BRIEF REPORT

Proposed Cutoff for Identifying Adult Smokeless Tobacco Users With Urinary Total 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanonol: An Aggregated Analysis of NHANES 2007–2010 Data

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ABSTRACT

Introduction: NNAL [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanonol] is a valid biomarker of tobacco use. However, no study has assessed its use in distinguishing current smokeless tobacco (SLT) users from nonusers. Therefore, this study used aggregated data from the 2007–2008 and 2009–2010 waves of the National Health and Nutrition Examination Survey to determine an optimal threshold for identifying SLT users with NNAL.

Methods: Optimal urinary total NNAL concentrations for discriminating SLT-only users from nonusers of any tobacco were determined using receiver operating characteristic analysis. Percentage agreement between self-reported SLT use status and NNAL levels was calculated overall and by sociodemographic characteristics. All analyses were weighted and performed with Stata, Version 11, and MedCalc for Windows, Version 9.5.0.0.

Results: In total, 264 individuals reported exclusively using SLT (and no other combustible tobacco product) within the past 5 days, whereas 14,824 were self-reported nonusers of any combustible or smokeless tobacco product. The optimal NNAL cutoff point was 34.0 pg/ml, which was associated with a high sensitivity (95.2%), specificity (93.4%), and overall correct classification rate (93.5%). The area under the curve was 98.3% and the corresponding Youden's Index was 88.7%. There was high agreement between the proposed NNAL cutoff point and self-reported SLT-only use (95.6%) and self-reported SLT nonuse (93.9%).

Conclusion: The proposed cutoff point of 34.0 pg/ml had high sensitivity and specificity and may be used by clinicians and researchers to verify or detect recent SLT use. This study also indicated that self-reported SLT use among adults is a reliable measure and has high agreement with biochemical assessment.

INTRODUCTION

Tobacco use remains the single largest preventable cause of morbidity and mortality in the United States (U.S. DHHS, 2010). As overall U.S. cigarette sales continue to decline (Connolly & Alpert, 2008) and the number of states and localities with comprehensive smoke-free laws increase (Centers for Disease Control and Prevention [CDC], 2011), the tobacco industry has actively promoted the use of smokeless tobacco (SLT) as an alternative to cigarettes in situations where smoking is not allowed (Carpenter, Connolly, Ayo-Yusuf, & Wayne, 2009; Curry, Pederson, & Stryker, 2011). Despite declines in smoking rates, little change in the use of SLT products has been observed in recent years. In 2011, 3.2% of persons aged 12 years or more in the United States reported current use of SLT products.

Surveillance of tobacco use is important to monitor existing and emerging trends. The most common population-based tobacco surveillance tools in the United States include use of tobacco sales data filed by tobacco manufacturers and self-reported tobacco use (as is the case in most national surveys). Cotinine is a major metabolite of nicotine and is generally preferred over nicotine for validation or verification of self-reported tobacco use because of its relatively longer half-life (15–20 hours vs. 0.5–3 hours for cotinine and nicotine, respectively) (Benowitz, Hukkanen, & Jacob, 2009; CDC, 2007; Jarvis, Russell, Benowitz, & Feyerabend, 1988). However, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanonol (NNAL), with its half-life of 10–16 days, and with a washout of 92% after 42 days of cessation (Carmella et al., 2009), may provide a substantially longer window of exposure in comparison with cotinine.

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NNAL is a metabolite of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco-specific nitrosamine and carcinogen formed from tobacco alkaloids during the curing, fermentation, and ageing of tobacco leaves (Hatsukami et al., 2004; Stanfill et al., 2011; Upadhyaya, Carmella, Guengerich, & Hecht, 2000). Significant levels of NNAL have been found in body fluids of SLT users (Hecht et al., 2002, 2007a, 2008; Kresty et al., 1996). Research has also suggested that NNAL levels may be higher among SLT users compared with those among smokers, possibly due to the direct swallowing of tobacco-heavy spittle associated with some SLT products, coupled with the long duration in which habitual SLT users place tobacco products in the mouth (Hecht et al., 2007a).

Despite the potential of NNAL as a biochemical compound useful for detecting or verifying SLT use, no study has assessed its use in distinguishing current SLT users from nonusers. To fill this gap in the literature, data from the 2007–2008 and 2009–2010 National Health and Nutrition Examination Surveys (NHANES; CDC, 2007) were analyzed to assess the levels of urinary total NNAL among U.S. adult SLT-only users and to identify a potential threshold for identifying SLT users from nonusers.

METHODS

Data Sources

Aggregated data from the 2007–2008 and 2009–2010 cycles of NHANES were analyzed for 15,088 respondents aged 18 years or more for whom data on NNAL and past-5-day SLT use were available and who also reported not using any other tobacco product such as a cigarette, cigar, or pipe within the past 5 days. NHANES is a household interview and examination survey that uses a complex, multistage probability sampling design to select participants from the noninstitutionalized U.S. population (CDC, 2007). Overall interview response rates were 78.4% (2007–2008) and 79.4% (2009–2010).

Measures

Smokeless Tobacco Use

Recent SLT use ad libitum was assessed using the following questions: “During the past 5 days (including today), on how many days did you use snuff such as Skoal, Skoal Bandits, or Copenhagen?” and “During the past 5 days (including today), on how many days did you use chewing tobacco such as Redman, Levi, Garrett, or Beech-nut?” Analysis was restricted to SLT-only users, defined as respondents who reported using chewing tobacco or snuff during the past 5 days but no combustible tobacco product (pipes, cigars, and cigarettes). SLT nonusers were defined as respondents who reported not using any SLT, pipes, cigars, or cigarettes within the past 5 days. To control for misclassification, self-reported tobacco nonusers were cross-referenced with their serum cotinine levels, using a cutoff of less than 15 ng/ml.

Urinary Total NNAL

Urinary total NNAL was measured by using liquid chromatography linked to tandem mass spectrometry (LC/MS/MS; CDC, 2007).

Sociodemographic Characteristics

Sociodemographic characteristics included within the analyses were gender (male or female); age (18–24; 25–44; 45–64, or ≥65 years); race/ethnicity (non-Hispanic White; non-Hispanic Black; Hispanic; or non-Hispanic other race); education (less than 9th grade; 9th–11th grades; high school graduate/General Educational Development Certificate; or more than high school); Poverty Index Ratio (lower than federal poverty level or equal to or above federal poverty level); marital status (married/living with partner; widowed/divorced/separated; or single, never married); and presence of any smoker in household as a proxy for secondhand smoke exposure in the home (≥1 smoker or none). Poverty index ratio was based on self-reported family income and federal poverty thresholds (U.S. Census Bureau, 1995).

Statistical Analysis

NNAL levels are presented using geometric means, medians, and ranges because of data skewness. The partial correlation between the number of days (within the past 5 days) during which the respondent used SLT and natural log-transformed NNAL levels was assessed, while linear regression was used to compare levels across groups, adjusting for sex, age, race/ethnicity, education, poverty status, marital status, and second-hand smoke exposure in the home ($p < .05$).

Receiver operating characteristic (ROC) analysis was used to estimate the optimal threshold for discriminating SLT-only users from nonusers, with SLT use status as the classifier and NNAL level as the criterion. We estimated the optimal threshold by finding the cutoff that maximized the sum of the sensitivity and specificity. For the ROC analysis, sensitivity was defined as the proportion of self-reported SLT-only users that had NNAL levels above the given threshold, while specificity was defined as the proportion of self-reported SLT nonusers that had NNAL levels below the given threshold. The variability of the estimated optimal cutoff was assessed by bootstrapping 3000 times from the data to yield 95% confidence intervals (CIs).

The area under the curve (AUC) and the corresponding Youden's Index for the performance of NNAL in discriminating self-reported SLT use were calculated. Concordance analysis was used to assess the extent of agreement between self-reports and NNAL levels overall and among subpopulation groups, presented as percentages with 95% CIs. All analyses were conducted with MedCalc for Windows, Version 9.5.0.0 (MedCalc Software, Mariakerke, Belgium) and Stata, Version 11 (StataCorp. 2009; Stata Statistical Software, College Station, TX).

RESULTS

Within the aggregated 2007–2008 and 2009–2010 NHANES data, 264 individuals reported exclusively using SLT (and no other tobacco product) within the past 5 days, whereas 14,824 respondents were self-reported nonusers of any tobacco product. Among those who exclusively used SLT products, the mean number of days within the past 5 days during which respondents used SLT products was 4.3/5 for chewing tobacco and 4.1/5 days for snuff, indicating almost daily use. Overall median NNAL levels in SLT-only users were about 2200 times higher than the levels in non-tobacco users (Table 1). Average NNAL levels by type of SLT product

Table 1. Distribution of Urinary Total NNAL Among Adults Aged ≥18 Years Who Reported Using Smokeless Tobacco Only Within the Past 5 Days, NHANES, 2007–2010

Characteristic	Smokeless tobacco-only users		Non-tobacco users	
	N	Median NNAL levels, pg/ml (range)	N	Median NNAL levels, pg/ml (range)
Overall	264	880.0 (2.0–33,000.0)	14,824	0.4 (0.4–65,200.0)
Gender				
Male	249	862.0 (2.0–28,300.0)	6,744	0.4 (0.4–65,200.0)
Female	15	3,480.0 (243.7–33,000.0)	8,080	0.4 (0.4–16,000.0)
Age, years				
18–24	27	233.0 (6.7–2,240.0)	1,709	1.7 (0.4–1,770.0)
25–44	104	1,060.0 (14.2–18,800.0)	4,412	0.4 (0.4–1,710.0)
45–64	86	756.0 (2.0–33,000.0)	4,623	0.4 (0.4–16,900.0)
≥65	47	1,660.0 (45.9–20,700.0)	4,080	0.4 (0.4–65,200.0)
Race/ethnicity				
White, non-Hispanic	222	862.0 (6.7–20,700.0)	6,700	0.4 (0.4–65,200.0)
Black, non-Hispanic	28	1,469.0 (2.0–33,000.0)	2,721	1.5 (0.4–16,900.0)
Hispanic	10	209.0 (14.2–3,180.0)	4,601	0.4 (0.4–2,590.0)
Other race, non-Hispanic	4	28,300.0 (80.4–28,300.0)	802	0.4 (0.4–13,700.0)
Marital status				
Married/living with partner	158	1,090.0 (2.0–28,300.0)	8,670	0.4 (0.4–16,900.0)
Widowed/divorced/separated	60	418.0 (14.2–33,000.0)	3,226	0.4 (0.4–16,000.0)
Single, never married	35	345.0 (6.7–27,100.0)	2,236	0.9 (0.4–65,200.0)
Educational attainment				
<9th grade	28	880.0 (80.4–20,700.0)	1,941	0.4 (0.4–65,200.0)
9th–11th grades	45	2,310.0 (2.0–33,000.0)	2,277	1.6 (0.4–16,900.0)
High school graduate/GED	72	1,040.0 (20.9–10,500.0)	3,290	0.6 (0.4–3,000.0)
>High school	119	602.0 (6.7–18,800.0)	7,280	0.4 (0.4–10,300.0)
Poverty index ratio ^a				
Below	59	682.0 (2.0–25,200.0)	2,594	1.5 (0.4–16,900.0)
Above	196	862.0 (18.2–33,000.0)	10,698	0.4 (0.4–65,200.0)
Unknown	9	1,340.0 (105–3,020.0)	1,532	0.4 (0.4–10,300.0)
Exposure to secondhand smoke in the home				
Yes (≥1 smokers in household)	37	473.0 (2.0–22,600.0)	1,124	19.1 (0.4–13,700.0)
No (0 smokers in household)	225	882.0 (6.7–33,000.0)	13,594	0.4 (0.4–65,200.0)

Note. Data were weighted to account for the complex survey design and to adjust for differential nonresponse and selection.

NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanonol; GED = General Educational Development Certificate;

NHANES = National Health and Nutrition Examination Surveys.

^aPoverty status was defined based on a measure developed by the U.S. Bureau of the Census. Members of families whose incomes were equal to or greater than poverty thresholds were categorized as “at or above federal poverty level”; those with family incomes below the poverty thresholds were considered “below federal poverty level.”

used were as follows: snuff-only users ($n = 74$; geometric mean = 1,243.6 pg/ml, 95% CI: 885.6–1746.4); chewing tobacco-only users ($n = 186$; geometric mean = 711.6 pg/ml; 95% CI: 550.9–919.2); and combined chewing tobacco and snuff users ($n = 4$; geometric mean = 607.5 pg/ml; 95% CI: 2.6–140,156.6). After adjusting for potential confounders, there was no significant difference in mean NNAL levels by type of SLT product used. NNAL levels correlated with the number of days out of the past 5 days on which the respondent used chewing tobacco ($\rho = 0.48$, $p < .001$) and snuff ($\rho = 0.65$, $p < .001$).

ROC analysis indicated that the optimal cutoff point for identifying adult SLT-only users was 34.0 pg/ml, which was associated with a high sensitivity (95.2%), specificity (93.4%), and overall correct classification rate (93.5%). The AUC was 98.3% (95% CI: 98.0–98.5) and the corresponding Youden’s Index was 88.7% (95% CI: 85.8–90.3). Overall, as depicted in Table 2, there was high agreement between the proposed

NNAL level and self-reported SLT-only use (95.6%) and self-reported SLT nonuse (93.9%); however, differences in concordance were noted between age groups, racial/ethnic backgrounds, and educational and marital characteristics, as well as by poverty index ratio and exposure status with regard to household secondhand smoke.

DISCUSSION

The current study assessed the aggregated 2007–2010 NHANES data and proposed a potential cutoff to discriminate SLT-only users from nonusers, using urinary total NNAL, a long-term tobacco-specific biomarker. Based on the analyses, we propose a cutoff of 34.0 pg/ml, which was indicated to have high sensitivity and specificity. Furthermore, assessment of NNAL concentrations by type of SLT product use (snuff vs. chewing tobacco) did not show any significant difference.

Table 2. Concordance Between Self-Reported Smokeless Tobacco Use Status and Urinary Total NNAL Using the Proposed Cutoff of 34.0pg/ml Among U.S. Adults Aged ≥18 Years, NHANES 2007–2010

Characteristic	Self-reported SLT-only users, % (95% CI)	Self-reported SLT nonusers, % (95% CI)
Overall	95.6 (91.4–99.8)	93.9 (93.1–94.6)
Sex		
Male	95.5 (91.3–99.8)	92.7 (91.4–94.0)
Female	100.0	94.8 (93.9–95.8)
Age, years		
18–24	84.0 (52.6–100.0)	89.5 (86.4–92.6)
25–44	96.2 (90.7–100.0)	93.2 (91.9–94.5)
45–64	97.9 (94.9–100.0)	94.2 (92.9–95.6)
≥65	100.0	96.9 (95.9–97.9)
Race/ethnicity		
White, non-Hispanic	96.5 (92.4–100.0)	94.3 (93.4–95.2)
Black, non-Hispanic	91.5 (74.7–100.0)	87.5 (84.0–91.0)
Hispanic	59.8 (16.5–100.0)	95.7 (94.6–96.7)
Other race, non-Hispanic	100.0	95.1 (93.1–97.2)
Marital status		
Married/living with partner	99.4 (98.2–100.0)	94.8 (93.9–95.6)
Widowed/divorced/separated	84.6 (64.7–100.0)	92.9 (91.3–94.5)
Single, never married	88.2 (67.1–100.0)	92.2 (90.4–93.9)
Educational attainment		
<9th grade	100.0	94.7 (93.1–96.3)
9th–11th grades	97.0 (90.4–100.0)	85.9 (82.8–89.1)
High school graduate/GED	98.7 (96.1–100.0)	90.6 (89.0–92.1)
>High school	93.3 (85.7–100.0)	96.4 (95.5–97.3)
Poverty index ratio ^a		
Below	84.7 (71.2–98.2)	86.8 (83.2–90.4)
Above	97.1 (92.7–100.0)	95.3 (94.5–96.1)
Unknown	100.0	89.8 (86.4–93.1)
Exposure to secondhand smoke in the home		
Yes (≥1 smokers in household)	91.8 (77.1–100.0)	63.7 (58.5–68.8)
No (0 smokers in household)	96.0 (91.4–100.0)	96.1 (95.6–96.6)

Note. Data were weighted to account for the complex survey design and adjust for differential nonresponse and selection.

NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanonol; 95% *CI* = 95% confidence interval; GED = General Educational Development Certificate; NHANES = National Health and Nutrition Examination Surveys; SLT = smokeless tobacco.

^aPoverty status was defined based on a measure developed by the U.S. Bureau of the Census. Members of families whose incomes were equal or greater than poverty thresholds were categorized as “at or above federal poverty level”; those with family incomes below the poverty thresholds were considered “below federal poverty level.”

The utility of NNAL in distinguishing cigarette smokers from passive smokers has been previously assessed, and a relatively similar but slightly higher cutoff of 47.3 pg/ml was identified as an optimal threshold to discriminate between the two groups, a fact that could be attributable to the different study populations and type of tobacco use (Goniewicz et al., 2011). In our study, overall concordance was estimated at 95.6% for the entire population; however, this cutoff would predominantly be applicable to Caucasian male SLT-only users, as approximately 85% were Caucasian and less than 5% were female. These characteristics are common of SLT users, and this pattern is consistent with what has been reported by other researchers (Bhattacharyya, 2012; Timberlake & Huh, 2009).

Although cotinine may remain the tobacco exposure biomarker of choice for various clinical or research purposes (Haufroid & Lison, 1998; Kim & Jung, 2013), qualitative (detection of presence) or quantitative (measurement of the concentration) assessment of NNAL may be used together, or in lieu of cotinine in situations where the relatively short half-life

of cotinine may limit its use or in the presence of other nontobacco nicotine exposures. For example, NNAL may be useful in the case of occasional SLT users whose last episode of tobacco use exceeds a week (i.e., greater than the half-life of cotinine) and in detecting tobacco use among persons who may possibly have elevated cotinine levels ab initio from nontobacco sources such as nicotine replacement therapy and pesticide exposure (e.g., occupational exposures such as agricultural and pesticide industry workers; Haufroid & Lison, 1998; Sexton et al., 2011).

The proposed cutoff point was calculated only among respondents who reported using traditional smokeless tobacco products such as snuff, chewing and dipping tobacco products, and did not include users of low nitrosamine novel smokeless tobacco products such as snus and dissolvable tobacco products because of unavailability of data. However the average NNAL levels observed among users of these novel smokeless tobacco products as indicated in previous research (Hatsukami et al., 2004), fall within the range observed for users of snuff, chewing and dipping tobacco in our study. Hence, the proposed

cutoff point may be applicable to novel smokeless tobacco users also.

To our knowledge, this study is the first to propose a cutoff to identify SLT-only users from nonusers using urinary total NNAL. Strengths of the study include the use of nationally representative data available across multiple survey years and the relatively large sample size available. Nonetheless, the findings are subject to at least three limitations. First, the analysis was limited to SLT-only users and did not include concurrent users of SLT and combustible tobacco. However, this is not likely to affect the validity of our findings because the threshold calculated from a sample of SLT-only users may be more conservative and sensitive compared with that from a sample of SLT users who concurrently smoke combustible tobacco, under the assumption of similar SLT topography. Second, given the potential of selection bias among the volunteers of our study, the cutoff values we generated should be interpreted with caution as they are mostly male Caucasian SLT users. Finally, NHANES data assessed only recent 5-day SLT use, and thus the proposed cutoff may not be generalizable to populations experimenting or infrequently using SLT but more likely can be extrapolated to current, almost-daily SLT users.

CONCLUSION

This study indicated that among U.S. adults, an optimal cutoff for discriminating SLT users from nonusers using urinary total NNAL is 34.0 pg/ml, which has a high degree of both sensitivity and specificity. Self-reported SLT-only use and nonuse have high concordance with urinary total NNAL levels. These findings may have useful implications in clinical and epidemiological studies assessing recent SLT use or verifying self-reported use.

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DECLARATION OF INTERESTS

None declared.

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