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 al1
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 tech-
nique 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 al6 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 al7
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 Fiereck8 which
was the reference method used by Rawbone et al7
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%
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 spectro-
photometer by the method of Tietz and Fiereck. Blood was haemolysed by dilution with 0.4%
ammonium hydroxide and oxyhaemoglobin was
reduced with sodium dithionate. 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 concentra-
tion 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 cal-
culated 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 com-
pared with the end-expired estimation of Jones et
al. After a 30 second breathhold at total lung
capacity the subject performed a maximal ex-
piration. Approximately the first 500 ml of the
expired gas was discarded and the carbon
monoxide level was measured in the remainder of
the expire.

The first three methods were compared in four
non-smokers and 25 smokers using three esti-
mations by each method. These results were used
to assess the variation of each method of esti-
mation. 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 Fiereck is less than the other methods.
When the COHb level of the blood was raised to
40-60% the standard deviation of the spectro-
photometric 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.
Evaluation of methods used to estimate inhaled dose of carbon monoxide

**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 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 \( \pm 2 \) 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 Fiereck 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.
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 \(\text{PACO}\) and \(\text{COHb}\) are similar to those found previously and confirm a curved correlation which probably reflects the interference of oxygen with the \(\text{PACO}:\text{COHb}\) relationship when levels of around 10\% \(\text{COHb}\) are reached.

We have used the mixed-expired \(\text{PACO}\) 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 \(\text{PACO}\) is the simplest, reliable method available.

An additional benefit is that a subject who decreases smoking is able to see a decreasing \(\text{PACO}\) 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 \(\text{PACO}\) 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 \(\text{COHb}\) 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 Cole\(^12\) 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 \(\text{COHb}\) 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 \(\text{COHb}\), and its ease of use without the necessity of venipuncture makes it suitable for general epidemiological use as well as monitoring results of advice about smoking.

We thank Imperial Tobacco Ltd for support.

References

Evaluation of methods used to estimate inhaled dose of carbon monoxide

Evaluation of methods used to estimate inhaled dose of carbon monoxide.
P J Rees, C Chilvers and T J Clark

Thorax 1980 35: 47-51
doi: 10.1136/thx.35.1.47

Updated information and services can be found at:
http://thorax.bmj.com/content/35/1/47

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/