont, Hamilton, and Warren counties) and Northern Kentucky (Campbell county) to participate in a longitudinal pregnancy and birth cohort study. Sixty-seven women dropped out in pregnancy during the run-in phase of a randomized controlled trial of residential lead and injury hazard controls nested within the cohort. From 2003 to 2014, we conducted up to 11 in-person follow-up visits on 410 eligible children (390 singleton and 10 twin sets) at the delivery hospital, our study clinic, or participant's homes when children were approximately 1 day, 4 weeks, and 1, 2, 3, 4, 5, and 8 years of age; follow-up rates ranged from 94% (age 4 weeks) to 48% (age 4 years). A detailed description of the cohort is published elsewhere (20).

Patient and Public Involvement

A community advisory board provided feedback on the original design of the HOME Study before the study began. Before initiating any new follow-up, we conducted pilot testing to ensure that the visit length and types of assessments were appropriate. At more recent childhood follow-up visits, we collected information regarding the visit length and experience from participants and used this to inform the development of subsequent visits. We previously reported back concentrations of environmental chemical biomarkers to participants while also providing contextual information. Finally, we reported clinically significant findings to participants and their medical providers.

Assessment of SHS exposure

Maternal-reported tobacco consumption and SHS exposure

Maternal-reported tobacco consumption and children's SHS exposure was obtained by using standardized face-to-face interviews administered by a trained interviewer. The questionnaire was administered during pregnancy and at five different points during the follow-up: 4-weeks after birth and when children were ages 12, 24, 36 and 48 months. At each interview, trained research staff surveyed the women about their smoking of cigarettes, cigars and pipes as well as the smoking of these products by other

members of the household. Women were also asked about their SHS exposure and that of their child at home (living with a smoker who smokes at home), in other frequently visited homes, and in the car. Each mother was classified as either a smoker, exposed (non-smoker with SHS exposure), or unexposed (non-smoker with no SHS exposure). Each child was classified as exposed if the mother reported either being a smoker or living with a smoker who smokes at home or if the mother reported that her child was exposed to SHS in the car or in other homes and places (such as grandmother´s home or daycare). Otherwise, we classified children as unexposed.

Serum biomarkers of SHS exposure

We collected venous serum samples from children (umbilical cord) at delivery and at 12, 24, 36, and 48 months. The samples were stored at or below -80ºC until analysis. Serum cotinine concentrations were determined by the Centers for Disease Control and Prevention Environmental Health Laboratories using high performance liquid chromatography atmospheric pressure tandem mass spectrometry. The assay LOD threshold for cotinine was 0.015 ng/ml (19). We classified women as unexposed if the cotinine concentration from newborn´s umbilical cord blood after birth was < LOD, SHS exposed if the value was ≥ LOD but ≤ 3 ng/ml, and smokers if the cotinine concentration was > 3 ng/ml (11). Children were classified as unexposed if their cotinine concentration was < LOD and exposed if it was ≥ LOD. After calculating age-specific cut-points for children (described below), we classified them as unexposed if their cotinine concentration was lower than the new cut-points and exposed if it was equal or higher than the new cut-points.

Statistical Analysis

We calculated descriptive analysis for maternal sociodemographic characteristics, serum cotinine concentrations and SHS exposure prevalence in children using maternal-reported information and serum cotinine concentrations considering both assay LOD derived cut-point and age-specific cut-points. The concordance between maternal-reported SHS exposure and categories of serum cotinine concentrations were calculated using Cohen's Kappa index for two observers, considering 2 categories (exposed/unexposed). Correlation and agreement between children's log-base transformed serum cotinine concentrations at different moments between 12 and 48 months of age were estimated using Pearson correlation coefficients (r) and intraclass correlation coefficient (ICC).

We used receiver operating characteristic (ROC) curves to identify the optimal serum cotinine concentration to distinguish SHS exposure from non-exposure in children at 12, 24, 36 and 48 months. For this calculation, we considered several information reported by mothers regarding their children´s exposure to SHS to validate the discriminatory capacity of the ROC curve. Exposed

category comprised children whose mothers reported either being a smoker or living with a smoker who smokes at home and those whose mothers reported they were exposed in the car or in other homes and places (such as grandmother´s home or daycare). Area under ROC curves (AUC) was calculated besides age-specific cut-points. The optimal serum cotinine cut-points at each age were the concentrations at which the difference between sensitivity and specificity was minimum. Specificity-sensitivity and positive-negative predictive values (PPV-NPV) were calculated for each age-specific cut-point. Estimations were accompanied by confidence intervals of 95% (CI 95%). Analysis is restricted to children with mother-reported information and serum sample. The analysis was performed by using Stata v14.2.

RESULTS

Sample characteristics

A total of 384 women had complete data on their tobacco smoke exposure at delivery, while 336 (87.5%) children had complete self-report data at 12 months, 280 (72.9%) at 24 months, 258 (67.2%) at 36 months, and 187 (48.7%) at 48 months. At baseline, 31% of women were between ages 30-34 years, 62% were non-Hispanic white, 75% had greater than high school education, 81% were employed, 78% lived with a spouse or partner and 71% had private health insurance. The attrition rate of women from pregnancy to the 48 month-age period was 51.3%; loss to follow-up was not related to any sociodemographic characteristics (Supplementary Table 1).

We restricted the analyses to children with both maternal self-reported tobacco consumption and SHS exposure information and cotinine measures. Information was available for 280 newborns, 270 children at 12 months, 197 at 24 months, 196 at 36 months, and 150 at 48 months (Supplementary Table 2). The attrition rate of these children with both self-reported data and serum biomarkers of SHS exposure was 46.4% from delivery to age 48 months (Supplementary Table 1).

Serum cotinine distribution

Children's geometric mean (GM) serum cotinine concentrations from 12-48 months was higher than newborn´s GM umbilical cord serum concentrations (Table 1). Serial measures of children's serum cotinine concentrations from 12-48 months were highly correlated with correlation coefficients between log-transformed children´s serum cotinine concentrations in consecutive periods ranging from 0.81 (24-36 months) to 0.72 (12-36 months). The ICC between repeated serum cotinine concentrations (analysis restricted to 73 children with cotinine measures at 12, 24, 36 and 48 months) was 0.72 (CI 95% 0.63-0.80) reflecting good agreement between measurements.

Prevalence of exposure to SHS

The prevalence of children exposed to SHS based on maternal report varied between 26.8%-31.3%. (Figure 1). The prevalence of SHS exposure based on cord serum cotinine after applying the assay LOD derived cut-point of 0.015 ng/ml, was double the self-reported prevalence. The prevalence of SHS exposure based on children´s serum cotinine concentrations decreased from 86.7% at 12 months to 74.7% at 48 months (Figure 1 upper). The difference between maternal-reported prevalence of exposure and

that estimated from children´s serum cotinine concentrations, excluding newborns and using LOD as cut-point, was nearly 50 percentage points at any age.

Children whose mothers reported SHS exposure had higher serum cotinine concentrations than children whose mothers reported no exposure to SHS (Figure 2). Fifty percent of the newborns born to self-reported smokers had cord serum cotinine concentrations > 3 ng/ml. At 12 and 24 months of age, 83% and 80% of children whose mothers reported that they were not exposed had cotinine values higher than the LOD, and at 36 and 48 months of age, the percentage was 66% and 65%. Moreover, the distribution of serum cotinine concentration was similar among children whose mothers were active smokers and non-smoking mothers who reported SHS exposure (Figure 2); for this reason, these categories were combined in further analysis.

Estimation of age-specific cut-points for distinguishing SHS exposure

The AUC of various serum cotinine thresholds ranged from 0.80 and 0.89 (Supplementary Figure 1). Cut-points for distinguishing SHS exposure from non-exposure decreased with child age and were set at 0.11 ng/ml at 12 months, 0.08 ng/ml at 24, 0.05 ng/ml at 36 and at 0.04 ng/ml at 48 months (Table 2). The sensitivity and specificity corresponding to these cut-points were above 72% and NPV was over 87%. Using the optimal serum cotinine age-specific cut-points, the prevalence of SHS was highest at 12 months (39.3%) and lowest at 48 months (38.0%). The greatest difference between maternal self-reported prevalence and serum cotinine estimated prevalence of SHS exposure is nearly 13 percentage points at 12 months (Figure 1 bottom).

Concordance between self-reported exposure and serum cotinine measures

The concordance between maternal-reported SHS exposure and serum cotinine improved considerably after applying age-specific cut-points. The Kappa coefficient between mother-reported exposure and child´s serum cotinine concentrations, using the LOD as threshold, was below 0.22 in each of the four time periods. In contrast, when age-specific cut-points were used, the kappa coefficient improved from 0.39 at 12 months to 0.62 at 48 months (Table 3). Taking Landis and Koch criteria into account in assessing the Kappa index, when using the assay LOD of 0.015 ng/ml as cut-point, the concordance between maternal-reported and children´s serum cotinine concentrations after delivery was insignificant at 12, 24 and 36 months and low at 48 months. When using the new age-specific cut-points, concordance improved with age, being low at 12 months, but moderate at 24, 36 and high at 48 months.

DISCUSSION

In this cohort, pre-school aged children whose mothers reported SHS exposure had higher serum cotinine concentrations than children whose mothers reported no SHS exposure. When using the serum cotinine assay LOD as the threshold for distinguishing SHS exposure from no exposure, concordance between serum cotinine concentrations and maternal-reported exposure was non-significant. In contrast, after deriving age-specific serum cotinine cut-points, the concordance between serum cotinine and mother-reported exposure to SHS improved, with increasing concordance as child age increased.

Various studies conclude that serum cotinine concentrations in children vary as a function of age, sex, or race (2, 21, 22). With respect to age, children seem to have higher cotinine concentrations than adults at similar exposures. This could be due to metabolic differences, or to the fact that children have a faster respiration rate and inhale larger quantities of SHS contaminants than adults (23). Previous studies found differences in cotinine concentrations among children in different age groups, with higher concentrations among the youngest children (1, 2, 24). Our results are consistent with this as GM serum cotinine concentrations increased from birth to 12 months and then declined again, possibly reflecting decreased respiratory rates as children aged. Differences between cord blood and later concentrations could be explained by the higher metabolism and faster elimination of cotinine in their mothers (25). Thus, results of prior studies and developmental appropriate changes in child behavior, anatomy, and physiology support the need for age-specific cut-points for children. Indeed, when the serum cotinine assay LOD threshold was used to distinguish SHS exposure, 65-83% of children classified as unexposed by mother report were re-classified as SHS exposed. In contrast, when we used the new age-specific cut-points, only 17-27% were re-classified as SHS exposed. Our results (data not shown) show that among the children whose mothers declared that they were not smokers at the 4 follow-up periods and that the children were not exposed to SHS, the cotinine concentration decreased as age increased. This decrease is unlikely to be due to misclassification of self-reported SHS by the mother.

Other investigators have concluded that the prevalence of SHS exposure obtained from maternal-report consistently underestimates actual exposure. Presumably, some of this estimate is because mothers might not report SHS exposure because of recall bias, social desirability bias or ignorance about their children's exposure in other settings (12, 21). Children's exposure may be so negligible that mothers are not able to identify or quantify it (6). Our results indicate that some of the discordance was because of failure to account for age-related differences in exposure or metabolism.

Based on cotinine derived from the LOD threshold, the prevalence of SHS exposure was 73.5% and 74.7% at 3 and 4 years-old, respectively. Taking into account the estimates obtained in population studies, about 4 out of 10 U.S. children aged 3–11 years

(40.6%) are exposed to secondhand smoke (2). These large differences, however, fail to assess the influence of other factors such as the sensitivity and suitability of the cut-points used to classify exposure to SHS. The assay LOD has become much more sensitive over time. Earlier studies set it at 0.05 ng/ml (4, 12, 17), while more recent studies, including ours, have a LOD of 0.015 ng/ml. This lower LOD could mean that low serum cotinine concentrations reflect transient SHS exposure or exposure from other sources, such as food (tomatoes, potatoes, cauliflower and black tea). Food consumption levels of dietary nicotine are insignificant compared with moderate SHS exposure, but consumption of high quantities of nicotine-containing foodstuffs might contribute to low-level elevations in serum cotinine (e.g. 80 g of eggplant is equivalent to approximately 0.01 ng/ml of serum cotinine) (7). Future studies could verify this hypothesis using studies with detailed dietary information and sensitive cotinine biomarkers.

Despite the improvement in the concordance between maternal-reported SHS and children's serum cotinine concentrations using the age-specific cut-points, misclassification is still a problem. This misclassification has been observed in prior studies, including those of pregnant women and their children younger than 5 (26-28) and it could be due to maternal concealment to avoid social judgment or ignorance about negligible and transient low level nicotine exposures quantified with sensitive cut-points.

This study has some limitations worth nothing. First, we had a modest sample size. Still, it was one of the largest samples of children younger than 5 years to validate SHS exposure using parental report and biomarkers (12, 27, 29, 30). Another limitation is the attrition of study participants. Yet loss to follow-up was not associated with any measured sociodemographic characteristic. Third, we did not include questions to evaluate third-hand smoke exposure, such as involuntary inhalation or cutaneous absorption of nicotine particles deposited on clothing and furniture, or dietary intake. Fourth, we lacked information about the duration of exposure to SHS, which could have been used to provide more valid and reliable cotinine thresholds, and we did not account for factors such as the size of the home, the intensity of exposure, or the proximity to smokers (7, 31, 32). Fifth, to identify the optimal serum cotinine cut-points we assumed maternal-report was the gold standard. We note that we expect any misreporting to predominately affect the sensitivity of maternal report and not the specificity; few women would report exposure in its absence. It should be noted that maternal self-report does not refer solely to whether the mother declares that the child is exposed, but it also takes into consideration if the mother is a smoker and if the child lives with other smokers who smoke at home. Also, while different methods are available to identify the optimal cotinine cut-points, we have chosen the one that minimizes the difference between sensitivity and specificity since our objective was to minimize misclassification of exposed or unexposed based on cotinine concentration; the differences between the methods were negligible. Finally, our findings may not be generalizable to other populations as our eligibility criteria were not designed to ensure that our cohort was representative of births in the study region. However, most chemical biomarker concentrations

among HOME Study participants are similar to pregnant women and children in the USA during the time of enrolment and follow-up (20).

This study also has several strengths. First, we had longitudinal measures of SHS exposure derived from both maternal report and children's serum cotinine concentrations over the first four years of life. Second, our cohort was relatively higher SES, with 75% of the mothers having greater than high school education. Thus, misreporting of SHS exposure is likely reduced as previous studies have shown that higher educational level is associated with more accurate SHS exposure reporting (15).

CONCLUSIONS

Previous investigators have concluded that maternal reports dramatically underestimate children's SHS exposure. When we used age-specific cut-points, we found that many fewer children were re-classified as SHS exposed. Thus, maternal report may be a better indicator of children's SHS than previous estimates. The age specific cut-points should be validated in other cohorts.

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FOOTNOTES

- **Contributors:** MPR, MIS and JB planned the study and supervise it with contribution from all the other authors. MPR and MIS performed the analysis with substantive contribution from all the other authors, MPR and NM wrote the first draft of the manuscript and MIS, JB, BP and KY provided critical comments and contributed on the drafts to successive versions of the manuscript. All the authors approved the final version paper. MPR is the guarantor.

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- **Competing interests:** JMB received an honoraria from Quest Diagnostic for serving on an expert panel related to endocrine disrupting chemicals. JMB's institution was financially compensated for his services as an expert witness for plaintiffs in litigation related to PFAS-contaminated drinking water; these funds were not paid to JMB directly. The other authors declare no competing interests.

- **Patient consent for publication:** not required.

- **Ethics approval:** The institutional review boards of Cincinnati Children's Hospital Medical Center, the participating delivery hospitals, and the CDC approved this study. During face-to-face visit, research assistants explained study protocols to each prospective participant and completed a checklist to ensure women were fully informed about the study. All mothers provided written informed consent for both themselves and their children prior to enrolment. ID of approval: 01-8-5.

- **Provenance and peer review:** Not commissioned; externally peer reviewed.

- **Data availability statement:** data are available upon reasonable request.

The HOME Study Principal Investigators welcome new collaborations with other

investigators and have actively engaged in collaborative data sharing projects.

Interested investigators should contact Drs Joseph M. Braun (joseph_ braun_ 1@brown. edu) and Kimberly Yolton (kimberly. yolton@ cchmc. org) to obtain additional information about The HOME Study, discuss collaborative opportunities,

and request a project proposal form. The HOME Study Protocol Review Committee reviews proposed research projects to ensure that they do not overlap with extant projects and are an efficient use of scarce resources (eg, biospecimens).

- Word count: 3,319.

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TABLES

Table 1. Descriptive statistics of serum cotinine concentrations (ng/ml) at birth and at 12, 24, 36 and 48 months: n, range, quartiles and geometric mean with CI 95%.

			Quartiles			Geometric mean		
	n	Range	P25	P50	P75	Mean	95% CI	
Newborn´s cotinine [a]	280	0.00000 - 261.0	0.003	0.017	0.088	0.022	0.015	0.032
Child´s cotinine [a]								
12 months	270	0.00030 - 35.3	0.023	0.063	0.357	0.093	0.073	0.118
24 months	197	0.00126 - 10.5	0.020	0.046	0.212	0.070	0.053	0.092
36 months	196	0.00032 - 21.6	0.012	0.033	0.199	0.046	0.034	0.064
48 months	150	0.00024 - 14.9	0.013	0.027	0.249	0.047	0.033	0.067

ng/ml, nanogram/milliliter; n, number of observations; CI, confidence interval; P, percentile.

[a] Analysis is restricted to participants with both maternal-reported data and cotinine measures.

Table 2. AUC, new age-specific cut-points for each age (ng/ml) with its sensitivity, specificity, PPV and NPV. Estimations are accompanied by 95% CI.

	12 months	24 months	36 months	48 months
n	270	197	196	150
Exposed to SHS	72 (26.7%)	54 (27.4%)	51 (26.0%)	47 (31.3%)
AUC (95% CI)	0.80 (0.74 - 0.86)	0.83 (0.76 - 0.90)	0.84 (0.77 - 0.91)	0.89 (0.82 - 0.95)
Cut-points (ng/ml)[a]	0.11	0.08	0.05	0.04
Sensitivity (95% CI)	72.20 (60.40 - 82.10)	75.90 (62.40 - 86.50)	74.50 (60.40 - 85.70)	83.00 (69.20 - 92.40)
Specificity (95% CI)	72.70 (66.00 - 78.80)	76.20 (68.40 - 82.90)	74.50 (66.60 - 81.40)	82.50 (73.80 - 89.30)
PPV (95% CI)	49.10 (39.20 - 59.00)	54.70 (42.70 - 66.20)	50.70 (38.90 - 62.40)	68.40 (54.80 - 80.10)
NPV (95% CI)	87.80 (81.80 - 92.40)	89.30 (82.50 - 94.20)	89.30 (82.30 - 94.20)	91.40 (83.80 - 96.20)

AUC, area under receiver operating characteristic curves; ng/ml, nanogram/milliliter; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; SHS, secondhand tobacco smoke.

[a] Age-specific cut-point values are those that maximized the AUC, that is to say, those which minimize the difference between sensitivity and specificity . These values were calculated with receiver operating characteristic curves and children´s SHS exposure reported by their mothers was considered the gold standard. Children´s serum cotinine concentrations above these cut-point values will reflect SHS exposure.

Table 3. Kappa concordance coefficient between maternal-reported SHS exposure (exposed/ unexposed) and child´s serum cotinine concentrations accompanied with the percentage of agreement when using the assay LOD threshold and age-specific cut-points at 12, 24, 36 and 48 months of age.

	12 months	24 months	36 months	48 months
Assay LOD threshold[a]				
Agreement (%)	38.52	42.13	49.49	54.00
Kappa (95% CI)	0.08 (0.04 - 0.13)	0.12 (0.07 - 0.17)	0.18 (0.10 - 0.25)	0.22 (0.13 - 0.32)
Age-specific cut-points[b]				
Agreement (%)	72.59	76.14	74.49	82.67
Kappa (95% CI)	0.39 (0.28 - 0.50)	0.47 (0.34 - 0.59)	0.43 (0.30 - 0.56)	0.62 (0.49 - 0.75)

LOD, limit of detection; CI, confidence interval.

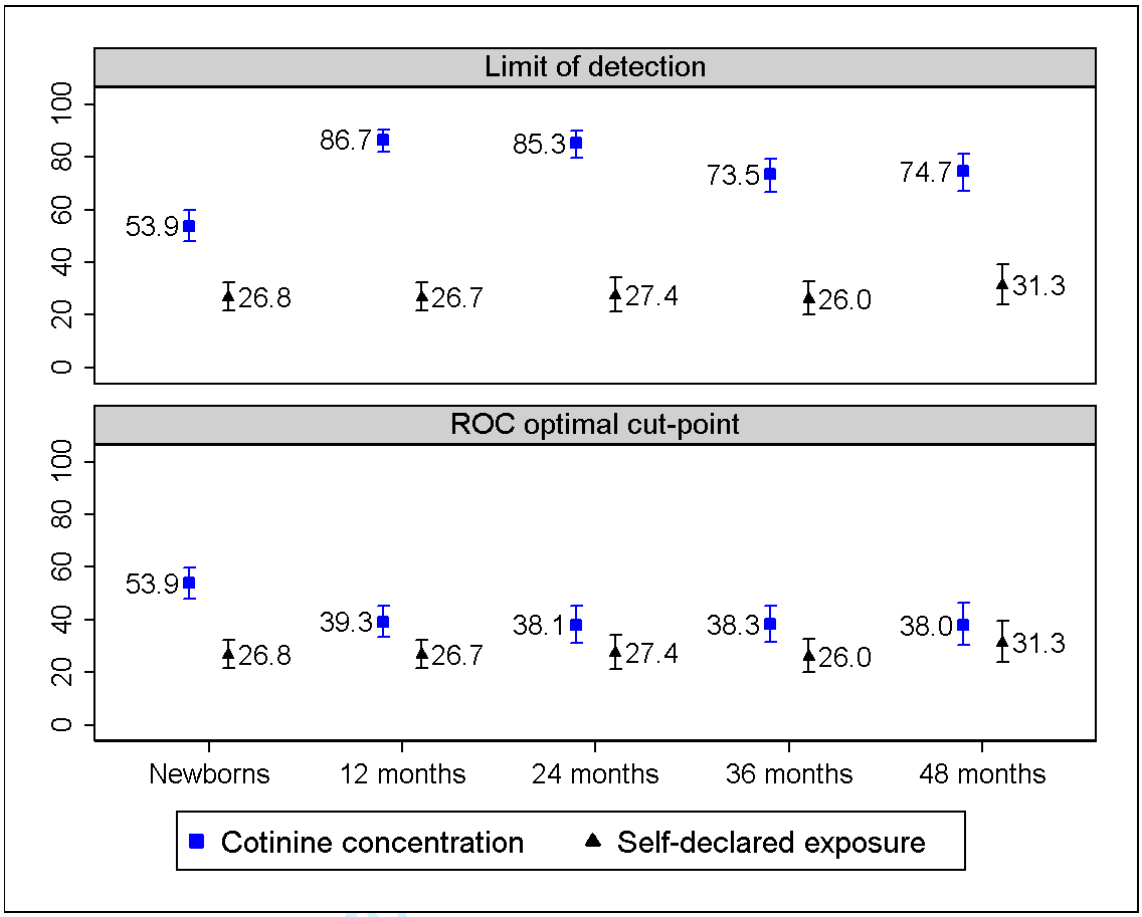
[a] Assay LOD threshold to discriminate between children exposed and unexposed to SHS: 0.015 ng/ml.

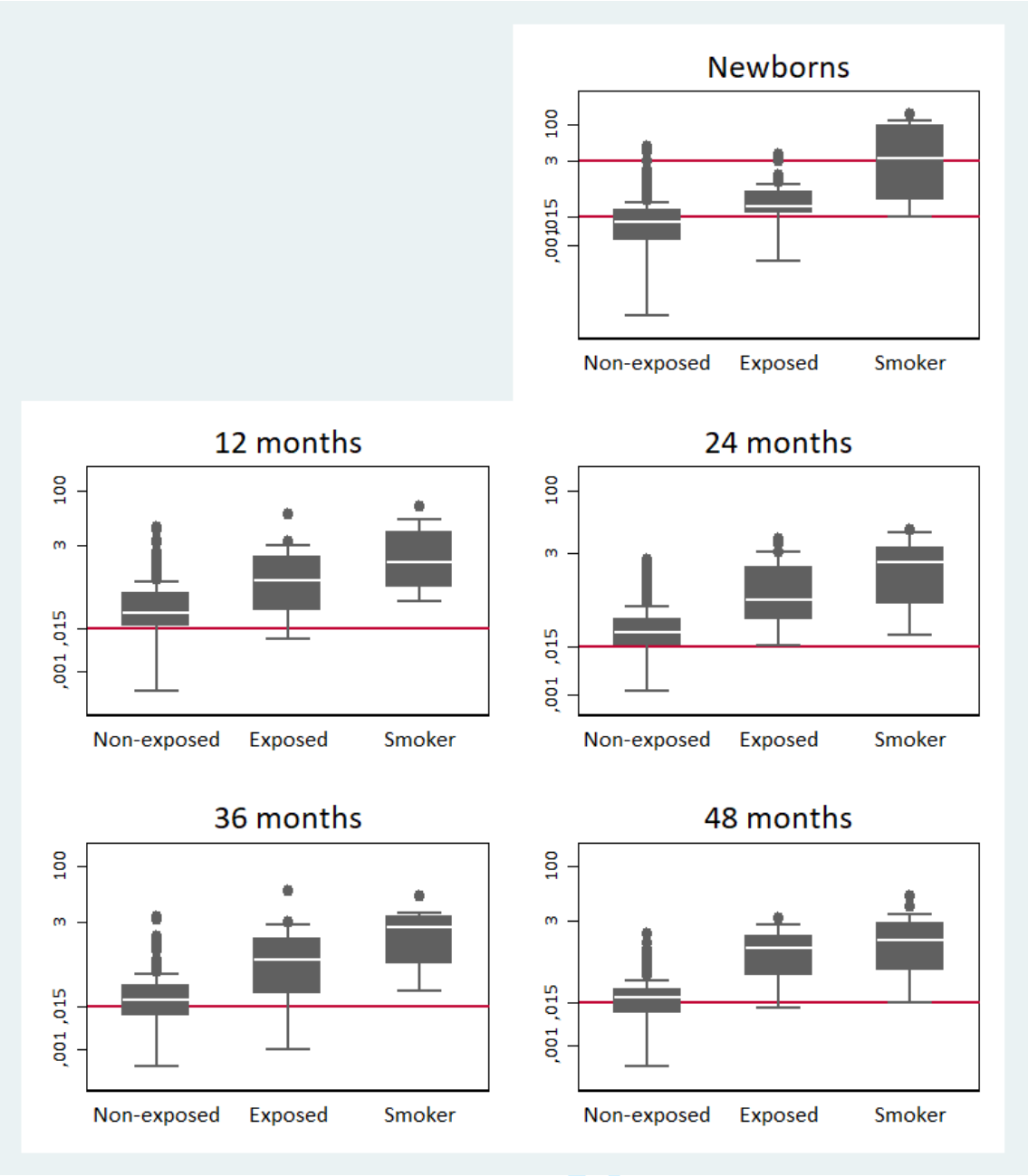
[b] Age-specific cut-points (ng/mL) to discriminate between children exposed and unexposed to SHS calculated with receiver operating characteristic curves, 12 months: 0.11; 24 months: 0.08; 36 months: 0.05; 48 months: 0.04.

FIGURE LEGENDS

Figure 1. Prevalence of SHS exposure among children is derived from maternal self-report (exposed/unexposed), depicted with a triangle, and also from serum cotinine concentrations, depicted with a square, applying assay LOD derived cut-point of 0.015 ng/ml (upper) and age-specific cut-points of 0.11 ng/ml at 12 months; 0.08 ng/ml at 24 months; 0.05 ng/ml at 36 months and 0.04 ng/ml at 48 months (bottom).

Figure 2. The box plots depict the distribution of serum cotinine concentrations (ng/ml), as logarithm, from neonatal umbilical cord (upper line=3ng/ml and bottom line=0.015 ng/ml) and child at 12, 24, 36 and 48 months (line=0.015 ng/ml) depending on children´s SHS exposure reported by mothers (unexposed/ exposed/ mother smoker). If using the LOD derived cut-point of 0.015 ng/ml to distinguish between SHS exposure/non-exposure, all the children, including those from the non-exposure category, had serum cotinine concentrations comparable to SHS exposure.

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Supplementary Table 1. Baseline sociodemographic characteristics of HOME Study women at pregnancy and at 12, 24, 36 and 48 months after delivery accompanied with their children sex.

	Pregnancy	12 months	24 months	36 months	48 months
n	384(100.0)	336 (87.5)	280 (72.9)	258 (67.2)	187 (48.7)
Age group (years)[a]					
under 25	93 (24.2)	67 (19.9)	50 (17.9)	45 (17.4)	36 (19.3)
25-29	109 (28.4)	97 (28.9)	79 (28.2)	80 (31.0)	59 (31.6)
30-34	120 (31.3)	115 (34.2)	106 (37.9)	90 (34.9)	61 (32.6)
35 and over	62 (16.2)	57 (17.0)	45 (16.1)	43 (16.7)	31 (16.6)
Race/ethnicity[a]					
Non-Hispanic white	238 (62.0)	226 (67.3)	197 (70.4)	183 (70.9)	128 (68.5)
Non-Hispanic black	121 (31.5)	89 (26.5)	66 (23.6)	58 (22.5)	47 (25.1)
Other	25 (6.5)	21 (6.3)	17 (6.1)	17 (6.6)	12 (6.4)
Level of education[a]					
Less than high school/high school	95 (24.7)	67 (19.9)	50 (17.9)	49 (19.0)	34 (18.2)
Some college	98 (25.5)	85 (25.3)	66 (23.6)	60 (23.3)	49 (26.2)
College	191 (49.7)	184 (54.8)	164 (58.6)	149 (57.8)	104 (55.6)
Employment[b]					
Not working	74 (19.3)	74 (22.0)			
Working	310 (80.7)	262 (78.0)			
Living with partner					
Yes	300 (78.1)	273 (81.3)	234 (83.6)	218 (84.5)	155 (82.9)
No	84 (21.9)	63 (18.8)	46 (16.4)	40 (15.5)	32 (17.1)
Income ($/year)					
Until 25,000	102 (26.6)	69 (20.7)	48 (17.3)	47 (18.4)	37 (19.9)
Over 25,000	282 (73.4)	264 (79.3)	229 (82.7)	209 (81.6)	149 (80.1)
Health insurance[b]					
Public	102 (26.6)	87 (25.9)	59 (21.1)	57 (22.1)	
Private	272 (70.8)	245 (72.9)	216 (77.1)	198 (76.7)	
None	10 (2.6)	4 (1.2)	5 (1.8)	3 (1.2)	
Child[c]					
n	384 (100.0)	336 (87.5)	280 (72.9)	258 (67.2)	187 (48.7)
Male	178 (46.4)	151 (44.9)	129 (46.1)	119 (46.1)	78 (41.7)
Female	206 (53.7)	185 (55.1)	151 (53.9)	139 (53.9)	109 (58.3)
Child[d]					
n	280 (100.0)	270 (96.4)	197 (70.4)	196 (70.0)	150 (53.6)
Male	130 (46.4)	122 (45.2)	90 (45.7)	86 (43.9)	65 (43.3)
Female	150 (53.6)	148 (54.8)	107 (54.3)	110 (56.1)	85 (56.7)

n, number of observations.

[a] Unchanged sociodemographic characteristics obtained at baseline and further adjusted to the distribution of mothers remaining at each time period of the HOME Study.

[b] Data not available at all the time periods.

[c] Child with maternal self-reported data.

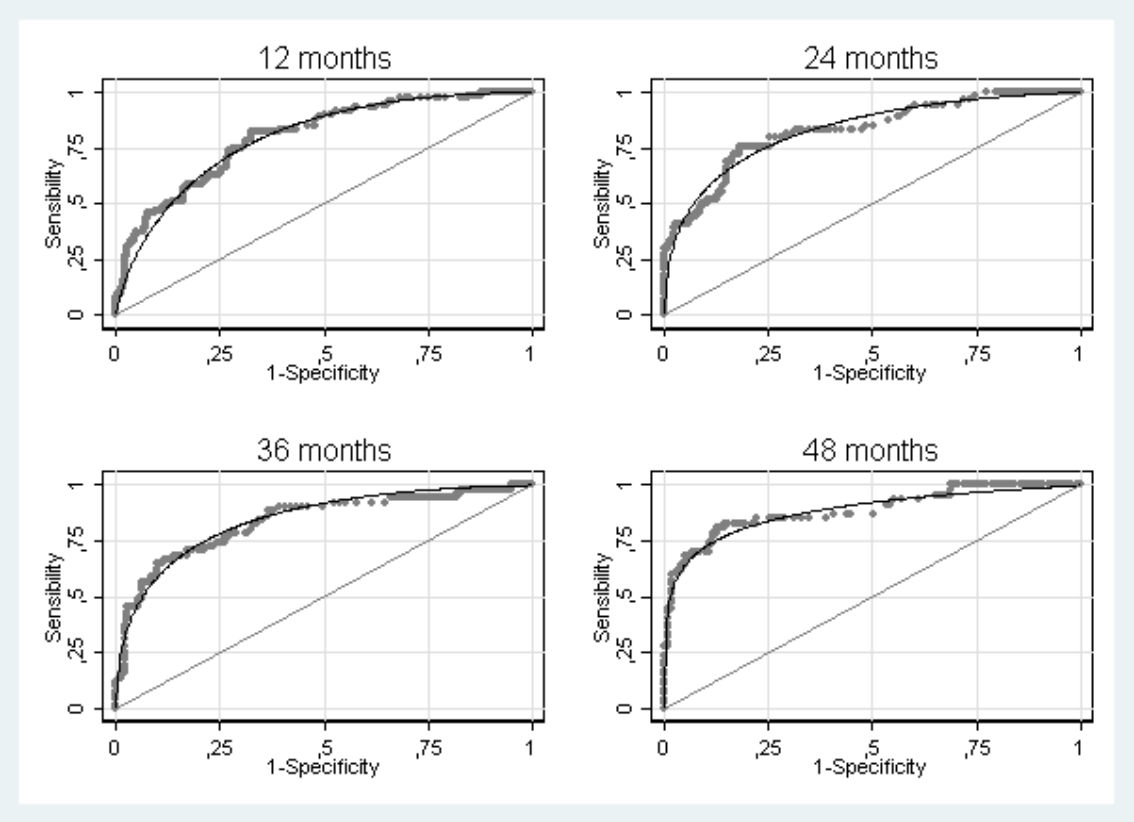
[d] Child with both maternal self-reported data and serum cotinine measures.

Supplementary Table 2. Prevalence of maternal tobacco consumption and exposure to SHS during pregnancy and at 12, 24, 36 and 48 months among participants with maternal-reported data and among participants with both maternal-reported data and cotinine measures.

	Pregnancy	12 months	24 months	36 months	48 months
Participants with self-reported data					
n	384	336	280	258	187
Mother active smoker	48 (12.5%)	35 (10.4%)	26 (9.3%)	23 (8.9%)	23 (12.3%)
Mother/Child Exposed to SHS	59 (15.4%)	55 (16.4%)	50 (17.9%)	41 (15.9%)	36 (19.3%)
Mother/Child Unexposed to SHS	277 (72.1%)	246 (73.2%)	204 (72.9%)	194 (75.2%)	128 (68.5%)
Participants with both self-reported data and cotinine measures					
n	280	270	197	196	150
Mother active smoker	34 (12.1%)	25 (9.3%)	17 (8.6%)	22 (11.2%)	21 (14.0%)
Mother/Child Exposed to SHS	41 (14.6%)	47 (17.4%)	37 (18.8%)	29 (14.8%)	26 (17.3%)
Mother/Child Unexposed to SHS	205 (73.2%)	198 (73.3%)	143 (72.6%)	145 (74.0%)	103 (68.7%)

SHS, secondhand tobacco smoke; n, number of observations.

Supplementary Figure 1. Receiver operating characteristic curves, empirical and binormal, for child´s serum cotinine concentrations and maternal-reported SHS exposure at each age.



Supplementary Figure 1. Sensitivity is represented on the y-axis and the complementary of specificity (1-specificity), which is the ratio of false positives, on the x-axis. The area under receiver operating characteristic curves was above 0.80 at all times. This value reflects the excellent diagnostic ability of the serum cotinine to classify SHS exposure among the participating children.

STROBE (Strengthening The Reporting of OBservational Studies in Epidemiology) Checklist

A checklist of items that should be included in reports of observational studies. You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

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 www.strobe-statement.org.

Section and Item	Item No.	Recommendation	Reported on Page No.
Title and Abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	
Introduction			
Background/Rationale	2	Explain the scientific background and rationale for the investigation being reported	
Objectives	3	State specific objectives, including any prespecified hypotheses	
Methods			
Study Design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	

Section and Item	Item No.	Recommendation	Reported on Page No.
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	
Bias	9	Describe any efforts to address potential sources of bias	
Study Size	10	Explain how the study size was arrived at	
Quantitative Variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical Methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
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	
		(b) Give reasons for non-participation at each stage	
		(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	
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome Data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	

Section and Item	Item No.	Recommendation	Reported on Page No.
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	
Other Analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key Results	18	Summarise key results with reference to study objectives	
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	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	
Generalisability	21	Discuss the generalisability (external validity) of the study results	
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	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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SECONDHAND TOBACCO SMOKE EXPOSURE AMONG CHILDREN UNDER 5 YEARS OLD; QUESTIONNAIRES VERSUS COTININE BIOMARKERS: A COHORT STUDY

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SECONDHAND TOBACCO SMOKE EXPOSURE AMONG CHILDREN UNDER 5 YEARS OLD; QUESTIONNAIRES VERSUS COTININE BIOMARKERS: A COHORT STUDY

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Supplementary Tables/Figures: 3

ABSTRACT

Objectives: Cotinine is the gold standard to estimate prevalence of secondhand tobacco smoke exposure (SHS), and assay limit of detection (LOD) cut-points are typically used regardless of age. Our aim was to compare the concordance between mother-reported SHS exposure and serum cotinine categorizing children as exposed with the assay LOD or age-specific cut-points.

Design: Data from the Health Outcomes and Measures of the Environment (HOME) Study, a prospective pregnancy and birth cohort.

Setting: hospital or participant's homes.

Participants: 389 pregnant women aged 18 years and older, between 13 and 19 weeks of gestation, living in a 5 county region of the Cincinnati, OH metropolitan area and with follow-up on their children at birth and ages 12, 24, 36 and 48 months.

Primary and secondary outcome measures: Children´s serum cotinine, mother-reported active smoking and SHS exposure were available at birth and during follow-up visits. We used Cohen's Kappa index to assess concordance between maternal self-report and child´s serum cotinine concentrations. We estimated optimal age-specific cut-points, its sensitivity-specificity and positive-negative predictive values with receiver operating characteristic curves.

Results: Self-reported exposure and cotinine data were available for 280 women who gave birth to singleton child. When applying the assay LOD (0.015 ng/ml), concordance between maternal report and serum cotinine, without accounting for age, was below 0.23 at all times. When using age-specific cut-points (12 months: 0.11 ng/ml; 24 months: 0.08 ng/ml; 36 months: 0.05 ng/ml and 48 months: 0.04 ng/ml), concordance improved, being low at 12 months (0.39), moderate at 24 and 36 months (0.47 and 0.43), and high at 48 months (0.62).

Conclusions: Concordance between mother-reported SHS exposure among children under 5 years and serum cotinine improved considerably after applying the cohort- and age-specific cut-points. Future studies are necessary to verify these results.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- First study estimating serum cotinine age-specific cut-points for children under 5 years old.
- This study has one of the largest samples of children younger than 5 years to validate SHS exposure using parental report and biomarkers.
- We have longitudinal measures of SHS exposure derived from both maternal report and children's serum cotinine concentrations over the first four years of life.
- We do not include questions to evaluate third-hand smoke exposure or dietary intake.
- We use concordance as another way of validating the discriminatory capacity of the ROC curve.

BACKGROUND

Exposure to secondhand tobacco smoke (SHS), or passive smoking, is the involuntary inhalation of a complex mixture of tobacco smoke produced by the consumption of tobacco (1). SHS exposure is a global public health concern and there is no safe threshold of exposure (2). Children are especially vulnerable to the effects of SHS exposure due to their narrower airways, faster respiratory rate, and undeveloped immune system (3). The detrimental effects of SHS exposure on children's health, which have been documented since the 1970s, include an increased risk of sudden infant death syndrome, acute respiratory tract infections (bronchitis and pneumonia), asthma exacerbation, respiratory symptoms (cough, phlegm, wheeze and breathlessness) and ear infections (4). Over the last 50 years, more than 100,000 infants exposed to SHS have already died in the United States (U.S.) (5).

Parent-report SHS exposure, which is often used to estimate SHS exposure, suffers from recall bias, social desirability bias and lack of knowledge about the child's exposure in the parents' absence (6). As a result, having access to sensitive and specific biomarkers of SHS exposure, such as cotinine, is generally considered a more valid and reliable method for estimating exposure (1). Cotinine, which is the primary metabolite of nicotine, is considered the optimal biomarker for measuring SHS exposure. Cotinine has high specificity and sensitivity (7), as well as a prolonged half-life, relative to nicotine, which ranges from 16-20 hours in children (1). Cotinine can be quantified in serum, urine, hair, saliva, maternal milk, amniotic fluid and meconium (8, 9). While there are validated cotinine cut-points which can differentiate between active smokers and non-smokers among adults, such cut-points are not well established for distinguishing SHS exposure among children (10-12). Most studies use the analytical technique limit of detection (LOD) to define SHS exposure.

Various investigators who have evaluated the validity of information collected from questionnaires using cotinine as the gold standard, have found that the concordance between parental self-report and cotinine measured in child serum is inconsistent (13-16). These inconsistencies could be related to poor validity of parental self-report, age-related differences or a lack of adequate cut-points for cotinine concentrations among children (17, 18). Given that assays for cotinine are so sensitive, the values above the LOD might be derived from other sources of nicotine, such as diet (7, 19). Thus, the establishment of valid cut-points for distinguishing SHS exposure from non-exposure among children could reduce exposure misclassification.

The purpose of this study was to characterize the concordance between mother-reported SHS exposure and serum cotinine concentrations in children younger than 5 years and assess the utility of age-specific serum cotinine cut-points to characterize children's SHS exposure compared with the serum cotinine assay LOD.

METHODS

Study participants

We used data from the Health Outcomes and Measures of the Environment (HOME) Study, a prospective cohort study that enrolled pregnant women from the Cincinnati, Ohio from 2003-2006 (20). The principal objective of the HOME Study was to evaluate the association of pre- and post-natal exposure to environmental toxicants with health and neurobehavioral outcomes in infants and children. The inclusion criteria were: ≥ 18 years old, between 13 to 19 weeks of gestation, single pregnancy, residing in a house built before 1978 within the study area, HIV-negative, not taking thyroid or epilepsy medication and not undergoing chemotherapy or radiation therapy. From March 2003 to January 2006, we recruited 468 pregnant women living in a five county region of the Cincinnati, OH metropolitan area (Butler, Clermont, Hamilton, and Warren counties) and Northern Kentucky (Campbell county) to participate in a longitudinal pregnancy and birth cohort study. Sixty-seven women dropped out in pregnancy during the run-in phase of a randomized controlled trial of residential lead and injury hazard controls nested within the cohort. From 2003 to 2014, we conducted up to 11 in-person follow-up visits on 410 eligible children (390 singleton and 10 twin sets) at the delivery hospital, our study clinic, or participant's homes when children were approximately 1 day, 4 weeks, and 1, 2, 3, 4, 5, and 8 years of age; follow-up rates ranged from 94% (age 4 weeks) to 48% (age 4 years). A detailed description of the cohort is published elsewhere (20).

Patient and Public Involvement

A community advisory board provided feedback on the original design of the HOME Study before the study began. Before initiating any new follow-up, we conducted pilot testing to ensure that the visit length and types of assessments were appropriate. At more recent childhood follow-up visits, we collected information regarding the visit length and experience from participants and used this to inform the development of subsequent visits. We previously reported back concentrations of environmental chemical biomarkers to participants while also providing contextual information. Finally, we reported clinically significant findings to participants and their medical providers.

Assessment of SHS exposure

Maternal-reported tobacco consumption and SHS exposure

Maternal-reported tobacco consumption and children's SHS exposure was obtained by using standardized face-to-face interviews administered by a trained interviewer. The questionnaire was administered during pregnancy and at five different points during the follow-up: 4-weeks after birth and when children were ages 12, 24, 36 and 48 months. At each interview, trained research staff surveyed the women about their smoking of cigarettes, cigars and pipes as well as the smoking of these products by other

members of the household. Women were also asked about their SHS exposure and that of their child at home (living with a smoker who smokes at home), in other frequently visited homes, and in the car. Each mother was classified as either a smoker, exposed (non-smoker with SHS exposure), or unexposed (non-smoker with no SHS exposure). Each child was classified as exposed if the mother reported either being a smoker or living with a smoker who smokes at home or if the mother reported that her child was exposed to SHS in the car or in other homes and places (such as grandmother´s home or daycare). Otherwise, we classified children as unexposed.

Serum biomarkers of SHS exposure

We collected venous serum samples from children (umbilical cord) at delivery and at 12, 24, 36, and 48 months. The samples were stored at or below -80ºC until analysis. Serum cotinine concentrations were determined by the Centers for Disease Control and Prevention Environmental Health Laboratories using high performance liquid chromatography atmospheric pressure tandem mass spectrometry. The assay LOD threshold for cotinine was 0.015 ng/ml (19). We classified women as unexposed if the cotinine concentration from newborn´s umbilical cord blood after birth was < LOD, SHS exposed if the value was ≥ LOD but ≤ 3 ng/ml, and smokers if the cotinine concentration was > 3 ng/ml (11). Children were classified as unexposed if their cotinine concentration was < LOD and exposed if it was ≥ LOD. After calculating age-specific cut-points for children (described below), we classified them as unexposed if their cotinine concentration was lower than the new cut-points and exposed if it was equal or higher than the new cut-points.

Statistical Analysis

We calculated descriptive analysis for maternal sociodemographic characteristics, serum cotinine concentrations and SHS exposure prevalence in children using maternal-reported information and serum cotinine concentrations considering both assay LOD derived cut-point and age-specific cut-points. The concordance between maternal-reported SHS exposure and categories of serum cotinine concentrations were calculated using Cohen's Kappa index for two observers, considering 2 categories (exposed/unexposed). Correlation and agreement between children's log-base transformed serum cotinine concentrations at different moments between 12 and 48 months of age were estimated using Pearson correlation coefficients (r) and intraclass correlation coefficient (ICC).

We used receiver operating characteristic (ROC) curves to identify the optimal serum cotinine concentration to distinguish SHS exposure from non-exposure in children at 12, 24, 36 and 48 months. For this calculation, we considered several information reported by mothers regarding their children´s exposure to SHS to validate the discriminatory capacity of the ROC curve. Exposed

category comprised children whose mothers reported either being a smoker or living with a smoker who smokes at home and those whose mothers reported they were exposed in the car or in other homes and places (such as grandmother´s home or daycare). Area under ROC curves (AUC) was calculated besides age-specific cut-points. There are various methods to optimize cotinine cut-points. We tested the three criteria most often used in biostatistics: maximizing the Youden index, the identification of the point on the curve with minimum distance from the left-upper corner of the unit square, and minimizing the difference between sensitivity and specificity; while the three methods provided similar results, we chose the third approach to be able to identify the optimal age-specific cut-point which maximizes both sensitivity and specificity, to minimize misclassification either as exposed or unexposed based on cotinine concentrations. Specificity-sensitivity and positive-negative predictive values (PPV-NPV) were calculated for each age-specific cut-point. Estimations were accompanied by confidence intervals of 95% (CI 95%). Analysis is restricted to children with mother-reported information and serum sample. The analysis was performed by using Stata v14.2.

RESULTS

Sample characteristics

A total of 384 women had complete data on their tobacco smoke exposure at delivery, while 336 (87.5%) children had complete self-report data at 12 months, 280 (72.9%) at 24 months, 258 (67.2%) at 36 months, and 187 (48.7%) at 48 months. At baseline, 31% of women were between ages 30-34 years, 62% were non-Hispanic white, 75% had greater than high school education, 81% were employed, 78% lived with a spouse or partner and 71% had private health insurance. The attrition rate of women from pregnancy to the 48 month-age period was 51.3%; loss to follow-up was not related to any sociodemographic characteristics (Supplementary Table 1).

We restricted the analyses to children with both maternal self-reported tobacco consumption and SHS exposure information and cotinine measures. Information was available for 280 newborns, 270 children at 12 months, 197 at 24 months, 196 at 36 months, and 150 at 48 months (Supplementary Table 2). The attrition rate of these children with both self-reported data and serum biomarkers of SHS exposure was 46.4% from delivery to age 48 months (Supplementary Table 1).

Serum cotinine distribution

Children's geometric mean (GM) serum cotinine concentrations from 12-48 months was higher than newborn´s GM umbilical cord serum concentrations (Table 1). Serial measures of children's serum cotinine concentrations from 12-48 months were highly correlated with correlation coefficients between log-transformed children´s serum cotinine concentrations in consecutive periods ranging from 0.81 (24-36 months) to 0.72 (12-36 months). The ICC between repeated serum cotinine concentrations (analysis restricted to 71 children with cotinine measures at 12, 24, 36 and 48 months) was 0.72 (CI 95% 0.63-0.80) reflecting good agreement between measurements.

Prevalence of exposure to SHS

The prevalence of children exposed to SHS based on maternal report varied between 26.8%-31.3%. (Figure 1). The prevalence of SHS exposure based on cord serum cotinine after applying the assay LOD derived cut-point of 0.015 ng/ml, was double the self-reported prevalence. The prevalence of SHS exposure based on children´s serum cotinine concentrations decreased from 86.7% at 12 months to 74.7% at 48 months (Figure 1 upper). The difference between maternal-reported prevalence of exposure and

that estimated from children´s serum cotinine concentrations, excluding newborns and using LOD as cut-point, was nearly 50 percentage points at any age.

Children whose mothers reported SHS exposure had higher serum cotinine concentrations than children whose mothers reported no exposure to SHS (Figure 2). Fifty percent of the newborns born to self-reported smokers had cord serum cotinine concentrations > 3 ng/ml. At 12 and 24 months of age, 83% and 80% of children whose mothers reported that they were not exposed had cotinine values higher than the LOD, and at 36 and 48 months of age, the percentage was 66% and 65%. Moreover, the distribution of serum cotinine concentration was similar among children whose mothers were active smokers and non-smoking mothers who reported SHS exposure (Figure 2); for this reason, these categories were combined in further analysis.

Estimation of age-specific cut-points for distinguishing SHS exposure

The AUC of various serum cotinine thresholds ranged from 0.80 and 0.89 (Supplementary Figure 1). Cut-points for distinguishing SHS exposure from non-exposure decreased with child age and were set at 0.11 ng/ml at 12 months, 0.08 ng/ml at 24, 0.05 ng/ml at 36 and at 0.04 ng/ml at 48 months (Table 2). The sensitivity and specificity corresponding to these cut-points were above 72% and NPV was over 87%. Using the optimal serum cotinine age-specific cut-points, the prevalence of SHS was highest at 12 months (39.3%) and lowest at 48 months (38.0%). The greatest difference between maternal self-reported prevalence and serum cotinine estimated prevalence of SHS exposure is nearly 13 percentage points at 12 months (Figure 1 bottom).

Concordance between self-reported exposure and serum cotinine measures

The concordance between maternal-reported SHS exposure and serum cotinine improved considerably after applying age-specific cut-points. The Kappa coefficient between mother-reported exposure and child´s serum cotinine concentrations, using the LOD as threshold, was below 0.22 in each of the four time periods. In contrast, when age-specific cut-points were used, the kappa coefficient improved from 0.39 at 12 months to 0.62 at 48 months (Table 3). Taking Landis and Koch criteria into account in assessing the Kappa index, when using the assay LOD of 0.015 ng/ml as cut-point, the concordance between maternal-reported and children´s serum cotinine concentrations after delivery was insignificant at 12, 24 and 36 months and low at 48 months. When using the new age-specific cut-points, concordance improved with age, being low at 12 months, but moderate at 24, 36 and high at 48 months.

DISCUSSION

In this cohort, pre-school aged children whose mothers reported SHS exposure had higher serum cotinine concentrations than children whose mothers reported no SHS exposure. When using the serum cotinine assay LOD as the threshold for distinguishing SHS exposure from no exposure, concordance between serum cotinine concentrations and maternal-reported exposure was non-significant. In contrast, after deriving age-specific serum cotinine cut-points, the concordance between serum cotinine and mother-reported exposure to SHS improved, with increasing concordance as child age increased.

Various studies conclude that serum cotinine concentrations in children vary as a function of age, sex, or race (2, 21, 22). With respect to age, children seem to have higher cotinine concentrations than adults at similar exposures. This could be due to metabolic differences, or to the fact that children have a faster respiration rate and inhale larger quantities of SHS contaminants than adults (23). Previous studies found differences in cotinine concentrations among children in different age groups, with higher concentrations among the youngest children (1, 2, 24). Our results are consistent with this as GM serum cotinine concentrations increased from birth to 12 months and then declined again, possibly reflecting decreased respiratory rates as children aged. Differences between cord blood and later concentrations could be explained by the higher metabolism and faster elimination of cotinine in their mothers (25). Thus, results of prior studies and developmental appropriate changes in child behavior, anatomy, and physiology support the need for age-specific cut-points for children. Indeed, when the serum cotinine assay LOD threshold was used to distinguish SHS exposure, 65-83% of children classified as unexposed by mother report were re-classified as SHS exposed. In contrast, when we used the new age-specific cut-points, only 17-27% were re-classified as SHS exposed. Our results (data not shown) show that among the children whose mothers declared that they were not smokers at the 4 follow-up periods and that the children were not exposed to SHS, the cotinine concentration decreased as age increased. This decrease is unlikely to be due to misclassification of self-reported SHS by the mother.

Other investigators have concluded that the prevalence of SHS exposure obtained from maternal-report consistently underestimates actual exposure. Presumably, some of this estimate is because mothers might not report SHS exposure because of recall bias, social desirability bias or ignorance about their children's exposure in other settings (12, 21). Children's exposure may be so negligible that mothers are not able to identify or quantify it (6). Our results indicate that some of the discordance was because of failure to account for age-related differences in exposure or metabolism.

Based on cotinine derived from the LOD threshold, the prevalence of SHS exposure was 73.5% and 74.7% at 3 and 4 years-old, respectively. Taking into account the estimates obtained in population studies, about 4 out of 10 U.S. children aged 3–11 years

(40.6%) are exposed to secondhand smoke (2). These large differences, however, fail to assess the influence of other factors such as the sensitivity and suitability of the cut-points used to classify exposure to SHS. The assay LOD has become much more sensitive over time. Earlier studies set it at 0.05 ng/ml (4, 12, 17), while more recent studies, including ours, have a LOD of 0.015 ng/ml. This lower LOD could mean that low serum cotinine concentrations reflect transient SHS exposure or exposure from other sources, such as food (tomatoes, potatoes, cauliflower and black tea). Food consumption levels of dietary nicotine are insignificant compared with moderate SHS exposure, but consumption of high quantities of nicotine-containing foodstuffs might contribute to low-level elevations in serum cotinine (e.g. 80 g of eggplant is equivalent to approximately 0.01 ng/ml of serum cotinine) (7). Future studies could verify this hypothesis using studies with detailed dietary information and sensitive cotinine biomarkers.

Despite the improvement in the concordance between maternal-reported SHS and children's serum cotinine concentrations using the age-specific cut-points, misclassification is still a problem. This misclassification has been observed in prior studies, including those of pregnant women and their children younger than 5 (26-28) and it could be due to maternal concealment to avoid social judgment or ignorance about negligible and transient low level nicotine exposures quantified with sensitive cut-points.

This study has some limitations worth nothing. First, we had a modest sample size. Still, it was one of the largest samples of children younger than 5 years to validate SHS exposure using parental report and biomarkers (12, 27, 29, 30). Another limitation is the attrition of study participants. Yet loss to follow-up was not associated with any measured sociodemographic characteristic. Third, we did not include questions to evaluate third-hand smoke exposure, such as involuntary inhalation or cutaneous absorption of nicotine particles deposited on clothing and furniture, or dietary intake. Fourth, we lacked information about the duration of exposure to SHS, which could have been used to provide more valid and reliable cotinine thresholds, and we did not account for factors such as the size of the home, the intensity of exposure, or the proximity to smokers (7, 31, 32). Fifth, to identify the optimal serum cotinine cut-points we assumed maternal-report was the gold standard. We note that we expect any misreporting to predominately affect the sensitivity of maternal report and not the specificity; few women would report exposure in its absence. It should be noted that maternal self-report does not refer solely to whether the mother declares that the child is exposed, but it also takes into consideration if the mother is a smoker and if the child lives with other smokers who smoke at home. Finally, our findings may not be generalizable to other populations as our eligibility criteria were not designed to ensure that our cohort was representative of births in the study region. However, most chemical biomarker concentrations among HOME Study participants are similar to pregnant women and children in the USA during the time of enrolment and follow-up (20).

This study also has several strengths. First, we had longitudinal measures of SHS exposure derived from both maternal report and children's serum cotinine concentrations over the first four years of life. Second, our cohort was relatively higher SES, with 75% of the mothers having greater than high school education. Thus, misreporting of SHS exposure is likely reduced as previous studies have shown that higher educational level is associated with more accurate SHS exposure reporting (15).

CONCLUSIONS

Previous investigators have concluded that maternal reports dramatically underestimate children's SHS exposure. When we used age-specific cut-points, we found that many fewer children were re-classified as SHS exposed. Thus, maternal report may be a better indicator of children's SHS than previous estimates. The age specific cut-points should be validated in other cohorts.

ACKNOWLEDGMENTS

We thank the HOME Study staff, patient advisers and participants for their years of dedication to the study.

FOOTNOTES

- **Contributors:** MPR, MIS and JB planned the study and supervise it with contribution from all the other authors. MPR and MIS performed the analysis with substantive contribution from all the other authors, MPR and NM wrote the first draft of the manuscript and MIS, JB, BP and KY provided critical comments and contributed on the drafts to successive versions of the manuscript. All the authors approved the final version paper. MPR is the guarantor.

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- **Competing interests:** JMB received an honoraria from Quest Diagnostic for serving on an expert panel related to endocrine disrupting chemicals. JMB's institution was financially compensated for his services as an expert witness for plaintiffs in litigation related to PFAS-contaminated drinking water; these funds were not paid to JMB directly. The other authors declare no competing interests.

- **Patient consent for publication:** not required.

- **Ethics approval:** The institutional review boards of Cincinnati Children's Hospital Medical Center, the participating delivery hospitals, and the CDC approved this study. During face-to-face visit, research assistants explained study protocols to each prospective participant and completed a checklist to ensure women were fully informed about the study. All mothers provided written informed consent for both themselves and their children prior to enrolment. ID of approval: 01-8-5.

- **Provenance and peer review:** Not commissioned; externally peer reviewed.

- **Data availability statement:** data are available upon reasonable request.

The HOME Study Principal Investigators welcome new collaborations with other

investigators and have actively engaged in collaborative data sharing projects.

Interested investigators should contact Drs Joseph M. Braun (joseph_ braun_ 1@brown. edu) and Kimberly Yolton (kimberly. yolton@ cchmc. org) to obtain additional information about The HOME Study, discuss collaborative opportunities,

and request a project proposal form. The HOME Study Protocol Review Committee reviews proposed research projects to ensure that they do not overlap with extant projects and are an efficient use of scarce resources (eg, biospecimens).

- Word count: 3,339.

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TABLES

Table 1. Descriptive statistics of serum cotinine concentrations (ng/ml) at birth and at 12, 24, 36 and 48 months: n, range, quartiles and geometric mean with CI 95%.

			Quartiles			Geometric mean		
	n	Range	P25	P50	P75	Mean	95% CI	
Newborn´s cotinine [a]	280	0.00000 - 261.0	0.003	0.017	0.088	0.022	0.015	0.032
Child´s cotinine [a]								
12 months	270	0.00030 - 35.3	0.023	0.063	0.357	0.093	0.073	0.118
24 months	197	0.00126 - 10.5	0.020	0.046	0.212	0.070	0.053	0.092
36 months	196	0.00032 - 21.6	0.012	0.033	0.199	0.046	0.034	0.064
48 months	150	0.00024 - 14.9	0.013	0.027	0.249	0.047	0.033	0.067

ng/ml, nanogram/milliliter; n, number of observations; CI, confidence interval; P, percentile.

[a] Analysis is restricted to participants with both maternal-reported data and cotinine measures.

Table 2. AUC, new age-specific cut-points for each age (ng/ml) with its sensitivity, specificity, PPV and NPV. Estimations are accompanied by 95% CI.

	12 months	24 months	36 months	48 months
n	270	197	196	150
Exposed to SHS	72 (26.7%)	54 (27.4%)	51 (26.0%)	47 (31.3%)
AUC (95% CI)	0.80 (0.74 - 0.86)	0.83 (0.76 - 0.90)	0.84 (0.77 - 0.91)	0.89 (0.82 - 0.95)
Cut-points (ng/ml)[a]	0.11	0.08	0.05	0.04
Sensitivity (95% CI)	72.20 (60.40 - 82.10)	75.90 (62.40 - 86.50)	74.50 (60.40 - 85.70)	83.00 (69.20 - 92.40)
Specificity (95% CI)	72.70 (66.00 - 78.80)	76.20 (68.40 - 82.90)	74.50 (66.60 - 81.40)	82.50 (73.80 - 89.30)
PPV (95% CI)	49.10 (39.20 - 59.00)	54.70 (42.70 - 66.20)	50.70 (38.90 - 62.40)	68.40 (54.80 - 80.10)
NPV (95% CI)	87.80 (81.80 - 92.40)	89.30 (82.50 - 94.20)	89.30 (82.30 - 94.20)	91.40 (83.80 - 96.20)

AUC, area under receiver operating characteristic curves; ng/ml, nanogram/milliliter; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval; SHS, secondhand tobacco smoke.

[a] Age-specific cut-point values are those that maximized the AUC, that is to say, those which minimize the difference between sensitivity and specificity . These values were calculated with receiver operating characteristic curves and children´s SHS exposure reported by their mothers was considered the gold standard. Children´s serum cotinine concentrations above these cut-point values will reflect SHS exposure.

Table 3. Kappa concordance coefficient between maternal-reported SHS exposure (exposed/ unexposed) and child´s serum cotinine concentrations accompanied with the percentage of agreement when using the assay LOD threshold and age-specific cut-points at 12, 24, 36 and 48 months of age.

	12 months	24 months	36 months	48 months
Assay LOD threshold[a]				
Agreement (%)	38.52	42.13	49.49	54.00
Kappa (95% CI)	0.08 (0.04 - 0.13)	0.12 (0.07 - 0.17)	0.18 (0.10 - 0.25)	0.22 (0.13 - 0.32)
Age-specific cut-points[b]				
Agreement (%)	72.59	76.14	74.49	82.67
Kappa (95% CI)	0.39 (0.28 - 0.50)	0.47 (0.34 - 0.59)	0.43 (0.30 - 0.56)	0.62 (0.49 - 0.75)

LOD, limit of detection; CI, confidence interval.

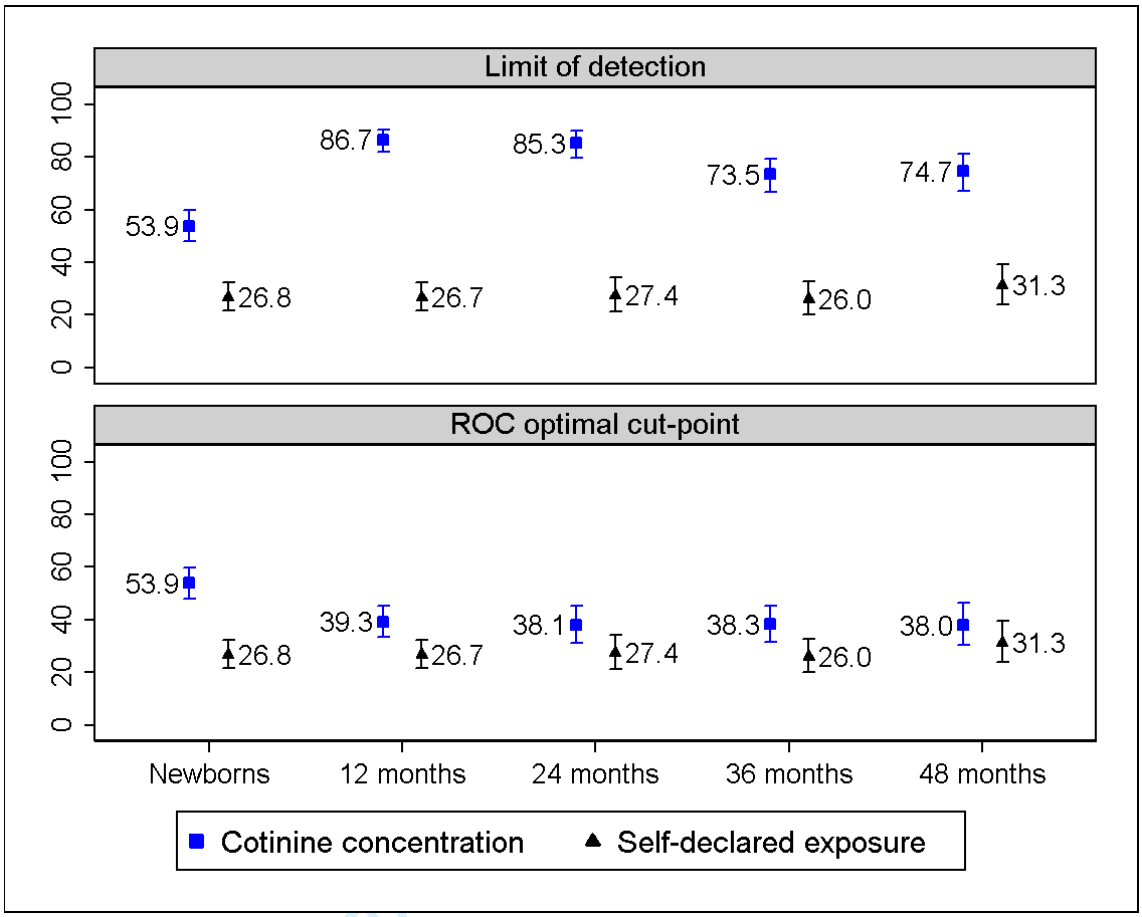
[a] Assay LOD threshold to discriminate between children exposed and unexposed to SHS: 0.015 ng/ml.

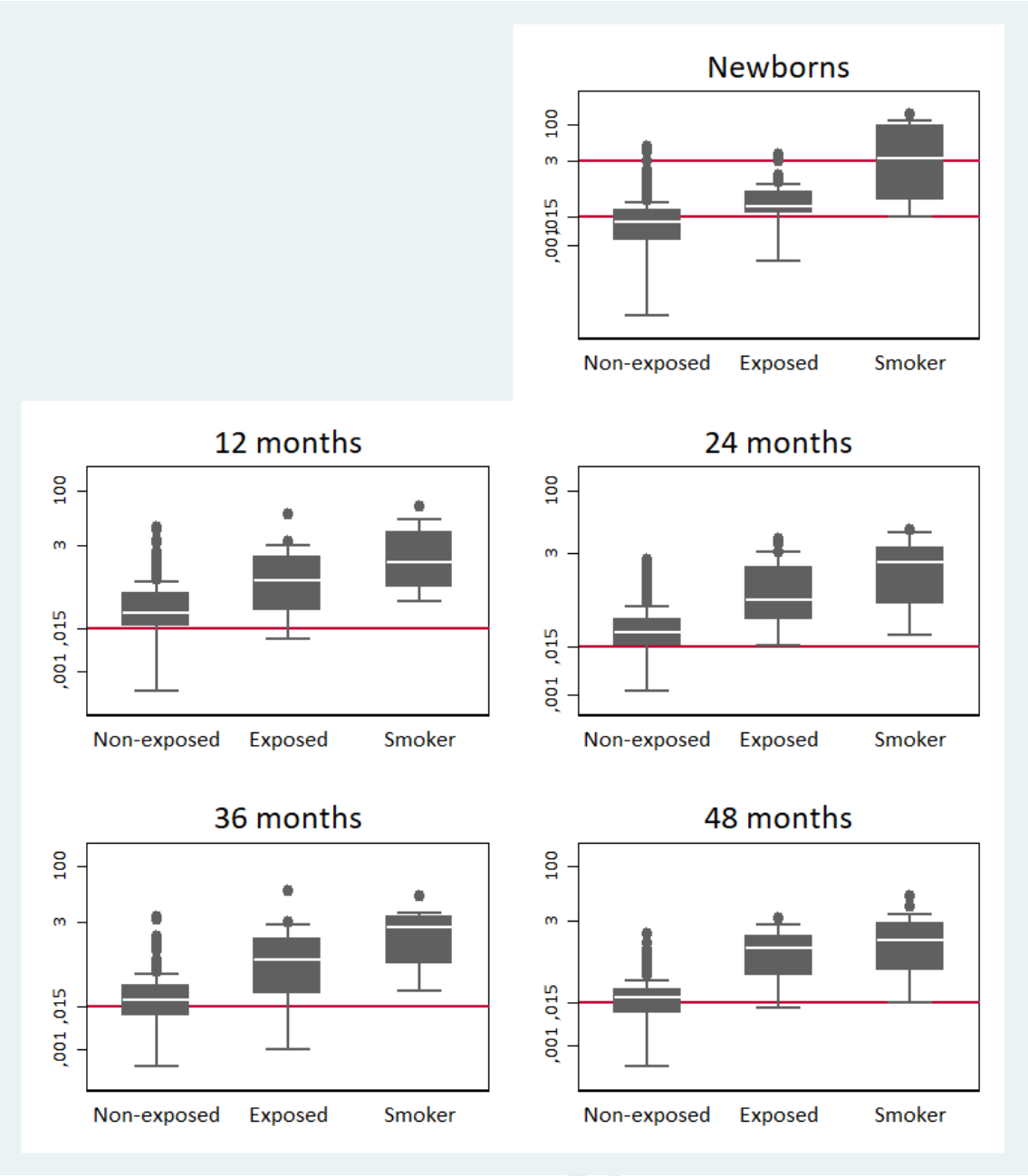
[b] Age-specific cut-points (ng/mL) to discriminate between children exposed and unexposed to SHS calculated with receiver operating characteristic curves, 12 months: 0.11; 24 months: 0.08; 36 months: 0.05; 48 months: 0.04.

FIGURE LEGENDS

Figure 1. Prevalence of SHS exposure among children is derived from maternal self-report (exposed/unexposed), depicted with a triangle, and also from serum cotinine concentrations, depicted with a square, applying assay LOD derived cut-point of 0.015 ng/ml (upper) and age-specific cut-points of 0.11 ng/ml at 12 months; 0.08 ng/ml at 24 months; 0.05 ng/ml at 36 months and 0.04 ng/ml at 48 months (bottom).

Figure 2. The box plots depict the distribution of serum cotinine concentrations (ng/ml), as logarithm, from neonatal umbilical cord (upper line=3ng/ml and bottom line=0.015 ng/ml) and child at 12, 24, 36 and 48 months (line=0.015 ng/ml) depending on children´s SHS exposure reported by mothers (unexposed/ exposed/ mother smoker). If using the LOD derived cut-point of 0.015 ng/ml to distinguish between SHS exposure/non-exposure, all the children, including those from the non-exposure category, had serum cotinine concentrations comparable to SHS exposure.

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Supplementary Table 1. Baseline sociodemographic characteristics of HOME Study women at pregnancy and at 12, 24, 36 and 48 months after delivery accompanied with their children sex.

	Pregnancy	12 months	24 months	36 months	48 months
n	384(100.0)	336 (87.5)	280 (72.9)	258 (67.2)	187 (48.7)
Age group (years)[a]					
under 25	93 (24.2)	67 (19.9)	50 (17.9)	45 (17.4)	36 (19.3)
25-29	109 (28.4)	97 (28.9)	79 (28.2)	80 (31.0)	59 (31.6)
30-34	120 (31.3)	115 (34.2)	106 (37.9)	90 (34.9)	61 (32.6)
35 and over	62 (16.2)	57 (17.0)	45 (16.1)	43 (16.7)	31 (16.6)
Race/ethnicity[a]					
Non-Hispanic white	238 (62.0)	226 (67.3)	197 (70.4)	183 (70.9)	128 (68.5)
Non-Hispanic black	121 (31.5)	89 (26.5)	66 (23.6)	58 (22.5)	47 (25.1)
Other	25 (6.5)	21 (6.3)	17 (6.1)	17 (6.6)	12 (6.4)
Level of education[a]					
Less than high school/high school	95 (24.7)	67 (19.9)	50 (17.9)	49 (19.0)	34 (18.2)
Some college	98 (25.5)	85 (25.3)	66 (23.6)	60 (23.3)	49 (26.2)
College	191 (49.7)	184 (54.8)	164 (58.6)	149 (57.8)	104 (55.6)
Employment[b]					
Not working	74 (19.3)	74 (22.0)			
Working	310 (80.7)	262 (78.0)			
Living with partner					
Yes	300 (78.1)	273 (81.3)	234 (83.6)	218 (84.5)	155 (82.9)
No	84 (21.9)	63 (18.8)	46 (16.4)	40 (15.5)	32 (17.1)
Income ($/year)					
Until 25,000	102 (26.6)	69 (20.7)	48 (17.3)	47 (18.4)	37 (19.9)
Over 25,000	282 (73.4)	264 (79.3)	229 (82.7)	209 (81.6)	149 (80.1)
Health insurance[b]					
Public	102 (26.6)	87 (25.9)	59 (21.1)	57 (22.1)	
Private	272 (70.8)	245 (72.9)	216 (77.1)	198 (76.7)	
None	10 (2.6)	4 (1.2)	5 (1.8)	3 (1.2)	
Child[c]					
n	384 (100.0)	336 (87.5)	280 (72.9)	258 (67.2)	187 (48.7)
Male	178 (46.4)	151 (44.9)	129 (46.1)	119 (46.1)	78 (41.7)
Female	206 (53.7)	185 (55.1)	151 (53.9)	139 (53.9)	109 (58.3)
Child[d]					
n	280 (100.0)	270 (96.4)	197 (70.4)	196 (70.0)	150 (53.6)
Male	130 (46.4)	122 (45.2)	90 (45.7)	86 (43.9)	65 (43.3)
Female	150 (53.6)	148 (54.8)	107 (54.3)	110 (56.1)	85 (56.7)

n, number of observations.

[a] Unchanged sociodemographic characteristics obtained at baseline and further adjusted to the distribution of mothers remaining at each time period of the HOME Study.

[b] Data not available at all the time periods.

[c] Child with maternal self-reported data.

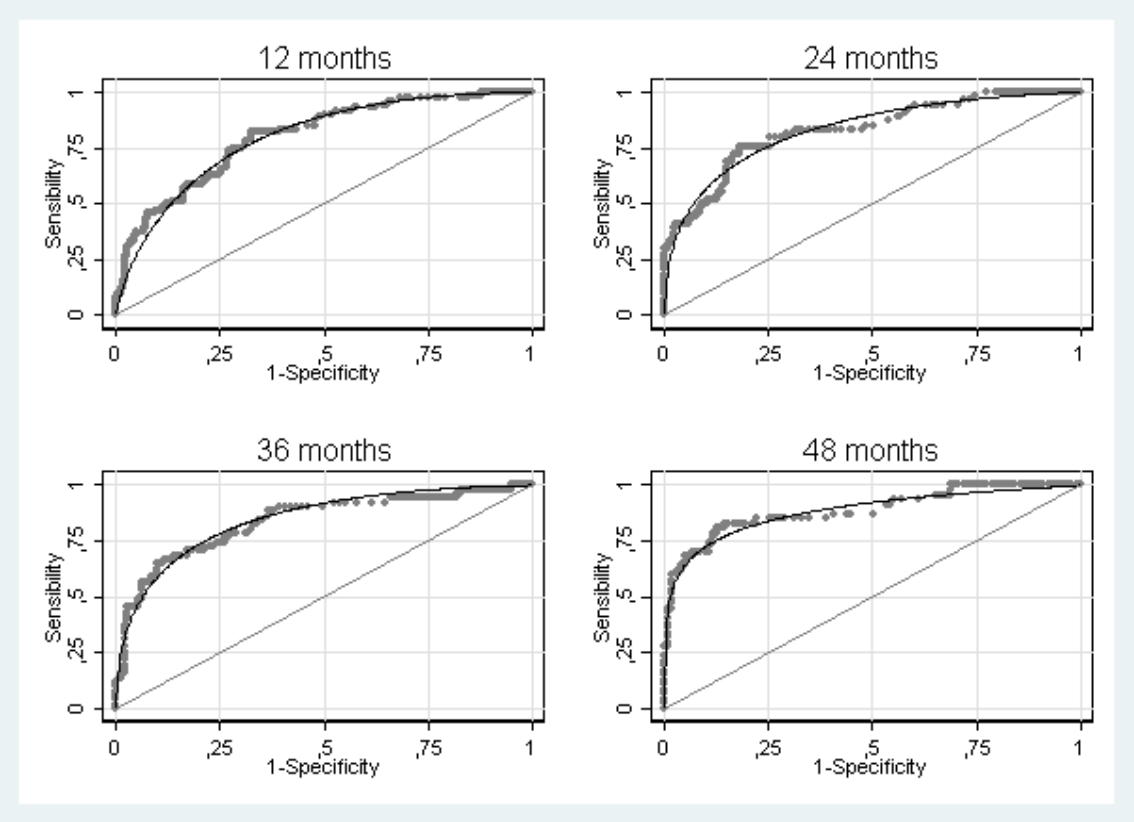
[d] Child with both maternal self-reported data and serum cotinine measures.

Supplementary Table 2. Prevalence of maternal tobacco consumption and exposure to SHS during pregnancy and at 12, 24, 36 and 48 months among participants with maternal-reported data and among participants with both maternal-reported data and cotinine measures.

	Pregnancy	12 months	24 months	36 months	48 months
Participants with self-reported data					
n	384	336	280	258	187
Mother active smoker	48 (12.5%)	35 (10.4%)	26 (9.3%)	23 (8.9%)	23 (12.3%)
Mother/Child Exposed to SHS	59 (15.4%)	55 (16.4%)	50 (17.9%)	41 (15.9%)	36 (19.3%)
Mother/Child Unexposed to SHS	277 (72.1%)	246 (73.2%)	204 (72.9%)	194 (75.2%)	128 (68.5%)
Participants with both self-reported data and cotinine measures					
n	280	270	197	196	150
Mother active smoker	34 (12.1%)	25 (9.3%)	17 (8.6%)	22 (11.2%)	21 (14.0%)
Mother/Child Exposed to SHS	41 (14.6%)	47 (17.4%)	37 (18.8%)	29 (14.8%)	26 (17.3%)
Mother/Child Unexposed to SHS	205 (73.2%)	198 (73.3%)	143 (72.6%)	145 (74.0%)	103 (68.7%)

SHS, secondhand tobacco smoke; n, number of observations.

Supplementary Figure 1. Receiver operating characteristic curves, empirical and binormal, for child´s serum cotinine concentrations and maternal-reported SHS exposure at each age.



Supplementary Figure 1. Sensitivity is represented on the y-axis and the complementary of specificity (1-specificity), which is the ratio of false positives, on the x-axis. The area under receiver operating characteristic curves was above 0.80 at all times. This value reflects the excellent diagnostic ability of the serum cotinine to classify SHS exposure among the participating children.

STROBE (Strengthening The Reporting of OBservational Studies in Epidemiology) Checklist

A checklist of items that should be included in reports of observational studies. You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

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 www.strobe-statement.org.

Section and Item	Item No.	Recommendation	Reported on Page No.
Title and Abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	
Introduction			
Background/Rationale	2	Explain the scientific background and rationale for the investigation being reported	
Objectives	3	State specific objectives, including any prespecified hypotheses	
Methods			
Study Design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	

Section and Item	Item No.	Recommendation	Reported on Page No.
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	
Bias	9	Describe any efforts to address potential sources of bias	
Study Size	10	Explain how the study size was arrived at	
Quantitative Variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical Methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
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	
		(b) Give reasons for non-participation at each stage	
		(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	
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome Data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	

Section and Item	Item No.	Recommendation	Reported on Page No.
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	
Other Analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key Results	18	Summarise key results with reference to study objectives	
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	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	
Generalisability	21	Discuss the generalisability (external validity) of the study results	
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	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Once you have completed this checklist, please save a copy and upload it as part of your submission. DO NOT include this checklist as part of the main manuscript document. It must be uploaded as a separate file.