Research Article

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Comparison of Urine 4-(Methylnitrosamino)-1-(3) Pyridyl-1-Butanol and Cotinine for Assessment of Active and Passive Smoke Exposure in Urban Adolescents

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Abstract

Background: Many adolescents are exposed to tobacco smoke, from either active smoking (CS) or secondhand smoke (SHS) exposure. Tobacco-specific biomarkers of exposure include cotinine (detects use in past 2–4 days) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL; detects use for a month or longer). NNAL is expected to detect more intermittent tobacco exposure. We compared NNAL and cotinine as biomarkers of exposure to tobacco in urban adolescents and determined the optimal NNAL cutoff point to distinguish CS from SHS exposure.

Methods: Surplus urine samples, collected from 466 adolescents attending pediatric well or urgent care visits at Zuckerberg San Francisco General Hospital in 2013 to 2014, were assayed for cotinine and NNAL.

Results: Ninety-four percent of adolescents had measurable levels of NNAL compared with 87% for cotinine. The optimal NNAL cutoff point to distinguish CS from SHS was 9.6 pg/mL by latent class or 14.4 pg/mL by receiver-operating characteristic analysis. Cotinine and NNAL were strongly correlated, but the correlation slopes differed for active versus SHS-exposed adolescents. Among nonsmokers, NNAL levels were significantly higher in African American (median, 3.3 pg/mL) compared with other groups (0.9–1.9 pg/mL), suggesting greater exposure to SHS.

Conclusions: Urine NNAL screening finds a large majority (94%) of urban adolescents are exposed to tobacco. African Americans are exposed to higher levels of SHS than other ethnic/racial groups.

Impact: SHS is associated with significant medical morbidity in adolescents. Routine biochemical screening with NNAL or cotinine detects high prevalence of SHS exposure and should be considered as a tool to reduce SHS exposure in high-risk populations. *Cancer Epidemiol Biomarkers Prev; 27(3); 254–61. ©2018 AACR.*

Introduction

Secondhand smoke (SHS) exposure poses a significant health risk to adolescents, particularly for respiratory problems and infections (1). SHS exposure also increases the likelihood of a nonsmoker becoming an active tobacco user (2). Biochemical assessment of tobacco smoke exposure could be a useful tool to identify and reduce SHS exposure and related disease. We recently reported a high prevalence of tobacco smoke exposure in adolescents screened using urine cotinine testing for tobacco smoke exposure while attending an urban hospital pediatric clinic (3). We found that 87% had evidence of nicotine exposure, with 12% having cotinine levels consistent with active smoking. Cotinine is the proximate metabolite of nicotine with a half-life averaging 16 to 24 hours, and as such, is sensitive to exposure to nicotine over the preceding 2 to 4 days (4, 5).

4-(Methylnitrosamino)-1-(3)pyridyl-1-butanol (NNAL) is a metabolite of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3)pyridyl-1-butanone (NNK; ref. 6). NNK, a potent lung carcinogen, is formed from nicotine in the curing and pyrolysis of tobacco. NNAL is a urinary metabolite that is specific for tobacco use, has a long half-life of 10 to 16 days, and is sensitive to tobacco exposure for a month or longer (7). Thus, NNAL would detect tobacco smoke exposure over a much longer period of time compared with cotinine. Many adolescents are intermittently exposed to tobacco smoke, such that exposure might not be detected using a biomarker with a short window of detection, such as cotinine. One set of aims of our study was to compare levels of NNAL and cotinine in urine of adolescents and to determine the optimal NNAL cutoff point to distinguish active versus passive smoking. By passive smoking, we include both SHS and thirdhand smoke (THS) exposure, the latter referring to exposure from tobacco smoke residues that contaminate homes and other indoor environments long after SHS has dissipated (8).

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Racial differences in cotinine levels in people exposed to tobacco smoke have been reported, with higher levels in African Americans smokers and nonsmokers compared with whites (9, 10). The reason for differences in cotinine levels has not been established but could be due to racial differences in cotinine metabolism and/or due to different levels of SHS exposure. African Americans on average metabolize nicotine and cotinine more slowly than Caucasians due to a higher prevalence of poor metabolism variants in the *CYP2A6* and *UGTB10* genes (11, 12). As a result, for a given level of nicotine intake, cotinine levels are on average higher in African Americans compared with Caucasians (13). African Americans are also reported to be more likely to live in homes with a smoker, and therefore may have higher SHS exposure compared to nonsmokers of other racial/ethnic groups (14). A second aim of our study was to compare NNAL as a marker of level of SHS exposure in African Americans compared to other racial groups.

We and others have reported previously that the ratio of NNAL to cotinine is higher in people exposed to SHS compared with active smokers (15–17). The reason is that NNK is formed from nicotine as SHS ages, while nicotine is rapidly lost by adsorption to room surfaces (8). Thus, the NNK/nicotine ratio in the air increases as smoke ages. This results in nonsmokers inhaling aged SHS with a higher ratio of NNK versus nicotine, and accordingly higher urine NNAL/cotinine ratios compared with active smokers inhaling mainstream smoke. A third aim of our study was to determine how well the ratio of NNAL/cotinine might serve as a diagnostic test to separate active versus passive smoking.

Materials and Methods

Study procedures

Details of the study procedures have been reported previously (3). In brief, 466 adolescents, ages 13 to 19, who received pediatric care at the Children's Health Center (CHC) at Zuckerberg San Francisco General Hospital (ZSFG) and who had surplus urine after collection for other clinical indications during a 12-month interval (June 2013–May 2014) were studied. The CHC is the primary pediatric outpatient clinic at ZSFG, a county hospital serving an economically disadvantaged population of San Francisco. Of our subjects, 91.4% had public insurance and 7.9% were uninsured. Ethnically, the adolescent population treated in the CHC is Latino 58.1%, African American 19.1%, Asian 11.0%, white 6.5%, and other 5.3%. At the CHC, 11.6% of active patients aged 13 to 18 who reported smoking status stated they were currently smoking, and 18.6% of active patients of all ages reported secondhand smoke exposure.

Adolescents presented to the clinic for both well and sick care, where urine samples were collected routinely for clinical indications including but not limited to urinary tract or sexually transmitted infection (STI) screening and diagnosis, abdominal pain evaluation, pregnancy screening and trauma. Samples were collected by clinic nurses and frozen for later analysis.

Information on subjects' race/ethnicity, gender, age, medical diagnosis, and self-reported tobacco use history (reported to the care provider) was retrieved from the hospital electronic database. This was an unconsented study approved by the Committee on Human Research at the University of California, San Francisco. There was no direct patient contact; and after the chart review was completed, all patient identifiers were deleted from the database and research charts.

Analytical chemistry

Urine samples were analyzed for free cotinine and total (free plus conjugated) NNAL by liquid chromatography–tandem mass spectrometry (18, 19). The lower limit of quantitation (LOQ) for cotinine was 0.05 ng/mL and for NNAL it was 0.25 pg/mL. Concentrations were analyzed both without and with creatinine normalization.

Data analysis/statistics

To determine the optimal NNAL concentration that discriminates between active smoking and SHS exposure, we used both latent class analysis (LCA) and receiver-operating characteristic (ROC) approaches. In the first approach, we performed density estimation and LCA on log-transformed urine NNAL levels using the R (www.R-project.org) package mclust (20, 21). This allowed us to fit models of the NNAL distribution as a set of Gaussian mixtures. Using Bayes Information Criterion maximum-likelihood methods, the 2-cluster model was selected as the best-fitting model. This approach makes no assumptions about smoking status, but rather looks at discontinuities in the frequency distribution curves.

The second approach was to construct ROC curves and compare the performance of multiple cutoff points. In our recent publication on this study population, we developed three cotinine groups using LCA to describe detectable cotinine levels in urine (3). Active smoking was determined to be >30 ng/mL, significant SHS exposure as 0.25 to 30 ng/mL, and low-level SHS and/or THS exposure as 0.05 to 0.25 ng/mL. In the ROC analysis, we used a cotinine concentration of >30 ng/mL as the gold standard for active smoking. ROC analyses were performed for urine NNAL concentration with and without creatinine correction, and for the urine NNAL/cotinine ratio. Optimal cutoff points were determined both as that which equalized sensitivity and specificity and that which maximized the Youden index (J). The Youden index, with a range of values from 0 to 1, provides a single number for summarizing a test's accuracy. A value of 1 represents a test that minimizes both false positives and false negatives. A value of 0 represents a test with no ability to discriminate between true and false positives (22, 23). We also performed ROC analyses using urine cotinine cutoff points of 10, 15, and 20 ng/mL to assess the impact of overestimating the optimal discriminating cotinine value to determine active smoking.

To examine the effects of race/ethnicity, sex (male, female), and age (13–14, 15–16, 17–19 years), we tested differences in NNAL detection frequency with the χ^2 test. We used Kruskal–Wallis tests to evaluate whether absolute measured values NNAL or the ratio of NNAL/cotinine differed across covariates.

Both NNAL and NNAL/cotinine were log-transformed given their approximate lognormal distribution. In correlational analyses, subjects with a cotinine or NNAL value below the limit of quantitation were excluded. Other than the LCA, all statistical analyses were carried out using SAS v. 9.4 (SAS Institute, Inc.). Statistical tests were considered statistically significant at $P < 0.05$.

Results

Overall, 94% of adolescents had NNAL levels above the limit of quantitation, compared with 87% for cotinine (Table 1). Supplementary Table S1 shows the high concordance of classification of smokers and nonsmokers by urine cotinine and NNAL. Median values for urine NNAL and cotinine in all subjects and by active

Table 1. Comparison of NNAL and cotinine exposure rates (urine NNAL > 0.25 pg/mL; urine cotinine > 0.05 ng/mL) by gender, ethnicity, and age

		NNAL		Cotinine	
	n	Frequency (%)	*P*	Frequency (%)	*P*
All	469	441 (94)		407 (87)	
Gender					
Male	184	173 (94)		159 (86)	
Female	285	268 (94)	1	248 (87)	1
Ethnicity					
Asian	51	47 (92)		42 (82)	
Latino	246	226 (92)		201 (82)	
Black	102	101 (99)		97 (95)	
White	15	13 (84)		15 (100)	
Mixed/other	55	54 (98)	0.05	52 (95)	<0.01
Age					
13–14	102	93 (91)		80 (78)	
15–16	203	192 (95)		180 (89)	
17–19	164	156 (95)	0.4	147 (90)	0.02

smoking status, gender, ethnicity, and age, based on volume and with correction for creatinine, are shown in Tables 2 and 3. A frequency histogram for urine NNAL is shown in Fig. 1. Log urine NNAL and log cotinine were significantly correlated ($R^2 = 0.38$ for active smokers, $R^2 = 0.33$ for passive smokers, both $P < 0.01$), but the slopes of the regression lines (0.51 vs. 0.72) differed for active versus passive smokers (Fig. 2).

Based on modeled density plots, LCA found an optimal cutoff point of 9.6 pg/mL (95% CI, 5.5–14.2) and 7.0 pg/mg creatinine (4.1–11.0). Assuming that a cotinine level of 30 ng/mL indicates active smoking, an NNAL cutoff point of 9.6 pg/mL would correctly classify 94.5% smokers as smokers and 88.5% of nonsmokers as nonsmokers. A cutoff point of 7.0 pg/mg creatinine would correctly classify 96.3% of smokers as smokers and 90.4% of nonsmokers as nonsmokers.

Based on ROC analysis using a urine cotinine of 30 ng/mL to define active smoking, the optimal cutoff point for NNAL was 14.4 pg/mL for both equality and Youden index (J = 0.88) with a sensitivity of 94.6% and specificity of 93.4%. For creatinine-normalized NNAL, the equality cutoff point was 10.2 pg/mg creatinine (sensitivity 92.6% and specificity 92.7%) and Youden-based cutoff point 6.6 pg/mg creatinine (sensitivity 98.1% and specificity 90.4%, with J = 0.88).

To examine the impact of overestimating the cotinine cutoff point to define active smoking, ROC analyses were performed using cotinine cutoff points of 10, 15, and 20 ng/mL for active smoking. Results are shown in Table 4. The optimal NNAL cutoff point using the Youden index was similar when active smoking was defined as 15, 20, or 30 ng/mL, and similar using the equality

Table 2. Median urine NNAL values by smoking status and gender, ethnicity, and age

	Active smokers (urine cotinine >30 ng/mL)			Nonsmokers		
	n	Median (IQR)	*P*	*n*	Median (IQR)	*P*
A. All	55	80.9 (42.6–151.3)		410	1.2 (0.5–3.2)	
Gender						
Male	20	84.3 (59.6 -99.8)		163	1.1 (0.4–2.9)	
Female	35	62.0 (38.4–183.9)	0.8	247	1.2 (0.5–3.4)	0.5
Ethnicity						
Asian	0	—		51	0.9 (0.3–3.0)	
Latino	8	29.3 (5.0–57.0)		235	0.9 (0.4–2.1)	
Black	32	82.7 (45.4–146.1)		70	3.3 (1.4–10.9)	
White	5	140.7 (80.8–145.1)		10	1.9 (0.5–4.9)	
Mixed/Other	10	128.3 (50.0–192.6)	<0.01	44	1.4 (0.6–3.9)	<0.01
Age						
13–14	5	89.2 (85.4–183.9)		95	0.9 (0.4–2.3)	
15–16	21	62.0 (42.6–82.3)		181	1.3 (0.5–3.0)	
17–19	29	105.3 (45.4–208.5)	0.14	134	1.2 (0.5–4.1)	0.2
B. All	54	43.6 (23.7–74.1)		384	1.0 (0.5–2.4)	
Gender						
Male	20	45.4 (39.2–54.5)		152	0.8 (0.5–2.0)	
Female	34	40.3 (22.3–83.9)	1	232	1.0 (0.5–3.1)	0.13
Ethnicity						
Asian	0	-		47	0.8 (0.4–3.4)	
Latino	7	14.2 (8.4–47.1)		217	0.7 (0.4–1.5)	
Black	32	41.3 (27.4–71.6)		69	2.3 (1.0–7.9)	
White	5	55.4 (51.9–92.0)		8	1.9 (0.7–2.7)	
Mixed/other	10	56.4 (39.8–83.9)	0.02	43	1.1 (0.5–3.9)	<0.01
Age						
13—14	5	43.7 (43.5–74.1)		87	0.8 (0.5–2.1)	
15—16	20	40.3 (23.9–61.4)		171	1.0 (0.5–2.4)	
17—19	29	51.9 (23.7–77.0)	0.7	126	1.0 (0.4–3.2)	0.9

A, Volume-based NNAL (pg/mL); B, creatinine-adjusted NNAL (pg/mg creatinine).

Table 3. Median urine cotinine values by smoking status and gender, ethnicity, and age

	Active smokers (urine cotinine >30 ng/mL)			Nonsmokers		
	n	**Median (IQR)**	***P***	***n***	**Median (IQR)**	***P***
A. All	55	155.2 (68.8–579.2)		410	0.3 (0.1–1.3)	
Gender						
Male	20	146.3 (73.6–390.2)		163	0.3 (0.1–1.1)	
Female	35	155.2 (57.0–791.0)	0.9	247	0.3 (0.1–1.5)	0.6
Ethnicity						
Asian	0	—		51	0.3 (0.1–1.7)	
Latino	8	50.3 (36.6–101.7)		235	0.2 (0.1–0.6)	
Black	32	139.3 (73.6–657.5)		70	1.3 (0.3–4.8)	
White	5	282.9 (250.4–406.1)		10	0.8 (0.2–2.0)	
Mixed/other	10	311.6 (161.7–811.8)	< 0.01	44	0.4 (0.2–1.8)	< 0.01
Age						
13–14	5	283.3 (78.4–800.2)		95	0.2 (0.1–1.1)	
15–16	21	104.9 (56.2–250.4)		181	0.3 (0.1–1.1)	
17–19	29	238.8 (111.5–791.0)	0.12	134	0.3 (0.1–2.7)	0.02
B. All	54	104.6 (40.0–263.0)		384	0.2 (0.1–0.9)	
Gender						
Male	20	98.9 (41.8–186.8)		152	0.2 (0.1–0.7)	
Female	34	115.8 (31.6–298.6)	0.8	232	0.3 (0.1–1.0)	0.3
Ethnicity						
Asian	0	-		47	0.3 (0.1–1.1)	
Latino	7	26.2 (12.7–99.1)		217	0.1 (0.1–0.5)	
Black	32	84.8 (41.6–310.62)		69	0.7 (0.2–3.2)	
White	5	261.9 (111.4–285.1)		8	1.1 (0.1–2.4)	
Mixed/other	10	157.3 (110.1–249.6)	0.03	43	0.3 (0.1–0.9)	< 0.01
Age						
13–14	5	138.2 (40.0–322.6)		87	0.2 (0.1–0.8)	
15–16	20	66.9 (35.7–201.3)		171	0.2 (0.1–0.9)	
17–19	29	116.0 (56.6–298.6)	0.4	126	0.2 (0.1–1.7)	0.06

A, Volume-based cotinine (ng/mL); B, creatinine-adjusted cotinine (ng/mg creatinine).

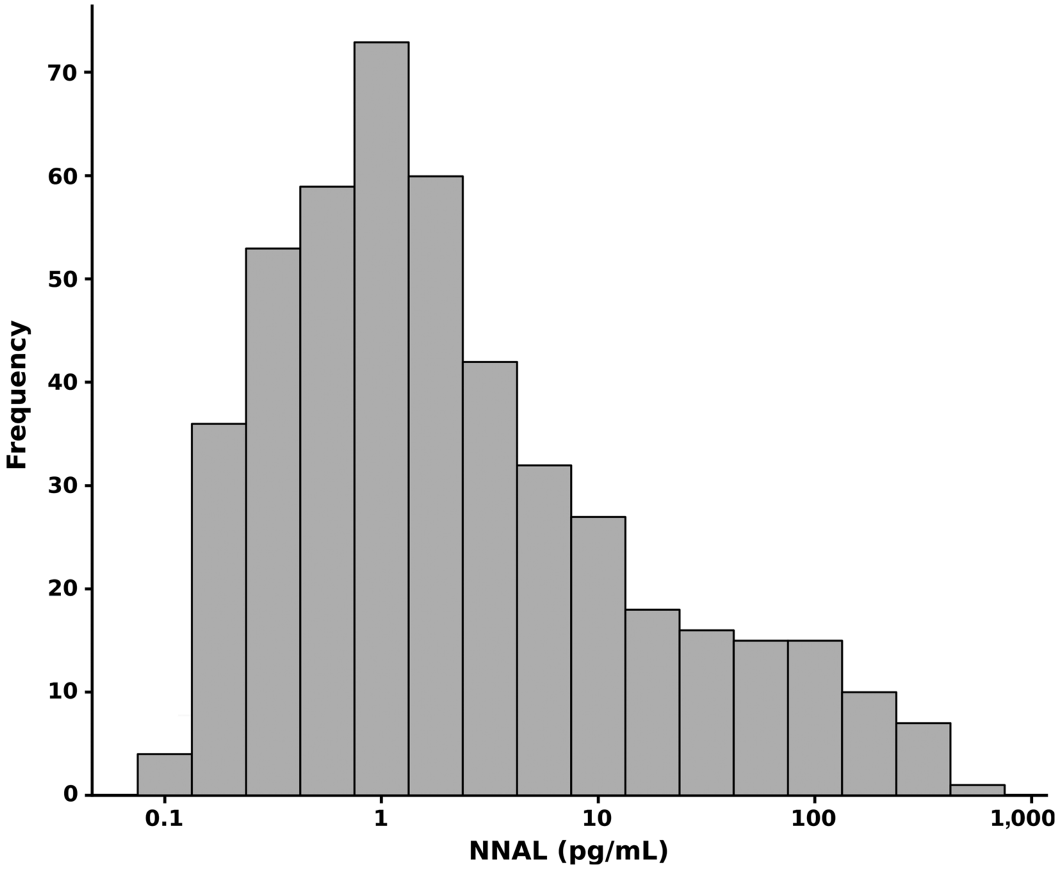

Figure 1.
Histogram: NNAL. The figure shows the frequency distribution of urine NNAL.

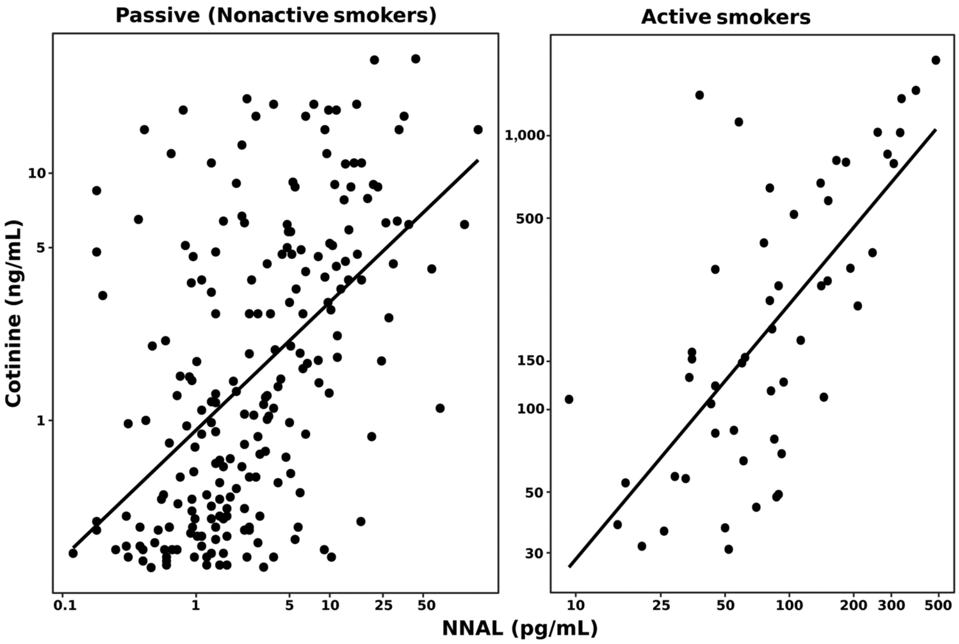

Figure 2.
Log-log plots: NNAL and cotinine. The figure includes scatterplots of NNAL and cotinine in two panels. The first panel includes participants with cotinine values below the established urine cotinine cutoff point and a fitted regression [(ln (COT) = 0.72 * ln(NNAL) + 3.1); R^2: 0.33]. The second panel plots the same for participants with cotinine values > 30 ng/mL with a fitted regression [ln(COT) = 0.51 * ln(NNAL) – 0.9; R^2: 0.38].

measure at 20 and 30 ng/mL. The optimal NNAL cutoff point was lower (6.2–6.6 pg/mL) when smoking was defined as a cotinine of >10 ng/mL.

Among all subjects who were not active smokers (defined as cotinine levels of 30 ng/mL or less), NNAL was detected in 93.4%, with a trend of higher prevalence in African American and older adolescents (Supplementary Table S1). There was no detectable difference in NNAL by age or sex. Median concentrations of NNAL were much higher in smokers compared with nonsmokers, as expected (Table 3). Among nonsmokers, NNAL levels were significantly higher in African American adolescents compared with those in other groups ($P < 0.01$).

Ratios of NNAL/cotinine were higher in nonsmokers compared with smokers ($P < 0.01$), but was not different by sex, race/ethnicity, or age (Supplementary Table S2). ROC analysis of the NNAL/cotinine ratio, indicated an equality-based cutoff point of 1.07×10^{-3} (sensitivity 85.0% and specificity 85.0%), and a Youden-based cutoff point was 1.85×10^{-3} (sensitivity 100% and specificity 73.4%).

Discussion

The overall aim of our research is to determine optimal biomarkers for routine screening of adolescents for tobacco smoke exposure. We recently reported extraordinarily high prevalence (87%) of tobacco exposure using a high-sensitivity urine cotinine assay (3). NNAL is a tobacco-specific biomarker, which has previously been shown to be a sensitive indicator of both active and passive cigarette smoke exposure (24–28). Now we find even higher prevalence (94%) of tobacco smoke exposure using a high-sensitivity NNAL assay. Because NNAL is entirely specific for tobacco exposure, this finding dispels any speculation that the high prevalence of low-level cotinine positivity in the population might be due to the consumption of foods like

Table 4. ROC analyses for the optimal urine NNAL cutoff point using different urine cotinine criteria for active smoking

Positive cotinine cutoff	AUC	Cutoff point	Noted for	Sensitivity	Specificity	Youden
		Volume based—NNAL pg/mL				
Cotinine > 10 ng/mL	0.917	6.2	Equality	0.872	0.873	0.743
		6.6	Youden	0.872	0.886	0.758
Cotinine > 15 ng/mL	0.942	8.2	Equality	0.884	0.884	0.768
		15.5	Youden	0.841	0.949	0.790
Cotinine > 20 ng/mL	0.955	13.9	Equality	0.931	0.931	0.862
		15.5	Youden	0.931	0.941	0.872
Cotinine > 30 ng/mL	0.971	14.4	Equality, Youden	0.946	0.934	0.882
		Creatinine adjusted—NNAL (pg/mg creatinine)				
Cotinine > 10 ng/mL	0.929	4.1	Equality	0.870	0.870	0.740
		5.6	Youden	0.857	0.917	0.774
Cotinine > 15 ng/mL	0.960	5.7	Equality	0.912	0.911	0.822
		6.6	Youden	0.912	0.924	0.836
Cotinine > 20 ng/mL	0.971	8.5	Equality	0.93	0.921	0.851
		6.6	Youden	0.965	0.908	0.873
Cotinine > 30 ng/mL	0.972	10.2	Equality	0.926	0.927	0.853
		6.6	Youden	0.981	0.904	0.885

tomatoes, potatoes, eggplant, and black tea that contain low levels of nicotine, or due to the use of other nicotine-containing products (29).

The subjects of our study were mostly minority, economically disadvantaged adolescents attending pediatric clinics at a public hospital in San Francisco. San Francisco is a city with relatively low smoking prevalence in 2012 to 2014 of 10.1% in the general population (30). In California, as elsewhere, the smoking prevalence in low socioeconomic status groups is much higher than the population average. In comparison with our findings, data from the nationally representative National Health and Nutrition Examination Survey (NHANES) from 2007 to 2008 and 2011 to 2012 found detectable NNAL in urine of 41% and 62% of all non-tobacco users, respectively, but used an assay with limit of detection of 0.6 pg/mL, which is considerably less sensitive than the assay used here (limit of quantitation 0.25 pg/mL; refs. 25, 27). Hecht and colleagues (31), using an assay with a detection limit of ~0.6 pg/mL, found NNAL in urine of 33% of schoolchildren in grades 2 to 5 (mean age 6.9 years) in Minneapolis. Due to adolescents frequenting more locations and social groups where smoking occurs, it is not surprising that rates of NNAL detected are higher in our sample.

As reported by others, we found a high overall correlation between urine cotinine and urine NNAL (25, 27, 31). However the quantitative relationship between NNAL and cotinine differed for active and passive smokers, as the ratio of NNAL to cotinine is more than 10 times higher with passive exposure compared with active smoking (15–17). As mentioned previously, this is because the aging of exhaled mainstream smoke (SHS) is associated with rapidly declining levels of nicotine and increasing levels of NNK, thereby increasing the ratio of NNK/nicotine in SHS compared with mainstream smoke.

We examined the use of urine NNAL as a biomarker to distinguish active versus passive smoke exposure. Using an LCA based on the distribution of NNAL in our subjects, the optimal NNAL cutoff point was 9.6 pg/mL or 7.0 pg/mg creatinine. This estimate makes no assumptions about who the active smokers are, but simply looks for likely partitions in the frequency distribution.

An ROC analysis requires a definition of who is an active smoker. Because our study was unconsented, we did not have concurrent self-report about smoking status. Instead, we used urine cotinine to define active smoking. Urine cotinine appeared to be a robust marker of active smoking based on an LCA of cotinine levels (3) and that analysis identified a urine cotinine of 30 ng/mL. Of note is that a urine concentration of 30 ng/mL corresponds to serum cotinine of about 6 ng/mL, which is similar to the cutoff point for active smoking in large national studies (32, 33). Using ROC analysis, with cotinine as the gold standard for active smoking, we found an optimal urine NNAL cutoff point of 14.4 pg/mL, and cutoff points of 6.6 and 10.2 pg/mg creatinine depending on preferred discriminant. Similar estimates were found when ROC analyses using lower urine cotinine cutoff points as gold standards for activity smoking, indicating that our estimates are robust.

In a prior study of adults, comparing those heavily exposed to SHS versus active smokers, we found an optimal NNAL cutoff point of 47.3 pg/mL (16). The higher cutoff point in our previous study is expected as nonsmoker samples were selected based on substantial SHS exposure and the active smokers were adult regular smokers, while in the present study we compared adolescent light smokers to all nonsmokers in a population in which cigarette smoke in active smokers is relatively low and passive exposure is light. Data from NHANES from 2007 to 2008 found in nonsmokers a 95th percentile urine NNAL of 24.4 pg/mL (25); and data from 2011 to 2012 found 11.7 pg/mL (27). Thus, selection of the optimal urine NNAL cutoff point to discriminate smokers from nonsmokers appears to vary from approximately 10 to 50 pg/mL, depending on the extent of SHS exposure and intensity of active smoking. Creatinine-corrected cut points are estimated to range from 5 to 40 pg/creatinine. The cutoff point will be higher in a population in which active smokers smoke more cigarettes per day and more people are exposed to higher levels of SHS.

The urine NNAL cutoff points showed excellent sensitivity and specificity for detecting active smoking. For example, the cutoff point of 14.4 pg/mL from ROC analysis provided 94.6% sensitivity and 93.4% specificity. For comparison, analysis of serum cotinine data from NHANES collected in 1999 to 2004, the optimal cutoff point in adolescents was 3 ng/mL with a sensitivity of 86.5% and specificity of 93.1% (32). While the populations of adolescents and the gold standards for smoking (urine cotinine in our study vs. self-report in NHANES) were different in the two studies, our analysis suggests that urine NNAL will perform as well or possibly better than serum cotinine as a discriminator of smoking versus nonsmoking.

Among nonsmokers, we found that the absolute NNAL concentration was higher in African American compared with Latino nonsmokers. This suggests that the prevalence of exposure to tobacco smoke is greater among black adolescents. Others have reported similar findings with NNAL levels comparing African Americans and whites (27).

We reported previously that in San Francisco, African American young children and adolescents have higher levels of cotinine compared with children of other race/ethnicity (3, 34). Higher cotinine levels could be due to greater SHS exposure and/or due to metabolic differences. Blacks carry a higher frequency of slow metabolism gene alleles for cotinine oxidation (*CYP2A6*) and cotinine glucuronidation (*UGT2B10*; refs. 11–13). These genetic differences could result in higher levels of cotinine for any given daily uptake of nicotine. The finding of much higher NNAL levels in African Americans without a difference in the NNAL/cotinine ratio supports that idea that African American adolescent nonsmokers are exposed to greater levels of tobacco smoke than those in other racial/ethnic groups rather than having higher cotinine levels due to genetic metabolic differences. In support of the idea of greater exposure, surveillance data from another California study found that the prevalence of smoking in the home is higher in homes of African Americans compared with white, Latino, or Asian homes (14).

The prevalence of active smoking assessed by urine cotinine in our African American subjects was 32%, which is surprisingly high. The overall smoking prevalence in African Americans in San Francisco from 2014 to 2015 was 13.2% (35). We were unable to find data on smoking prevalence among adolescents in San Francisco. However, the California Department of Public Health reported the state-wide smoking prevalence for high school students (grades 9–12) in 2012 was 9.5% for blacks, compared with 13% for white non-Hispanic, 10.4% for Hispanics, and 5.9% for Asians (30). The high active smoking rates in our African Americans subjects may be related to lower socioeconomic status as mentioned previously, and may also be related to the use of blunts—hollowed-out cigars filled with marijuana (36). Blunt use is more common in African Americans compared with other racial/ethnic groups and could contribute to more African American youth being classified as both active and heavy SHS exposure.

The NNAL/cotinine ratio can also be used as a biomarker to distinguish active versus passive smoking (16). The present study found that the optimal ratio cutoff points were 1.07 or 1.85 $\times 10^{-3}$ by ROC analysis. This compares to the ratio cutoff point of 0.74×10^{-3} that we determined in a prior comparison of adults who were regular smokers versus those with heavy SHS exposure (16). Differences in the optimal cutoff point are expected because, at present, we are trying to distinguish any active smoking from all nonsmoking, while in our prior study we tried to distinguish active from heavy passive smoking. In any case, comparing sensitivity and specificity, the performance of urine NNAL alone was superior to that of the NNAL/cotinine ratio, indicating no advantage of the latter in discriminating between smokers and nonsmokers.

While analysis of urine NNAL detected exposure in most of our study's adolescents, the health consequences of low-level passive smoke exposure are still unclear. Many of the constituents of SHS and THS have the potential to cause adverse health effects, and a threshold level for toxicity has not been determined. With respect to NNAL as a biomarker for human disease from SHS, in nonsmokers with chronic obstructive lung disease who are exposed to SHS, urine NNAL was a better predictor of symptoms and impaired quality of life than was cotinine (37). Presumably this is because NNAL has a long half-life and provided a measure of exposure over long periods of time. Furthermore, urine NNAL was linearly related to COPD severity, without evidence of a threshold level for harm. The U.S. Surgeon General has indicated that there is no safe level of SHS exposure (38). Further epidemiologic research is needed to determine the particular health consequences of low level passive smoke exposure among adolescents.

Limitations of our study include that our subjects were patients in one urban public hospital in a city where the overall smoking prevalence was relatively low. Many attended a hospital clinic for medical care, which could be a marker for greater smoke exposure risk. In addition, our subjects were primarily Latino and African American, thereby reducing generalizability. A strength of study is that because the study was nonconsented, there was no subject self-selection bias. We think our data are likely representative of urban economically disadvantaged adolescents and our findings are expected to be relevant to similar populations in other cities.

In conclusion, using urine NNAL as a biomarker of tobacco smoke exposure, we confirm a prior finding based on urine cotinine of nearly ubiquitous tobacco exposure in adolescents seen in pediatric clinics in an urban public hospital. We find that urine NNAL detects a higher prevalence of exposure than cotinine. This could be due to difference in assay sensitivity to a given level of exposure, or to the fact that NNAL detects exposure over a longer duration of time, and would be expected to be more sensitive to intermittent exposure. We present novel data on the optimal NNAL cutoff point to distinguish active from passive smoking. We find that among nonsmokers urine NNAL exposure is more frequent and NNAL levels are higher in African Americans compared with other racial/ethnic groups, indicating higher levels of SHS or other sources of tobacco exposure.

Given the high level of tobacco exposure in our population of economically disadvantaged adolescents and the well-established risks to health of tobacco smoke exposure, we suggest that routine biochemical screening be considered to identify and reduce exposure of such individuals. Our data indicate that either cotinine or NNAL would be suitable analytes for biochemical screening, although NNAL would detect more exposed adolescents.

Disclosure of Potential Conflicts of Interest

N.L. Benowitz is a consultant/advisory board member for Pfizer, and has served as a paid expert witness in litigation against tobacco companies. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

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Comparison of Urine 4-(Methylnitrosamino)-1-(3)Pyridyl-1-Butanol and Cotinine for Assessment of Active and Passive Smoke Exposure in Urban Adolescents

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