Validation of the end-expired method for measuring carboxyhaemoglobin levels for the use in occupational and environmental exposure studies

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Carbon monoxide is one of the most common toxins encountered in work settings, the gas being emitted in situations where there is incomplete combustion of carbon-containing substances. Its acute and chronic health effects have been well-documented. While identification of dangerous situations and evaluation of control measures are conducted by environmental monitoring, the body burden due to inhalation of carbon monoxide is measured by an individual's blood carboxyhaemoglobin level. Carboxyhaemoglobin level can be measured directly from a blood sample or, indirectly, by measuring the end-expired carbon monoxide level and using the charts provided to read the corresponding carboxyhaemoglobin level. As the end-expired method is not an intervention method, and is therefore easy to conduct, it is being used widely in epidemiological studies and it could also be used for individual measurements. This study presents a better statistical method for validating the end-expired method than the correlation method used and described in previous studies.

Key words: Carbon monoxide; carboxyhaemoglobin; end-expired method; epidemiological studies; validation.

INTRODUCTION

Some of the previous studies measuring the carboxyhaemoglobin (COHb) levels of workers and smokers have used the end-expired method, i.e., measuring the CO level in the expired air, to evaluate the blood COHb levels. These studies have used the correlation coefficient (R) as the statistic to denote the degree of agreement between the COHb readings obtained by the two methods of measurements, i.e., the direct readings using blood and the indirect readings using end-expired air. However, this method of demonstrating the agreement between two different methods of measurements has been criticized for the following reasons.

- The correlation coefficient measures the strength of the association; i.e., relationship between two variables and not the agreement between them.
- A change in the scale of measurement does not affect the correlation, but it certainly affects the agreement.
- Correlation depends on the true quantity in the sample. If this is wide, the correlation will be greater than if it is narrow. Since investigators usually try to compare two methods over the whole range of values typically encountered, a high correlation is guaranteed.
- The test of significance may show that the two methods are related, but it would be surprising if two methods designed to measure the same quantity were not related. The test of significance is irrelevant to the question of agreement.
- Data which seem to be in poor agreement can produce quite high correlations.

As such, we have, in a study measuring COHb levels using the two methods mentioned, also used an alternative statistical analysis to examine the agreement between the readings, designed by Altman and Bland.
It is most unlikely that different methods of measuring a biological variant will agree exactly, and give an identical result for all individuals studied. Some factors which are responsible for differences in readings are due to errors in precision and accuracy. Precision, and related concepts of reliability and consistency, are affected by random error. Three main sources of errors are observer variability, subject variability and instrument variability. Accuracy is a function of systematic error or bias. Three main classes of measurement error noted are observer bias, subject bias and the instrument bias. As far as the instrument bias is concerned, a systematic error could arise from one or both types of instruments. Further, biological measurements of parameters in different compartments may give varying results in certain circumstances, e.g., interpretation of blood glucose and urinary glucose in relation to diabetes. In this instance an increase in urinary levels may be secondary to renal leakage in tubular dysfunction rather than a reflection of changes in blood sugar. In the current situation of interest, however, the assumption is that the lung function is normal to facilitate a free diffusion of CO between the lungs and the vascular compartment. Therefore, the aim of this study was to find out by how much the COHb level read by the end-expired method was likely to differ from the direct measurement using blood samples. If the disagreement was not enough to cause problems in clinical interpretations, the end-expired method was then considered acceptable to measure the COHb levels in epidemiological studies or in an individual person.

To examine the agreement between two methods of measuring the same value, a scatter plot of the difference in readings between the methods against the mean of the two readings has been considered as a more informative approach. It would be a mistake to plot the difference between the methods against either value separately because the difference will be related to each, a well-known statistical artefact. In the method used, agreement is examined by calculating the bias of lack of agreement, i.e., by estimating the mean difference \( (d) \) and the standard deviation \( (sd) \) of the differences. If the differences are normally distributed, as they usually are, 95% of the differences will be within a distance of 2 Sds (+0.4) (termed 'limits of agreement') from the mean of the differences. This meant that 95% of

CO levels in the samples were measured using the end-expired CO measuring instrument, a Model 211 CO Monitor, called an 'Ecolyser' by the manufacturer. The corresponding carboxyhaemoglobin level in the blood was then read from the chart provided with the instrument. Blood samples from these patients, taken at the same time, were used to measure their blood COHb levels directly, using an IL 482 CO-Oximeter. The paired measurements (COHb from the blood sample and COHb read from chart) recorded were then statistically analysed to estimate the correlation coefficient. The distribution of the differences between the readings around the mean of the differences, the method of interest described in the introductory paragraph, was examined by plotting the differences between the two COHb readings against the mean COHb values, i.e., the mean of the two readings (Figure 2). The differences in readings were normally distributed.

RESULTS

Figure 1 demonstrates a strong association between COHb values read indirectly from end-expired CO levels and the blood COHb levels, with a correlation coefficient \( (R) \) of 0.99 (i.e., a coefficient of determination of 0.98). This result is in agreement with results of similar validations conducted in other studies mentioned in the introductory paragraph.

Figure 2 demonstrates the distribution of the differences between the readings around the mean of the differences in readings. Ninety-five per cent of the differences between the readings were within a distance of 2 Sds (+0.4) (termed 'limits of agreement') from the mean (0.04) of the differences. This meant that 95% of

MATERIALS AND METHODS

With approval from the Ethics Committees of the Royal Adelaide Hospital and the University of Adelaide, 20 volunteer outdoor patients (sample size obtained using standard tables) presenting at the Institute of Medical and Veterinary Studies (IMVS) in Adelaide, Australia, for blood tests were requested to blow air at the end of an expiration (end-expired air) into rubber balloons. The
the readings were only 0.40% COHb away from the mean value while 90% of the values only differed by a value of less than 0.30% COHb.

CONCLUSION

The differences between the readings obtained from the two methods used to measure COHb were clinically acceptable for the purposes of epidemiological studies. Further, the maximum difference between the readings recorded in this study was 0.44, i.e., in less than 5% of the study sample. Such a difference is also acceptable for individual measurements in clinical practice where the main reason for use is usually in cases of CO poisoning; at higher COHb levels such a small difference would not alter the method of treatment. However, it must be noted that most patients admitted with CO poisoning are not in a state to blow a sample of expired air into a balloon, and thus taking a sample of blood is the only method by which the carboxyhaemoglobin level can be measured in such a situation. The end-expired method can be also be used to measure moderate and low COHb levels in individuals as suggested by Galvin et al. We conclude that (1) the statistical method suggested by Altman and Bland is a better way of determining the agreement between the COHb readings obtained by the end-expired method and the COHb levels read in blood and (2) the end-expired method can be used to measure the blood COHb level in epidemiological studies and in individuals who are, clinically, in a state to blow a sample of expired air into a collecting device.

REFERENCES
