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## Validation of Self-Reported Smokeless Tobacco Use by Measurement of Serum Cotinine Concentration Among US Adults

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### Abstract

Although investigators have assessed the relationship between self-reported cigarette smoking and biomarker levels, the validity of self-reported information on smokeless tobacco (SLT) use is uncertain. We used aggregated data from the 2003–2004, 2005–2006, 2007–2008, and 2009–2010 administrations of the National Health and Nutrition Examination Survey (NHANES) to compare self-reported SLT use with serum concentrations of cotinine, a metabolite of nicotine, among US adults aged ≥18 years. Receiver operating characteristic analysis was used to determine the optimal serum cotinine cutpoint for discriminating SLT users from nonusers of tobacco, and concordance analysis was used to compare self-reported SLT use with cotinine levels. Among the 30,298 adult respondents who completed the NHANES during 2003–2010, 418 reported having exclusively used SLT and no other type of tobacco (cigarettes, cigars, or pipes) during the past 5 days, while 23,457 reported not using any tobacco. The optimal cotinine cutpoint for discriminating SLT users from non-tobacco users was 3.0 ng/mL (sensitivity = 97.0%, specificity = 93.0%), which was comparable to a revised cutpoint recommended for identifying adult cigarette smokers. Concordance with cotinine was 96.4% and 93.7% for self-reported SLT use and tobacco nonuse, respectively. These findings indicate that self-reported SLT use among adults correlates highly with serum cotinine levels and that the optimal cutpoint for minimizing misclassification of self-reported use is a serum cotinine concentration of 3.0 ng/mL.

### Keywords

biomarkers; cotinine; epidemiologic methods; smokeless tobacco; smoking; snuff; tobacco

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Despite recent declines in cigarette smoking prevalence in the United States, little change has occurred in smokeless tobacco (SLT) use (1). In 2011, 3.5% of the US population aged 12 years currently used SLT (1). High levels of carcinogenic tobacco-specific nitrosamines have been found in SLT products, especially those formed by fermentation, such as moist snuff and chewing tobacco (2).

Surveillance of recent tobacco use is commonly based on population-based self-report surveys, which may be subject to misreporting because of inaccurate recall of information and can lead to underestimation of tobacco use (3). The degree of underreporting is particularly high among groups in which tobacco use is perceived as particularly undesirable, including persons with smoking-related diseases and pregnant women (4).

The most frequently utilized biomarker for validation of self-reported smoking data is cotinine, a metabolite of nicotine, which can be assessed in serum (5, 6), urine (7, 8), and saliva (9, 10). Cotinine has a half-life of 15–20 hours and thus is a useful biomarker for assessing recent tobacco use (11). Although investigators have assessed the relationship between self-reported smoking and cotinine levels (3), no study has validated self-reported SLT use, to our knowledge. Therefore, in this study we used data from the 2003–2010 administrations of the National Health and Nutrition Examination Survey (NHANES) to compare self-reported, past-5-day exclusive SLT use with measured serum cotinine levels among US adults aged 18 years. Our objectives were to identify the optimal threshold for discriminating SLT users from non-tobacco users and to determine the agreement between self-reported SLT use and cotinine levels.

## METHODS

### Data source

NHANES is a household interview and examination survey of the noninstitutionalized US population that utilizes a complex, multistage probability sampling design (12). Interviews are administered in English or Spanish in respondents' homes using computer-assisted technology. Health examinations and measurements are performed in specially designed and equipped mobile examination centers, which travel to various locations throughout the country.

We analyzed aggregated data from the 2003–2004, 2005–2006, 2007–2008, and 2009–2010 waves of the NHANES. Response rates were 79.0%, 80.5%, 78.4%, and 79.4%, respectively.

### Sample

The study was limited to 2003–2010 NHANES respondents aged 18 years for whom data on serum cotinine and past-5-day SLT use were available, and who also reported not having used a cigarette, cigar, or pipe within the past 5 days. The sample included 23,875 respondents (418 exclusive SLT users and 23,457 non-tobacco users).

## Measures

**SLT use**—Exclusive SLT users were respondents who reported having used chewing tobacco or snuff but no other type of tobacco (cigarettes, cigars, or pipes) during the past 5 days. Respondents were considered nonusers of any tobacco product if they reported not having used SLT, cigarettes, cigars, or pipes during the past 5 days.

**Serum cotinine assays**—Blood samples were obtained by venipuncture, and serum cotinine concentration was assayed using isotope dilution, liquid chromatography, and tandem mass spectrometry (13). There were no changes in the NHANES cotinine protocol during 2003–2010.

**Respondent characteristics**—Assessed respondent characteristics included: race/ethnicity (non-Hispanic white, non-Hispanic black, or Mexican-American); age (18–24, 25–44, 45–64, or 65 years); education (less than 9th grade, 9th–11th grade, high school graduation/General Educational Development certificate, or more than high school); poverty status (less than poverty level or at or above poverty level); marital status (married or living with a partner; widowed, divorced, or separated; or never married); and whether a smoker lived in the home (yes or no).

## Analysis

Cotinine levels were assessed using descriptive statistics (median values and ranges). Partial correlation between the number of days of SLT use during the past 5 days and cotinine level was also assessed, adjusting for respondent characteristics ( $\alpha = 0.05$ ).

Receiver operating characteristic curve analysis was used to estimate the optimal threshold for discriminating exclusive SLT users from nonusers of any tobacco product (5). Sensitivity was defined as the proportion of persons self-reporting exclusive SLT use who had cotinine levels at or above the given threshold, while specificity was defined as the proportion of persons not reporting tobacco use who had cotinine levels below the given threshold (5). The variability of each threshold was assessed using 95% confidence intervals. For comparison, we calculated the one-time sensitivity and specificity for all integer values within the 95% confidence intervals of the optimal cutpoint, as well as for the fixed cutpoint commonly used to discriminate self-reported cigarette smokers from nonsmokers (15 ng/mL) (14, 15). We assessed the variability of our estimated optimal cutpoint by bootstrapping 2,000 times from the data to obtain 95% confidence intervals, with weights determined by the survey design. Agreement between self-reports and cotinine levels was assessed using concordance analysis, which was performed overall and according to respondent characteristics. All analyses were conducted using R, version 2.12.0 (R Foundation for Statistical Computing, Vienna, Austria) and Stata, version 11 (Stata-Corp LP, College Station, Texas).

## RESULTS

Median cotinine concentrations were 293 ng/mL (range, 0.1–1,438) among self-reported exclusive SLT users and 0.03 ng/mL (range, 0.01–1,639) among self-reported nonusers of

tobacco. Among exclusive SLT users, cotinine levels were correlated with the numbers of days on which snuff ( $\rho = 0.51$ ,  $P < 0.001$ ) and chewing tobacco ( $\rho = 0.52$ ,  $P < 0.001$ ) had been used (data not shown).

The optimal cotinine cutpoint that separated self-reported exclusive SLT users from non-tobacco users was 3.0 ng/mL (95% confidence interval: 1.4, 9.1), with a correct classification rate (concordance) of 93.2%, sensitivity of 97.0%, and specificity of 93.0% (Table 1). Across cutpoints, concordance ranged from 90.1% (at the 1-ng/mL cutpoint, sensitivity = 98% and specificity = 90%) to 94.5% (at the 10-ng/mL cutpoint, sensitivity = 94% and specificity = 95%). At the cutpoint commonly used for discriminating cigarette smokers from nonsmokers (15 ng/mL), the concordance for exclusive SLT users and nonusers was 94.8% (sensitivity = 92%, specificity = 95%).

Overall concordance between self-reports and cotinine levels was higher for self-reported exclusive SLT users (96.4%) than for non-tobacco users (93.7%) (Table 2). Among self-reported exclusive SLT users, concordance was higher among females (100%) than among males (96.3%). By age, concordance was highest among persons aged  $\geq 65$  years (100%) and lowest among persons aged 45–64 years (95.4%). By race/ethnicity, concordance ranged from 78.5% (Mexican Americans) to 96.7% (non-Hispanic whites). By marital status, concordance was highest among persons who were married or living with a partner (96.8%) and lowest among persons who had never married (94.0%). By education, concordance was highest among those with less than a 9th-grade education (100%) and lowest among those with more than a high school education (94.7%). Concordance was lower among persons living below the poverty level (88.5%) than among persons living at or above this level (97.7%) and higher among persons who lived with a smoker (96.8%) than among those who did not (96.3%).

Among self-reported non-tobacco users, concordance was higher among females (95.0%) than among males (92.0%) (Table 2). By age, concordance was highest among persons aged  $\geq 65$  years (96.8%) and lowest among persons aged 18–24 years (89.0%). By race/ethnicity, concordance ranged from 87.1% (non-Hispanic blacks) to 94.9% (Mexican Americans). By marital status, concordance was highest among those married or living with a partner (94.8%) and lowest among those never married (90.5%). By education, concordance was highest among those with more than a high school education (95.8%) and lowest among those with a 9th- to 11th-grade education (86.7%). Concordance was lower among persons living below the poverty level (87.2%) than among persons living at or above this level (94.8%), and it was lower among persons who reported living with a smoker (65.0%) than among those who did not (96.0%).

## DISCUSSION

These study findings reveal that the optimal cotinine cut-point for discriminating between exclusive SLT users and non-tobacco users is 3.0 ng/mL. This cutpoint had a high degree of sensitivity and specificity among US adults, and it is comparable to a recently revised cutpoint for identifying US adult cigarette smokers (3.08 ng/mL) that was revised because of declines in population-level secondhand smoke exposure (5). Considerable secondhand

smoke exposure can produce serum cotinine levels greater than 10 ng/mL in non-smokers (16, 17); thus, a higher cutpoint was required to exclude most nonsmokers (5).

Despite high levels of concordance between past-5-day SLT use and cotinine concentration, agreement was lower among self-reported nonusers than among users. This finding may be due to changing social norms related to tobacco use and respondents' reluctance to identify themselves as SLT users (5), tobacco smoke exposure among nonusers, or the use of emerging tobacco products not assessed by the questionnaire (e.g., snus, dissolvable tobacco products, and electronic cigarettes) (18–21).

Disparities in concordance were observed across subpopulations. For instance, concordance was lowest among persons aged 18–24 years, which may be a result of young adults being more likely to underreport behavior perceived as socially undesirable (9, 22) or the use of novel tobacco products among these persons (18–21). This may also be due to higher secondhand smoke exposure from peers who smoke; adults aged 18–24 years had one of the highest smoking rates of any age group during the study period, ranging from 20.1% in 2010 to 24.4% in 2005 (23, 24). Disparities were also observed by race/ethnicity. For example, concordance between self-reported exclusive SLT use and cotinine level was lowest among Mexican Americans. This may be due to the lower prevalence and intensity of tobacco consumption among Hispanics than among persons of other races/ethnicities and, hence, lower cotinine levels that may overlap those of nonusers (5). Differences in nicotine metabolism and secondhand smoke exposure may also account for some of the observed racial/ethnic differences. Cotinine metabolism is slower among blacks than among persons of other races/ethnicities (25, 26), which may explain the relatively low concordance between self-reported non-tobacco use and cotinine levels in blacks. Concordance between self-reported nonuse and cotinine concentration was also lower among persons living with smokers, which may be due to elevated cotinine levels from secondhand smoke exposure (17, 27).

With a half-life of 15–20 hours (11), cotinine can only detect recent tobacco use, and thus it may not accurately capture occasional use. Additionally, cotinine may not be the ideal biomarker for persons who have elevated nicotine levels from secondhand smoke exposure or other, nontobacco sources, such as nicotine replacement therapy, or those with occupational exposure to pesticides (28, 29). In these situations, an alternative biomarker may be utilized for biochemical detection or confirmation of tobacco use. With a half-life of 10–16 days, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanonol (NNAL) may provide a longer window of exposure than cotinine in these situations (30, 31).

The observed optimal cutpoint for biochemical confirmation of self-reported exclusive SLT use and non-tobacco use (3.0 ng/mL) can have utility in both clinical and research settings. Researchers can use this cutpoint for studies of SLT use. Additionally, in settings where SLT use is the sole focus of interest, clinicians can use the cutpoint for verification or detection of recent use in the absence of other tobacco use. However, this cutpoint would predominantly be applicable to white male SLT users, since approximately 91% of the SLT users in this study were non-Hispanic white and 97% were male, which is consistent with previous studies (32, 33).

To our knowledge, this study was the first to assess the validity of self-reported SLT use. Nonetheless, at least 3 limitations should be noted. First, the sample included only US adults and was predominately comprised of non-Hispanic white males; thus, the optimal cotinine cutpoint found here may not be generalizable to other countries and subpopulations. Second, certain subpopulations had few SLT users, which may have yielded imprecise concordance estimates for these groups. Third, the exclusive SLT definition did not account for use of nicotine replacement therapy, electronic cigarettes, snus, or dissolvable tobacco products; in NHANES, nicotine replacement therapy was assessed only among persons who had tried to quit cigarette smoking, while data were unavailable for the then-emerging noncombustible tobacco products. However, most of the study period preceded the availability of these products in the United States. Additionally, novel SLT products such as snus and dissolvable tobacco products account for only a fraction of US SLT use, whereas moist snuff accounted for 86.5% of total SLT sales in 2011 (34). As these new SLT products continue to be marketed and increase in popularity, it will be important to measure use and to assess response validity with future cotinine validation studies.

In conclusion, this study found that among US adults, self-reported SLT use had high concordance with cotinine levels, and the optimal cutpoint for discriminating self-reported SLT users from non-tobacco users was 3.0 ng/mL. This cutpoint had a high degree of sensitivity and specificity and thus may be useful for researchers investigating the relationship between SLT use and health, as well as clinicians seeking to verify SLT use.

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## Abbreviations

NHANES	National Health and Nutrition Examination Survey
SLT	smokeless tobacco

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**Table 1**

Sensitivity, Specificity, and Correct Classification Rates for Classification of Smokeless Tobacco Use by Cotinine Level Among US Adults Aged 18 Years in the National Health and Nutrition Examination Survey, 2003–2010

Cotinine Cutpoint, ng/mL	Sensitivity	Specificity	% Correctly Classified
1	0.9779	0.8993	90.08
2	0.9681	0.9228	92.36
3 <sup>a</sup>	0.9657	0.9313	93.20
4	0.9559	0.9361	93.65
5	0.9510	0.9385	93.88
6	0.9485	0.9406	94.08
7	0.9485	0.9420	94.21
8	0.9461	0.9435	94.35
9	0.9412	0.9448	94.47
10	0.9363	0.9456	94.54
15 <sup>b</sup>	0.9240	0.9484	94.80

<sup>a</sup>Optimal cutpoint (95% confidence interval: 1.4, 9.1).

<sup>b</sup>Threshold commonly used in discriminating cigarette smokers from nonsmokers.



Characteristic	Self-Reported Exclusive SLT Use ( <i>n</i> = 418) <sup>d</sup>			Self-Reported Tobacco Nonuse ( <i>n</i> = 23,457) <sup>d</sup>				
	No. of Persons	% <sup>b</sup>	Concordance With Cotinine Level, <sup>c</sup> %	95% CI	No. of Persons	% <sup>b</sup>	Concordance With Cotinine Level, <sup>c</sup> %	95% CI
No	350	85.6	96.3	93.3, 99.3	21,284	92.1	96.0	95.5, 96.4
Yes	65	13.6	96.8	91.8, 100.0	1,965	7.3	65.0	60.6, 69.3
Overall	418	100.0	96.4	93.7, 99.1	23,457	100.0	93.7	93.0, 94.3

Abbreviations: CI, confidence interval; GED, General Educational Development; N/A, not applicable; SLT, smokeless tobacco.

<sup>a</sup> Self-reported SLT users were persons who reported having used snuff or chewing tobacco at least once within the past 5 days. This excluded persons who reported using any cigarettes, pipes, or cigars within the previous 5 days. Self-reported nonusers were persons who reported not having used any tobacco product (including snuff, chewing tobacco, cigarettes, pipes, or cigars) within the previous 5 days.

<sup>b</sup> Weighted percentage. Proportions may not total 100% because of missing/unknown values for some covariates.

<sup>c</sup> A serum cotinine cutpoint of 3.0 ng/mL was used to identify SLT users.

<sup>d</sup> Results for persons of other races/ethnicities are not shown separately but are included in the total.

<sup>e</sup> Poverty status was defined in accordance with methods used by the US Census Bureau (35).