Expired Air Carbon Monoxide and Serum Thiocyanate As Objective Measures of Cigarette Exposure

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Abstract: Expired air carbon monoxide (CO) and serum thiocyanate (SCN) were used to assess exposure to cigarettes in 139 middle-aged men. Subjects who reported smoking cigarettes generally had CO levels exceeding 8ppm and SCN levels exceeding 100umol/L; non-smokers had lower levels. For both tests the mean concentration among men smoking more than one pack daily was three times that of non-smokers. There is a high correlation between the two tests (r = .571 for smokers), an association that was largely independent of the smaller correlations between either test and reported smoking frequency (r = .476 for CO; r = .479 for SCN).

The ability to distinguish between individuals who reported "typical" smoking habits and non-smokers was best when the CO and SCN analyses were used together to take advantage of their separate sources of variance; it was 99 per cent when the two tests were mutually concordant (91 per cent of cases). The CO and SCN measurements allowed 16 individuals who reported light smoking habits to be categorized into high and low presumptive tobacco exposure groups. The two tests are inexpensive and suitable for use in epidemiologic and health care delivery programs. (Am. J. Public Health 67:545–549, 1977)

Introduction

Habitual exposure to cigarettes has traditionally been assessed by questionnaire. The accuracy of this approach is limited by conscious false reporting and by inadequate recognition of the true degree of exposure. Two biochemical tests for objectively measuring smoking exposure have been available for years but have not been widely used in epidemiologic or health care delivery settings.

One is the measurement of serum thiocyanate (SCN) concentration,[1–7] elevated in smokers as a consequence of trace amounts of cyanide in tobacco. The long biologic half-life of SCN, about two weeks,[4] is suitable for assessing habitual smoking behavior. The main disadvantage of this approach is the overlap that is observed in the distributions of the SCN levels of smokers and non-smokers.[1–7]

A second test is the measurement of blood carboxyhemoglobin concentration,[8–12] elevated in smokers because cigarette smoke contains carbon monoxide (CO). Technical problems limit the general applicability of analyzing whole blood for carboxyhemoglobin; fortunately, the concentration of CO in expired air, which is directly related to the blood carboxyhemoglobin concentration,[9–12] can be easily measured.

The expired air method is rapid and inexpensive, but its reliability as an index of *habitual* smoking behavior needs testing, particularly in view of the relatively short half-life of carboxyhemoglobin (about four hours).[9] In this paper the results of such validation studies are presented, and expired CO and serum SCN levels are used in combination to discriminate cigarette smokers from non-smokers.

Materials and Methods

The subjects were males aged 35–57 who were enrolled at the San Francisco clinic of the Multiple Risk Factor Intervention Trial (MRFIT), a six-year multi-center heart disease prevention study.[13] One hundred forty-eight persons received SCN and CO testing. The nine who reported smoking pipes or cigars were excluded from this report so that the results and conclusions would reflect only cigarette tobacco usage. Of the remaining 139, 45 subjects claimed to be non-smokers and 94 to smoke only cigarettes.

Each participant's smoking history was determined in an oral interview. CO measurements were performed with the Ecolyzer (Energetics Science, Inc., New York), an instrument that measures the rate of conversion of CO to CO_2 while it is passed over a catalytically active electrode. The subject was asked to hold his breath for 10 seconds, then ex-

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pire half the breath to eliminate dead space air and fill a polyethylene bag with the remaining alveolar air. The bag was immediately attached to the Ecolyzer for CO concentration determination. The procedure was performed twice on each individual, and the results reported as the average of the two readings.

Thiocyanate was measured using the automated method of Butts et al.;[7] ferric nitrate is reacted with SCN to produce ferric thiocyanate, the concentration being determined colorometrically. The analyses were carried out on serum obtained after a 12-hour fast.

Results

The mean expired CO and serum SCN concentrations are plotted against habitual cigarette frequency, assessed by questionnaire, in Figure 1. Mean levels of these tests are closely correlated with reported smoking frequency. The levels of expired air CO and serum SCN in subjects smoking more than 20 cigarettes per day are three times those of non-smokers.

Correlation coefficients among smokers for the variables measuring the time elapsed since last smoking, the number of cigarettes smoked per day, expired CO and serum SCN are given in Table 1. The correlation between CO and SCN is greater than that between either test and the frequency of smoking, and it is relatively independent of the recency and frequency of smoking; the partial CO-SCN correlation coefficient after the influence of the time interval has been removed is .529. With the number of cigarettes per day removed as well, the partial correlation between CO and SCN is further reduced but still strong (r = .430).

The short biologic half-life of expired CO does not substantially diminish its usefulness as an index of habitual smoking behavior. This is a consequence of covariance between the reported time interval since last smoking and the habitual cigarette frequency (r = −.314). The partial correlation between CO and the time elapsed since the last cigarette, with the linear influence of cigarette frequency removed, is only −.100.

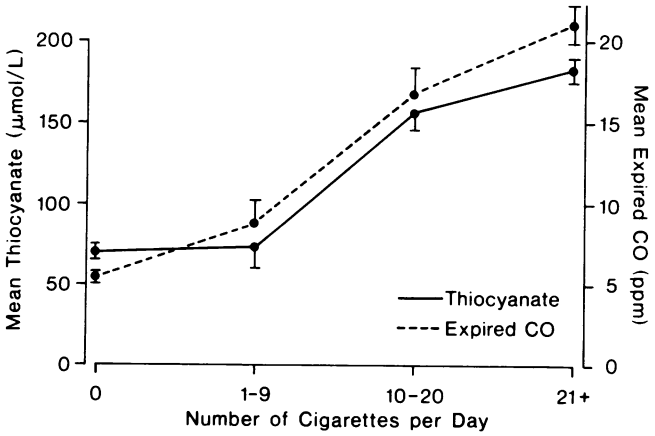

FIGURE 1—Mean expired air CO and serum SCN plotted against number of cigarettes smoked per day. The brackets represent one standard error.

TABLE 1—Pearson Correlation Coefficients among Smokers.

	Serum SCN	Reported Number Cigarettes/day	Time Since Last Smoking
Expired CO	0.571	0.476	−0.416*
Serum SCN		0.479	−0.255
Reported number of cigarettes/day			−0.314

All correlation coefficients are significant at p ≤ .001.
*Log transformation of the expired CO values, performed because the carboxyhemoglobin decay curve is a log function, raised the correlation with time since smoking from −.416 to −.469.

Expired air CO is plotted against serum SCN in Figure 2. Only one of the persons falling in the upper right quadrant denied smoking. Eight smokers, however, appeared in the lower left quadrant with most of the non-smokers. When records of these eight individuals were examined it was found that all had marginal smoking histories: they reported not inhaling and/or smoking less than nine cigarettes per day and/or not smoking within 24 hours of testing.

Comparable marginal smoking histories were reported by eight other individuals who had levels of CO and/or SCN in the smoking range. The questionnaire did not distinguish the eight "atypical" smokers with objective findings of sub-

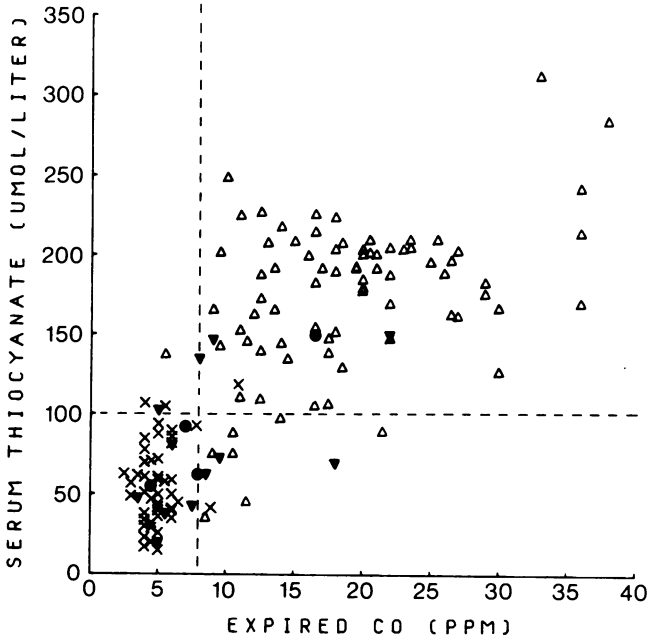

FIGURE 2—Expired air CO plotted against serum SCN for all subjects. The dashed lines are the values for each test that best discriminates smokers from non-smokers.
Δ = "Typical" smoker
x = Non-smoker
▼ = "Atypical" smoker (reports <9 cigarettes/day and/or no inhaling)
● = "Atypical" smoker (reports no cigarette for 24 hours)
Pipe and cigar smokers are excluded from these analyses.

stantial tobacco exposure from the eight with no such evidence (Table 2). Tobacco exposure in such persons is not adequately defined by responses to the questionnaire and the objective tests seem helpful in classifying this group of subjects.

Figure 3 is a plot of expired air CO against serum SCN for all subjects except the "atypical" smokers. From this figure the optimal cutoff values for dichotomizing smokers from non-smokers are 8ppm for expired CO and 100umol/L for serum SCN.

Table 3 allows a comparison of smoking history by CO and SCN levels. The substantial heterogeneity of the biochemical results among subjects reporting atypically light smoking habits is evident. For distinguishing "typical" smokers from non-smokers, the accuracy is greater when both objective tests are used together than when either is employed alone; there was only one false classification out of the 108 subjects who had concordant objective tests.

TABLE 2—Characteristics of the 16 "Atypical" Smokers

Subject	Questionnaire Variables: Daily Cigarette Frequency	Depth of Inhalation*	Hours Since Last Smoking	Expired CO (ppm)	Serum SCN (μmol/L)
1	2	0	5	8	62
2	5	0	6	10	72
3	15	0	7	8	145
4	8	1	5	8	42
5	7	0	9	5	19
6	5	1	>24	6	37
7	40	0	4	16	150
8	20	2	>24	6	138
9	8	2	6	22	142
10	3	1	>24	5	102
11	2	3	1	18	69
12	1	3	>24	8	134
13	8	3	5	9	146
14	8	0	>24	4	54
15	5	1	12	6	53
16	12	0	6	5	92

*Depth of inhalation is graded on a 4 point scale from 0 (none) to 3 (deeply into chest).

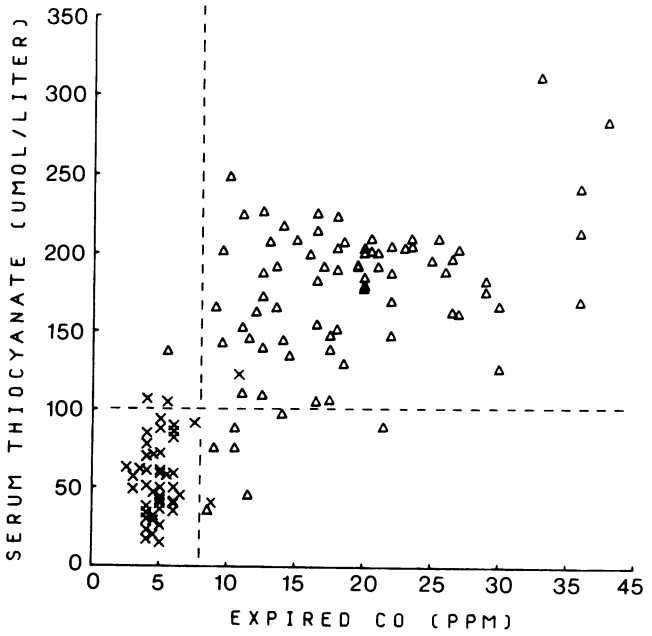

FIGURE 3—Expired air CO plotted against serum SCN for "typical" smokers (Δ) and non-smokers (x). The dashed lines are the values for each test that best discriminates smokers from non-smokers.

Discussion

Three measures of habitual exposure to cigarettes have been examined. Questionnaire responses have subjective sources of bias, both intended and unconscious. Serum SCN and expired CO are objective, but each has biologic and technical variance that contributes to the overlap in the distributions of test results when cigarette smokers are compared with non-smokers.[1-7, 9-12] There are no previous reports of using the two tests in conjunction to take advantage of their separate sources of variance. In the current study a knowledge of both CO and SCN values allowed smokers to be discriminated from non-smokers with greater accuracy than when either test was used alone.

Nearly all "errors" in categorization involved persons with marginal ("atypical") smoking histories. If this group is removed from the analysis, the remaining smokers are identified by the two tests with an accuracy of 99 per cent. The "atypical" smokers had unpredictable CO and SCN results ranging from non-smoking to heavy exposure levels. It is inferred that while many "atypical" smokers may be at little or no increased risk of disease, some individuals (identifiable only by biochemical testing) may have the same risk as con-

TABLE 3—Smoking History by CO and SCN Levels.

		Smokers	
	Non-smokers (N = 45)	"Typical" (N = 78)	"Atypical" (N = 16)
CO concentration			
> 8ppm	2	77	6
≤ 8ppm	43	1	10
SCN concentration			
> 100μmol/L	3	71	5
≤ 100μmol/L	42	7	11
CO and SCN concentrations			
Concordant			
CO > 8ppm; SCN > 100μmol/L	1	70	3
CO ≤ 8ppm; SCN ≤ 100μmol/L	41	0	8
Discordant			
CO > 8ppm; SCN ≤ 100μmol/L	1	7	3
CO ≤ 8ppm; SCN > 100μmol/L	2	1	2

When CO and SCN are concordant typical smokers are distinguished from non-smokers with 99 per cent accuracy (dashed line rectangle).

*"Atypical" smokers are those reporting unusually light smoking habits; less than 9 cigarettes daily and/or no inhalation and/or no cigarettes for 24 hours.

ventional heavy smokers. The discovery of a group of persons whose tobacco exposure is not adequately categorized by smoking history is a potentially useful outcome of CO and SCN testing.

This report contains findings from the baseline period of a voluntary smoking cessation trial, a point in time chosen to avoid the emotional investment in appearing successful that participants develop later in the course of such a project. Nevertheless, the argument presented thus far suffers from a circular reasoning problem; the questionnaire responses that have been used to validate the biochemical indices may themselves be suspect. The validity of the biochemical tests as measures of tobacco exposure is supported by three lines of reasoning.

The first is the nearly perfect way that the tests separate smokers from non-smokers. Second, the CO and SCN levels of smokers are more strongly correlated with each other ($r = 0.571$) than is either with reported smoking frequency ($r = 0.476$ and 0.479 respectively) (it is possible that the biochemical tests may be better measures of smoking behavior than is self-reported smoking history). Third, partial correlation coefficients provide a measure of the amount of information obtained from CO and SCN that is not present in the questionnaire data. When the cigarette frequency and recency components are removed, the remaining CO-SCN partial correlation is still large (.430). The magnitude of this correlation is remarkable when it is considered that the biochemical tests have separate sources of variance, with tobacco exposure the single known commonality.

Several factors other than tobacco smoking are known to affect expired CO and serum SCN. CO is raised by exposure to the products of incomplete combustion; gas heaters and automobile engines are common sources of exposure.[14] The factors in addition to smoking that may elevate serum SCN levels include industrial exposure to cyanides in electroplating, precious metal refining, case hardening of steel, and gas manufacturing.[15] Some foods also produce SCN, including the cabbage family, turnips, garlic, horseradish, mustard, and almonds.[3] Vegetarians have slightly higher SCN concentrations than non-vegetarians but the increase is not as large or consistent as that seen in smokers.[5]

Both expired air CO and serum SCN are elevated threefold when the mean levels of subjects smoking more than 20 cigarettes per day are compared with those of non-smokers. The mean expired CO level of these relatively heavy smokers, 21ppm, is compatible with previous reports[9, 11, 12, 14] and corresponds to a blood carboxyhemoglobin concentration at 5–6 per cent of the total hemoglobin level.[9–11, 14] The observed correlation between expired CO level and habitual smoking frequency is notable in view of the relatively rapid dissociation of carboxyhemoglobin. This reliability of CO as a measure of *chronic* smoking behavior is due to an association between cigarette smoking frequency and time elapsed since last smoking; those who habitually smoke heavily are more likely to have smoked shortly before testing. This covariance also accounts for the existence of a correlation between serum SCN and the time elapsed since smoking ($r = -.255$) despite the two week half-life of the SCN.

The expired CO test has great potential as a tool in smoking intervention programs; the instrument is portable and virtually without operating costs once it has been purchased, and the analysis can be witnessed by the patient to provide immediate and tangible feedback. The common knowledge that CO and cyanide are poisons* contributes to the usefulness of both CO and SCN as incentive devices.

Expired CO and serum SCN are also well suited for use in epidemiologic studies of tobacco exposure and disease. They have great potential for better defining factors that influence dosage of tobacco exposure, such as cigarette frequency, cigarette brand, depth of inhalation, and the use of pipes and cigars.

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*The dosage of CO resulting from cigarettes is in fact toxic; there are changes in neurologic reflexes, altered psychomotor test results, changes in sensory discrimination and electrocardiographic abnormalities, as well as fatigue, headache, irritability, dizziness, and disturbed sleep.[14] Chronic elevation of CO levels has been associated with the development of atherosclerotic disease, and there is speculation that the relationship is causal.[12, 14, 16–18] The role of trace amounts of SCN in human health is less clear, and the levels observed among smokers are not known to be toxic to the average person. Individuals suffering from tobacco amblyopia may have a metabolic defect preventing the conversion of cyanide to SCN at a rapid enough rate to prevent toxic buildup of cyanide levels in the body.[4, 19]

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On the Counting of Indians for the Census

"The instructions of the First Census (1790) provided for the exclusion of Indians not taxed, and no Indians were reported. The data relating to Indians go back only to 1890, when one-fourth of a million Indians were returned in a population of 60 million."

Taeuber, Conrad and Taeuber, Irene. The Changing Population of the United States. NY: John Wiley and Sons Inc., 1958.

"Chairman Jo-Jo Hunt told the hearing that the major problem facing Indians in this region is being accurately counted by census takers and gaining Federal recognition for tribes not recognized by the US Bureau of Indian Affairs for historic reasons. . . . Many Indians do not respond to white census enumerators because of their distrust of them."

Meier, Mary. O'Neill calls for state housing authority for Indians. Boston Globe, April 10, 1976. p. 2.

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