Published in final edited form as:
Hum Psychopharmacol. 2010 January ; 25(1): 80–83. doi:10.1002/hup.1078.

Breath Carbon Monoxide and Semiquantitative Saliva Cotinine as Biomarkers for Smoking

Gina F. Marrone, **Mane Paulpillai**, **Rebecca J. Evans**, **Edward G. Singleton**, and **Stephen J. Heishman***
Nicotine Psychopharmacology Section, Intramural Research Program, National Institute on Drug Abuse, Baltimore, MD, USA

Abstract

Objective—As a biomarker of smoking, semiquantitative analysis of cotinine (NicAlert®) offers several advantages over breath carbon monoxide (CO) and quantitative analysis of cotinine. Recent studies have used urine NicAlert® and breath CO in combination to verify abstinence. However, no studies have evaluated the performance of saliva NicAlert® against or in combination with breath CO.

Method—Breath CO, saliva NicAlert®, and smoking history were compared in an urban population of daily smokers (n = 24) and nonsmokers (n = 25).

Results—Saliva NicAlert® predicted self-reported smoking with 100% sensitivity and 96% specificity. At a cutoff of > 5 ppm, breath CO had 100% sensitivity and 100% specificity in predicting self-reported smoking. Breath CO was positively correlated with saliva NicAlert® and negatively correlated with minutes since last cigarette.

Conclusion—Saliva NicAlert® had high sensitivity and specificity in identifying daily smokers. Compared to saliva NicAlert®, breath CO level was more indicative of recent smoking. Future treatment studies should evaluate the performance of saliva NicAlert® as an alternative to the urine test.

Keywords

Cigarette smoking; Nicotine; Cotinine; Carbon monoxide; Biomarker

Introduction

Breath carbon monoxide (CO) is the most rapid, noninvasive, and easily measured biochemical indicator of smoking. However, several factors can affect its sensitivity and specificity (Benowitz, 2002). Breath CO measurement is only sensitive to recent smoking because CO has a short half-life (2–3 hours). Thus, breath CO readings following short and long term abstinence can be indistinguishable (Acosta *et al.*, 2004; Gariti *et al.*, 2002). The specificity of CO as a biomarker of smoking is limited by environmental sources of CO from pollution, motor vehicle exhaust, and passive smoke exposure (Jarvis and Russell, 1984). Thus, breath CO levels of nonsmokers exposed to environmental sources of CO (e.g. urban areas) can fall within the range of intermittent smokers (Cunnington and Hormbrey, 2002).

*Corresponding author: Stephen J. Heishman, Ph.D. NIDA Intramural Research Program, 251 Bayview Blvd. Baltimore, MD 21224, USA, Phone: 443.740.2458, Fax: 443.740.2855, heishman@nih.gov.

An ideal biomarker for smoking is cotinine, the major metabolite of nicotine, because it has a half-life of 10–30 hours (Benowitz, 2002). Quantitative analysis of cotinine is the "gold standard" for verifying smoking status, but it can be costly and time consuming. The recent development of NicAlert® colorimetric immunoassay tests allows for relatively inexpensive, easily measured, and rapid semiquantitative analysis of cotinine in saliva and urine. The major limitation of cotinine is that, due to its long half-life, it cannot discriminate between short term abstinence (< 24 hr) and continued smoking.

Because breath CO and cotinine differ in their ability to identify short and long term smoking abstinence, studies with inclusion criteria that require specific periods of abstinence, such as overnight tobacco deprivation or long term smoking abstinence, may require different biomarkers or biomarker combinations. For example, recent treatment studies have used NicAlert® tests in combination with breath CO to verify abstinence (Acosta *et al.*, 2004; Schepis *et al.*, 2008). In smokers attempting abstinence, CO [cutoff < 8 parts per million (ppm)] had a sensitivity of 83.1% and a specificity of 100%, whereas urine NicAlert® had a sensitivity of 98.5% and a specificity of 58.5% compared to cotinine assayed by gas chromatography/mass spectrometry (GC/MS) (Acosta *et al*., 2004). These data suggest that CO and urine NicAlert® may be more effective together than either measure alone in verifying self-reported abstinence (Acosta *et al.*, 2004).

To our knowledge, no study has compared the relative sensitivities and specificities of breath CO and saliva NicAlert®. We compared saliva NicAlert® and breath CO to self-reported smoking status in daily smokers and nonsmokers to determine the feasibility of using saliva NicAlert® as an alternative to urine NicAlert®. We were interested in the performance of the NicAlert® saliva test because, relative to urine, saliva samples are easy to collect and may be considered less invasive.

Methods

Participants

Smokers (n = 24) and nonsmokers (n = 25) were recruited from the Baltimore metropolitan area via newspaper and radio advertisements. Participants were over the age of 18, had no chronic pulmonary disease, and reported no use of marijuana within 24 hr. Smokers were included if they reported smoking at least 5 cigarettes per day for the past 6 months and were not seeking treatment. Nonsmokers were included if they had not used any tobacco or nicotine products within the past 6 months. Of the smokers, 11 were Black (8 male, 3 female) and 13 were White (8 male, 5 female). Of the nonsmokers, 12 were Black (5 male, 7 female), 12 were White (4 male, 8 female), and one was Asian (male). One smoker was excluded because of poor forced vital capacity. Participants gave written informed consent according to guidelines for the protection of research volunteers of the U.S. Department of Health and Human Services and were paid for their participation. The NIDA Institutional Review Board approved the study.

Procedure

Participants completed a single 20-min experimental session. After giving written informed consent, participants completed a brief smoking history questionnaire. Participants then gave a breath CO sample (Vitalograph, Lenexa, KS). Vitalograph readings range from 0 to 199 ppm with accuracy of ± 3 ppm. Finally, participants provided a saliva sample via passive drool that was tested with a NicAlert® dipstick (Nymox Corp., Hasbrouck Heights, NJ). The NicAlert® test yields a semiquantitative measure of cotinine based on a colorimetric immunoassay reaction. The test strip displays seven zones that represent a range of cotinine

levels from 0 (0–10 ng/ml) to 6 (> 1000 ng/ml). Results were recorded as values from 0 to 6; a result ≥1 indicated tobacco use (see Table 1).

Data Analysis

We used Receiver Operating Characteristic (ROC) analyses (Metz, 1978; Zweig and Campbell, 1993) to evaluate predictive accuracy between self-reported smoking and breath CO and between self-reported smoking and saliva NicAlert®. The CO cutoff level that optimally discriminated between smokers and nonsmokers was the value corresponding to the maximum of the Youden index (Youden, 1950): $J = \max[SEi + SPi - 1]$, where SEi and SPi are the sensitivity and specificity, respectively, over all possible cutoff values. ROC analyses were performed using MedCalc for Windows, version 9.3.9.0. We used *t*-tests to compare demographic and smoking history variables between smokers and nonsmokers, Whites and Blacks, and males and females. Pearson product-moment correlations were calculated between breath CO, saliva NicAlert®, and items on the smoking history questionnaire. All statistical tests were two-tailed, and results were considered significant at $p < .05$.

Results

Participant characteristics

Mean age of smokers was 36.2 ± 8.5 yr (mean ± SD). They currently smoked 19.2 ± 9.0 cigarettes per day, had smoked daily for 14.2 ± 9.2 yr, had made 4.0 ± 5.4 lifetime quit attempts, and smoked their last cigarette 106.9 ± 105.8 min before the test session. Mean age of nonsmokers was 34.1 ± 9.7 yr. Twelve percent (3/25) reported smoking daily in the past; years smoking ranged from 1 to 15, and years since last cigarette ranged from 3 to 10. The remaining 22 nonsmokers reported smoking < 20 cigarettes in their life.

In smokers, mean breath CO was 21.4 ± 12.0 ppm (range 6–45); in nonsmokers, mean breath CO was 1.8 ± 1.0 ppm (range 1–5). Table 1 shows the distribution of NicAlert® readings in smokers and nonsmokers. As expected, breath CO and cotinine levels were significantly higher in smokers compared with nonsmokers (*p*'s < 0.001).

There were several differences between White and Black smokers. Compared with Blacks, Whites smoked more cigarettes per day (21.9 ± 7.5 vs. 15.9 ± 9.9, $p = 0.05$), reported more quit attempts (5.9 ± 6.8 vs. 1.9 ± 2.0, $p < 0.05$), and smoked more recently before the test session (51.9 ± 60.6 vs. 171.8 ± 113.1 min, $p < 0.01$). Consistent with these reports, White smokers had higher breath CO (25.2 ± 12.8 vs. 17.0 ± 9.7 ppm, $p < 0.05$) and saliva NicAlert® levels (5.3 ± 1.0 vs. 4.4 ± 1.2, $p < 0.05$). There were no differences between males and females with respect to demographic and smoking history variables.

ROC analyses

Table 2A shows the results of ROC analysis using breath CO to predict self-reported smoking (prevalence = 49%). The cutoff indicating the optimal equilibrium between sensitivity and specificity was > 5 ppm. Table 2B shows the results of ROC analysis using saliva NicAlert® to predict self-reported smoking. The cutoff indicating the optimal equilibrium between sensitivity and specificity was > 0, which is the manufacturer's recommended cutoff value.

Correlational analyses

Among all participants, there was a significant positive correlation between breath CO and saliva NicAlert® ($r = 0.81$, $p < 0.001$). For smokers, there was a significant negative correlation between breath CO and time since last cigarette ($r = -0.56$, $p < 0.01$). However,

breath CO and number of cigarettes per day were not significantly correlated ($r = 0.20$, $p > 0.1$). Correlations between saliva NicAlert® and cigarettes per day ($r = 0.34$, $p = .10$) and time since last cigarette ($r = -0.30$) were not significant.

Discussion

This was the first study to compare the sensitivities and specificities of saliva NicAlert® and breath CO to determine the feasibility of using saliva NicAlert® as an alternative to urine NicAlert®. Saliva NicAlert® and CO performed well in predicting self-reported smoking status. Breath CO and saliva NicAlert® had the same sensitivity, whereas CO had better specificity.

ROC analyses indicated that breath CO > 5 ppm predicted self-reported smoking with 100% sensitivity and specificity. The SRNT Subcommittee on Biochemical Verification recommended a cutoff of 8 ppm (Benowitz, 2002), which has been used by previous studies comparing CO and urine NicAlert® (Acosta *et al.*, 2004; Schepis *et al.*, 2008). If we had used this cutoff, sensitivity would have been 91.7% (Table 2A).

Saliva NicAlert® predicted self-reported smoking with 100% sensitivity and 96% specificity. In previous studies, its sensitivity and specificity were similar when compared against self-report (100% and 100%, respectively) (Cooke *et al.*, 2008) and quantitative analysis of cotinine in urine (99% and 96%, respectively) (Montalto and Wells, 2007) and saliva (95% and 93%, respectively) (Cooke *et al.*, 2008).

Breath CO was negatively correlated with minutes since last cigarette, whereas saliva NicAlert® was not. This is consistent with findings that breath CO levels are more indicative of recent smoking than cotinine (Schepis *et al.*, 2008). In addition, compared with Black smokers, Whites smoked more cigarettes per day, had higher breath CO and saliva NicAlert® levels, and made more quit attempts. These findings are consistent with previous findings that racial differences in smoking behaviors and nicotine metabolism might contribute to variability in biomarkers of smoking (Andreski and Breslau, 1993; Cropsey *et al.*, 2006; Luo *et al.*, 2008; Perez-Stable *et al.*, 1998).

This study was limited by its small sample size; however, our results were consistent with previous studies. Because we wanted an equal number of smokers and nonsmokers to test the feasibility of using saliva NicAlert®, smoking prevalence was constrained to 49%, which is much higher than the current smoking prevalence of 20% among adults in the United States (CDC, 2008). We tested daily smokers not interested in treatment, whereas previous studies comparing breath CO to cotinine examined treatment seekers (Acosta *et al.*, 2004; Jatlow *et al.*, 2008; Schepis *et al.*, 2008). Breath CO and cotinine levels in individuals who recently began abstinence, relapsed, or were denying use during treatment might be different than in smokers not seeking treatment; such differences could impact the relative sensitivity and specificity of the biochemical methods. In addition, mean NicAlert® in smokers was 4.9, which seemed high (zone 4=200–500 ng/ml; zone 5=500–1000 ng/ml) compared with mean saliva cotinine levels previously measured by gas chromatography (e.g., 295 ng/ml; Jarvis et al., 2003). The NicAlert® manufacturer has reported that 3-hydroxycotinine showed 12–40% cross reactivity with cotinine in the NicAlert® assay in urine. Cross reactivity might explain the elevated NicAlert® levels observed among smokers in our study. In conclusion, our finding that saliva NicAlert® was highly sensitive and specific suggests that future treatment studies should evaluate the performance of saliva NicAlert® as an alternative to urine NicAlert®.

Acknowledgments

This research was supported by the Intramural Research Program of the NIH, National Institute on Drug Abuse. The authors have no conflicts of interest.

References

Acosta M, Buchhalter A, Breland A, et al. Urine cotinine as an index of smoking status in smokers during 96-hr abstinence: comparison between gas chromatography/mass spectrometry and immunoassay test strips. Nicotine Tob Res. 2004; 6:615–620. [PubMed: 15370157]

Andreski P, Breslau N. Smoking and nicotine dependence in young adults: differences between blacks and whites. Drug Alcohol Depend. 1993; 32:119–125. [PubMed: 8508722]

Benowitz NL. Biochemical verification of tobacco use and cessation. Nicotine Tob Res. 2002; 4:149–159. [PubMed: 12028847]

Centers for Disease Control and Prevention. Cigarette smoking among adults-- United States, 2007. MMWR Morb Mortal Wkly Rep. 2008; 57:1221–1226. [PubMed: 19008790]

Coburn RF, Forster RE, Kane PB. Considerations of Physiological Variables That Determine Blood Carboxyhemoglobin Concentration in Man. Journal of Clinical Investigation. 1965; 44:1899. [PubMed: 5845666]

Cooke F, Bullen C, Whittaker R, et al. Diagnostic accuracy of NicAlert cotinine test strips in saliva for verifying smoking status. Nicotine Tob Res. 2008; 10:607–612. [PubMed: 18418783]

Cropsey KL, Eldridge GD, Weaver MF, et al. Expired carbon monoxide levels in self-reported smokers and nonsmokers in prison. Nicotine Tob Res. 2006; 8:653–659. [PubMed: 17008192]

Cunnington AJ, Hormbrey P. Breath analysis to detect recent exposure to carbon monoxide. Postgrad Med J. 2002; 78:233–237. [PubMed: 11930027]

Gariti P, Alterman AI, Ehrman R, et al. Detecting smoking following smoking cessation treatment. Drug Alcohol Depend. 2002; 65:191–196. [PubMed: 11772480]

Jarvis MJ, Russell MAH. Measurement and Estimation of Smoke Dosage to Non-Smokers from Environmental Tobacco-Smoke. European Journal of Respiratory Diseases. 1984; 65:68–75. [PubMed: 6423402]

Jarvis MJ, Primatesta P, Erens B, et al. Measuring nicotine intake in population surveys: Comparability of saliva cotinine and plasma cotinine estimates. Nicotine Tob Res. 2003; 5:349–55. [PubMed: 12791530]

Jatlow P, Toll BA, Leary V, et al. Comparison of expired carbon monoxide and plasma cotinine as markers of cigarette abstinence. Drug Alcohol Depend. 2008; 98:203–209. [PubMed: 18650033]

Luo Z, Alvarado GF, Hatsukami DK, et al. Race differences in nicotine dependence in the Collaborative Genetic study of Nicotine Dependence (COGEND). Nicotine Tob Res. 2008; 10:1223–1230. [PubMed: 18629733]

Metz CE. Basic principles of ROC analysis. Semin Nucl Med. 1978; 8:283–298. [PubMed: 112681]

Montalto NJ, Wells WO. Validation of self-reported smoking status using saliva cotinine: a rapid semiquantitative dipstick method. Cancer Epidemiol Biomarkers Prev. 2007; 16:1858–1862. [PubMed: 17855706]

Perez-Stable EJ, Herrera B, Jacob P 3rd, et al. Nicotine metabolism and intake in black and white smokers. JAMA. 1998; 280:152–156. [PubMed: 9669788]

Schepis TS, Duhig AM, Liss T, et al. Contingency management for smoking cessation: enhancing feasibility through use of immunoassay test strips measuring cotinine. Nicotine Tob Res. 2008; 10:1495–1501. [PubMed: 19023841]

Youden WJ. Index for rating diagnostic tests. Cancer. 1950; 3:32–35. [PubMed: 15405679]

Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem. 1993; 39:561–577. [PubMed: 8472349]

Table 1

NicAlert® zones, corresponding concentrations, and distribution of participant readings.

Zone	Cotinine (ng/ml) (manufacturer)	Indicates	Nonsmokers (%)total= 25	Smokers (%)total= 24
0	0–10	nonsmoker	24 (96%)	
1	10–30	smoker	1 (4%)	
2	30–100	smoker		1 (4.17%)
3	100–200	smoker		3 (12.5%)
4	200–500	smoker		2 (8.33%)
5	500–1000	smoker		10 (41.67%)
6	1000+	smoker		8 (33.33%)

Table 2

A. Receiver Operating Characteristic analysis showing optimal cutoff for breath CO (> 5 ppm) to predict self-reported smoking				
Criterion	**Sensitivity (%)**	**95% CI**	**Specificity (%)**	**95% CI**
≥ 1	100.00	85.6 – 100.0	0.00	0.0 – 13.8
> 1	100.00	85.6 – 100.0	40.00	21.2 – 61.3
> 2	100.00	85.6 – 100.0	92.00	73.9 – 98.8
> 4	100.00	85.6 – 100.0	96.00	79.6 – 99.3
> 5	100.00	85.6 – 100.0	100.00	86.2 – 100.0
> 6	95.83	78.8 – 99.3	100.00	86.2 – 100.0
> 8	91.67	73.0 – 98.7	100.00	86.2 – 100.0
> 9	83.33	62.6 – 95.2	100.00	86.2 – 100.0
> 10	79.17	57.8 – 92.8	100.00	86.2 – 100.0

B. Receiver Operating Characteristic analysis showing optimal cutoff for saliva NicAlert® (> 0) to predict self-reported smoking				
Criterion	**Sensitivity (%)**	**95% CI**	**Specificity (%)**	**95% CI**
≥ 0	100.00	86.2 – 100.0	0.00	0.0 – 14.4
> 0	100.00	85.6 – 100.0	96.00	79.6 – 99.3
> 1	0.00	0.0 – 13.8	100.00	85.6 – 100.0