Evaluation of methods used to estimate inhaled dose of carbon monoxide

P J REES, CLAIR CHILVERS, AND T J H CLARK

From the Clinical Science Laboratories, Guy's Hospital Medical School, London

ABSTRACT We have compared estimation of alveolar carbon monoxide measured either as end-expired or mixed-expired carbon monoxide tension with two spectrophotometric estimations of venous blood carboxyhaemoglobin (COHb). Estimation of mixed-expired carbon monoxide proved more convenient for patients than the end-expired method, and this estimation of carbon monoxide exposure discriminated between non-smokers and smokers as well as measurements of COHb. The technique is bloodless, economical, and simple to perform. It should prove a valuable method for validating reported smoking habits of people attending smoking advisory centres and for epidemiological surveys of smoking.

The reliability of reported cigarette consumption of subjects attending for follow-up in smoking advisory centres is notoriously poor. Sillett *et al*¹ found that 44 out of 133 such subjects who claimed to have stopped smoking had levels of venous blood carboxyhaemoglobin (COHb) which made their claims doubtful. In order to assess the success rates in such clinics and for other epidemiological work it is desirable to have a simple means of confirming smoking habits.

Carboxyhaemoglobin levels are generally higher in smokers than non-smokers although the ranges overlap. The COHb levels reflects exposure to carbon monoxide in cigarette smoke which is determined by the number and type of cigarette as well as the manner of smoking. It is also raised by exposure to carbon monoxide in ambient air, and its range of removal from the blood is slow and depends in part on the degree of physical activity.

Most reported measurements of COHb have used a venous blood sample and recently have been performed spectrophotometrically on an IL 182 CO-Oximeter. Many workers have found the IL 182 CO-Oximeter unstable² but the technique is simple and applicable to the analysis of large numbers of samples. The earlier spectrophotometric method of COHb estimation described by Commins and Lawther³ requires only a finger prick sample and is more reliable but it is tech-

nically more difficult to perform. Other methods available use gas chromatography⁴ and infrared spectrophotometry.⁵

Jones *et al*⁶ showed that the level of carbon monoxide in the end-expired gas after a breathhold of 20 seconds at total lung capacity correlated well with the venous COHb level. This method requires an apparatus to collect the end-expired gas after discarding its first part. The level of end-expired carbon monoxide is thought to approximate to alveolar carbon monoxide (PACO). Rawbone *et al*⁷ have described an alternative estimation of PACO in which carbon monoxide in mixed-expired gas is measured and an allowance made for estimated dead space. In our experience subjects have found this latter mixed-expired measurement of PACO easier to perform than the earlier end-expired measurement.

We have compared the mixed-expired and end-expired estimations of PACO and have evaluated the mixed-expired method against COHb estimated by the IL 182 CO-Oximeter and by the spectrophotometric method of Tietz and Fioreck⁸ which was the reference method used by Rawbone *et al*⁷ in their original description of the mixed-expired estimation.

Methods

IL 182 CO-OXIMETER

The IL 182 CO-Oximeter was calibrated at the beginning of the study with blood at 0% and 100%

Address for reprint requests: Professor TJH Clark, Respiratory Function Unit, Guy's Hospital, London SE1 9RT.

COHb. Lithium heparin was used as an anti-coagulant and all samples were analysed within one hour of being taken. After the initial calibration a regular check was made that the same non-smokers' blood at the same time of day produced a consistent value.

SPECTROPHOTOMETRY

Samples were analysed on a Beckman spectrophotometer by the method of Tietz and Fiereck.⁸ Blood was haemolysed by dilution with 0.4% ammonium hydroxide and oxyhaemoglobin was reduced with sodium dithionite. The absorbance of the sample was read at 541 nm and 555 nm and the ratio of these two absorbances compared with that for 0% and 100% COHb. Tietz and Fiereck showed that the relationship between the absorbance ratio and the COHb percentage was linear over the entire range and this was used to calculate COHb from the absorbance ratio.

MIXED-EXPIRED ESTIMATION OF ALVEOLAR CARBON MONOXIDE

Alveolar carbon monoxide was estimated by the mixed-expired method using an infrared carbon monoxide analyser (Analytical Development Company Ltd). The subject breathed tidally through a one-way valve with a dry gas meter on the inspiratory side. The expired gas was sampled after passing through a container of soda lime and calcium chloride to absorb carbon dioxide and water vapour. This container also served as a mixing chamber for the expired gas. The subject breathed through the apparatus until a steady level was recorded on the carbon monoxide analyser (usually between 10 and 20 breaths). Mean tidal volume was calculated from the volume of inspired air measured on the dry gas meter and the number of breaths. The concentration of carbon monoxide in alveolar air was calculated from the equation:

$$F_{ACO} = \frac{V_T \times F_{eco} - V_D \times F_{ico}}{V_T - V_D}$$

where F_{ACO} is the fractional concentration of carbon monoxide in alveolar air, F_{ico} the concentration in inspired air, F_{eco} the mixed-expired concentration, V_T the mean tidal volume, and V_D the dead space estimated as 1 ml per lb of ideal body weight. Alveolar carbon monoxide was calculated from the fractional concentration and barometric pressure assuming an alveolar partial pressure of carbon dioxide of 5.32 kPa (40 mmHg). The calibration of the CO meter was checked several times a day with gas mixtures containing 0 ppm CO and 98 ppm CO (PK Morgan Ltd).

END-EXPIRED ESTIMATION OF ALVEOLAR CARBON MONOXIDE

The mixed-expired estimation of PACO was compared with the end-expired estimation of Jones *et al.*⁶ After a 30 second breathhold at total lung capacity the subject performed a maximal expiration. Approximately the first 500 ml of the expired gas was discarded and the carbon monoxide level was measured in the remainder of the expireate.

The first three methods were compared in four non-smokers and 25 smokers using three estimations by each method. These results were used to assess the variation of each method of estimation. The results of 70 estimates of mixed-expired PACO and COHb by CO-Oximeter were used to produce prediction of COHb from PACO.

In a further 28 subjects, composed of 14 smokers and 14 non-smokers, single mixed-expired and end-expired estimations of PACO were compared to study their correlation. Finally 160 subjects were recruited from hospital staff and a smokers' clinic for a comparison to be made between mixed-expired PACO and their reported smoking habits.

Results

COMPARISON OF CO-OXIMETER AND SPECTROPHOTOMETRIC ASSESSMENTS OF COHb WITH MIXED-EXPIRED PACO

Three measurements by each method were made on four non-smokers and 25 smokers, so that in all nine measurements were made on each subject. Analysis of variance was carried out using the data from each measurement method in turn. This separated out variability between subjects (which, as expected, was considerable) from intra-subject variability. The CO-Oximeter and mixed-expired PACO each had a coefficient of variation of 6% compared with 22% for the spectrophotometric method. With the latter method only 80% of total variability was attributable to variability between subjects compared with 97% and 98% for PACO and CO-Oximeter respectively. Therefore, the repeatability of the spectrophotometric method of Tietz and Fiereck⁸ is less than the other methods. When the COHb level of the blood was raised to 40–60% the standard deviation of the spectrophotometric method was slightly less (1.7% COHb cf 1.9% COHb) with a coefficient of variation of 3% which is close to the value of 2.4% found by Tietz and Fiereck at a COHb level of 40%. The repeatability of the CO-Oximeter is comparable to that reported by Mass *et al.*⁵

PREDICTION OF COHb FROM MIXED-EXPIRED PACO
In order to transform the results of the estimation to COHb%, a prediction equation was calculated using regression methods for 70 comparative values from the mixed-expired method and the CO-Oximeter. These results produced the following prediction equation:

$$Y = 3.95x - 0.32x^2 - 2.4$$

where x is PACO in pascals (Pa) and Y is %COHb. The results are shown graphically in fig 1.

COMPARISON OF MIXED-EXPIRED WITH END-EXPIRED PACO

Subjects who were not accustomed to performing physiological measurements sometimes found the end-expired PACO estimation after 30 seconds breathhold difficult to perform. The two methods of obtaining PACO were compared in 28 subjects and were in good agreement (fig 2). The maximum discrepancy was 0.36 Pa (0.003 mmHg) and there was no significant difference between the two estimates using a t test.

MIXED-EXPIRED PACO IN SMOKERS AND NON-SMOKERS

Alveolar carbon monoxide was estimated by mixed-expired method in 161 subjects. Forty-nine

Fig 1 Venous carboxyhaemoglobin (%COHb) estimated by IL 182 CO-Oximeter related to PACO estimated by the mixed-expired method.

Fig 2 PACO estimated by mixed-expired and end-expired methods. Solid line shows the line of identity between the two estimations.

of these were non-smokers whose claim of non-smoking was thought to be reliable. The other 112 smokers were carefully questioned about their current smoking habit and although no account was taken of the time since their last cigarette all had smoked in the previous 24 hours. The values of PACO found in the different groups are shown in fig 3. Our upper limit for non-smokers based on mean +2 SD is 1.57 Pa which is equivalent to 3.0% COHb using our prediction equation. The highest level observed in a non-smoker was 1.64 Pa.

Five out of 14 smokers of 10 or less cigarettes per day had a PACO level of less than 1.57 Pa compared to two out of 59 smokers of 11–20 cigarettes per day, and none of the 39 smokers of more than 20 cigarettes per day. It seems unlikely that PACO of greater than 1.6 Pa will be found in a non-smoker unless they have recently been exposed to high ambient levels of carbon monoxide.

Discussion

The comparison of two estimations of venous carboxyhaemoglobin with the mixed-expired estimation of alveolar carbon monoxide indicates that the spectrophotometric method of Tietz and Fiereck⁸ is too variable for reliable estimation of COHb levels in the 0–12% range found in smokers. When the COHb level was raised to 40% the coefficient of variation approximated to that found by Tietz and Fiereck, indicating that our technique was comparable to that originally described.

Fig 3 PACO in non-smokers and smokers of different numbers of cigarettes. Horizontal bars indicate limits of the ranges.

Despite its problems the IL 182 CO-Oximeter produces results which were more reproducible and the measurement was much simpler to perform. The IL 282 which has now replaced the IL 182 is claimed to be a more stable instrument but it is considerably more expensive at a cost of £7830.

The infrared carbon monoxide analyser used for the estimations of PACO costs less (£2250), and its other attraction is that it does not require a blood sample—the mixed-expired estimation merely requires subjects to breathe normally into a mouth-piece for about one minute. We have found that the PACO obtained by the mixed-expired methods

is in good agreement with that estimated by the end-expired method which has been more widely used.^{6,9} Our results for the relation between PACO and COHb are similar to those found previously and confirm a curved correlation which probably reflects the interference of oxygen with the PACO-COHb relationship when levels of around 10% COHb are reached.

We have used the mixed-expired PACO technique at smokers' clinics in the Guy's Health District and have been impressed by its usefulness. In order to assess the response to treatment in such a clinic it is essential to have an objective assessment to confirm statements of current smoking habit which are often unreliable. We believe that the mixed-expired estimation of PACO is the simplest, reliable method available.

An additional benefit is that a subject who decreases smoking is able to see a decreasing PACO level which provides an added incentive compared with simple respiratory function tests which generally fail to show any change in the short run. Judging by our results a claim to non-smoking in a subject with a PACO level of above 1.6 Pa should be treated with caution. The overlap between smokers and non-smokers is similar to that seen using venous carboxyhaemoglobin levels.¹⁰ The range of COHb levels found in non-smokers varies in different studies. Both Wald *et al*¹¹ and Sillett *et al*¹ found lower ranges for non-smokers but our upper limit is close to that found by Cole¹² in a hospital outpatient department and a London office, and the range is similar to that found by other workers in Finland,¹³ England,¹⁴ and America.¹⁰ The differences reflect technique and subject differences and indicate that each laboratory should establish its own range for non-smokers, and its own prediction equation if equivalent levels of COHb are required.

Although the CO meter requires at least an hour to warm up it is relatively portable. Its variability is similar to the most commonly used method of estimating venous COHb, and its ease of use without the necessity of venepuncture makes it suitable for general epidemiological use as well as monitoring results of advice about smoking.

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