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Carboxyhemoglobin, Cotinine, and Thiocyanate Assay Compared for Distinguishing Smokers from Non-Smokers

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We compared cotinine, carboxyhemoglobin, and thiocyanate concentrations in blood sampled from 187 cigarette smokers and 181 non-smokers. All three differed significantly between smokers and non-smokers. Cotinine performed best as a test for assessing smoking status, with a sensitivity of 98% as compared with 94% for carboxyhemoglobin and 80% for thiocyanate, all at a specificity of 95%. These differences were statistically significant. Results by none of these three methods correlated well with number of cigarettes smoked per day.

Additional Keyphrases: *validating smoking claims · cutoff value*

Several biochemical methods are used to distinguish smokers from non-smokers in health surveys, anti-smoking programs, and heart-disease prevention studies (*1–3*). These methods—which include plasma cotinine, blood carboxyhemoglobin, plasma thiocyanate, and expired-air carbon monoxide—are a valuable aid to interpretation of the questionnaires used in smoking-cessation programs. Information supplied solely by the volunteers in these programs is often unreliable (*5, 6*), and to validate results independent measures of cigarette smoking are necessary.

Thus it seems important to document and inter-compare the performance of these tests, but there are few such studies adequately comparing the discriminating power of the various biochemical methods (*1, 2*). Here, we examine the performance of three of these tests: cotinine, carboxyhemoglobin, and thiocyanate, and compare them, both with each other and with the number of cigarettes reportedly smoked per day. We attempted to fulfill the criteria suggested by Zweig and Robertson (*7*): subject selection, independent classification, performance of all tests on all subjects, and comparison of tests at the same specificities. We have also performed statistical tests to validate impressions of difference in performance.

Subjects and Methods

The study population consisted of 187 smokers and 181 non-smokers, the former being participants in a voluntary smoking-reduction campaign in an suburban general practice, the latter being unselected patients attending the clinic

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for various reasons, who agreed to take part in the development of counselling methods for smokers. The proportions of men and women were the same in the smoker and non-smoker groups, and the ages in the two groups were comparable. The smokers consumed 22.8 ± 12.5 (mean ± SD) cigarettes per day. Blood was sampled for analysis from the volunteers at the start of the program, when information on smoking habits was obtained from them. Each sample was then analyzed for each of the three analytes without the analysts' knowledge of the smoking habits of the participant. The data were correlated at the conclusion of all analyses by use of the SPSS (*8*).

Plasma thiocyanate was determined colorimetrically after ion-exchange chromatography on Amberlyst A21 resin (B.D.H. Chemicals Ltd., Poole, England) (*9, 10*). Carboxyhemoglobin was measured in whole blood by an automated spectrophotometric method, with a CO-Oximeter (Instrumentation Laboratory, Lexington, MA) (*11*). Cotinine in plasma was determined by gas chromatography on a 12.5 m × 0.3 mm (i.d.) Carbowax capillary column (Hewlett Packard, Avondale, PA) (*12, 13*).

The analytical precision (CV) for the three methods was 0.17% at a mean concentration of 1.9% for carboxyhemoglobin, 27 nmol/L at 247 nmol/L for cotinine, and 3.6 μmol/L at 52 μmol/L for thiocyanate.

Results

Figures 1, 2, and 3 illustrate the frequency distributions for results for carboxyhemoglobin, cotinine, and thiocyanate for the study population, classified according to their responses to the questionnaire. The mean respective values (and SD) for non-smokers and smokers were 0.93 (0.52)% and 4.36 (2.09)%, 25 (78) and 1905 (1321) nmol/L, and 33 (15) and 109 (47) μmol/L. For each, the differences between smokers and non-smokers were highly significant ($p < 0.001$).

The value for carboxyhemoglobin that misclassifies the fewest subjects overall is 2.0%, and this figure gives a sensitivity of 87.7% and a specificity of 97.8%. For thiocyanate the best discriminating value is 70 μmol/L, which yields a sensitivity of 75.9% and a specificity of 96.7%. Cotinine, with a cutoff point of 250 nmol/L, has a sensitivity of 95.2% and a specificity of 98.3%. Figure 4 shows the sensitivity and specificity of the three tests at various cutoff values and compares the sensitivities of the three assays at a constant 95% specificity.

Inter-comparison of the sensitivity of the three tests at constant specificity (Table 1) showed the three tests to be significantly different.

Table 2 shows coefficients of correlation for the relation between results of each of the three tests and the number of cigarettes smoked, and of the methods with each other. It

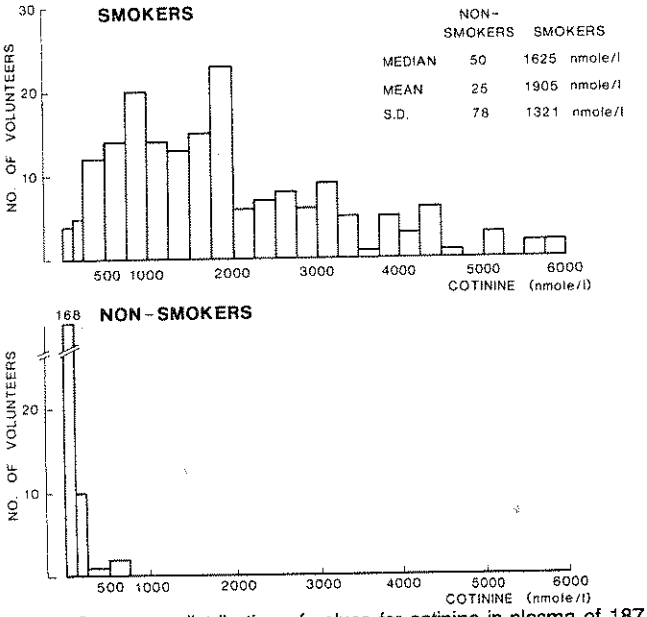

Fig. 2. Frequency distribution of values for cotinine in plasma of 187 smokers and 181 non-smokers

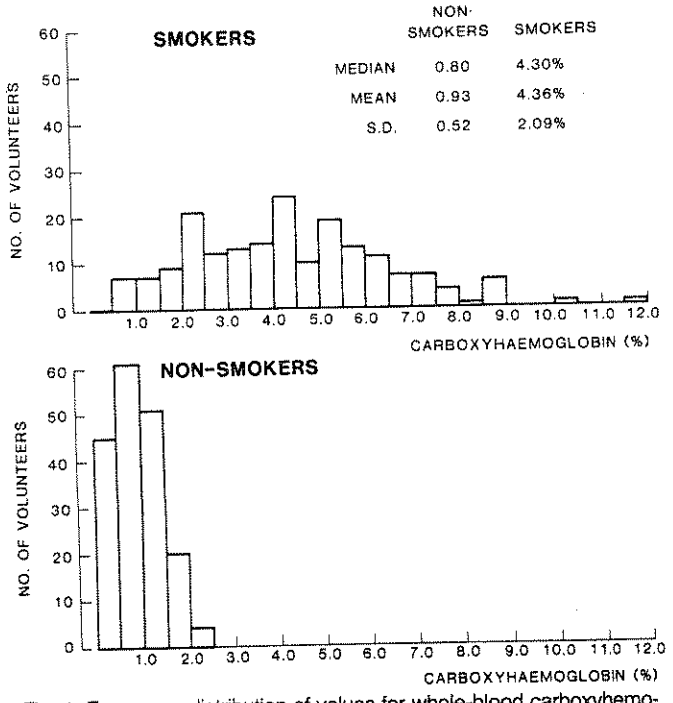

Fig. 1. Frequency distribution of values for whole-blood carboxyhemoglobin in 187 smokers and 181 non-smokers

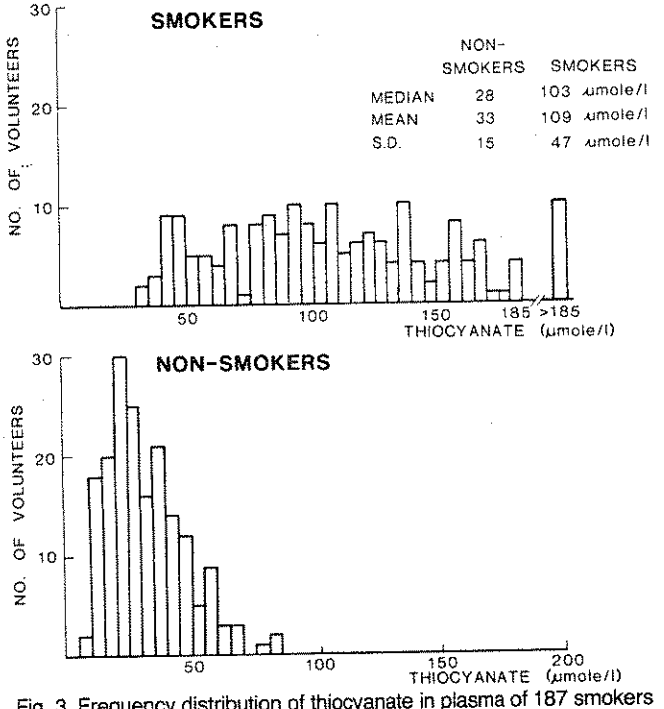

Fig. 3. Frequency distribution of thiocyanate in plasma of 187 smokers and 181 non-smokers

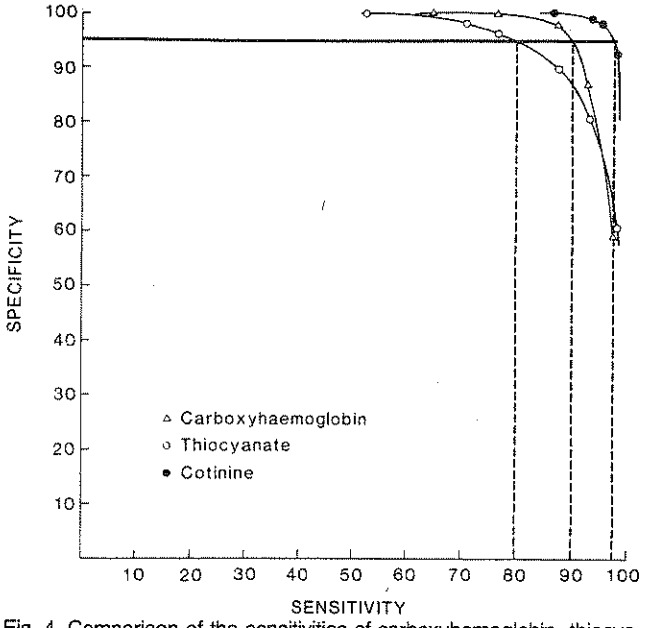

Fig. 4. Comparison of the sensitivities of carboxyhemoglobin, thiocyanate, and cotinine assay at comparable specificities

The curves were generated by calculating sensitivity and specificity at various cutoff points for each test

Table 1. Intercomparison of Sensitivity of the Three Assays[a]

True positive	False positive	$(Chi)^2$	p
Cotinine vs carboxyhemoglobin			
181	6	8.10	0.005
167	20		
Carboxyhemoglobin vs thiocyanate			
167	20	7.26	0.007
148	39		
Thiocyanate vs cotinine			
148	39	27.5	<0.001
181	6		

[a] Comparisons of numbers of true- and false-positive results at cutoff points chosen to give an equal specificity (95%) for the three tests for smoking: 1.9% for carboxyhemoglobin, 160 nmol/L for cotinine, and 67 μmol/L for thiocyanate.

Table 2. Correlation Data[a]

	CoHB/Cot	CoHB/SCN	Cot/SCN
Correlation	0.69	0.58	0.46
Partial correlation, controlling for no. of cigarettes	0.64	0.56	0.43
	CoHb	**Cot**	**SCN**
Correl. with no. cigarettes smoked per day	0.41	0.34	0.19

[a] Correlations and partial correlations between test results (smokers only). All correlations except that between number of cigarettes consumed and thiocyanate ($p = 0.01$) are significant at the $p < 0.001$ level.

also shows the partial correlations for each of these methods with each other, after the effect of the control variable (number of cigarettes smoked per day) is removed.

Discussion

We conclude that any of the three assays will distinguish cigarette smokers from non-smokers. This concurs with other studies (*1–3*). Whereas a previous study (*2*) suggested that thiocyanate may be a more sensitive test than carboxyhemoglobin, but equally specific, we find thiocyanate to be a less sensitive test than carboxyhemoglobin, and we find cotinine to be a far more sensitive and specific test than either of them. This agrees with the study by Haley et al. (*4*), who found cotinine to be a better marker to determine smokers than thiocyanate. This difference is well illustrated by comparing the relative sensitivities of the three tests at a fixed specificity of 95% (Figure 4). We have also been able to establish statistically significant differences in the sensitivities of the three tests, the most likely explanation for which is that factors other than cigarette smoking can affect the concentrations of these three analytes in the blood. For example, carboxyhemoglobin can be affected by atmospheric pollution, particularly from motor exhaust fumes (*1*), and thiocyanate can be increased after consumption of certain vegetables or after industrial exposure to cyanides (*1*). Cotinine is not subject to these extraneous influences.

In vivo, nicotine from cigarette smoker is converted to cotinine (*14*). Gas-chromatographic estimation of nicotine can be very difficult (*15*), and nicotine is an ubiquitous contaminant of our laboratory air-conditioning system. Thus we elected to assay cotinine as an indirect measure of nicotine intake. Cotinine is not widely distributed in the environment, and falsely increased cotinine concentrations in non-smokers arise only by passive smoking, i.e., by passive inhalation of cigarette smoke—and even this effect reportedly is small (*16*). We have also found that passive intake of cigarette smoke by a non-smoker rarely results in plasma cotinine concentrations > 100 nmol/L (data not shown). These facts may account for the superior performance of cotinine as compared with carboxyhemoglobin and thiocyanate. In the same way as it has previously been suggested that measurement of carboxyhemoglobin is a better test than plasma nicotine (*17*), cotinine would be expected to be a better discriminating test than a direct assay for nicotine.

We found poor correlation between values for the three analytes and stated daily cigarette consumption. This agrees with previous studies (*17, 18*) and is probably caused by such variables as different patterns of smoke inhalation, differences in the subject's biochemical responses, or inaccurate survey information supplied by the subjects. It would seem from the pattern of correlation between them that factors affecting the relationship between number of cigarettes and test results affect each of the three tests similarly. Partial correlation estimates the strength of this association between the test results, after correcting for a factor that may influence each of them—in this case the number of cigarettes inhaled. Evidently the correlations between results in the three tests in the smokers are only slightly ascribable to differences in number of cigarettes (*8*).

In most previous studies, values for either plasma thiocyanate or blood carboxyhemoglobin were used to distinguish smokers from non-smokers, but we find the performance of these two tests to be quite different. Neither test appears to be as good as plasma cotinine. In fact, cotinine would appear to be the best single biochemical test with which to distinguish smokers from non-smokers in clinical and epidemiological studies; the high sensitivity of cotinine and the correlation between results of all three tests suggest that using two tests would not be advantageous.

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