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Assessing tobacco use in an African population: Serum and urine cotinine cut-offs from South Africa

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ABSTRACT

Background: Cotinine, a nicotine metabolite, is used to measure tobacco use and exposure, but recommended cut-offs to differentiate tobacco users from those exposed through the environment range from 3 to 58 ng/ml in serum, and 2.5 to 550 ng/ml in urine. Cut-offs may differ by ethnicity, sex and age. As data from adults in Africa are scarce, our aim was to evaluate cut-offs for serum and urine cotinine that best predict self-reported tobacco use in South African adults.

Methods: Two datasets were explored: African-PREDICT (n = 941 black and white healthy young adults, 20–30 years, serum cotinine); and WHO SAGE Wave 2 (n = 604 adults, 18–102 years, urine cotinine). Population specific cut-offs (ROC analyses) were compared with published cut-offs and self-reported tobacco use.

Results: Overall, 19% (293 of 1545) reported current tobacco use. The following cotinine cut-offs showed the highest sensitivity and specificity: serum ≥15 ng/ml in black and white men, and white women; serum ≥10 ng/ml in black women; urine ≥300 ng/ml for black, mixed ancestry, and older adults (50-plus years); urine ≥500 ng/ml for younger adults (18–49 years). Specificity was lower for urine than for serum cotinine.

Conclusion: Our study suggests that a serum cotinine level of ≥15 ng/ml and a urine cotinine level of ≥300 ng/ml best distinguish current tobacco users from non-users generally in the South African adult population.

1. Introduction

Cotinine has proven to be a valid and reliable biomarker of tobacco use and exposure, and is most often determined in saliva, serum and urine, with a longer half-life (16h) than its precursor nicotine (2h) (Benowitz et al., 2009b). Both nicotine and cotinine levels increase in a dose-dependent manner with the degree of tobacco use or exposure (Benowitz, 1984; Lawson et al., 1998; Rickert and Robinson, 1981), with cotinine detectable in urine, saliva and serum up to four days following nicotine intake (Jarvis et al., 1988). As such, various cotinine cut-offs have been proposed to differentiate between (a) individuals not using tobacco and not exposed to environmental tobacco smoke (ETS); (b) individuals not using tobacco but exposed to ETS; and (c) individuals actively using tobacco products.

ETS, including all forms of involuntary tobacco smoke exposure (Samet and Wang, 2000), causes a significant disease burden in the African region. Previous research suggests 16% of adults in South Africa are current smokers, while up to 30% are exposed to ETS as assessed by blood cotinine ≥10 ng/ml (SANHANES-1, 2013). However, determining appropriate cut-offs to distinguish between these categories of tobacco use or exposure is often complicated by significant overlap between the categories (Fustinoni et al., 2013). The recommended cut-

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offs to identify active tobacco users from non-users already cover a vast range from 3 to 58 ng/ml in serum (Assaf et al., 2002; Jarvis et al., 1987); and from 2.47 to 550 ng/ml in urine (Balhara and Jain, 2013; Zielińska-Danch et al., 2007). Furthermore, age, sex and ethnicity appear to influence the metabolism of nicotine and cotinine such that older adults, men and African Americans may exhibit higher cotinine levels with the same level of tobacco exposure (Benowitz et al., 2006, 1999; Caraballo et al., 1998; Johnstone et al., 2006; Kandel et al., 2007; Molander et al., 2001; Perez-Stable et al., 1998; Wagenknecht et al., 1990).

To determine the level of serum and urine cotinine biomarker that best predicts tobacco use in African populations, we analyzed data from two large South African cohort studies including older and younger men and women of different ethnicities.

2. Materials and methods

2.1. Study populations

This study combines two adult South African research cohorts. The first, African-PREDICT (PRospective study on the Early Detection and Identification of Cardiovascular disease and hyperTension), is performed in the North-West province of South Africa. The study includes black and white, apparently healthy, young adults (age 20–30 years) who are normotensive and HIV-negative at screening, and has been described in detail elsewhere (Thompson et al., 2016). Cross-sectional data on self-reported tobacco use were collected between January 2013 and August 2017 with serum cotinine measured in 941 respondents. The second group is a nested cohort within the World Health Organization Study on global AGEing and adult health (WHO SAGE; data available from www.who.int/healthinfo/sage/en/). This is also a longitudinal study in six countries focusing on adults 50 years old and above, with a smaller comparison group aged 18–49 years (age range 18–102 years). Cohorts are located within China (n ≈ 14,800), Ghana (n ≈ 5100), India (n ≈ 11,200), Mexico (n ≈ 2800), Russian Federation (n ≈ 4400) and South Africa (n ≈ 4200). Data was collected from August to December 2015 from a subsample nested within SAGE South Africa Wave 2 (described in detail previously; Charlton et al., 2016). Data on self-reported tobacco use were captured and spot urine cotinine measured in 604 respondents. All participants signed a written informed consent prior to taking part. WHO SAGE was approved by the WHO Ethics Review Committee [RPC149], and the University of the Witwatersrand Human Research Ethics Committee (Johannesburg, South Africa). Both studies were approved by the North-West University Human Research Ethics Committee (Potchefstroom, South Africa).

2.2. Study measures

The collection of spot urine and blood samples for cotinine analysis has been described previously (Charlton et al., 2016; Thompson et al., 2016). Briefly, spot urine was collected without preservative by WHO SAGE fieldwork teams from the participants homes and kept in a cool box until shipped to the central Durban laboratory within three days of collection, maintaining the cold chain. The spot sample was taken from the second morning urine void. Fasting blood samples were drawn from the antecubital fossa by trained African-PREDICT nurses within the Hypertension in Africa Research Unit at NWU. Serum cotinine in African-PREDICT was measured by immunoassay (Immulite, Siemens Healthcare, Germany) and urine cotinine in WHO SAGE was measured on the Beckman Coulter Unicel DXC (Beckman Coulter, Brea, CA, USA) and a Siemens Immulite system. The Beckman analyzer was necessary for urine samples exceeding the Siemens upper limit of detection (500 ng/ml). Urine Cotinine values below the limit of detection (LOD, < 10 ng/ml) were substituted with a constant value (LOD/2) as this has been shown to introduce less bias than omission of data below the LOD (Bergstrand and Karlsson, 2009). African-PREDICT collected data on self-reported smoking status to determine previous and current tobacco use, frequency of use and type of use (cigarettes with filter; rolled cigarettes known locally as beedies or zol; chewing tobacco; pipe; water pipe/hookah; snuff; cannabis with tobacco, known as dagga; or other). WHO SAGE collected similar data using questions from the Tobacco Questions for Surveys (TQS), a subset of key questions from the Centre for Disease Control (CDC) Global Adult Tobacco Survey (2011) (Centers for Disease Control and Prevention (CDC, 2011). Current tobacco use in both cohorts consisted of both daily and non-daily tobacco users. Covariates captured in both cohorts included ethnicity, education, markers of socioeconomic status, current employment status, alcohol consumption, blood pressure, body weight and height. While African-PREDICT included black and white, apparently healthy, urban dwelling, young adults (aged 20–30 years); SAGE included all South African ethnic groups, and both urban and rural dwelling younger and older adults (aged 18–102 years).

2.3. Data management and analysis

Data for the African-PREDICT study was collected and managed using the REDCap electronic data capture tools hosted at the North-West University, Potchefstroom Campus (Harris et al., 2009). The Computer Assisted Personal Interview program used for SAGE South Africa was developed in multi-mode interviewing capability. Differences between self-reported current tobacco users, previous tobacco users and never tobacco users were examined for each cohort using independent samples Kruskal- Wallis tests, with non-normal distribution of data confirmed using the Kolmogorov- Smirnov test and visual inspection of histograms. Receiver operator characteristic (ROC) curves were plotted to determine serum and urine cotinine cut-off values for tobacco users, following the exclusion of 'occasional tobacco users' i.e., non-daily, as cotinine levels may vary considerably day to day within this group. Various ROC cut-offs were selected based on combined sensitivity and specificity to predict current tobacco use in all respondents. These cut-offs were compared with those identified through literature review (Supplementary Table 1), with the sensitivity and specificity to detect tobacco users from non-users presented for the whole sample (including occasional tobacco users) and by subgroups of sex, ethnicity and age where subgroups were 100 or more participants. Articles reporting cotinine cut-offs expressed as ng/ml were included. Articles reporting cotinine cut-offs as a ratio to creatinine or to detect tobacco use solely in pregnant women, adolescents or children were excluded. All data were analyzed using SPSS version 24 (IBM Corp., New York, USA).

3. Results

3.1. Tobacco use is common especially in men and frequent alcohol consumers

While the two cohorts were quite different, reflecting the different aims of each cohort study, together there was a broad range of age, ethnicity, educational and household income levels represented. Across the combined sample of South African adults, 19% (293 of 1545) reported current tobacco use, with the highest rate observed in the African-PREDICT cohort (22.4%, n = 211 of 941; aged 20–30 years), followed by the older WHO-SAGE cohort (15.5%, n = 57 of 367; 50-plus years) and lastly the younger WHO-SAGE cohort (10.5%, n = 25 of 237; 18–49 years). In African-PREDICT, filtered cigarettes were the most commonly used form of tobacco (82% of tobacco users), followed by water pipe (hubbly; 27%), cannabis with tobacco (dagga; 13%), rolled cigarettes (beedies/zol; 9%), and cigars (8%) with infrequent use of pipes, chewing tobacco, snuff or other types of tobacco use. Among the adults in WHO-SAGE, filtered cigarettes were again the most commonly used form of tobacco (70% of tobacco users), followed by rolled cigarettes (beedies/zol; 12%), and snuff (mostly via the nose, 12%),

Table 1
Characteristics of never, previous and current tobacco users from the two study cohorts.

	African PREDICT Tobacco Use (n = 941)				WHO SAGE Tobacco Use (n = 604)			
	Never n = 624	Previous n = 106	Current n = 211	*p*	Never n = 502	Previous n = 20	Current n = 82	*p*
Age, years	25 (4)	24 (6)	24 (6)	0.004	52 (27)	57 (15)	55 (15)	0.246
Sex female, n (%)	414 (66)	58 (55)	66 (31)	< 0.001	381 (76)	15 (75)	39 (48)	< 0.001
Ethnicity, n (%)*								
Black	344 (55)	49 (46)	116 (55)	0.226	372 (74)	9 (45)	43 (52)	< 0.001
White	280 (45)	57 (54)	95 (45)		8 (2)	4 (20)	3 (4)	
Mixed ancestry (colored)	–	–	–		72 (14)	4 (20)	31 (38)	
Indian	–	–	–		25 (5)	3 (15)	2 (2)	
Missing data	–	–	–		25 (5)	0	3 (4)	
Urban, n (%)	all	all	all		317 (63)	14 (70)	62 (76)	0.073
Highest level of education, n (%)								
College/university	381 (61)	54 (51)	74 (35)	< 0.001	21 (4)	3 (15)	3 (4)	0.065
Highschool	226 (36)	50 (47)	126 (60)		220 (44)	7 (35)	31 (38)	
Primary school or less	17 (3)	2 (2)	11 (5)		138 (27)	8 (40)	33 (40)	
Missing data	–	–	–		123 (25)	2 (10)	15 (18)	
Currently working, n (%)	426 (68)	70 (67)	109 (52)	< 0.001	90 (18)	5 (25)	16 (20)	0.332
Monthly Household income, n(%)								
20,000 ZAR plus	141 (23)	23 (22)	32 (15)	0.136	3 (1)	0	1 (1)	0.654
10-19,999 ZAR	124 (20)	25 (24)	48 (23)		15 (3)	1 (5)	2 (2)	
5-9999 ZAR	110 (18)	25 (24)	36 (17)		37 (7)	0	3 (4)	
Below 5,000	248 (39)	33 (30)	95 (45)		253 (50)	14 (70)	43 (52)	
Missing data	–	–	–		194 (39)	5 (25)	33 (40)	
Body mass index, kg/m$^+$	24.8 (7.0)	24.2 (6.7)	22.6 (7.8)	< 0.001	29.4 (9.0)	30.5 (11.1)	24.1 (7.4)	< 0.001
Systolic blood pressure, mmHg	115 (16)	118 (17)	119 (17)	0.003	128 (29)	140 (29)	127 (23)	0.082
Diastolic blood pressure, mmHg	78 (10)	78 (11)	80 (11)	0.178	80 (14)	84 (21)	80 (19)	0.238
Serum cotinine, ng/ml	1.0 (0.0)	1.0 (0.0)	188 (260)	< 0.001	–	–	–	
Urine cotinine, ng/ml	–	–	–		17 (674)	49 (925)	1075 (1766)	< 0.001
Alcohol use (≥1 d/week), n (%)	317 (51)	66 (62)	155 (74)	< 0.001	15 (3)	1 (5)	22 (27)	< 0.001

All data shown are median (IQR, interquartile range) unless otherwise stated. *Classifications used by the South African Population Census. Continuous variables compared using Independent Samples Kruskal-Wallis test; categorical variables compared using the Chi-Square test.

with infrequent use of cigars, pipes, chewing tobacco, or other types of tobacco use. Specific use of water pipes (hubbly/hookah) and dagga were not investigated. Comparing the older with the younger group within WHO-SAGE, tobacco type use was similar with the exception that all snuff use occurred in the older tobacco users.

In both cohorts, current tobacco users were more often men and had a lower BMI (Table 1). In the African-PREDICT cohort, tobacco use was associated with lower education levels, current employment and a higher systolic blood pressure. In the WHO-SAGE cohort, prevalence of self-reported tobacco use was almost three times higher in the mixed ancestry group (29%), compared to the black group (10%), while the white and Indian groups were too small to draw inference. Across both cohorts, current tobacco use was also associated with alcohol use on one or more days per week ($p < 0.001$).

3.2. A serum cotinine cut-off of 15 ng/ml best predicts tobacco use

ROC analysis (excluding occasional tobacco users; n = 76) showed that serum cotinine was a statistically significant marker of self-reported daily tobacco use (area under the curve 0.943; $p < 0.001$; Fig. 1a). Based on this ROC curve, a serum cotinine level of 20 ng/ml was associated with the highest level of combined sensitivity (0.92) and specificity (0.93) to detect self-reported daily tobacco use. Comparison of this cut-off with those previously reported in the literature to predict tobacco use in the whole group (including occasional tobacco users; Table 2), showed that 15 ng/ml performed best, correctly classifying 83% of tobacco users and 92% of non-users.

3.2.1. Lowering the serum cut-off to 10 ng/ml marginally improves sensitivity to detect black female tobacco users

The data are shown in Table 2. In both black and white men, the same cotinine cut-off of 15 ng/ml showed the highest sensitivity (0.87 and 0.96 respectively) and specificity (0.92 and 0.93 respectively) to detect self-reported tobacco use. At this cut-off, although specificity remained high (> 0.90), sensitivity was lower for both white (0.70) and black women (0.58) compared to men. Reducing the cut-off to 10 ng/ml or less improved sensitivity in black women (to 0.62) but not white women, with little change in specificity. However, ≥30% of female tobacco users remained misclassified as non-tobacco users. As female sex hormones may influence nicotine and cotinine metabolism, the frequency of hormonal contraception use (pills, injection, implant, or hormone releasing intrauterine device use) was compared between black and white women and was not significantly different. A significant difference was found in the prevalence of self-reported tobacco use, which was twice as high in white women (16.5%) compared to black women (8.8%; $p < 0.01$).

3.3. A urine cotinine cut-off of 300 ng/ml best predicts tobacco use

ROC analysis (excluding occasional tobacco users; n = 9) showed that urine cotinine was also a statistically significant marker of self-reported daily tobacco use (area under the curve 0.747; $p < 0.001$; Fig. 1b). Based on this ROC curve, a urine cotinine level of 300 ng/ml was associated with the highest level of combined sensitivity (0.82) and specificity (0.67) to detect self-reported daily tobacco use. Comparison of this cut-off with those previously reported in the literature showed that the ROC identified cut-off of 300 ng/ml performed best to predict tobacco use in the whole group (including occasional tobacco users; Table 3), correctly classifying 81% of tobacco users and 67% of non-users. Specificity was generally lower for urine than serum cotinine.

3.3.1. A higher urine cutoff of 500 ng/ml performs better in younger or mixed ancestry individuals

The data are shown in Table 3. While the ROC identified cut-off of 300 ng/ml, gave the highest combined sensitivity and specificity in both black and mixed ancestry adults, a higher cut-off of 500 ng/ml also

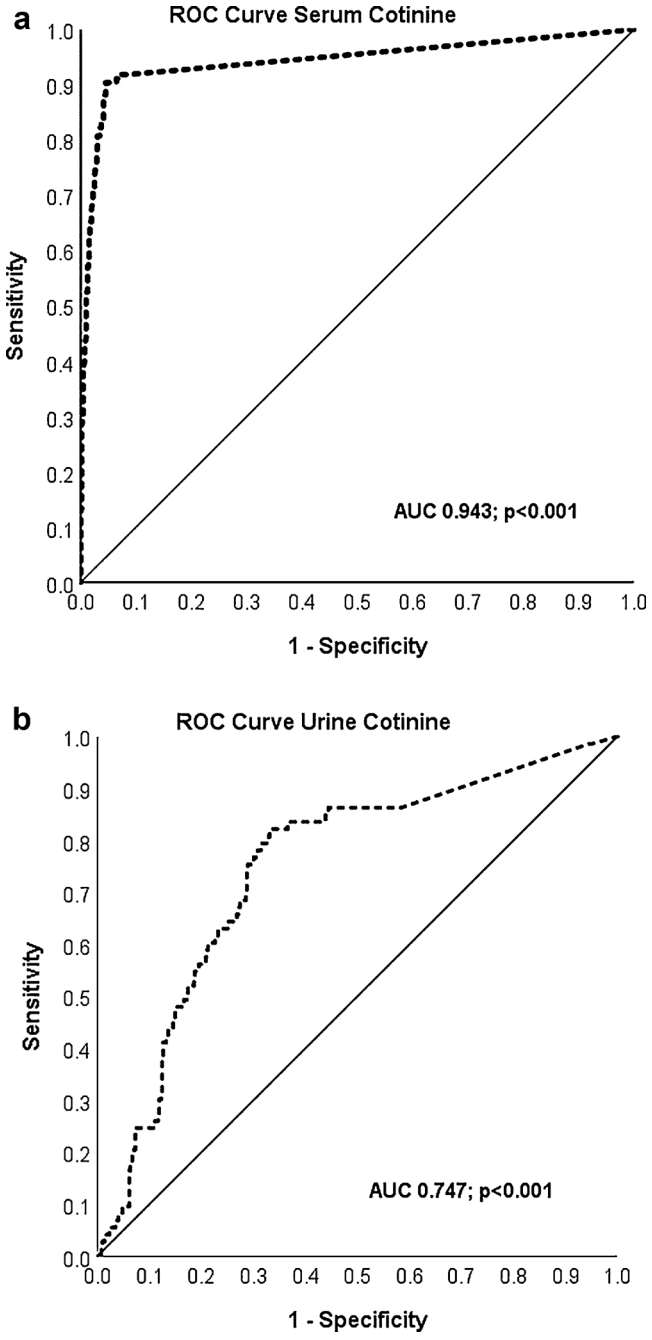

Fig. 1. Receiver Operating Characteristics (ROC) analysis of cotinine to predict current self-reported daily tobacco use for: **(a)** Serum cotinine (current self-reported daily tobacco use (n = 132) compared to non-tobacco users (n = 733); excludes smokers reporting only occasional tobacco use (n = 76)); and **(b)** Urine cotinine (current self-reported daily tobacco use (n = 73) compared to non-tobacco users (n = 522); excludes smokers reporting only occasional tobacco use (n = 9)).

performed well in the mixed ancestry group with a lower sensitivity but higher specificity. The prevalence of tobacco use was much higher in the mixed ancestry compared to the black group, predominantly due to increased self-reported tobacco use in mixed ancestry women (30.5%) compared to black women (4.0%; $p < 0.001$).

Comparing the younger (18–49 years) to the older group (50-plus), showed the higher cut-off of 500 ng/ml also performed better in the younger group, while 300 ng/ml gave the highest combined sensitivity and specificity in the older group. Prevalence of self-reported tobacco use was not significantly different between younger and older adults, but it was between younger and older women (5.3% and 11.3% respectively; $p = 0.022$) although the numbers were small.

4. Discussion

Our study suggests that a serum cotinine level of ≥15 ng/ml and a urine cotinine level of ≥300 ng/ml best distinguish current tobacco users from non-users generally in the South African adult population. These cut-offs may differ in specific subgroups (lower in black women, higher in younger adults), although it is not clear if this is due to age, ethnicity, sex, mis-reporting or other factors.

Previous studies suggest that age, sex and ethnicity influence the metabolism of nicotine and cotinine such that older adults, men and African Americans may exhibit higher cotinine levels with the same level of tobacco exposure (Benowitz et al., 2006, 1999; Caraballo et al., 1998; Johnstone et al., 2006; Kandel et al., 2007; Molander et al., 2001; Perez-Stable et al., 1998; Wagenknecht et al., 1990). However, our study suggests that cotinine levels are higher in younger adults compared to older adults, despite lower self-reported tobacco use in the younger group, especially in women. The prevalence of tobacco use in a population can influence the cut-off, although there is disagreement whether a lower prevalence is associated with a higher cut-off (Cummings and Richard, 1988) or lower cut-off (Kim, 2016).

In a reanalysis of data from London adults in 1988 with a self-reported smoking rate of 43% (Jarvis et al., 1987), Cummings and Richard suggested a serum cotinine cut-off of 14 ng/ml to detect smokers when smoking prevalence was 20%, reducing to 13 ng/ml when prevalence increased to 50% (1988). This is presumably due to the impact of background environmental tobacco smoke exposure. However, our data in the mixed ancestry population suggest that both prevalence of tobacco use and the cut-off to detect tobacco use are increased, while the lower cut-off in black women is accompanied by lower self-reported tobacco use.

Many of the recommended cut-offs for cotinine come from populations in Europe, the UK, and the US (Supplementary Table 1), where significant reductions in smoking prevalence have taken place over the last 30 years (Ng et al., 2014). As far as we are aware, the largest study in Africa to use serum cotinine as a marker of tobacco use has been the South African National Health and Nutrition Examination Survey (SANHANES; n = 5500) (Shisana et al., 2014). In this survey, a cut-off of 10 ng/ml was applied to differentiate between no tobacco exposure and tobacco use or ETS. The authors of the SANHANES report discuss how the cotinine cut-off may change with prevalence of tobacco use and for different groups, stating that 10 ng/ml was chosen as it was between the previously suggested cut-offs of 14 ng/ml (Jarvis et al., 1987)and 3 ng/ml (Benowitz et al., 2009a), although the ethnicity and sex specific cut-offs recommended by the latter study were not applied. SANHANES showed that, overall, 35% of men and 25% of women had serum cotinine ≥ 10 ng/ml. Of note, the self-reported tobacco use rates were much lower (29.2% of men and 7.3% of women) (Reddy et al., 2015) and the difference was primarily ascribed to low ETS exposure awareness (Shisana et al., 2014) while misreporting of tobacco use was not addressed. In contrast, Hong (2016) assessed the concordance between self-reported smoking rates and serum cotinine verified smoking in men and women in Korea, suggesting the lower concordance in women was due to social and cultural denigration of women smokers resulting in lower reporting rates (Hong, 2016).

While it may be common to use self-reported tobacco use as the "gold standard" against which to assess the usefulness of a biomarker for that behavior, this approach can be limited by reporting bias. Indeed, a systematic review of studies comparing self-report to cotinine measures suggests that smoking prevalence is often underestimated when based on self-report alone, especially in populations such as pregnant women, where smoking is perceived as a socially undesirable behavior (Gorber et al., 2009). Within South African cultures, such as the Nguni, the use of tobacco or other substances among black girls is

Table 2
Serum cotinine cut-offs to detect self-reported tobacco users from non-users by sex and ethnicity, in comparison with reported prevalence of tobacco use, occasional use and hormonal contraceptive use (African-PREDICT young adults, aged 20–30 years).

	All (n = 941)		Black women (n = 296)		White women (n = 242)		Black men (n = 213)		White men (n = 190)	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Cotinine cut-off	*n (%)*		*n (%)*		*n (%)*		*n (%)*		*n (%)*	
58 ng/ml	157 (74)	696 (95)	13 (50)	251 (93)	25 (63)	198 (98)	74 (82)	116 (94)	45 (82)	131 (97)
47 ng/ml	159 (75)	691 (95)	13 (50)	249 (92)	26 (65)	195 (97)	74 (82)	116 (94)	46 (84)	131 (97)
40.35 ng/ml	161 (76)	687 (94)	13 (50)	247 (92)	26 (65)	194 (96)	75 (83)	116 (94)	47 (86)	130 (96)
30 ng/ml	164 (78)	683 (94)	13 (50)	247 (92)	26 (65)	194 (96)	76 (84)	116 (94)	49 (89)	126 (93)
25 ng/ml	166 (79)	683 (94)	13 (50)	247 (92)	26 (65)	194 (96)	77 (86)	116 (94)	50 (91)	126 (93)
20ng/ml (ROC)	171 (81)	680 (93)	15 (58)	246 (91)	27 (68)	194 (96)	77 (86)	114 (93)	52 (95)	126 (93)
15 ng/ml (BA)	**174 (83)**	**674 (92)**	15 (58)	244 (90)	**28 (70)**	**192 (95)**	**78 (87)**	**113 (92)**	**53 (96)**	**125 (93)**
14 ng/ml	174 (83)	671 (92)	15 (58)	243 (90)	28 (70)	190 (94)	78 (87)	113 (92)	53 (96)	125 (93)
13.2 ng/ml	174 (83)	669 (92)	15 (58)	242 (90)	28 (70)	190 (94)	78 (87)	112 (91)	53 (96)	125 (93)
12.5 ng/ml	174 (83)	668 (92)	15 (58)	241 (89)	28 (70)	190 (94)	78 (87)	112 (91)	53 (96)	125 (93)
3 to 10 ng/ml	175 (83)	666 (91)	**16 (62)**	**241 (89)**	28 (70)	189 (94)	78 (87)	112 (91)	53 (96)	124 (92)
Self-report data, n (%)										
Current tobacco use	211 (22.4)		26 (8.8)		40 (16.5)[+]		90 (42.3)		55 (28.9)[+]	
Occasional use[*]	76 (36.0)		8 (30.8)		18 (45.0)		33 (36.7)		17 (30.9)	
Hormonal Contraception	–		142 (48.0)		102 (42.1)		–		–	

BA – Cut-off recommended for black adults; Hormonal contraception includes pill, injection, implant, or hormone releasing intrauterine device use.
Values in bold are those with the highest sensitivity and specificity combined.
[*] Occasional tobacco users calculated as % of all tobacco users.
[+] $P < 0.01$ (Chi-square test). See Supplementary Table 1 for description of studies and references.

highly stigmatized (Reddy et al., 2007). Increased exposure to cigarettes and altered social networks, as a consequence of urbanization, may moderate these more traditional attitudes towards smoking practices amongst black African women (Williams et al., 2008). However, the influence of traditional attitudes on underreporting in this group cannot be discounted.

Recent data regarding the relative social desirability and perceived harm of different forms of tobacco use may further confound validity of self-reported tobacco use. For example, the use of water pipes (hookah) is highly prevalent among South African students and is initiated at a young age (Combrink et al., 2010), with use perceived as socially acceptable and safer than cigarettes (Daniels and Roman, 2013). To date, there is limited research on electronic nicotine delivery systems (ENDS or e-cigarette) use, behavior or perceptions within South Africa, though evidence from other countries suggests over half of study participants perceive e-cigarettes as healthier than regular cigarettes (Xu et al., 2016).

Similarly, many South African tobacco users are unaware of the relative harm of snuff compared to cigarettes (Ayo-Yusuf and Agaku, 2014). As we and others have found, snuff is commonly used by older adults, and black South African women (Ayo-Yusuf et al., 2006). The type of tobacco product (smoked or smokeless) may also influence the pharmacokinetics of nicotine and cotinine (Benowitz et al., 1988).

In addition to sex differences in smoking prevalence and tobacco type, the enzymes involved in nicotine and cotinine metabolism (e.g., CYP2A6) are influenced by sex hormones, so that nicotine metabolism

Table 3
Urine cotinine cut-offs to detect self-reported tobacco users from non-users by sex, ethnicity and age (WHO-SAGE).

	All (n = 604)		[*]Black (n = 424)		Mixed ancestry (n = 107)		18–49yrs (n = 237)		50yrs plus (n = 367)	
	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
Cotinine cut-off	*n (%)*		*n (%)*		*n (%)*		*n (%)*		*n (%)*	
550 ng/ml	57 (70)	372 (71)	30 (70)	279 (73)	22 (71)	50 (66)	18 (72)	159 (75)	39 (68)	213 (69)
500 ng/ml	60 (73)	367 (70)	31 (72)	276 (72)	**24 (77)**	**48 (63)**	**19 (76)**	**156 (74)**	41 (72)	211 (68)
300 ng/ml (ROC)	**66 (81)**	**347 (67)**	**36 (84)**	**260 (68)**	**25 (81)**	**45 (59)**	**20 (80)**	**147 (69)**	**46 (81)**	**200 (65)**
200 ng/ml	66 (81)	344 (66)	36 (84)	258 (68)	25 (81)	44 (58)	20 (80)	145 (68)	46 (81)	199 (64)
164 ng/ml	66 (81)	338 (65)	36 (84)	252 (66)	25 (81)	44 (58)	20 (80)	141 (67)	46 (81)	197 (64)
100 ng/ml	66 (81)	331 (63)	36 (84)	245 (64)	25 (81)	44 (58)	20 (80)	140 (66)	46 (81)	191 (62)
50 ng/ml	67 (82)	308 (59)	36 (84)	226 (59)	26 (84)	43 (57)	20 (80)	125 (59)	47 (83)	183 (59)
31.5 ng/ml	67 (82)	293 (56)	36 (84)	215 (56)	26 (84)	42 (55)	20 (80)	116 (55)	47 (83)	177 (57)
25 ng/ml	70 (85)	286 (55)	36 (84)	210 (55)	27 (87)	41 (54)	22 (88)	111 (52)	48 (84)	171 (55)
22.5 ng/ml	70 (85)	276 (53)	36 (84)	203 (53)	27 (87)	39 (51)	22 (88)	111 (52)	48 (84)	165 (53)
20 ng/ml	70 (85)	264 (51)	36 (84)	194 (51)	27 (87)	36 (47)	22 (88)	109 (51)	48 (84)	155 (50)
2.47 ng/ml	80 (98)	26 (5)	42 (98)	21 (6)	30 (97)	2 (3)	24 (96)	15 (7)	56 (98)	11 (3.5)
Self-reported current tobacco use										
All	82 (13.6)		43 (10.1)		31 (29.0)		25 (10.5)		57 (15.5)	
Men	43 (25.4)		31 (25.2)		6 (24.0)		16 (23.9)		27 (26.5)	
Women	39 (9.0)		12 (4.0)		25 (30.5)		9 (5.3)		30 (11.3)	
Occasional use[*]										
All	9 (11)		3 (7.0)		6 (19.4)		2 (8.0)		7 (12.3)	
Men	4 (9.3)		2 (6.5)		2 (33.3)		1 (6.3)		3 (11.1)	
Women	5 (12.8)		1 (8.3)		4 (16)		1 (11.1)		4 (13.3)	

Values in bold are those with the highest sensitivity and specificity combined.
[*] Occasional tobacco users calculated as % of all tobacco users. [1]Black men and women combined as cut-offs performed with similar sensitivity and specificity in both groups (200 ng/ml – women: 83% sensitivity, 69% specificity; men: 84% sensitivity, 63% specificity). See Supplementary Table 1 for references.

is faster in women compared to men, and accelerated by oral contraceptive use (Benowitz et al., 2006). As the cotinine cut-off to detect tobacco use was lower in black women than in white women, we assessed hormonal contraception use and found no difference. However, the interaction between ethnicity, sex, hormone contraceptives and tobacco use requires further investigation within African populations.

In countries such as South Africa where the prevalence of tobacco use, particularly in men, remains high (Reddy et al., 2015), higher background ETS exposure may contribute to higher cotinine cut-offs. Although, in relation to other chemicals in cigarette smoke, exposure to nicotine from second-hand smoke may be markedly reduced depending on the environment (Singer et al., 2002). Additionally, several foods contain small amounts of nicotine, including several commonly eaten in the region, so that nicotine intake is not entirely specific to tobacco. However, the impact of these foods is likely to be minimal, such that a person would need to eat between 1 and 3 kg of either cauliflower, potatoes or tomatoes to produce even 1 ng/ml of cotinine in urine, or almost a kilogram of eggplant to produce cotinine levels usually observed with ETS (Benowitz, 1996). As such, Benowitz suggests that serum or urine cotinine is a good marker for tobacco exposure in population or epidemiological studies, with ETS exposed non-smokers typically showing serum and urine cotinine levels much lower than our suggested cut-offs (Benowitz, 1996). The inclusion of occasional smokers did influence the serum but not the urine cut-off value. Others have highlighted the difficulties of developing biomarker cut-offs in populations with high levels of occasional use, suggesting new ways to detect tobacco use may be warranted in these groups (Vartiainen et al., 2002).

Previous studies have found differences in nicotine and cotinine metabolism in African Americans compared to other US racial groups (Benowitz et al., 2009a, 2016). While we did not find a marked difference between black and white men in serum cotinine, we did find a difference between black and white women in serum cotinine and in black and mixed ancestry adults in the urine cotinine cut-off. However, significant differences were observed in smoking prevalence in these groups. Well controlled nicotine dose-response studies are needed to evaluate if relevant genetic polymorphisms are more prevalent in some ethnic groups and influence nicotine metabolism within the South African context.

The higher area under the curve for serum compared to urine cotinine suggests serum cotinine may be a better marker for self-reported tobacco use in this combined South African population. This could be due to the influence of urine flow rate and pH on variability in the urine cotinine measure (Benowitz et al., 1983). However, urine is often easier to collect than blood and urine cotinine levels are typically 4–5 times higher than those in serum, providing a better biomarker for detecting lower levels of tobacco exposure (Benowitz et al., 2009b, 2009c). In selecting the biomarker and the cut-off to use, the intended use should be considered. Many of the recommended cotinine cut-offs in serum have been used to detect true prevalence of tobacco cessation (Cummings and Richard, 1988; Jatlow et al., 2008). As Cummings and Richard point out, the reference cut-off value needs to consider the prevalence of deception, with higher cut-offs producing higher 'validated' tobacco cessation rates (1988).

Limitations of this study are that tobacco use was self-reported and we cannot be sure of the potential levels of underreporting, especially in subgroups where tobacco use may be stigmatized. While the overall sample was relatively large and included different ethnic, age and sex groups within South Africa, the subgroup comparisons were smaller. A further limitation is the lack of sufficient data from other ethnic groups (whites and Indians) within South Africa and the use of adults only, providing no data for adolescent or younger tobacco users. Identification of previous cotinine cut-offs from the literature was not designed to be a systematic review but to give representation across the reported ranges. As such, this may not include all recommended cotinine thresholds. Furthermore, different analytical techniques used by different studies may influence the comparison of results, though the two main techniques, ELISA and LC/MS, have been shown to be comparable (Park et al., 2010). Lastly, the ROC identified thresholds from this study require validation in other African groups, where the effects of sex, age, ethnicity, tobacco use, tobacco type and exposure can be further evaluated.

While traditional tobacco advertising, sponsorship and promotion has decreased over time in South Africa in line with the WHO Framework Convention on Tobacco Control (World Health Organization (WHO, 2015), our data show that approximately one in five South African adults uses tobacco. Additionally, tobacco users show more frequent alcohol consumption. As tobacco and alcohol use are shown to share common psychosocial risk factors amongst South African youths (Morojele et al., 2016), our results lend further support to interventions aimed at reducing the use of both substances simultaneously.

5. Conclusions

In conclusion, we found serum cotinine of ≥15 ng/ml and urine cotinine of ≥300 ng/ml to give the highest sensitivity and specificity to detect self-reported tobacco use in an adult South African population. A lower serum cotinine cut-off (≥10 ng/ml) may be warranted in black women, while a higher urine cotinine cut-off (≥500 ng/ml) may be appropriate for adults under 50 years of age. Further research is needed to inform the extent to which physiological (genetics, hormones, age) or behavioral factors (tobacco use, reporting accuracy) underlie these differences. However, the first step should be the systematic implementation of cotinine measurement within national surveys in the region with application of appropriate thresholds for assessing tobacco use. This will allow the comparison of datasets to understand better the sociocultural influences on tobacco use as the region rapidly transitions, and to direct tobacco cessation efforts.

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Contributors

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Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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