Carboxyhaemoglobin and plasma thiocyanate: complementary indicators of smoking behaviour?

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ABSTRACT Carboxyhaemoglobin and plasma thiocyanate concentrations were measured in 79 non-smokers and 360 cigarette smokers. The mean levels were 0.73% and 7.09% carboxyhaemoglobin and 40.2 and 133.8 μmol thiocyanate/l plasma respectively. With 1.6% carboxyhaemoglobin and 73.0 μmol thiocyanate/l plasma as critical values the concentrations of carboxyhaemoglobin in 96.6% of subjects and of thiocyanate in 93.4% were compatible with reported smoking status. This difference between the two tests is significant ($p < 0.005$). Statistical combination of the carboxyhaemoglobin and thiocyanate results, with the use of linear discrimination analysis, only marginally improved their diagnostic efficiency (96.8% of subjects were grouped correctly). This analysis did, however, successfully regroup 21 of 26 individuals with contradictory carboxyhaemoglobin and thiocyanate classifications. It is concluded that in this study determination of thiocyanate added little to the information obtained from carboxyhaemoglobin measurements alone.

Twenty to thirty-five per cent of smokers attending clinics and claiming to have stopped smoking have been shown not to have done so.¹⁻⁴ Consequently, to assess the impact of antismoking strategies biochemical tests are being increasingly used as an independent validation of such claims. Unfortunately even with these tests some smokers and non-smokers cannot be distinguished with certainty. Vogt⁵ has suggested that discrimination between the groups may be improved by combining two of the tests—namely, estimation of alveolar carbon monoxide and serum thiocyanate. Indeed, he reported that when the two measurements agreed 99% of the predictions proved correct. In one-tenth of his population, however, the two tests disagreed and assigned subjects to opposite groups. In these instances a means of interpreting the contradictory results is necessary or the tests become meaningless. No such method has yet been suggested.

We have reassessed the ability of carboxyhaemoglobin and plasma thiocyanate estimations to distinguish between smokers and non-smokers, and have also used the statistical technique of discriminant analysis to weight these measures to predict the group to which subjects with conflicting values are most likely to belong.

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Methods

All the subjects were volunteers who had no reason to conceal their true smoking habits. They were smoking and non-smoking members of staff of St Bartholomew's Hospital and smokers attending the addiction research unit at the Maudsley Hospital. The subjects were assigned to one of two groups on the basis of their reported smoking habits. The first group consisted of 360 cigarette smokers (178 men and 182 women) who had not altered their usual smoking behaviour. In the second group there were 79 non-smokers (51 men and 28 women).

Venous blood was taken from each subject, usually in the afternoon or late morning, into a heparinised syringe and kept at 4°C. Carboxyhaemoglobin was measured within 48 hours with an IL282 CO-Oximeter, the accuracy of which was cross-checked with a gas chromatographic method.⁶ The remaining blood was centrifuged and the plasma separated and stored at -20°C until analysed for thiocyanate. In this estimation plasma (0.5 ml) was mixed with 5% trichloroacetic acid (4.5 ml) and the thiocyanate measured in the protein-free supernatant by an automated modification of the Aldridge technique.^{7,8} All carboxyhaemoglobin and thiocyanate estimations were performed in duplicate. Statistical calculations were made on the ICL 2980 com-

puter at Queen Mary College, London. The linear discrimination function ("z") was calculated as described by Armitage.⁹

Results

The means and standard deviations and ranges of carboxyhaemoglobin and plasma thiocyanate concentrations in the two groups are shown in table 1. After normalisation of the distribution by logarithmic transformation, the upper 95% confidence limits (mean + 2 SD) in the non-smoking group were 2.0% carboxyhaemoglobin and 78.0 $\mu\text{mol/l}$ plasma thiocyanate respectively. These limits and the alternative values of 1.6% carboxyhaemoglobin and 73.0 $\mu\text{mol/l}$ plasma thiocyanate were used to separate the population into smoking and non-smoking groups (table 2). Since 1.6% carboxyhaemoglobin and 73.0 $\mu\text{mol/l}$ thiocyanate/l plasma gave fewer misclassifications these values were used in all subsequent analysis.

The figure shows the scatter of carboxyhaemoglobin (COHb) versus thiocyanate (SCN) values and the line of discrimination based on both measurements ($z = \log \text{COHb} + 0.777 \log \text{SCN}$). The allocation rule would predict that a subject was a smoker if z was greater than 1.74 and a non-smoker if it was less than 1.74. The data were log transformed before this analysis to stabilise the variances in the two groups.

The predictions of the biochemical measurements are compared with the reported smoking habits in table 2. The carboxyhaemoglobin value and z each gave essentially the same percentage of correct classifications (96.6% and 96.8% respectively),

which was significantly higher than that achieved with plasma thiocyanate (93.4%, $p < 0.005$). The carboxyhaemoglobin and thiocyanate values were in agreement in the classification of 94% of our population. They placed 26 of the 439 subjects in opposite groups, however; of this number, six had carboxyhaemoglobin levels and 20 had plasma thiocyanate concentrations incompatible with their reported smoking habits. With the use of z four carboxyhaemoglobin and 17 thiocyanate misclassifications were corrected (table 3). Subdividing the smokers into classes according to cigarette consumption shows that errors in prediction are most likely to occur in the light smoking categories (table 4).

Discussion

To distinguish between smokers and non-smokers an index compound should ideally satisfy the following criteria: (1) be specific for tobacco smoke; (2) be unaffected by genetic, nutritional, or environmental differences; (3) have a long half-life; and (4) be easy to analyse accurately. The most common of such compounds in current use—carbon monoxide, thiocyanate, nicotine, and cotinine—do not completely fulfil these requirements.

Blood or urinary nicotine and cotinine are probably the most specific indicators of smoking available, and while these substances may be detected in non-smokers as a result of passive smoking high concentrations are unusual.^{10,11} They both, however, have a shorter half-life than thiocyanate and are not as easy to measure as carboxyhaemoglobin. Furthermore, where chewing gum containing nicotine is

Table 1 Mean ($\pm$ SD) and range of carboxyhaemoglobin (COHb) and plasma thiocyanate (SCN) concentrations in smokers and non-smokers

	n	% COHb			SCN ($\mu\text{mol/l}$)		
		Mean	$\pm$ SD	Range	Mean	$\pm$ SD	Range
Non-smokers	79	0.73	0.41	0 – 2.3	40.2	16.6	14–114
Smokers	360	7.09	2.9	0.3–15.5	133.8	45.5	33–287

Table 2 Percentage of self-reported non-smokers and smokers misclassified on the basis of the specified limiting values of carboxyhaemoglobin (COHb) or plasma thiocyanate (SCN) and by discriminant analysis (z) (subjects with values less than or equal to the cut-off point are considered to be non-smokers)

	COHb cut-off point (%)		SCN cut-off point ($\mu\text{mol/l}$)		z^*
	1.6	2.0	73.0	78.0	1.74
% non-smokers (n = 79)	2.53	1.27	5.06	2.53	0
% smokers (n = 360)	3.61	4.17	6.94	9.44	3.89
% all subjects	3.42	3.64	6.61	8.20	3.19

* $z = \log \text{COHb} + 0.777 \log \text{SCN}$.

Scatter diagram of carboxyhaemoglobin (COHb) v thiocyanate (SCN) values in 439 adults with the line of discrimination between smokers (□) and non-smokers (x). The dashed lines are the limiting values of 1.6% COHb and 73 μmol SCN/l.

Table 3 Cigarette consumption (cigarettes/day), carboxyhaemoglobin (COHb), and plasma thiocyanate (SCN) concentrations, and discriminant analysis (z) predictions in subjects with contradictory biochemical test classifications

(a) COHb misclassifications when SCN predictions were correct			
Cigarettes/day	COHb (%)	SCN (μmol/l)	z
0	1.9	23.0	Non-smoker
0	2.3	30.0	Non-smoker
10	0.7	90.0	Non-smoker
15	1.4	133.0	Smoker
20	1.3	85.0	Non-smoker
37	1.5	136.0	Smoker

(b) SCN misclassifications when COHb predictions were correct			
Cigarettes/day	COHb	SCN	z
0	0.7	74.0	Non-smoker
0	0.8	76.0	Non-smoker
0	0.5	89.0	Non-smoker
0	1.2	114.0	Non-smoker
2	3.4	65.0	Smoker
5	2.5	70.0	Smoker
6	3.7	58.0	Smoker
7	2.4	54.0	Smoker
8	2.0	54.0	Non-smoker
10	3.4	57.0	Smoker
13	1.9	55.0	Non-smoker
13	2.1	49.0	Non-smoker
16	5.3	67.0	Smoker
20	2.6	50.0	Smoker
20	4.8	69.0	Smoker
23	5.6	42.0	Smoker
28	2.5	60.0	Smoker
30	3.7	49.0	Smoker
38	5.6	55.0	Smoker
40	3.9	52.0	Smoker

Table 4 The number of subjects in each class of daily cigarette consumption misclassified by predictions based on carboxyhaemoglobin (COHb) or plasma thiocyanate (SCN) concentrations and on discriminant analysis (z)

Smoking class (cigarettes/day)	Total No in class	No misclassified		
		COHb	SCN	z
1- 5	11	6	9	6
6-15	43	4	7	6
16-25	108	2	5	2
26-35	90		2	
36-45	75	1	2	
≥46	33			

Table 5 Sex, daily cigarette consumption (cigarettes per day), and carboxyhaemoglobin (COHb) and plasma thiocyanate (SCN) levels of smokers misclassified by all tests including z

Sex	Cigarettes/day	COHb (%)	SCN (μmol/l)
F	1	0.5	42
F	4	0.7	42
F	5	1.5	45
F	8	0.3	53
F	8	1.3	33
F	16	1.5	66
M	1	1.1	47
M	3	1.1	56
M	5	1.3	70

being used to assist people to stop smoking nicotine and its metabolite cotinine are obviously inappropriate for monitoring withdrawal.

Carboxyhaemoglobin and thiocyanate are less specific indicators of smoking. Carbon monoxide is produced endogenously from haem compounds and raised levels of carboxyhaemoglobin may occur in haemolytic anaemias.¹² Occupational exposure to environmental carbon monoxide will also raise the concentration.¹³ There are more exogenous sources of thiocyanate and diet is probably the main reason for the wide variations of this anion in the plasma, urine, and saliva of non-smokers.¹⁴ Smoking, however, remains the single most important cause of high carboxyhaemoglobin and thiocyanate concentrations in blood (table 1). Pyrolysis of tobacco produces up to 5% carbon monoxide and 0.16% hydrogen cyanide in the gas phase, as well as organic cyanide compounds (nitriles—about 1 mg a cigarette) in the particulate phase. Most of the carbon monoxide and hydrogen cyanide (and its compounds) is absorbed through the lungs. In addition, some hydrogen cyanide is absorbed through the buccal and nasal mucosa. Both the free hydrogen cyanide and that released during the metabolism of the nitriles is converted to thiocyanate by the mitochondrial enzyme rhodanese.

Carboxyhaemoglobin and plasma thiocyanate each identified correctly the group to which over 90% of our subjects belonged (table 2). These results are similar to those reported by Vogt⁵ and better than those of Cohen and Bartsch.¹⁵ The very high levels of carboxyhaemoglobin ($\geq 6\%$) and thiocyanate ($\geq 200 \mu\text{mol/l}$) found in some of the non-smoking subjects in the latter study make it possible that some of those claiming to be non-smokers were in fact smokers; this would explain the reduced specificity and sensitivity of the tests in this case.

In nine of our smoking subjects both carboxyhaemoglobin and thiocyanate levels were within the non-smoking range (table 5). Most of these people consumed relatively few cigarettes, while others might have been non-inhalers. These factors may account for the atypical results and no biochemical test is likely to identify such individuals.

The object of using discriminant analysis was to weight and linearly combine the discriminating variables (carboxyhaemoglobin and thiocyanate) in such a manner that the groups (smokers and non-smokers) are forced to be as statistically distinct as possible. The chief benefit of this analysis was in deciding the group to which subjects with contradictory carboxyhaemoglobin and thiocyanate classifications belonged. There were 26 such subjects and 21 of these were successfully grouped with

the help of the function z (table 3). But as z achieved only a marginally better overall classification than that obtained with carboxyhaemoglobin there appears to be no real advantage in making both biochemical measurements. Indeed, thiocyanate estimation is a more complicated procedure than the procedure for determining carboxyhaemoglobin levels with the CO-Oximeter; and the extra expense, analysis time, and labour required in measuring thiocyanate cannot be justified. The one major advantage of thiocyanate derives from the long half-life (14 days) of this anion in plasma.¹⁶ Carboxyhaemoglobin, on the other hand, has a half-life of only 3.1 hours in subjects engaged in light activity, increasing to 6.9 hours during sleep.¹⁷ Carboxyhaemoglobin is therefore more susceptible to the effects of short-term changes in smoking habits. Thus if subjects stop smoking for 24 hours before blood sampling carboxyhaemoglobin levels can fall to within the non-smoking range, whereas the plasma thiocyanate concentration will be virtually unaffected.

The cut-off points used in this study may not be applicable to studies in which different analytical procedures are used. There are no standardised techniques for measuring either carboxyhaemoglobin or thiocyanate and systematic errors between different methods may arise.^{18,19} Published means for these measures in non-smokers show considerable variation^{2,3,20,21} and each laboratory must therefore determine its own normal values. The CO-Oximeter gave results with excellent repeatability⁶ and is easy to use and rapid (an analysis takes only one minute). Our thiocyanate method also has a good precision; routine analysis of two batches of pooled plasma over three months gave means and standard deviations of 46.1 ± 2.4 and $117.8 \pm 6.4 \mu\text{mol SCN/l}$ plasma ($n=50$). This analysis, however, is slower (only 20 plasma samples are processed in an hour) and requires costly reagents.

In conclusion, our results indicate that the ability to distinguish between smokers and non-smokers is only marginally enhanced by combining carboxyhaemoglobin and thiocyanate measurements. In most instances carboxyhaemoglobin estimation alone is sufficient to decide whether a person is smoking. Measurement of thiocyanate, however, may be preferable, because of its longer half-life, in people who show erratic smoking patterns or are making intermittent attempts to stop smoking.

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