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ORIGINAL ARTICLE

Comparing measured total carbon dioxide and calculated bicarbonate

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Abstract

Introduction: The objective of the study is to determine the level of agreement between measured total carbon dioxide (TCO_2) and calculated bicarbonate (HCO_3^-) in our laboratory. *Materials and Methods:* TCO_2 and HCO_3^- values of 1820 samples drawn at the same time from the patient were compared. TCO_2 from venous samples was measured on Dimension RxL while HCO_3^- was obtained from arterial blood gas samples analyzed on Radiometer ABL 700. *Results:* The TCO_2 and HCO_3^- values correlated well ($r = 0.977$, $p<0.001$), with the correlation given by the equation, $y = 0.986x – 0.5335$. Using Bland-Altman analysis, the bias was 0.87 mmol/L (SD 1.42 mmol/L), and the limits of agreement (LOA) were -1.92 to 3.67 mmol/L. Story and Poustie's criteria were applied to study the agreement between these two methods. Based on the first criterion that the bias between TCO_2 and HCO_3^- should be less than ±1 mmol/L, the results for the two methods appear to be in good agreement. The second criterion requires that the LOA between the two methods should range between a bias of ± 2 mmol/L or a total span of 4 mmol/L; the LOA was exceeded in our study. Using the total allowable error in the Bland Altman plot also showed that the two values cannot be used interchangeably especially at the lower values. *Conclusions:* TCO_2 did not show good agreement with HCO_3^-. Clinicians should be aware of this discrepancy and hence should be cautious when using HCO_3^- for management of acid-base disorders.

Keywords: measured total carbon dioxide, calculated bicarbonate

INTRODUCTION

Bicarbonate concentration [HCO_3^-] is an analyte that is widely used to assess the acid-base status of patients, the values of which can be directly measured or derived from calculations using the Henderson-Hasselbalch equation. Bicarbonate ions make up ~ 95% of the total carbon dioxide of the plasma,[1] and hence both of them have been used interchangeably.

Most blood gas analyzers use the Henderson-Hasselbach equation to calculate bicarbonate values based on the assumption that the dissociation constant (pK′) and solubility coefficient (α) are invariant. However, pK′ is affected by changes in pH, ionic strength and temperature, while the values of α varies with the composition of the solution such as the presence of increased salts, proteins or lipids.[1] Therefore, the calculated bicarbonate values may have significant error under certain circumstances, making its reliability questionable.

Previous studies using different statistical methods to assess the agreement between measured and calculated bicarbonate have shown conflicting results, with some studies showing good agreement,[2-5] while some studies showed otherwise.[6-10] The objective of this study was to compare measured total carbon dioxide (TCO_2) and calculated bicarbonate (HCO_3^-) to assess the degree of agreement between these two values and hence whether they can be used interchangeably in our laboratory setting.

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MATERIAL AND METHODS

We examined 1820 records of measured total carbon dioxide (TCO_2) and calculated bicarbonate (HCO_3^-), drawn simultaneously from the same patient, between January and June 2008.

TCO_2 was obtained from venous samples analyzed on Dimension RxL (Dade Behring, Inc, Newark, DE, USA), which measures TCO_2 using an indirect potentiometry method. The principle is based on the release of carbon dioxide (CO_2) from acidified samples followed by measurement of the resultant pH change by the pH electrode, the assumption being that the pH change is proportional to the amount of TCO_2 in the sample.

HCO_3^- values were obtained from arterial blood gas samples analyzed on ABL 700 (Radiometer, Copenhagen, Denmark), which calculated HCO_3 from measured pH and pCO_2 values according to the Henderson-Hasselbalch equation:

$$pH = pK' + \log [HCO_3 / (\alpha \cdot pCO_2)]$$

where pK′ is the dissociation constant for carbonic acid (equal to 6.103 for blood at 37°C), and α is the solubility coefficient for CO_2 gas (equal to 0.0306 for plasma at 37°C).

Statistical analysis using least squares linear regression, correlation coefficient and Bland-Altman analysis were performed and Story and Poustie's criteria[6] were applied to evaluate the agreement between TCO_2 and HCO_3^-.

For the Bland-Altman analysis, the averages of TCO_2 and HCO_3^- were calculated and plotted on the x-axis, and the differences between TCO_2 and HCO_3^- (in mmol/L and percentage) were calculated and plotted on the y-axis. The upper and lower limits of agreement (LOA) were calculated from bias ± 1.96 SD. The span was calculated from the high limit and low limit values.

To determine whether the TCO_2 and HCO_3^- methods were clinically equivalent, we used Two Instrument Comparison (2IC) from the EP Evaluator software. Two methods are deemed clinically equivalent if the difference between them is less than the allowable error. We defined our allowable total error (TEa) for bicarbonate as 10%, based on The Royal College of Pathologists of Australasia (RCPA) Quality Assurance Program allowable limits of performance.[11] Error Index was calculated as the ratio of the difference (y-x) to allowable total error. An error index >1.00 or <-1.00 is considered unacceptable.

RESULTS

TCO_2 results ranged from 3 – 53 mmol/L (mean 21.32 mmol/L), while HCO_3^- ranged from 3 – 51 mmol/L (mean 20.44 mmol/L). The values of TCO_2 and HCO_3^- correlated well (r = 0.977, p<0.001), with the correlation given by the equation, y = 0.986x – 0.5335, standard error of estimate ($S_{y/x}$) of 1.422 (Figure 1).

Using Bland-Altman analysis, when the differences between TCO_2 and HCO_3^- in mmol/L were plotted against the average, the bias obtained was 0.87 mmol/L (SD 1.42 mmol/L),

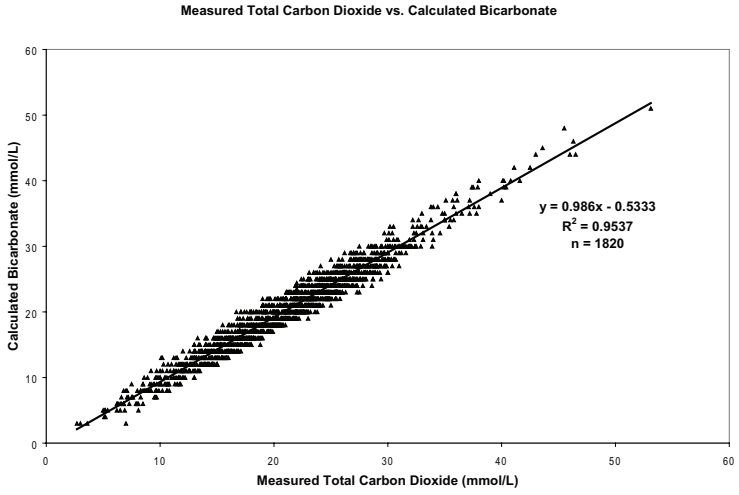

FIG. 1: Scatter plot of TCO_2 (analyzed with Dade Behring Dimension RxL) vs. HCO_3^- (analyzed with Radiometer ABL 700)

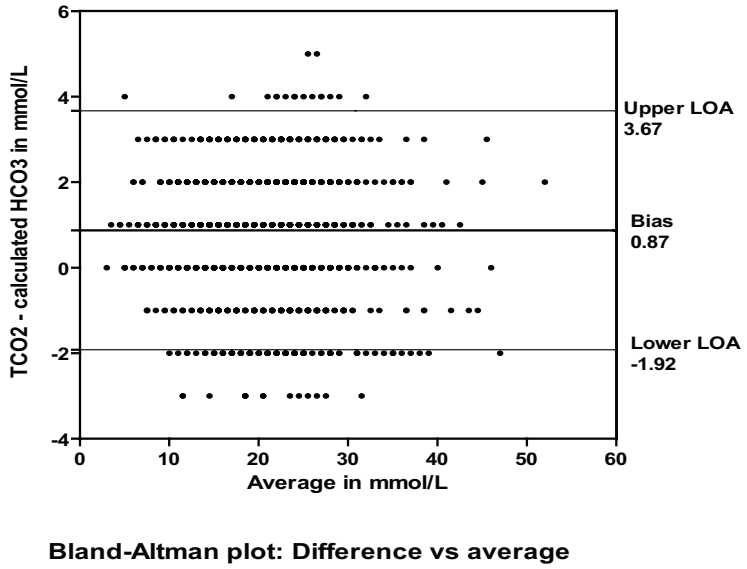

FIG. 2: Bland-Altman plot of average of TCO_2 and HCO_3^- vs. the difference between the two methods in mmol/L. LOA = limit of agreement

and the limits of agreement (LOA) were -1.92 to 3.67 mmol/L, with a span of 5.59 mmol/L. Out of the1820 values, 1738 (95.49%) were within the LOA (Figure 2).

Bland-Altman plot using differences between TCO_2 and HCO_3^- in percentage against the average revealed a bias of 4.8% (SD 8.19%), and LOA of -11.25 to 20.85%. We found that majority of the values that fell outside the LOA were for bicarbonate concentration ≤20 mmol/L (Figure 3).

Based on TEa of 10% and using 2IC, the difference between TCO_2 and HCO_3^- was within the TEa for 75.5% of the results (1375 out of 1820). The average error index, [(y-x)/TEa] was -0.44, with a range of

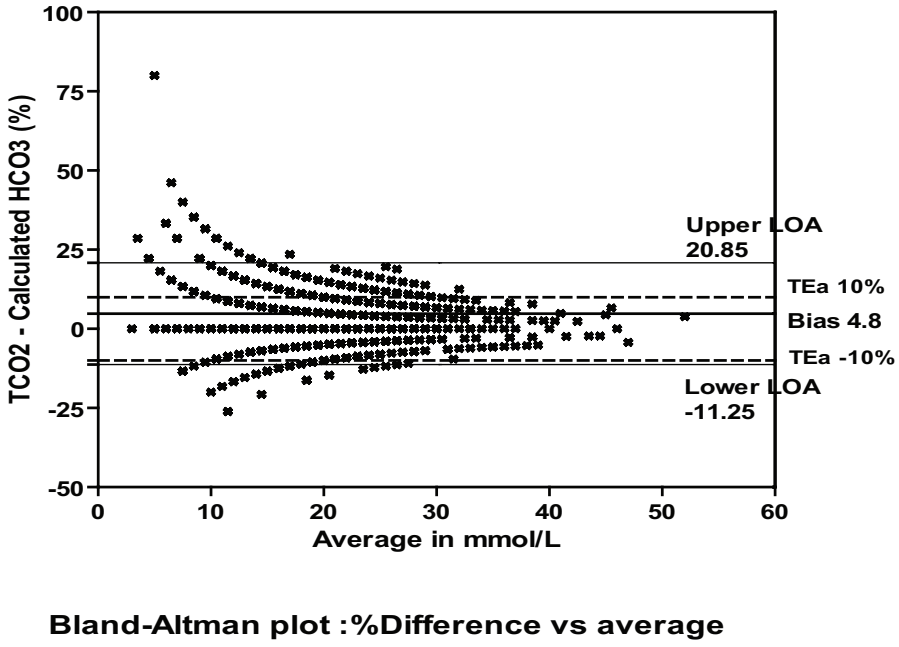

FIG. 3: Bland-Altman plot of average of TCO_2 and HCO_3^- vs. the difference between the two methods in percentage. LOA = limit of agreement, TEa = total allowable error

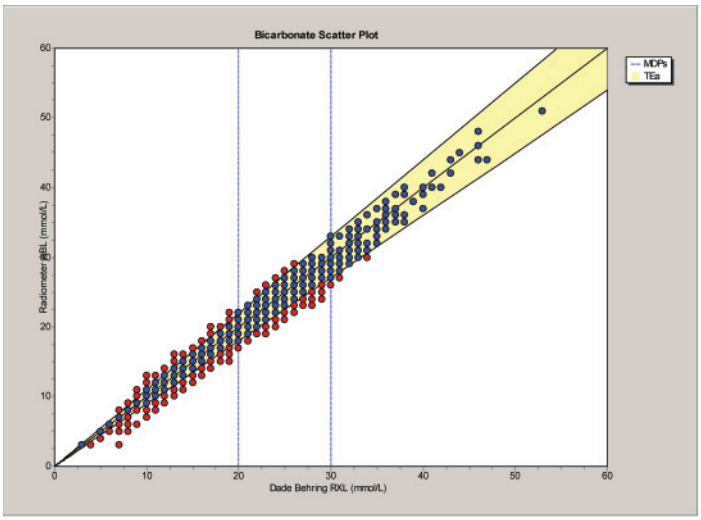

FIG. 4: Scatter plot showing TCO_2 (measured with Dade Behring RXL) vs. HCO_3^- (measured with Radiometer ABL 700) with TEa of 10%. MDP = medical decision points, TEa = total allowable error

-5.71 to 3.00. We found that most of the unacceptable error indexes were clustered at bicarbonate concentration ≤ 20 mmol/L and the largest error index occurred at bicarbonate concentration of 7 mmol/L (Figures 4 and 5).

DISCUSSION

The agreement or discrepancy between measured and calculated bicarbonate and whether both can be used interchangeably has long been discussed since the 80's,[4-10] without any concrete conclusion. With the advancement in the methodology, this issue seemed to have resurfaced, with a couple of articles written on it in the year 2008.[2,3]

The correlation coefficient between the measured and calculated bicarbonate revealed a good correlation ($r = 0.977$, $p<0.001$). However, the use of correlation coefficient alone to assess the agreement between two methods may not be appropriate, as correlation depends on the range of values in the sample; a wide range of values like ours will yield a high correlation

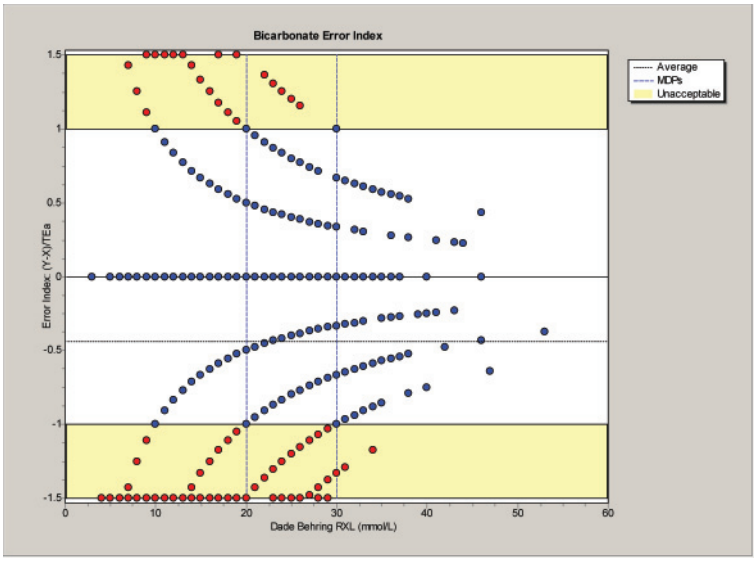

FIG. 5: Bicarbonate error index plot, showing most of the unacceptable error index occurring at bicarbonate concentration ≤ 20 mmol/L. MDP = medical decision points

coefficient. Therefore, values which seem to be in poor agreement can produce high correlations provided the range is wide enough.

Using Bland-Altman analysis, the bias obtained was 0.87 mmol/L (SD 1.42 mmol/L), and the LOA were -1.92 to 3.67 mmol/L, with a span of 5.59 mmol/L. Only 82 (4.51%) out of 1820 values fell outside the LOA. At a glance, it looks like HCO_3^- can be used interchangeably with TCO_2. Story and Poustie[6] proposed two criteria to assess the agreement derived for two different methods for bicarbonate using Bland-Altman analysis. They were i) the difference in means (bias) between TCO_2 and HCO_3^- should be less than ±1 mmol/L; ii) the LOA between the methods should range within a bias ±2 mmol/L or a total span of 4 mmol/L, to be clinically unimportant. Our results fulfilled the first but not the second criterion. Despite the excellent correlation, our findings did not show good agreement between TCO_2 and HCO_3^- when Story and Poustie's criteria were applied.

Stöckl *et al* demonstrated that the incorporation of confidence limits and predefined error limits in a Bland–Altman plot allowed easy visual interpretation of a method comparison study. The upper or lower 95% confidence limit of 1.96 SD of the differences between the methods (or LOA) should be equal to or smaller than a predefined limit for total error to be accepted.[12] We found that both our upper and lower limits of agreement itself exceeded the allowable total error (TEa) of 10%. The poor agreement between the two methods was also revealed by two instrument comparison (2IC) and especially at a lower bicarbonate concentration (error index >1).

Pre-analytical and analytical factors such as sample collection and handling, analytical imprecision and calibration errors may affect the degree of agreement between TCO_2 and HCO_3^-.[6,8] Inadequate mixing, acidification and dilution of arterial blood gas samples with excess heparin can decrease the pH and pCO_2, which will affect the values of HCO_3^-. Maintaining an anaerobic condition is important in sample processing for bicarbonate concentration analysis. The high volume processing and automated analysis used to measure TCO_2 may cause some dissolved gaseous carbon dioxide to be lost from the specimen, as preservation of anaerobic conditions are not practical between the time the specimen is placed on the instrument and the time it is sampled[1]. As arterial blood gas samples are usually considered as urgent specimens, they are rapidly processed and analyzed; therefore, the anaerobic conditions tend to be better preserved leading to smaller errors in HCO_3^-. The poor agreement between TCO_2 and HCO_3^- also could be due to the different sample types compared (arterial vs. venous). pH is 0.02-0.05 pH units lower and pCO2 is 2-8 mmHg higher in the venous blood when compared to arterial blood[1]. Both of these parameters are used to calculate HCO_3^-. Theoretically, there will be some difference of uncertain significance in the values of bicarbonate in arterial and venous blood. Ungerer et al mentioned that arterial and venous acid-base analytes can differ markedly in certain clinical conditions, with the difference in calculated bicarbonate being ~2 mmol/L on average.[9] The arterio-venous differences widened in patients with cardiac and circulatory failure, and those with cardiac arrest resuscitated by cardiopulmonary resuscitation and mechanically ventilated.[13,14] However, a few studies have stated otherwise, that venous bicarbonate estimation showed high level of agreement with arterial bicarbonate and can be used as a reliable substitute for arterial bicarbonate.[15-17]

The discrepancy between TCO_2 and HCO_3^- could be attributed to the variability of pK′ in the Henderson-Hasselbalch equation, which was used to calculate HCO_3^-. Flear *et al* found that pK′ values varied by considerably more than 0.06 in healthy volunteers and in very ill patients, and HCO_3^- calculated based on pK′ values of 6.1 could be in error by some ± 60%.[18] A study by O'Leary and Langton found a significant decrease of the pK′ in patients considered to have metabolic acidosis when compared to patients with bicarbonate concentration within the reference interval.[10] Our findings that majority of the values that fell outside the LOA and most of the unacceptable error indexes occurred at bicarbonate concentration ≤20 mmol/L seem to agree with O'Leary and Langton.

In locations where disturbances of acid-base status are common, such as the intensive care unit (ICU) and neonatal intensive care units (NICU), frequent and rapid blood gas analysis is needed. Clinicians may prefer to use the results of HCO_3^- from the automated blood gas analyzer, which can be placed in the ICU itself to facilitate the management of acid-base imbalances, rather than sending for TCO_2 that is measured in the laboratory. The finding of poor agreement between TCO_2 and HCO_3^- may have an impact on the utility of HCO_3^- by the clinicians, especially in patients with metabolic

acidosis. Parameters that are calculated using bicarbonate concentration such as base excess and anion gap may also be affected by this discrepancy.

CONCLUSION

Despite the excellent correlation, TCO_2 did not show good agreement with HCO_3^- when Story and Poustie's criteria were applied. Therefore, clinicians should be advised of this discrepancy and be cautious when using TCO_2 and HCO_3^- interchangeably in the assessment and management of acid base disorders, especially in patients with metabolic acidosis.

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