Comparison of Tests Used to Distinguish Smokers from Nonsmokers

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Abstract: Questionnaire and biochemical measures of smoking were studied in 211 hospital outpatients. Eleven different tests of smoke intake were compared for their ability to categorize smokers and nonsmokers correctly. The concentration of cotinine, whether measured in plasma, saliva, or urine, was the best indicator of smoking, with sensitivity of 96–97 per cent and specificity of 99–100 per cent. Thiocyanate provided the poorest discrimination. Carbon monoxide measured as blood carboxyhaemoglobin or in expired air gave sensitivity and specificity of about 90 per cent. Sensitivities of the tests were little affected by the presence among the claimed nonsmokers of a group of 21 "deceivers" who concealed their smoking. It is concluded that cotinine is the measure of choice, but for most clinical applications carbon monoxide provides an acceptable degree of discrimination and is considerably cheaper and simpler to apply. (*Am J Public Health* 1987; 77:1435–1438.)

Introduction

Self-reports of smoking status may not always be reliable, particularly in situations where smokers feel under strong pressure to give up smoking but have not been able to achieve this.[1–3] A number of biochemical markers have been used to validate claims of nonsmoking, including measures based on thiocyanate,[4–7] nicotine,[8] cotinine,[9–11] and carbon monoxide.[4,6,11,12] These measures differ widely in availability, cost, and ease of administration. Measures based on nicotine have the advantage of being specific to tobacco but require expensive laboratory instrumentation. Levels of thiocyanate and carbon monoxide are easier to determine but may be raised through exposures unrelated to smoking, such as traffic emissions and diet. Few studies have attempted to compare the various biochemical tests.[11] We report here a study in which all the markers of smoking currently in widespread use are compared for their ability to categorize smokers and nonsmokers correctly.

Methods

Subjects

The subjects for the study were 215 outpatients at St. Mary's Hospital, London. On arrival for their clinic appointment, they were asked to fill in a self-completion questionnaire giving details of smoking habits and to provide samples of blood, expired air, saliva, and urine. There was no prior warning of the survey, but consent for the biochemical tests was obtained before completion of the questionnaire. It was emphasized that the questionnaire responses were confidential and would not become part of hospital notes or be communicated to the medical staff. It was hoped that this would encourage accurate self-report. The present report is confined to 211 subjects who provided adequate questionnaire and biochemical data. There were 159 men (average age 56.0) and 52 women (average age 55.3); 119 attended afternoon cardiology clinics, and 92 a morning peripheral vascular clinic. A high proportion were suffering from smoking-related diseases. A total of 188 (89 per cent) reported having been cigarette smokers at some time and 90 (43 per cent) said that they were current smokers of cigarettes, pipes, or cigars. Reported mean cigarette consumption in the cigarette smokers was 13.2 cigarettes per day, and 97 per cent reported having smoked on the test day, with a mean time since last cigarette of 1.5 hours.

The concentration of nicotine and cotinine in plasma, saliva, and urine was determined by gas chromatography.[13,14] Carboxyhaemoglobin concentrations were measured with an IL282 CO-Oximeter and carbon monoxide in expired air after breath-holding with a portable CO analyzer incorporating an ethanol filter.[15] Thiocyanate was measured by an automated modification of the Aldridge technique.[16] Urinary concentrations of nicotine, cotinine, and thiocyanate were not adjusted for urine flow.

Self-reported Smoking Categories

Cigarette smokers were defined as those who answered "yes" to the question "Do you smoke cigarettes now?" Pipe and cigar smokers answered "no" to this question but "yes" to "Do you smoke a pipe?" or "Do you smoke cigars?" Nonsmokers were those who answered "no" to all these questions.

Analysis

The sensitivity (per cent of smokers detected) and specificity (per cent of nonsmokers correctly classified) of each test was first examined by selecting the value which, in relation to self-reported smoking, misclassified the fewest subjects. Subsequent analysis of sensitivity and specificity made allowance for claimed nonsmoking in active smokers ("deceivers"). Finally, a comparison between the tests was made by examining their sensitivity when specificity was standardized at 95 per cent.[17]

Results

The mean values for each marker are shown in Table 1 according to self-reported smoking status. As would be expected, concentrations both in the cigarette smokers and in those who smoked cigars or pipes but not cigarettes were greatly raised by comparison with the claimed nonsmokers. Table 2 gives the optimal cut-off value, sensitivity and specificity for each marker in relation to self-reported smoking status. With the exception of urinary thiocyanate, all the tests performed reasonably well in identifying smokers. Sensitivities were higher for measures based on nicotine than for CO or thiocyanate, and were slightly higher for identifying cigarette smoking rather than smoking of any tobacco product. Measures of cotinine correctly classified all but one or two of the self-reported smokers.

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TABLE 1—Mean Values of Biochemical Markers by Self-reported Smoking Status

Biochemical Markers	Nonsmokers (n = 121)	Smokers: Cigarettes (n = 75)	Smokers: Pipe or Cigars (n = 15)
Carbon Monoxide			
ECO (ppm)	7.5 (6.1)	21.3 (10.4)	18.3 (13.1)
COHb (%)	1.3 (1.3)	4.1 (2.0)	3.2 (2.2)
Nicotine (ng/ml)			
Plasma	2.9 (6.1)	15.7 (10.1)	10.9 (6.8)
Saliva	33.3 (103.3)	476 (895)	1602 (4881)
Urine	170.4 (642.6)	1843 (2802)	1463 (2385)
Cotinine (ng/ml)			
Plasma	46.1 (117.0)	294 (164)	210 (149)
Saliva	44.2 (117.7)	330 (190)	231 (171)
Urine	150.7 (415.2)	1448 (1024)	1185 (1071)
Thiocyanate			
Plasma (μmol/l)	70.0 (39.9)	123.9 (44.1)	121.7 (54.9)
Saliva (μmol/l)	1.5 (1.1)	2.5 (1.0)	2.2 (1.1)
Urine (μmol/l)	81.3 (44.1)	153.2 (86.2)	155.2 (99.2)

NOTE: Data shown are means (SD). Numbers for each variable may differ from the bases shown because of missing data.

Specificity was less satisfactory. About 15–20 per cent of claimed nonsmokers were classified as smokers by each test. For the measures of nicotine and cotinine this would be unexpected, since nicotine is specific to tobacco and passive exposure to other people's smoke has been found to have only a small effect in raising concentrations.[18] It therefore seemed probable that there was some denial of active smoking among the claimed nonsmokers. Inspection of the data for individual subjects lent support to this. The figure shows the distribution of values of plasma cotinine according to self-reported smoking. The distribution in the claimed nonsmokers was clearly bimodal, with 21 subjects having concentrations similar to those seen in the smokers and two orders of magnitude higher than in the rest of the nonsmokers. This pattern was repeated across the other nicotine-based measures, and also recurred with the non-nicotine tests. For example, 18 out of 21 apparent "deceivers" had expired air CO concentrations which exceeded the nonsmoker–smoker cut-off, and in 16 out of 21 the concentration of plasma thiocyanate was similarly raised.

TABLE 2—Sensitivity and Specificity of each Marker in Discrimination of Self-reported Smoking Status

Biochemical Markers	Cut-off Value	Sensitivity: % all smokers	Sensitivity: % cigarette smokers	Specificity: % non-smokers
Carbon Monoxide				
ECO (ppm)	10	84	88	84
COHb (%)	1.7	88	92	82
Nicotine (ng/ml)				
Plasma	2.3	91	93	86
Saliva	21.8	93	95	86
Urine	58.6	89	93	84
Cotinine (ng/ml)				
Plasma	13.7	94	97	81
Saliva	14.2	95	99	82
Urine	49.7	96	98	83
Thiocyanate				
Plasma (μmol/l)	78.0	85	86	79
Saliva (mmol/l)	1.64	82	86	63
Urine (μmol/l)	118.0	62	63	83

NOTE: The cut-off value was chosen to minimize the number of misclassifications, equal weight being given to the misclassification of smokers and nonsmokers.

TABLE 3—Mean Values of Biochemical Markers in True Nonsmokers and Deceivers

Biochemical Markers	Nonsmokers (n = 100)	"Deceivers" (n = 21)
Carbon Monoxide		
ECO (ppm)	5.6 (2.7)	16.4 (9.2)
COHb (%)	0.9 (0.7)	3.2 (1.9)
Nicotine (ng/ml)		
Plasma	0.9 (1.2)	12.2 (10.2)
Saliva	4.8 (5.8)	169 (203)
Urine	8.2 (15.1)	999 (1331)
Cotinine (ng/ml)		
Plasma	1.5 (2.3)	239 (166)
Saliva	1.7 (2.3)	244 (177)
Urine	4.8 (8.4)	888 (636)
Thiocyanate		
Plasma (μmol/l)	49.9 (22.9)	117.6 (53.3)
Saliva (mmol/l)	1.3 (0.9)	2.4 (1.3)
Urine (μmol/l)	75.2 (40.5)	113.4 (49.6)

NOTE: Data shown are means (SD). Numbers for each variable may differ from the bases shown because of missing data.

Table 3 shows mean values for each marker for true nonsmokers and for the 21 "deceivers". In each case the concentration in the deceivers was raised substantially by comparison with "true" nonsmokers. For eight out of 11 markers there was no real difference between "deceivers" and self-reported smokers. In subsequent analyses, the deceivers were therefore combined with the other smokers to form a true smoking group (n = 111).

Table 4 gives the cut-off, sensitivity and specificity for each marker in discriminating true smoking. Cut-offs were unchanged from self-report for all measures except carbon monoxide. Specificities were markedly improved, and approached 100 per cent for the nicotine-based measures.

TABLE 4—Optimal Cut-off, Sensitivity and Specificity for each Marker in Discriminating true Smoking Status

Biochemical Markers	Cut-off Value	Sensitivity: % Smokers Detected	Specificity: % Nonsmokers Detected	95% CI for % Accuracy
Carbon Monoxide				
ECO (ppm)	8	90	89	86.2–91.7
COHb (%)	1.6	86	92	83.0–89.2
Nicotine (ng/ml)				
Plasma	2.3	88	99	89.4–93.8
Saliva	21.8	90	99	91.6–95.2
Urine	58.6	89	97	93.3–96.3
Cotinine (ng/ml)				
Plasma	13.7	96	100	98.3–99.1
Saliva	14.2	96	99	98.5–99.3
Urine	49.7	97	99	98.4–99.2
Thiocyanate				
Plasma (μmol/l)	78.0	84	91	81.1–87.9
Saliva (mmol/l)	1.64	81	71	66.0–76.0
Urine (μmol/l)	118.0	59	89	67.0–77.0

NOTE: True smokers were those who reported smoking cigarettes, pipes, or cigars (n = 90) and the 21 "deceivers". Non-smokers were the self-reported non-smokers less the deceivers (n = 100).

Accuracy defined as overall % correct classification, and estimated for a population with equal proportions of smokers and nonsmokers.

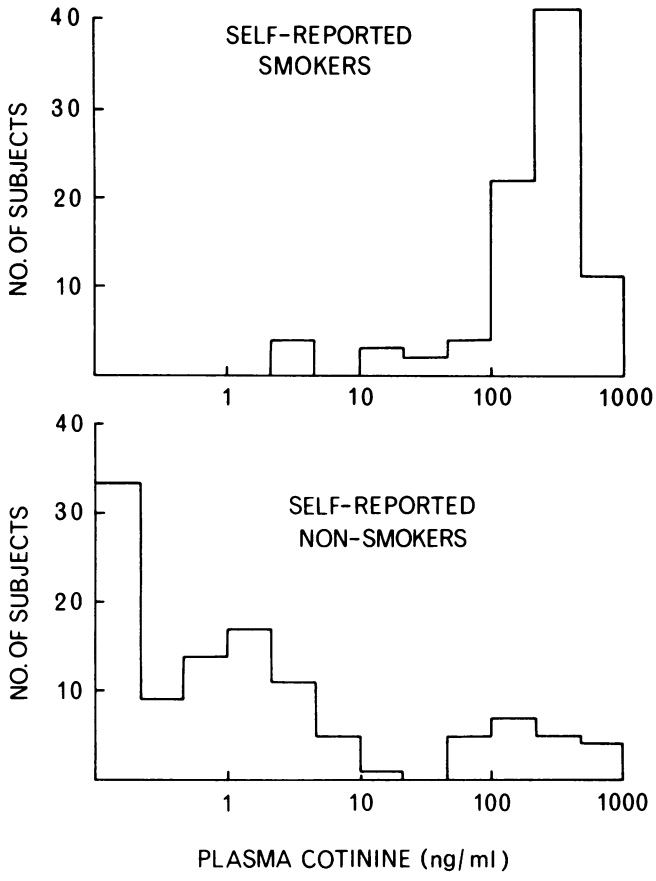

FIGURE 1—Frequency Distribution of Plasma Cotinine in Self-reported Smokers and Nonsmokers.
NOTE: Horizontal scale is logarithmic. Distribution for self-reported nonsmokers is bimodal, a group of 21 "deceivers" having values similar to the smokers

Comparison between Markers

In order to standardize comparison between tests, a cut-off for each was selected such that 95 per cent of nonsmokers were correctly classified. Table 5 gives the corresponding sensitivities. Measures of thiocyanate in saliva and urine performed worst, with sensitivities of only 25 per cent and 51 per cent, respectively, for detecting cigarette smoking. Plasma thiocyanate fared better (74 per cent), but still was substantially less sensitive than CO in expired air (88 per cent) or blood carboxyhaemoglobin (89 per cent). Measures based on nicotine showed the greatest sensitivity, with cotinine (99 per cent) consistently better than nicotine itself (93–95 per cent). All the tests were slightly less successful in detecting smoking of any tobacco product than in detecting cigarette smoking. Their failure to identify all smokers correctly reflected the presence in the sample of seven atypical light smokers. Four of these were cigarette smokers and three smoked cigars. They reported little or no inhalation, and most had not smoked on the day of the survey. Their marker concentrations were consistently at or near nonsmoker levels.

TABLE 5—Sensitivity at Specificity = 95%, and Corresponding Cut-off Value

Biochemical Markers	Cut-off Value	Sensitivity	
		% All Smokers Detected	% Cigarette Smokers Detected
Carbon Monoxide			
ECO (ppm)	10	82	88
COHb (%)	1.8	81	89
Nicotine (ng/ml)			
Plasma	1.6	89	93
Saliva	15.0	92	95
Urine	50.7	89	93
Cotinine (ng/ml)			
Plasma	6.2	96	99
Saliva	5.9	96	99
Urine	22.5	98	99
Thiocyanate			
Plasma (μmol/l)	91	73	74
Saliva (mmol/l)	3.07	25	25
Urine (μmol/l)	143	44	51

Discussion

The results of this study indicate that the concentration in the body of certain biochemical markers of smoke intake can give a categorization of smoking status which is substantially more accurate than self-report. Cotinine, because of its longer half-life, performed better than nicotine. Since both are specific to tobacco, it is not surprising that they were more successful in identifying smokers than measures of carbon monoxide or thiocyanate. However, no measure can be 100 per cent sensitive to active smoking or completely successful in identifying nonsmokers. This is because, on the one hand, exposure to other people's smoke leads to the absorption of smoke products in nonsmokers and, on the other, because some smokers may smoke so infrequently or inhale so little that their intakes cannot reliably be distinguished from passive smoking.

Our results were robust and not influenced in any significant way by the presence in the sample of a substantial minority of smokers who denied their habit. When cut-offs were optimized to discriminate smoking according to self-report, the "deceivers" were correctly categorized as smokers, and their reallocation to the smoking group caused very little shift in cut-off values. The optimal cut-offs for the nicotine, cotinine, and thiocyanate measures all remained unchanged, and there were only minimal shifts in the cutting points for expired air carbon monoxide and COHb.

When biochemical markers are used to discriminate smoking status the optimal cut-point for any particular application will depend on the prevalence of smoking in the study population and on whether differential weights are attached to misclassifications of smokers and nonsmokers. Our cut-points were optimized for one particular sample, and the precise values may not therefore be directly generalizable to all clinical and research situations. However, the cut-points we found are similar to those reported by other workers for each of the markers studied, and may therefore provide an approximate indication of cut-off points likely to be useful in a variety of situations.

Our findings have implications for clinical practice in hospitals and other settings where patients may present with smoking-related disease. Self-reported smoking rates are likely to give a substantial underestimate of the true prevalence of smoking. Among our sample, 19 per cent of smokers claimed to be nonsmokers. This misreporting could lead to an underestimate of the effects of smoking on the course of disease and could prejudicially affect decisions on patient management. At the same time, the success of objective tests

in identifying smokers means that the clinician can escape from dependence on unreliable self-report.

Which then, of the tests examined, can be recommended for routine clinical use? Cotinine provides the best discrimination and must be the marker of choice for situations where accuracy is paramount. Non-invasive specimens of saliva or urine give essentially the same information as blood samples. But high costs and the elaborate laboratory facilities necessary argue against cotinine for routine use. The same is true for nicotine, where difficulties of avoiding sample contamination can raise added problems. Carbon monoxide levels provide a more sensitive indication of smoking than measures of thiocyanate and are cheaper and easier to apply. They are also likely to be valuable for excluding smoking in people who admit to using smokeless tobacco products and who therefore would have elevated levels of nicotine and cotinine. The concentration of carbon monoxide in expired air can be measured non-invasively with a simple and inexpensive portable monitor by someone with minimal training, with essentially the same results as achieved by a more expensive and difficult assay requiring a blood sample.[19,20] Although in principle smokers could avoid detection by a day's abstinence before coming for test, in practice this seems unlikely to occur, particularly when there is repeated and regular contact. In the present sample, 97 per cent of self-reported cigarette smokers said they had smoked on the day of testing.

In conclusion, whether or not a person is a current smoker can be established accurately by objective tests of smoke intake. The few smokers who cannot be reliably identified smoke so infrequently or inhale so little that their habit is of minimal clinical significance. Although measures of cotinine perform best in discriminating smokers and non-smokers and are the tests of choice for research protocols where accurate categorization is essential, for routine clinical applications expired air carbon monoxide is a simple, cheap, and acceptably accurate measure.

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