Urinary Cotinine and Cotinine + Trans-3′-Hydroxycotinine (TNE-2) Cut-points for Distinguishing Tobacco Use from Nonuse in the United States: PATH Study (2013–2014)

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ABSTRACT

Background: Determine the overall, sex-, and racially/ethnically-appropriate population-level cotinine and total nicotine equivalents (TNE-2, the molar sum of the two major nicotine metabolites) cut-points to distinguish tobacco users from nonusers across multiple definitions of use (e.g., exclusive vs. polytobacco, and daily vs. non-daily).

Methods: Using Wave 1 (2013–2014) of the U.S. Population Assessment of Tobacco and Health (PATH) Study, we conducted weighted Receiver Operating Characteristic (ROC) analysis to determine the optimal urinary cotinine and TNE-2 cut-points, stratified by sex and race/ethnicity.

Results: For past 30-day exclusive cigarette users, the cotinine cut-point that distinguished them from nonusers was 40.5 ng/mL, with considerable variation by sex (male: 22.2 ng/mL; female: 43.1 ng/mL) and between racial/ethnic groups (non-Hispanic other: 5.2 ng/mL; non-Hispanic black: 297.0 ng/mL). A similar, but attenuated, pattern emerged when assessing polytobacco cigarette users (overall cut-point = 39.1 ng/mL, range = 5.5 ng/mL–80.4 ng/mL) and any tobacco users (overall cut-point = 39.1 ng/mL, range = 4.8 ng/mL–40.0 ng/mL). Using TNE-2, which is less impacted by racial differences in nicotine metabolism, produced a comparable pattern of results although reduced the range magnitude.

Conclusions: Because of similar frequency of cigarette use among polytobacco users, overall cut-points for exclusive cigarette use were not substantially different from cut-points that included polytobacco cigarette use or any tobacco use. Results revealed important differences in sex and race/ethnicity appropriate cut-points when evaluating tobacco use status and established novel urinary TNE-2 cut-points.

Impact: These cut-points may be used for biochemical verification of self-reported tobacco use in epidemiologic studies and clinical trials.

Introduction

Cigarette smoking prevalence has changed drastically in the United States, down from 40% in 1964 to 13.7% in 2018 (1, 2). Second-hand exposure has also been greatly impacted by the passage of smoke-free laws in restaurants, public spaces, public housing, and college campuses (3–10). Furthermore, as public health efforts in the United States are considering reducing the addictive potential of cigarettes by reducing their nicotine content (11), it is critical to accurately evaluate changes in cigarette smoking behavior. Large longitudinal and surveillance studies often rely on self-reported tobacco use. Some large studies [e.g., Population Assessment of Tobacco and Health (PATH) Study, National Health and Nutrition Examination Survey (NHANES)] also measure biomarkers such as cotinine and other nicotine metabolites, allowing biochemical verification of self-reported tobacco use. Previous analyses of NHANES data from the 1990s and early 2000s suggest that self-reported estimates may underestimate true smoking prevalence, but only minimally (12, 13). However, cigarette smoking prevalence as well as exposure to second-hand smoke has decreased considerably in the last two decades (3–10), and use of noncigarette tobacco products has grown in popularity (14). As such, there is a need to revisit the appropriate thresholds (or cut-points) for biochemical validation of tobacco use, in addition to cigarette smoking, as polytobacco use (use of more than one tobacco product) increases (14, 15).

Cotinine is the primary metabolite of nicotine and its detection in serum, urine, and saliva has been used to distinguish smokers from nonsmokers (16–19), as well as second-hand exposure versus active smoking (20, 21). Numerous cotinine cut-points (across various biological matrices) have been suggested for biochemical validation of smoking status (17, 22). Primary applications of these cut-points include validating abstinence in smoking cessation trials, as well as validating self-reported use for inclusion in research studies or in

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national surveillance surveys. One study evaluating cotinine cut-points using the NHANES data from 1999 to 2004 to distinguish recent cigarette smokers who have not used other tobacco products in the last five days from nonsmokers found optimal cotinine cut-points of approximately 5 ng/mL in serum and projected approximately 15 ng/mL free cotinine in urine (16). This study also found differences in optimal cut-point by sex and race/ethnicity (16). These differences are the result of considerable variability in nicotine metabolism (23, 24).

Nicotine is metabolized into cotinine primarily by the liver enzyme CYP2A6. Cotinine is metabolized by CYP2A6 and UGT2B10 into trans-3′-hydroxycotinine (3HC) and cotinine glucuronide, respectively (22, 24, 25). There is considerable genetic variability in CYP2A6 and UGT2B10 activity, with slow metabolism more common in Asians and African Americans (23, 25). Sex differences, driven by estrogen induction of CYP2A6 activity, results in faster metabolism in females (26). Although cotinine levels are variable due to these influences, they have been the primary mechanism for validating smoking status. Total nicotine equivalents (TNE), or the molar sum of nicotine and its metabolites, is considered the gold standard for estimating nicotine intake and is not affected by sex or race/ethnicity (22). TNE is measured by summing nicotine, cotinine, 3HC, four other minor metabolites, and their glucuronides (TNE-7; ref. 22). Analysis of TNE is more expensive than cotinine alone, and optimal TNE cut-points to distinguish tobacco users from nonusers have not yet been reported. Because nicotine tends to be ubiquitous in the environment and attempting to achieve lower urine blanks is not feasible; TNE-2 (the sum of cotinine and 3HC) is used when nonusers are included in analysis. TNE-2 is highly correlated with TNE-7 ($r = 0.99$) and is not affected significantly by race/ethnicity or sex (22).

Seventy-five percent of current smokers are daily users, and 19% use at least two tobacco products (14). Moreover, cigarette smokers are a heterogeneous group with distinct racial/ethnic profiles (as well as sex differences) that may interact with different patterns of use (i.e., daily vs. non-daily) to make a single cut-point misleading. Using data from Wave 1 of the PATH Study, the main goal of this study is to determine overall as well as sex and racially/ethnically appropriate cut-points using cotinine and TNE-2 to distinguish cigarette users from nonusers across multiple definitions of use (i.e., exclusive vs. polytobacco use; daily vs. nondaily). In addition, because nicotine is not a selective indicator of cigarette smoking but of overall tobacco exposure and polytobacco use continues to rise (14), determining sex and racially/ethnically appropriate cotinine and TNE-2 cut-points to distinguish any tobacco use (from no tobacco use) is essential for accurate prevalence estimates.

Materials and Methods

Data source

Adult interview

Data are from Wave 1 (September 12, 2013 to December 15, 2014) of the PATH Study, a nationally representative, longitudinal cohort study of adults (≥18 years) and youth (12–17 years) in the U.S. The PATH Study used audio-computer assisted self-interviews available in English and Spanish to collect information on tobacco-use patterns and associated health behaviors. Recruitment employed address-based, area-probability sampling, using an in-person household screener to select youths and adults. Adult tobacco users, young adults ages 18 to 24 and African Americans were oversampled relative to population proportions. The weighted response rate for the household screener was 54.0%. Among households that were screened, the overall weighted response rate was 74.0% for the adult interview. Further details regarding the PATH Study design, methods, and instruments are published elsewhere (27, 28). Details on survey interview procedures, questionnaires, sampling, weighting, and information on accessing the data are available at https://doi.org/10.3886/Series606. Westat's Institutional Review Board, in accordance with the Common Rule, approved the study design and data collection protocol. All respondents ages 18 and older provided written informed consent, with youth respondents ages 12 to 17 providing assent whereas each one's parent/legal guardian provided consent.

Biospecimen collection and analysis

All adult interview respondents ($N = 32,320$) at Wave 1 were asked to provide biospecimens. Full-void urine specimens were self-collected by 21,801 (67.5%) consenting participants. For more information on the collection procedures, materials, and aliquots created from the urine specimens please see the PATH Study Biospecimen Urine Collection Procedures document in the "Study Level" files (http://doi.org/10.3886/ICPSR36840.v5).

A stratified probability sample of 11,522 adults who completed the Wave 1 Adult Interview and who provided a urine specimen were selected for analyses. The sample was selected to ensure respondents represented diverse tobacco product use patterns, including users of multiple tobacco products, and never users of any tobacco product. The current analysis draws from the 11,504 Adult Interviews collected at Wave 1 who have urinary cotinine data available [Wave 1 Biomarker Restricted Use Files (http://doi.org/10.3886/ICPSR36840.v5); Wave 1 Adult Restricted Use Files (https://doi.org/10.3886/ICPSR36231.v20)].

See Supplementary Fig. 1 for a flow diagram indicating our final analytic sample. Of the past 30-day (P30D) tobacco users ($N = 8,963$) and nonusers ($N = 2,276$) with cotinine data, 3,010 P30D exclusive cigarette users, 3,592 P30D polytobacco cigarette users, and 2,209 nonusers were included in the analyses stratified by cigarette use. Given that not all respondents agreed to provide biospecimens, the resulting biomarker data represent a subsample of adults; therefore, specific urine weights are needed to account for potential differences between the full set of adult interview respondents in the specified tobacco product user groups and the set of adults with analyzed biospecimens. The weighting procedures adjusted for oversampling and nonresponse; combined with the use of a probability sample, weighted estimates are representative of never, current, and recent former (within 12 months) users of tobacco products in the U.S. civilian, noninstitutionalized adult population at the time of Wave 1 (https://www.icpsr.umich.edu/files/NAHDAP/36840-User_guide-Biomarker_Restricted_Use_Files_User_Guide.pdf).

Laboratory analysis

Total urinary nicotine metabolites, including the free and glucuronide conjugated forms, were measured by two separate isotope dilution high-performance LC/MS-MS (HPLC/MS-MS) methods based on the cotinine cut-point value of 20 ng/mL. For samples with cotinine levels above or equal to 20 ng/mL, the "Nicotine Metabolites and Analogs in Urine" method was used to measure nicotine, cotinine, 3HC, and 4 other metabolites as well as minor tobacco alkaloids (29). For samples with cotinine levels less than 20 ng/mL, the "Cotinine and Hydroxycotinine in Urine" method was applied to sensitively measure cotinine and 3HC using a modified version of the method of Bernert and colleagues (2005; ref. 30). The lower limit of detection (LOD) for cotinine and 3HC is 0.030 ng/mL. Result values that were below the LOD were replaced with LOD divided by the square root of 2. TNE-2

was calculated by taking the molar sum (nmol/mL) of cotinine and 3HC for all respondents. If a respondent was missing a value for either analyte, TNE-2 was treated as a missing.

Measures

Tobacco use groups

P30D exclusive cigarette use was defined as those who are P30D smokers of cigarettes (either every day or some days), and are not P30D users of other tobacco products. P30D exclusive cigarette use was then stratified into P30D daily cigarette use and P30D nondaily cigarette use for those who used "every day" or "some days," respectively.

P30D polytobacco cigarette use was defined as those who are P30D every day or some day users of cigarettes, and have also used at least one of the following tobacco products in the past 30 days: e-cigarettes, traditional cigar, cigarillo, filtered cigar, pipe, smokeless tobacco, snus pouches, and/or dissolvable tobacco. P30D polytobacco cigarette use was then stratified into P30D daily polytobacco cigarette use and P30D non-daily polytobacco cigarette use for those who used cigarettes "every day" or "some days," respectively.

P30D any tobacco use was defined as those who are P30D users of any tobacco product (cigarettes, e-cigarettes, traditional cigar, cigarillo, filtered cigar, pipe, smokeless tobacco, snus pouches, and dissolvable tobacco).

Nonuser (reference for P30D any tobacco use) was defined as those who are not P30D users of any tobacco product. See Supplementary Fig. 1 for more details.

Nonuser (reference for P30D exclusive and polytobacco cigarette use) was defined as those who did not report P30D use of any tobacco product, did not report being a current every day or someday cigarette user, and provided logically consistent responses to both past 30-day use and daily/non-daily cigarette use items.

To avoid confounding nicotine exposure, all tobacco use groups and the nonuser reference group excluded those who indicated any past 3-day use of nicotine replacement therapy (NRT) products. Product users were asked to confirm past 3-day use of a given tobacco product either in the questionnaire, or prior to biospecimen collection if collection occurred at least 4 hours after the questionnaire was completed. Instances where a respondent indicated no past 30-day use in the questionnaire but did indicate past 3-day use prior to collection were excluded.

All outliers were removed for the reference categories of the tobacco use groups. Outliers were removed in order to capture true nonusers and avoid potentially misclassifying self-reported users as nonusers, and to ensure that anomalies do not drive the cut-points higher. Values outside of the range of two standard deviations from the mean of urinary cotinine in the reference category were considered outliers. Similarly for TNE-2, values outside of the range of two SDs from the mean of TNE-2 in the reference category were considered outliers.

Demographics and other tobacco product characteristics

Demographic characteristics presented for each user group include age, sex, race/ethnicity, educational attainment, and household income. Missing data on age, sex, race, Hispanic ethnicity, education were imputed as described in the PATH Study Restricted Use Files User Guide (United States Department of Health and Human Services, 2019). Additional tobacco use characteristics presented for each user group include cigarettes used per month [amount of cigarettes used per day (on days used) multiplied by number of days used in the past 30 days], percentage of daily use, type of polytobacco use, recency of last cigarette use, and exposure to second-hand smoke. See **Tables 1** and **2**.

Statistical analysis

Weighted percentages and means were calculated for demographic and tobacco use characteristics for each user group. Statistical differences between user groups were calculated using χ^2 tests for categorical variables and independent sample t test for continuous variables.

Next, weighted Receiver Operating Characteristic (ROC) curves were calculated to determine the optimal cut-point using urinary cotinine or TNE-2 levels to distinguish P30D users from nonusers. The Wave 1 full sample and 100 replicate urine weights were incorporated in logistic regression models of urinary cotinine run against the tobacco use groups to estimate predicted probabilities. The predicted probabilities were then used to generate ROC curves and associated characteristics with the full sample urine weight. The 95% CIs of the weighted area under the curves (AUC) were calculated using a bootstrap approach incorporating the 200 replicate bootstrap weights (31).

Analyses were stratified by exclusive and polytobacco cigarette use, and then further stratified by daily and nondaily use among males and females and four race/ethnicity categories (non-Hispanic white, non-Hispanic black, non-Hispanic other race/multiple race, and Hispanic). This approach was repeated (without daily/non-daily stratification) to determine an ideal cut-point to distinguish any P30D tobacco users from nonusers. All cut-points were selected using Youden J-statistic.

Analyses were conducted using Stata software survey procedures, version 15.1 (StataCorp), and SAS software survey procedures, version 9.4 (SAS Institute, Inc.). Variances were estimated using the balanced repeated replication (BRR) method (32) with Fay adjustment set to 0.3 to increase estimate stability (33).

Results

Sample characteristics

As shown in **Table 1**, compared with exclusive cigarette smokers, polytobacco cigarette smokers were more likely to be male (poly: 62.9%, exclusive: 48.8%, $P < 0.001$) and younger (age 18–24, poly: 23.4%, exclusive 9.8%, $P < 0.001$). Exclusive cigarette users smoked more cigarettes per month (exclusive: 120, poly: 92, $P = 0.01$) and had greater daily use (exclusive: 80.7%, poly: 75.7%, $P = 0.01$) than polytobacco cigarette users. Nonusers were more likely to be female (nonuser: 61.0%, exclusive: 51.2%, poly: 37.1%, $P < 0.001$) and Hispanic (nonuser: 20.3%, exclusive: 14.0%, poly: 13.0%, $P < 0.001$) than exclusive or polytobacco cigarette users.

As shown in **Table 2**, compared with nonusers, any tobacco users were more likely to be male (any tobacco: 59.0%, nonusers: 39.1%, $P < 0.001$), had an income level of less than $25,000 a year (any tobacco: 43.0%; nonuser: 30.8%, $P < 0.001$), and had exposure to second-hand smoke (any tobacco: 85.3%, nonuser: 37.3%, $P < 0.001$).

Cotinine cut-points

Exclusive cigarette users

To compare our results to previous cut-points estimated using serum cotinine, we further extrapolated Benowitz et al.'s estimated cut-point of 15 ng/mL of free cotinine in urine to 30 ng/mL total cotinine in urine (as shown in **Fig. 1A**) because total cotinine estimates tend to be two times greater than free cotinine estimates (16, 24). For exclusive cigarette users the cotinine cut-point that distinguished P30D users from nonusers was 40.5 ng/mL (AUC = 0.98; 95% CI: 0.97–0.99). Females had a higher cut-point (43.1 ng/mL; AUC = 0.98; 95% CI: 0.97–0.99) than males (22.2 ng/mL; AUC = 0.98, 95% CI: 0.97–0.99; see **Table 3A**). There was considerable range among racial/

Table 1. Self-reported smoking prevalence, sociodemographic characteristics, and tobacco use characteristics of Wave 1 (2013–2014) past 30-day exclusive and polytobacco cigarette users.

	Wave 1 respondents with nonmissing cotinine data						Statistical differences between user groups[b]		
	Past 30-day exclusive cigarette use[a] (*N* = 3,010)		Past 30-day polytobacco cigarette use (*N* = 3,592)		No past 30-day tobacco use (*N* = 2,209)				
	Unweighted *N*	Weighted % (CI)[c]	Unweighted *N*	Weighted % (CI)	Unweighted *N*	Weighted % (CI)	Exclusive use vs. no use	Exclusive use vs. poly use	Poly use vs. no use
Age									
18–24	509	9.8 (8.3–11.5)	1,297	23.4 (21.3–25.6)	881	16.7 (15.3–18.2)	<0.001	<0.001	<0.001
25–39	934	30.9 (28.3–33.8)	1,175	37.7 (34.7–40.7)	564	27.9 (25.4–30.5)			
40–54	921	32.2 (29.6–35.0)	719	23.2 (21.2–25.3)	386	25.9 (23.2–28.8)			
55+	646	27.0 (24.4–29.9)	401	15.8 (13.4–18.5)	378	29.6 (26.7–32.6)			
Sex									
Male	1,409	48.8 (45.8–51.8)	2,184	62.9 (60.0–65.7)	903	39.0 (36.7–41.4)	<0.001	<0.001	<0.001
Female	1,601	51.2 (48.3–54.2)	1,408	37.1 (34.3–40.0)	1,306	61.0 (58.6–63.3)			
Race/ethnicity									
Non-Hispanic white	1,903	66.0 (62.7–69.1)	2,184	64.5 (61.3–67.5)	1,112	56.6 (53.1–60.1)	<0.001	0.53	<0.001
Non-Hispanic black	448	14.9 (12.5–17.6)	537	16.8 (14.0–20.0)	399	14.4 (12.3–16.8)			
Non-Hispanic other race/multiple race	207	5.2 (4.2–6.4)	313	5.8 (5.0–6.8)	189	8.7 (7.1–10.6)			
Hispanic	452	14.0 (11.8–16.4)	558	13.0 (11.5–14.6)	509	20.3 (17.9–23.1)			
Education									
Less than high school or some high school (no diploma) or GED	940	29.8 (27.4–32.4)	1,069	28.6 (25.9–31.5)	345	15.7 (13.8–17.8)	<0.001	0.03	<0.001
High school diploma	749	29.5 (26.8–32.5)	904	25.3 (23.1–27.6)	532	25.0 (21.7–28.7)			
Some college (no degree) or associate degree	1,033	30.9 (28.3–33.6)	1,349	36.9 (33.8–40.0)	809	28.3 (25.5–31.3)			
Bachelor's degree or more	288	9.7 (8.0–11.7)	270	9.3 (7.9–10.8)	523	30.9 (27.5–34.7)			
Income									
<$25,000	1,493	43.4 (40.4–46.3)	1,994	50.2 (47.6–52.9)	806	30.7 (27.8–33.8)	<0.001	<0.001	<0.001
$25,000–$74,999	1,012	35.4 (32.5–38.3)	1,077	31.9 (29.4–34.5)	750	33.5 (30.3–36.9)			
≥$75,000	302	11.1 (9.6–12.8)	327	11.6 (9.8–13.6)	457	26.0 (22.7–29.6)			
Not reported	203	10.2 (8.1–12.8)	194	6.3 (5.4–7.4)	196	9.8 (8.0–11.9)			
Tobacco use characteristics									
CPM (cigarettes per month)	785	120.4 (104.9–135.8)	1,110	92.2 (81.0–103.4)	N/A	N/A	N/A	0.01	N/A
Daily cigarette use	2,394	80.7 (78.0–83.2)	2,629	75.7 (73.3–77.9)	N/A	N/A	N/A	0.01	N/A
Polytobacco- combustible only	N/A	N/A	2,208	57.2 (54.1–60.2)	N/A	N/A	N/A	N/A	N/A
Polytobacco- combustible +noncombustible	N/A	N/A	1,384	42.8 (39.8–45.9)	N/A	N/A	N/A	N/A	N/A
Recent cigarette use									
Last used today	2,480	83.3 (80.6–85.7)[d]	2,717	77.4 (74.8–79.9)	N/A	N/A	N/A	0.02	N/A
Last used yesterday	246	7.4 (6.0–9.0)	423	9.6 (8.4–10.9)	N/A	N/A			
Last used ≥ the day before yesterday	237	7.6 (6.1–9.4)	373	9.9 (8.2–11.9)	N/A	N/A			
Nicotine exposure									
Geometric mean of urinary cotinine (ng/mL)	3,010	1,550.3 (1333.9–1801.9)	3,592	1,515.1 (1,391.5–1,649.7)	2,209	0.4 (0.4–0.5)	<0.001	0.78	<0.001
Exposure to second hand smoke	2,694	88.5 (86.2–90.4)	3,360	92.9 (91.5–94.0)	962	37.3 (33.4–41.3)	<0.001	0.02	<0.001

[a]Exclusive users could have no missing values on other tobacco product use. Polytobacco users could be missing on other products as long as they indicated using at least two products.
[b]Statistical differences between user groups were calculated using χ^2 tests for categorical variables and *t* tests for continuous variables. *P* values below 0.05 indicate statistical significance.
[c]For continuous variables mean and standard error are reported.
[d]Includes missing cases, therefore some column percentages add up to less than 100%.

Table 2. Self-reported smoking prevalence, sociodemographic characteristics, and tobacco use characteristics of Wave 1 (2013–2014) past 30-day any tobacco users.

	Wave 1 respondents with nonmissing cotinine data				
	Past 30-day any tobacco use (*N* = 8,963)		No past 30-day tobacco use (*N* = 2,276)		Statistical differences between user groups[a]
	Unweighted *N*	Weighted % (CI)[b]	Unweighted *N*	Weighted % (CI)	Any tobacco use vs. no tobacco use
Age					
18–24	2,710	18.5 (17.1–20.0)	907	16.8 (15.5–18.2)	<0.001
25–39	2,731	32.8 (30.9–34.8)	585	27.9 (25.5–30.5)	
40–54	2,116	26.9 (25.5–28.3)	400	25.9 (23.1–28.8)	
55+	1,406	21.8 (20.3–23.5)	384	29.4 (26.6–32.5)	
Sex					
Male	5,199	59.0 (57.0–61.0)	938	39.1 (36.9–41.5)	<0.001
Female	3,764	41.0 (39.0–43.0)	1,338	60.9 (58.5–63.2)	
Race/ethnicity					
Non-Hispanic white	5,578	65.7 (63.7–67.7)	1,139	56.5 (53.0–59.9)	<0.001
Non-Hispanic black	1,335	15.1 (13.7–16.7)	402	14.4 (12.3–16.7)	
Non-Hispanic other race/multiple race	697	5.7 (5.0–6.6)	195	8.71 (7.1–10.6)	
Hispanic	1,353	13.5 (12.4–14.6)	540	20.5 (18.0–23.2)	
Education					
Less than high school or some high school (no diploma) or GED	2,441	26.2 (24.7–27.7)	356	15.7 (13.8–17.8)	<0.001
High school diploma	2,255	27.1 (25.7–28.6)	548	25.0 (21.8–28.7)	
Some college (no degree) or associate degree	3,333	34.4 (32.8–36.0)	830	28.3 (25.5–31.3)	
Bachelor's degree or more	934	12.3 (11.2–13.5)	542	31.0 (27.5–34.7)	
Income					
<$25,000	4,399	43.0 (41.2–44.8)	832	30.8 (27.8–33.8)	<0.001
$25,000–$74,999	2,872	33.6 (31.8–35.5)	765	33.5 (30.3–36.8)	
≥$75,000	1,134	14.9 (13.7–16.2)	475	26.0 (22.7–29.6)	
Not reported	558	8.5 (7.5–9.7)	204	9.8 (8.0–11.9)	
Tobacco use characteristics					
Cigarette	7,196	81.6 (80.4–82.8)	N/A	N/A	N/A
E-cigarette	2,599	24.4 (23.0–25.9)	N/A	N/A	N/A
Cigar	2,663	25.5 (24.1–26.9)	N/A	N/A	N/A
Traditional cigar	1,241	13.4 (12.2–14.7)	N/A	N/A	N/A
Cigarillo	1,862	16.3 (15.2–17.5)	N/A	N/A	N/A
Filtered cigar	742	6.7 (5.7–7.7)	N/A	N/A	N/A
Pipe	351	3.0 (2.5–3.6)	N/A	N/A	N/A
Hookah	1,037	8.3 (7.5–9.3)	N/A	N/A	N/A
Smokeless	1,126	10.7 (9.7–11.7)	N/A	N/A	N/A
Snus	237	2.2 (1.7–2.9)	N/A	N/A	N/A
Dissolvable	36	0.2 (0.2–0.4)	N/A	N/A	N/A
Recent cigarette use					
Last used today	5,378	62.8 (61.0–64.5)[c]	N/A	N/A	N/A
Last used yesterday	686	6.5 (5.8–7.3)	N/A	N/A	
Last use ≥ the day before yesterday	693	7.2 (6.3–8.1)	62	0.8 (0.6–1.0)	
Nicotine exposure					
Geometric mean of urinary cotinine (ng/mL)	8,963	762.7 (692.2–840.4)	2,276	0.5 (0.4–0.5)	<0.001
Exposure to second hand smoke	7,765	85.3 (84.0–86.4)	988	37.3 (33.4–41.3)	<0.001

[a]Statistical differences between user groups were calculated using χ^2 tests for categorical variables and *t* tests for continuous variables. *P* values below 0.05 indicate statistical significance.
[b]For continuous variables mean and standard error are reported.
[c]Includes missing cases, therefore some column percentages add up to less than 100%.

ethnic groups, from 5.2 ng/mL (AUC = 0.98, 95% CI: 0.97–1.00) for non-Hispanic other race/multiple race users to 297.0 ng/mL (AUC = 0.99, 95% CI: 0.98–1.00) for non-Hispanic black users. For all cut-points, sensitivity ranged from 88.4% to 96.0% and specificity ranged from 95.2% to 99.0%. Characteristics that may impact exposure, i.e., cigarettes per month, are also included in **Table 3**. Our team explored the possibility that menthol smoking may play a role in the race/ethnicity differences. We examined whether menthol interacted with cotinine exposure among non-Hispanic black and white users differently. The menthol interaction term was not significant in either subgroup (*P*s > 0.15); therefore, there was not significant effect modification of menthol status on the cotinine cut-points.

When stratifying the sample by daily (N = 2,394) and nondaily (N = 655) cigarette use, the overall cut-point increased to 144.0 ng/mL,

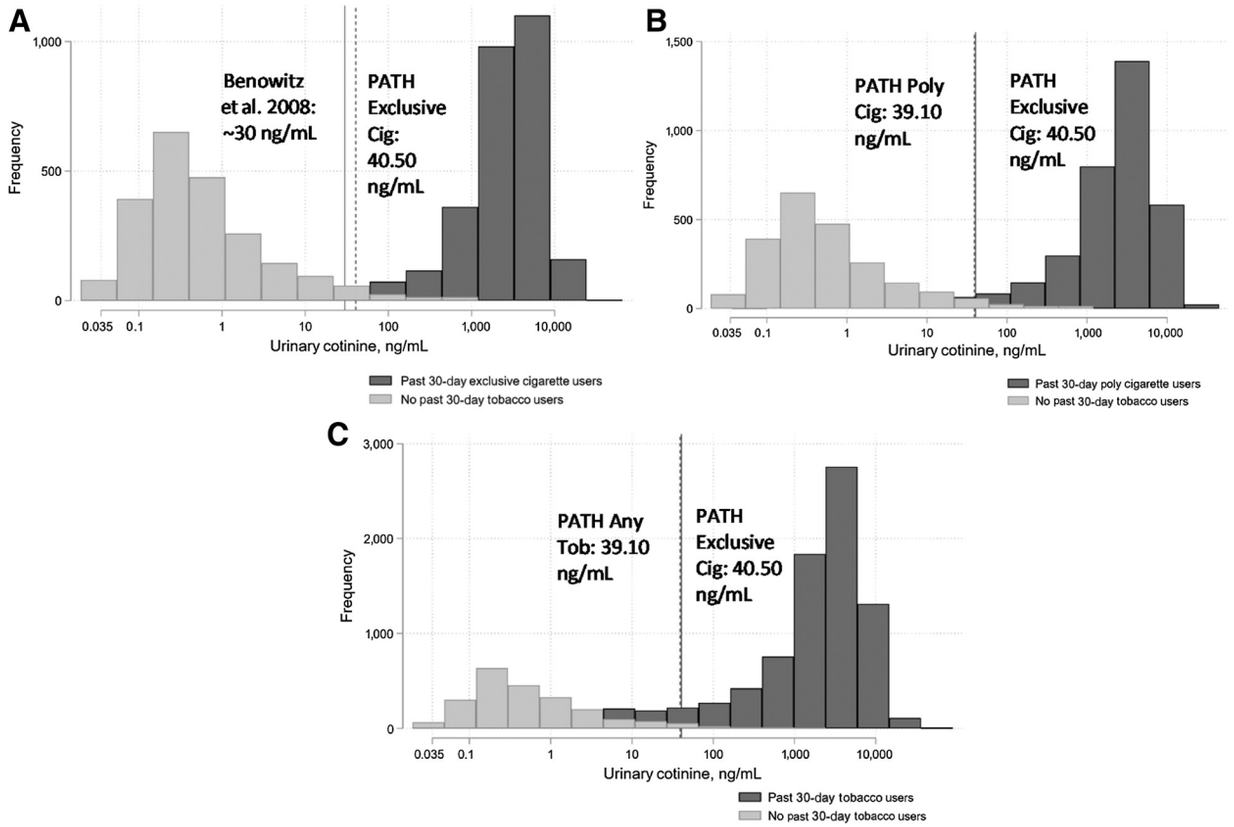

Figure 1.

Cotinine cut-points to distinguish past 30-day use. In **A**, past 30-day exclusive cigarette use vs. no past 30-day tobacco use, the reference cut-point (solid line) was extrapolated from Benowitz et al., 2008 who measured serum cotinine cut-points. In **B**, past 30-day polytobacco cigarette use vs. no past 30-day tobacco use and **C**, past 30-day any tobacco use vs. no past 30-day tobacco use, the reference cut-point (solid line) is from overall past 30-day exclusive cigarette use (**Fig. 1A**; **Table 3**). Histogram frequencies are unweighted.

AUC = 0.99, (95% CI: 0.99–1.00) for distinguishing daily users from nondaily/nonusers, and decreased to 4.8 ng/mL, AUC = 0.93 (95% CI, 0.91–0.95) for distinguishing nondaily users from nonusers (see Supplementary Table S1A and S1B). The large range in cut-points across racial/ethnic groups followed the same pattern for both daily and nondaily users, but in the daily and nondaily analyses males had higher cut-points than females.

Polytobacco cigarette users

The cotinine cut-points for polytobacco cigarette users were overall lower but followed a similar pattern as exclusive cigarette users (see **Fig. 1B**; **Table 3B**). The cotinine cut-point that distinguished P30D polytobacco cigarette users from nonusers was 39.1 ng/mL, AUC = 0.99 (95% CI: 0.98–0.99). Females had a higher cut-point (39.5 ng/mL; AUC = 0.99; 95% CI: 0.98–0.99) than males (19.5 ng/mL; AUC = 0.99, 95% CI: 0.98–0.99). The cut-points among racial/ethnic groups ranged from 5.5 ng/mL (AUC = 0.95, 95% CI: 0.94–0.97) for Hispanic users to 80.4 ng/mL (AUC = 0.99, 95% CI: 0.99–1.00) for non-Hispanic black users. For all cut-points, sensitivity ranged from 86.2%–96.2% and specificity ranged from 95.2%–98.7%.

When stratifying the sample by daily (N = 2,629) and nondaily (N = 963) cigarette use, the overall cut-point increased to 82.6 ng/mL, AUC = 1.00 (95% CI: 1.00–1.00) for distinguishing daily users from nondaily/nonusers, and decreased to 7.4 ng/mL, AUC = 0.95 (95% CI: 0.94–0.96) for distinguishing nondaily users from nonusers (see Supplementary Table S1C and S1D). The large range in cut-points across racial/ethnic groups followed the same pattern for both daily and nondaily users, but in the daily and nondaily analyses males had higher cut-points than females.

Any tobacco users

The cotinine cut-point that distinguished P30D any tobacco use from nonuse was 39.1 ng/mL [AUC = 0.96 (95% CI: 0.95–0.96); see **Fig. 1C**; **Table 3C**)]. Females had a higher cut-point (39.5 ng/mL; AUC = 0.96; 95% CI: 0.95–0.97) than males (7.4 ng/mL; AUC = 0.95, 95% CI: 0.95–0.96). The cut-points among racial/ethnic groups range from 4.8 ng/mL (AUC = 0.95, 95% CI: 0.93–0.97) for non-Hispanic other race/multiple race users to 40.0 ng/mL (AUC = 0.97, 95% CI: 0.96–0.97) for non-Hispanic white users. For all cut-points, sensitivity ranged from 78.5%–90.0% and specificity ranged from 94.6%–98.7%.

TNE-2 cut-points

Exclusive cigarette users

Using the molar sum of cotinine and 3HC (TNE-2), the cut-point for distinguishing P30D users from nonusers was 0.82 nmol/mL, AUC = 0.98 (95% CI: 0.98–0.99). As shown in **Table 4A**, similar to results using cotinine alone, females had a higher cut-point than males

Table 3. Receiver Operating Characteristics (ROC) and optimal cotinine cut-point to distinguish past 30-day cigarette users from nonusers, overall, and by sex and race/ethnicity.

	Unweighted *N*	Unweighted denominator	CPM	ROC optimal cut-point				
				Cut-point (ng/mL)	Sensitivity %	Specificity %	AUC	95% CI
A. Past 30-day exclusive cigarette use vs. no past 30-day tobacco use								
Overall	3,010	5,219	120.4	40.5	93.6%	98.1%	0.98	0.97–0.99
Sex								
Male	1,409	2,312	131.9	22.2	95.0%	96.9%	0.98	0.97–0.99
Female	1,601	2,907	107.3	43.1	93.7%	98.5%	0.98	0.97–0.99
Race/ethnicity								
Non-Hispanic white	1,903	3,015	134.9	53.2	95.1%	99.0%	0.99	0.98–0.99
Non-Hispanic black	448	847	150.1	297.0	94.3%	98.5%	0.99	0.98–1.00
Non-Hispanic other race/multiple race	207	396	103.4	5.2	96.0%	97.6%	0.98	0.97–1.00
Hispanic	452	961	75.4	5.5	88.4%	95.2%	0.93	0.90–0.97
B. Past 30-day polytobacco cigarette use vs. no past 30-day tobacco use								
Overall	3,592	5,801	92.2	39.1	93.3%	98.1%	0.99	0.98–0.99
Sex								
Male	2,184	3,087	90.8	19.5	94.8%	96.8%	0.99	0.98–0.99
Female	1,408	2,714	94.7	39.5	92.7%	98.3%	0.99	0.98–0.99
Race/ethnicity								
Non-Hispanic white	2,184	3,296	101.2	40.0	96.2%	98.7%	0.99	0.99–1.00
Non-Hispanic black	537	936	106.7	80.4	95.2%	96.1%	0.99	0.99–1.00
Non-Hispanic other race/multiple race	313	502	64.3	5.9	92.0%	97.9%	0.98	0.97–1.00
Hispanic	558	1,067	68.3	5.5	86.2%	95.2%	0.95	0.94–0.97
C. Past 30-day any tobacco use vs. no past 30-day tobacco use								
Overall	8,963	11,239	111.6	39.1	85.0%	98.0%	0.96	0.95–0.96
Sex								
Male	5,199	6,137	115.3	7.4	88.9%	94.6%	0.95	0.95–0.96
Female	3,764	5,102	106.4	39.5	85.4%	98.2%	0.96	0.95–0.97
Race/ethnicity								
Non-Hispanic white	5,578	6,717	123.2	40.0	87.7%	98.7%	0.97	0.96–0.97
Non-Hispanic black	1,335	1,737	135.7	39.8	90.0%	94.7%	0.97	0.96–0.98
Non-Hispanic other race/multiple race	697	892	83.7	4.8	85.8%	97.5%	0.95	0.93–0.97
Hispanic	1,353	1,893	76.6	5.5	78.5%	95.0%	0.90	0.88–0.92

Note: CPM values were winsorized at 95% to adjust for outlier values (all values above 95th percentile were recoded as the value at the 95th percentile). Cotinine was log-transformed. Reference group observations with cotinine values that were outside of the range of 2 times the standard deviation of the mean of the reference groups were classified as outliers and removed from analysis. Cut-points based off Youden's J statistic. Analyses are weighted.
Abbreviation: CPM, cigarettes per month.

(0.82 vs. 0.56 nmol/mL), and non-Hispanic black users had a higher cut-point than other racial ethnic groups (0.94 nmol/mL vs. 0.06–0.68 nmol/mL). For all cut-points, sensitivity ranged from 89.1%–97.3% and specificity ranged from 94.8%–99.2%.

Polytobacco cigarette users

Using TNE-2, the cut-point for distinguishing P30D users from nonusers was 0.61 nmol/mL, AUC = 0.99 (95% CI: 0.98–0.99). As shown in **Table 4B**, similar to results using cotinine alone, females had a higher cut-point than males (0.61 vs. 0.55 nmol/mL), and non-Hispanic black users had a higher cut-point than other racial ethnic groups (1.25 nmol/mL vs. 0.09–0.61 nmol/mL). For all cut-points, sensitivity ranged from 87.3%–96.6% and specificity ranged from 94.8%–99.0%.

Any tobacco users

Using TNE-2, the cut-point for distinguishing P30D any tobacco use from nonuse was 0.61 nmol/mL, AUC = 0.96 (95% CI: 0.95–0.96). As shown in **Table 4C**, similar to results using cotinine alone, females had a higher cut-point than males (0.82 vs. 0.17 nmol/mL), and non-Hispanic black users had a higher cut-point than other racial ethnic groups (0.80 nmol/mL vs. 0.04–0.61 nmol/mL). For all cut-points, sensitivity ranged from 79.4%–90.4% and specificity ranged from 94.3%–99.0%.

Discussion

Using nationally representative data of U.S. tobacco users, we found that cut-points to distinguish cigarette users from nonusers when focused on exclusive cigarette use compared with polytobacco cigarette use do not differ substantially (cotinine: 40.5 vs. 39.1 ng/mL; TNE-2: 0.82 vs. 0.61 nmol/mL). The number of cigarettes per month smoked by the exclusive versus polytobacco cigarette users was 120 vs. 92, respectively. Together, this indicates that cigarette use in these groups is the driver for nicotine exposure, regardless of other product use. Previous research exploring dual use of cigarettes and e-cigarettes, as well as cigarettes and cigars indicates that cigarette use was similar in the exclusive versus dual use groups (34, 35).

Table 4. Receiver Operating Characteristics (ROC) and optimal TNE-2 cut-point to distinguish past 30-day cigarette users from nonusers, overall, and by sex and race/ethnicity.

	Unweighted *N*	Unweighted denominator	CPM	ROC optimal cut-point				
				Cut-point (nmol/mL)	Sensitivity %	Specificity %	AUC	95% CI
A. Past 30-day exclusive cigarette use vs. no past 30-day tobacco use								
Overall	3,006	5,195	120.8	0.82	93.6%	98.6%	0.98	0.98–0.99
Sex								
Male	1,405	2,296	132.8	0.56	94.1%	98.0%	0.98	0.97–0.99
Female	1,601	2,899	107.3	0.82	93.4%	98.9%	0.98	0.97–0.99
Race/ethnicity								
Non-Hispanic white	1,901	3,002	135.2	0.68	95.5%	99.2%	0.99	0.99–0.99
Non-Hispanic black	448	844	150.1	0.94	97.3%	95.6%	0.99	0.98–1.00
Non-Hispanic other race/multiple race	207	395	103.4	0.06	96.2%	95.5%	0.98	0.96–1.00
Hispanic	450	954	76.2	0.08	89.1%	94.8%	0.94	0.91–0.98
B. Past 30-day polytobacco cigarette use vs. no past 30-day tobacco use								
Overall	3,592	5,781	92.2	0.61	93.5%	98.3%	0.99	0.98–0.99
Sex								
Male	2,184	3,075	90.8	0.55	94.0%	98.0%	0.99	0.98–0.99
Female	1,408	2,706	94.7	0.61	93.0%	98.4%	0.99	0.98–0.99
Race/ethnicity								
Non-Hispanic white	2,184	3,285	101.2	0.61	96.2%	99.0%	0.99	0.99–1.00
Non-Hispanic black	537	933	106.7	1.25	96.6%	96.2%	0.99	0.99–1.00
Non-Hispanic other race/multiple race	313	501	64.3	0.18	90.5%	98.1%	0.98	0.96–1.00
Hispanic	558	1,062	68.3	0.09	87.3%	94.8%	0.95	0.94–0.97
C. Past 30-day any tobacco use vs. no past 30-day tobacco use								
Overall	8,949	11,205	111.8	0.61	85.3%	98.2%	0.96	0.95–0.96
Sex								
Male	5,188	6,115	115.7	0.17	88.2%	95.3%	0.96	0.95–0.96
Female	3,761	5,090	106.4	0.82	85.0%	98.9%	0.96	0.95–0.97
Race/ethnicity								
Non-Hispanic white	5,568	6,695	123.3	0.61	88.0%	99.0%	0.97	0.96–0.97
Non-Hispanic black	1,335	1,734	135.7	0.80	90.4%	95.3%	0.97	0.96–0.98
Non-Hispanic other race/multiple race	696	891	83.7	0.04	87.2%	94.5%	0.95	0.93–0.97
Hispanic	1,350	1,885	77.0	0.08	79.4%	94.3%	0.91	0.89–0.93

Note: CPM values were winsorized at 95% to adjust for outlier values (all values above 95th percentile were recoded as the value at the 95th percentile). TNE2 was log-transformed. Reference group observations with TNE-2 values that were outside of the range of 2 times the SD of the mean of the reference groups were classified as outliers and removed from analysis. Cut-points based off Youden J statistic. Analyses are weighted.
Abbreviations: CPM, cigarettes per month; TNE-2, total nicotine equivalents-2.

Results revealed large variability in the sex and race/ethnicity specific cotinine cut-points. There are well-documented differences in nicotine metabolism in non-Hispanic black, non-Hispanic white, and Hispanic tobacco users (23, 36). Non-Hispanic black users have reduced CYP2A6 activity and metabolize nicotine more slowly than non-Hispanic white users (23). Therefore, with larger quantities of systemic nicotine and subsequently cotinine, their cotinine cut-points are much higher than for faster metabolizers (i.e., non-Hispanic Whites), which is consistent with our results. This was a consistent finding across various definitions of smoking status (i.e., exclusive vs. polytobacco use; daily vs. non-daily use). Furthermore, when examining cut-points using TNE-2, which is less impacted by differences in nicotine metabolism, the magnitude of the differences by race/ethnicity are lower than for cotinine cut-points among exclusive cigarette users. Studies seeking to use biochemical verification of smoking status should consider using race/ethnicity-specific cut-points.

Although the direction of race/ethnicity differences are consistent with previous literature, the magnitude of the racial/ethnic differences in cotinine cut-points is notable, particularly among exclusive users. Menthol smoking is much more prevalent in non-Hispanic black users than non-Hispanic white users (37). There is also previous research indicating that menthol may interact with CYP2A6 activity (38, 39). However, we did not find any significant interaction of menthol use and cotinine exposure. The differences in cut-point by sex are less consistent than those for race/ethnicity. Previous research indicates that females are faster metabolizers of nicotine (36), and despite smoking fewer cigarettes per day than their male counterparts, may experience greater behavioral dependence symptoms and increased difficulty quitting (40). This study found overall that females have a higher cotinine cut-point regardless of exclusive cigarette, polytobacco cigarette, or any tobacco use, but a lower cut-point when stratified by daily versus nondaily cigarette use. One limitation may be misclassification of self-reported smoking status or amount used per day. Future research can use more recent waves of data to further elucidate these findings.

Daily users have greater systemic intake of nicotine and non-daily users have lower, more variable levels of nicotine. Therefore, when

classifying daily versus nondaily, use the cut-point shifts higher, and conversely shifts lower when classifying nondaily from nonusers. When expanding our tobacco use population from cigarette users to users of any tobacco, we found the cut-point was no different than that of polytobacco cigarette users. This is likely due to the fact approximately 40% of our any tobacco users use cigarettes.

The cut-points determined in this study are slightly higher than the projected cut-points (∼30 ng/mL total urinary cotinine) from U.S. data in 1999–2004, although within the range of total urinary cotinine cut-point (34.5–46 ng/mL) suggested in the 2019 revised biochemical verification guidelines (22). We would have anticipated that cut-points would continue to decline over time due to decreased cigarette smoking prevalence and increases in tobacco-free policies. However, use of different biological specimens (Benowitz and colleagues 2008 publication used serum, and only projected urine cut-points), advances in laboratory methods, and continued high rates (∼75%) of daily smoking among users may contribute to the differences between their findings and this study.

Limitations of this study include the use of TNE-2 instead of TNE-3 because nicotine was not measured in our reference (nonuse) groups. We also did not exclude blunt (marijuana wrapped in tobacco leaf) use from the tobacco use or referent groups, which impacts overall nicotine exposure and is more prevalent in non-Hispanic black users (41). While this study was able to generate updated total cotinine cut-points and novel TNE-2 cut-points for different types of cigarette users and any tobacco users more generally, these findings may not generalize to exclusive users of noncigarette tobacco products. Future research could explore cut-points for noncigarette users, as well as geography/region-specific cut-points because patterns of tobacco use may differ by region (42). Studies may also wish to use the cut-points derived from this analysis to biochemically verify smoking status using subsequent waves of PATH Study data, or other types of data sources (e.g., clinical trials).

In conclusion, the overall cut-points defined by exclusive cigarette use were not substantially different from cut-points that include polytobacco cigarette use or any tobacco use. This may be a result of the high frequency of use of cigarettes among polytobacco users, particularly in 2013–2014. It will be important to continue to examine changes in cotinine/TNE-2 thresholds over time as new highly efficient nicotine delivery devices enter the market. Moreover, differences in sex and race/ethnicity cotinine cut-points were revealed and are critical to consider when using cotinine cutoffs to determine cigarette smoking status in epidemiologic studies and clinical trials. This study is the first to examine cut-points using TNE-2 that is less impacted by sex and race/ethnicity differences in nicotine metabolism, and a preferred validation mechanism if available. In practice, these findings can serve as a reference for validating smoking or tobacco use status for different demographic subgroups.

Authors' Disclosures

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Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the U.S. Department of Health and Human Services or any of its affiliated institutions or agencies.

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